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REVIEW

Progress report on new antiepileptic drugs: A summary of the Twelfth Eilat Conference (EILAT XII)

Meir Bialer[a,*], **Svein I. Johannessen**[b,c], **René H. Levy**[d,e], **Emilio Perucca**[f,g], **Torbjörn Tomson**[h], **H. Steve White**[i]

[a] *Institute for Drug Research, School of Pharmacy and David R. Bloom Centre for Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel*
[b] *The National Center for Epilepsy, Sandvika, Oslo University Hospital, Oslo, Norway*
[c] *Department of Pharmacology, Oslo University Hospital, Oslo, Norway*
[d] *Department of Pharmaceutics, University of Washington, Seattle, WA, USA*
[e] *Department of Neurological Surgery, University of Washington, Seattle, WA, USA*
[f] *Division of Clinical and Experimental Pharmacology, Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Italy*
[g] *C. Mondino National Neurological Institute, Pavia, Italy*
[h] *Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden*
[i] *Anticonvulsant Drug Development Program and Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT, USA*

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Summary The Twelfth Eilat Conference on New Antiepileptic Drugs (AEDs) — EILAT XII, took place in Madrid, Spain from August 31st to September 3rd 2014. About 130 basic scientists, clinical pharmacologists and neurologists from 22 countries attended the conference, whose main themes included ''Conquering pharmacoresistant epilepsy'', ''Innovative emergency treatments'', ''Progress report on second-generation treatment'' and ''New methods and formulations''. Consistent with previous formats of this conference, a large part of the program was devoted to a review of AEDs in development, as well as updates on AEDs introduced since 2004. Like the EILAT X and EILAT XI reports, the current article focuses on the preclinical and clinical pharmacology of AEDs that are currently in development. These include adenosine-releasing silk, allopregnanolone (SAGE-547), AMP-X-0079, brivaracetam, bumetanide, cannabidiol, cannabidivarin, 2-deoxy-glucose, everolimus, ganaxolone, huperzine A, imepitoin, minocycline, NAX 801-2, pitolisant, PRX 0023, SAGE-217, valnoctamide and its

* Corresponding author at: Institute for Drug Research, School of Pharmacy and David R. Bloom Centre for Pharmacy, Faculty of Medicine, Ein Karem, The Hebrew University of Jerusalem, 91120 Jerusalem, Israel. Tel.: +972 2 6758610; fax: +972 2 6757246.
E-mail address: bialer@md.huji.ac.il (M. Bialer).

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homologue *sec*-butyl-propylacetamide (SPD), and VLB-01. Since the previous Eilat conference, perampanel has been introduced into the market and twelve novel potential epilepsy treatments are presented for the first time.

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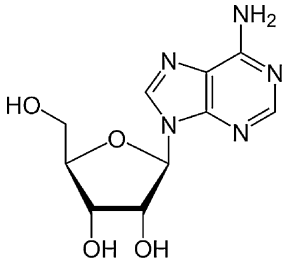
Adenosine-Releasing Silk

D. Boison

Robert Stone Dow Neurobiology Labs, Legacy Research Institute, Portland, OR, USA



Adenosine

Introduction and rationale for development

Adenosine is an endogenous anticonvulsant responsible for seizure arrest and postictal refractoriness (Boison, 2007). However, maladaptive changes in adenosine homeostasis occur during epileptogenesis and induce a deficiency in adenosine — a characteristic pathological hallmark of human temporal lobe epilepsy (Aronica et al., 2013). In addition, reduced adenosine drives epigenetic changes thought to be instrumental in epileptogenesis (Williams-Karnesky et al., 2013). Therefore, adenosine augmentation represents a rational approach to prevent seizures and the progression of epilepsy (Boison, 2012). Systemic, largely cardiovascular, side effects of adenosine require a local mode of adenosine delivery. To deliver a defined dose of adenosine with a known release kinetics locally to the brain, a silk biopolymer was used as drug delivery system. Purified silk fibroin presents a unique option for therapeutic adenosine delivery as it is biocompatible and biodegrades slowly. Degradation kinetics can be regulated to allow control of release from weeks to years. Both silk as well as adenosine are already FDA-approved for different indications and the frequent use of silk sutures in brain confirms the feasibility of implanting silk biomaterials into brain.

Pharmacology

Adenosine acts as endogenous ligand of four types of G protein coupled adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3) (Chen et al., 2013). In addition, adenosine provides biochemical feedback inhibition of DNA methylation (Williams-Karnesky et al., 2013). Adenosine homeostasis in the brain is largely

under the control of metabolic clearance through adenosine kinase (ADK) expression in astrocytes (Boison, 2013).

Anticonvulsant profile

Demonstration of the therapeutic efficacy of adenosine augmentation therapy (AAT) has been documented in at least 15 published research studies in two different species (mice and rats), and in four different models of epilepsy (mouse intrahippocampal and intraamygdaloid kainic acid (KA) model; rat hippocampal kindling model; rat systemic KA model) (Boison, 2012). In all four models robust seizure suppression or prevention in the absence of discernible side effects was achieved by focal AAT mediated by: (i) intraventricular implants of adenosine releasing polymeric devices; (ii) intraventricular or infrahippocampal implants of cells engineered to release adenosine; and (iii) AAV-based gene therapy designed to reduce ADK expression in astrocytes using antisense technology. Therapeutic efficacy has been demonstrated in a combined total of >150 different experimental epileptic animals. Dose response studies have shown that intraventricular doses of 50—500 ng adenosine per kg body weight per day provide effective seizure suppression in rodents, whereas doses of up to 5000 ng adenosine per kg per day were without any discernible side effects. To circumvent systemic side effects of global adenosine augmentation, a local (epileptogenic region) therapeutic approach becomes a necessity to harness the powerful anti-seizure potential of adenosine in the absence of side effects. Local AAT affords reliable bioavailability of adenosine within the epileptogenic brain region. AAT effectively suppressed seizures in three different models of epilepsy (Tables 1 and 2):

Mouse intra-hippocampal KA model: Pharmacological augmentation of adenosine signaling (ADK inhibitor or adenosine A_1R agonist) completely (0/1900sz) prevented seizures that were refractory to carbamazepine.
Mouse intra-amygdaloid KA model: Infra-hippocampal implants of adenosine-releasing mouse embryonic stem cell-derived neural precursor cells (releasing 20—40 ng adenosine per day) completely (0/400sz) prevented the development of seizures when cells were implanted 24 h after the SE and animals were assessed for spontaneous seizures at 3 weeks.
Rat kindling model: Adenosine releasing brain implants (polymers, encapsulated cells, stem cell derived implants) solidly suppressed epileptic seizures and retarded kindling epileptogenesis. Those studies demonstrated that the local paracrine release of adenosine resulting in adenosine concentrations <25 nM at the site of action is sufficient to suppress fully kindled seizures.

Antiictogenic and antiepileptogenic effects of silk-based adenosine delivery

In an effort to develop an AAT compatible with future clinical application, we developed a novel silk protein-based release system for adenosine (Dr. David Kaplan, Tufts University, Medford, MA). Adenosine releasing implants with target release doses of 0, 40, 200, and 1000 ng adenosine per day were prepared by embedding adenosine containing microspheres into nanofilm-coated silk fibroin scaffolds. *In vitro*, the respective polymers released 0, 33, 171, and 819 ng adenosine per day over 14 days. The therapeutic potential of the implants was validated in a dose—response study in the rat model of kindling epileptogenesis. Four days prior to the onset of kindling, adenosine releasing polymers were implanted into the infra-hippocampal fissure and progressive acquisition of kindled seizures was monitored over a total of 48 stimulations. We documented a dose-dependent retardation of seizure acquisition. In recipients of polymers releasing 819 ng each day, kindling epileptogenesis was delayed by 1 week (18 kindling stimulations). Our dose—response studies allowed us to estimate the minimal effective dose to be in the range of 50—200 ng adenosine per day (Wilz et al., 2008). Importantly, high doses of adenosine (>2000 ng adenosine per day) were well tolerated, without any gross behavioral changes of the animals. To study *antiepileptogenic* properties of adenosine, we used silk-based polymers designed to release 1000 ng adenosine per day during a limited time span of 10 days. When transplanted into the brain ventricle of fully kindled rats those implants completely suppressed kindled seizures only during the time span of active adenosine release. After expiration of adenosine release from the polymers seizures gradually resumed. When rats were kindled in the presence of adenosine releasing silk, seizure development was suppressed. Importantly, following a washout period of adenosine from the silk, kindling stimulations did not trigger any generalized seizures, indicating a novel antiepileptogenic effect of focal adenosine delivery (Szybala et al., 2009). To further substantiate the antiepileptogenic effect of transient adenosine release, we implanted silk-polymers releasing 250 ng adenosine/ventricle/day into the brain ventricles of epileptic rats 9 weeks after the systemic administration of KA. During active adenosine release (restricted to 10 days) the incidence of spontaneous recurrent seizures was markedly suppressed (by ~75%) in epileptic rats. Furthermore, this transient therapeutic intervention reversed the DNA hypermethylation seen in the epileptic brain, inhibited sprouting of mossy fibers in the hippocampus, and prevented the progression of epilepsy for at least 3 months. Thus, pathological changes in DNA methylation homeostasis may underlie epileptogenesis and reversal of these epigenetic changes with AAT may halt disease progression longterm (Williams-Karnesky et al., 2013).

Other pharmacological properties

In addition to seizure suppression and prevention, adenosine augmentation has neuroprotective, antipsychotic and pro-cognitive properties (Boison, 2013; Shen et al., 2012).

Mechanism(s) of action

Antiictogenic mechanisms

Binding of adenosine to pre- and postsynaptic A_1 receptors (K_i: 77 nM; high expression levels in the limbic system) inhibits adenylyl cyclase activity, activates potassium channels, blocks transient calcium channels and increases intracellular calcium and inositol-1,4,5-trisphosphate levels by activating phospholipase C (Table 3). Through these mechanisms A_1 receptors block transmitter release and reduce the neuronal firing rate (Chen et al., 2013).

Antiepileptogenic mechanisms
Adenosine is an obligatory metabolic endproduct of transmethylation reactions, which include DNA methylation. Increased adenosine shifts the S-adenosylhomocysteine (SAH) hydrolase reaction towards increased SAH production, which inhibits DNA methyltransferases through product inhibition (Williams-Karnesky et al., 2013). Transient therapeutic adenosine augmentation reduces DNA methylation status of the epileptic rodent brain and prevents disease progression long term through this epigenetic mechanism.

Toxicology

Adenosine is an endogenous nucleoside that occurs in all cells of the body and is subject to endogenous metabolic clearance mechanisms. Adenosine is FDA-approved for the treatment of supraventricular tachycardia and available commercially in a preservative-free formulation (3 mg/mL) for intravenous use. In addition, the safety of intrathecal (IT) adenosine in humans has been addressed: In a preclinical toxicity screening no side effects were observed in dogs with IT adenosine at 10 μL/kg/h (2.4 mg/day for 48 h, then 7.2 mg/day for 26 days). In reports using IT adenosine agonists for the treatment of neuropathic pain in humans, investigators demonstrated statistically significant reduction of neuropathic pain and allodynia by IT injection of the adenosine agonist R-PIA. No adverse effects were reported. In a Phase I clinical safety study 1 mg adenosine was injected IT in 12 volunteers with neuropathic pain. No adverse effects were noticed. Additional Phase I studies demonstrated the general safety of intrathecal adenosine administration in concentrations of up to 2 mg.

Pharmacokinetics and metabolic profile

Intravenously administered adenosine, in concentrations used for the treatment of supraventricular tachycardia in humans, is rapidly cleared from the circulation *via* cellular uptake, primarily by erythrocytes and vascular endothelial cells. Intracellular adenosine is rapidly metabolized either *via* phosphorylation to AMP by ADK, or *via* deamination to inosine by adenosine deaminase (ADA) in the cytosol (Boison, 2013). Since ADK has a lower K_m and V_{max} than ADA, deamination plays a significant role only when cytosolic adenosine saturates the phosphorylation pathway. Inosine formed by deamination of adenosine can leave the cell intact or can be degraded to hypoxanthine, xanthine, and finally uric acid. AMP formed by phosphorylation of adenosine is incorporated into the high-energy phosphate pool. In the brain, adenosine clearance is largely mediated by ADK activity (Boison, 2013). Extracellular adenosine is primarily cleared by cellular uptake with a half-life of less than 10 s in whole blood and less than 10 min in brain. Excessive amounts of adenosine can be deaminated by adenosine ecto-deaminase (ectoADA). Adenosine requires no hepatic or renal function for its activation or inactivation, and hepatic and renal failure would not be expected to alter its effectiveness or tolerability.

Drug interactions

The methylxanthines theophylline and caffeine competitively antagonize adenosine's effects on adenosine receptors. Dipyridamole potentiates the action of adenosine.

Efficacy data

Efficacy data of adenosine releasing silk are available from rodent models of epilepsy as described above. It is estimated that the minimally effective dose of adenosine delivered by a local brain implant is in the range of 50—500 ng adenosine per kg per day.

Tolerability and adverse effect profile

Adverse effects of *intravenous* adenosine (6 mg given as a rapid intravenous bolus administered over a 1—2 s period) in adult subjects for the treatment of supraventricular tachycardia have been found to affect a variety of systems. Cardiovascular effects included facial flushing (18%), headache (2%), sweating, palpitations, chest pain, hypotension (less than 1%). Respiratory effects included shortness of breath/dyspnea (12%), chest pressure (7%), hyperventilation, head pressure (less than 1%). Central nervous system (CNS) effects included lightheadedness (2%), dizziness, tingling in arms, numbness (1%), apprehension, blurred vision, burning sensation, heaviness in arms, neck and back pain (less than 1%). Gastrointestinal effects were nausea (3%), metallic taste, tightness in throat, pressure in groin (less than 1%). The local application of adenosine in the brain is expected to avoid any of the above-mentioned systemic non-CNS side effects.

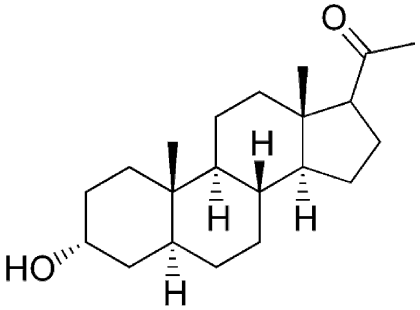
Planned studies

Further preclinical studies for the application ''antiepileptogenesis'' are planned. This includes determination of minimally effective antiepileptogenic doses of adenosine, optimization of the delivery procedure, further mechanistic studies and long-term efficacy studies in rodent models of epileptogenesis.

Allopregnanolone (SAGE-547) Injection

S.J. Kanes[1], J. Doherty[1], R. Hammond[1], M.A. Rogawski[2], A. Robichaud[1], J. Jonas[1]

[1] Sage Therapeutics, Cambridge, MA 02142, USA
[2] Department of Neurology, University of California, Davis, Sacramento, CA 95817, USA



Allopregnanolone (SAGE-547)

Introduction and rationale for development

SAGE-547 (allopregnanolone) Injection is a solution of 5 mg/mL allopregnanolone and 250 mg/mL β-cyclodextrin sulfobutyl ethers sodium salts, NF (Captisol®). Allopregnanolone Injection (SAGE-547) is being developed for the treatment of super-refractory SE (SRSE).

Allopregnanolone is an endogenously occurring neuroactive steroid and a principal metabolite of progesterone formed in the corpus luteum of the ovary, adrenal cortex and CNS (Paul and Purdy, 1992). Endogenous concentrations of allopregnanolone are at their highest in women during the third trimester of pregnancy, and approximate 157 nM at time of parturition (Luisi et al., 2000).

Allopregnanolone is a potent positive allosteric modulator of $GABA_A$ receptor responses (Lambert et al., 2003). Through $GABA_A$ receptor modulation, allopregnanolone possesses potent anxiolytic, sedative and anticonvulsant activity when studied in non-clinical *in vitro* and *in vivo* model systems (Belelli et al., 1989; Bitran et al., 1991; Wieland et al., 1991; Kokate et al., 1994).

Pharmacology

Anticonvulsant profile (animal models/electrophysiology) There is ample evidence indicating a role for allopregnanolone in the treatment of seizures and status epilepticus (SE) (Kokate et al., 1996; Reddy and Rogawski, 2012). Allopregnanolone has anticonvulsant properties in a variety of acute seizure models, including the pentylenetetrazol (PTZ), 6 Hz, bicuculline, and picrotoxin models (Rogawski et al., 2013; Tables 1 and 2). The acute anticonvulsant efficacy of allopregnanolone in the PTZ-induced seizure model in mice is maintained upon repeat dosing. Allopregnanolone produces complete suppression of generalized amygdala-kindled convulsions and protection against pilocarpine- or kainate-induced limbic seizures and SE, with higher protective index values than clonazepam. In the rat kainate-induced model of SE, allopregnanolone (30 mg/kg, i.p.) eliminated SE whether administered at either 10 min or at 70 min following kainate administration, whereas diazepam (5 mg/kg, i.p.) was only effective at 10 min following kainate administration (Rogawski et al., 2013). These results support the hypothesis that enhancement of extra-synaptic $GABA_A$ receptor function by allopregnanolone may provide anticonvulsant efficacy when prolonged seizure activity has become pharmaco-resistant to benzodiazepine treatment.

Mechanism(s) of action

Mammalian $GABA_A$ receptors are heteropentameric chloride-conducting ion channels that mediate fast inhibition of synaptic transmission *via* a reduction of neuronal membrane excitability. These ionotropic receptors are called $GABA_A$ receptors to distinguish them from metabotropic GABA receptor (G-protein coupled) ($GABA_B$) receptors that mediate a slower form of synaptic inhibition.

Allosteric potentiation of $GABA_A$ receptor function by allopregnanolone has been extensively documented (Belelli et al., 2005). Consistent with this literature, SAGE-547 potentiated GABA-mediated currents from recombinant human $GABA_A$ receptors expressed in heterologous mammalian cell lines. SAGE-547 produced a potent, concentration-dependent enhancement of GABA-evoked currents recorded using whole-cell patch electrophysiology from Ltk-1 cells expressing $\alpha_1\beta_2\gamma_2$ receptors, with a half maximal effective concentration (EC_{50}) of 60 nM and a maximal potentiation of 380% for agonist (EC_{20})-induced currents (Table 3). SAGE-547 also produced a potent enhancement of $\alpha_4\beta_3\delta$ receptors, with an EC_{50} of 80 nM and a maximal potentiation of 418% for agonist (EC_{20})-induced currents.

In addition to allosteric enhancement of channel function, allopregnanolone modulates the binding of a variety of other ligands to the $GABA_A$ receptor, including the picrotoxin/convulsant site labeled by [^{35}S]-t-butylbicyclophosphorothionate (TBPS) (Concas et al., 1996). Consistent with this literature, SAGE-547 potently inhibited [^{35}S]-TBPS binding, displacing the radioligand from rat cerebral cortex membranes with an inhibition constant (K_i) of 18 nM.

Toxicology

The minimum lethal doses for allopregnanolone in mice, rats, and rabbits are 20, 15, and 7 mg/kg intravenously, respectively. Based on the correction for body surface area across the species, these are equivalent to human doses of 1.6, 2.4, and 2.25 mg/kg intravenously, or 112, 168, and 158 mg in a 70 kg human (Gyermek et al., 1968).

A recently published abstract presented the determination of maximum tolerated doses (MTD) of allopregnanolone in neonatal beagle dogs after bolus and i.v. infusions (Steinmetz and Rausch, 2013). One pup administered allopregnanolone *via* i.v. bolus (50 mg/kg), was not responsive to stimuli and exhibited, shallow rapid breathing but fully recovered without intervention. One pup administered 20 mg/kg allopregnanolone over a 4 h i.v. infusion, fell asleep approximately 30 min post dose and recovered without intervention. No allopregnanolone-related adverse effects on chemistry or hematology labs were observed. Of note, studies with allopregnanolone by the same investigators suggested that the presence of isoflurane anesthesia resulted in an MTD of <3 mg/kg bolus i.v. injection to unconscious neonatal Beagle dogs (Steinmetz and Rausch, 2013).

Pharmacokinetics and metabolic profile

The pharmacokinetic parameters of i.v. allopregnanolone have been evaluated in two studies (Balgård et al., 2006; Timby et al., 2006; van Broekhoven et al., 2007). In the first study (Timby et al., 2006), 10 non-pregnant healthy women were administered allopregnanolone by i.v. injection during the follicular phase of the menstrual cycle. After receiving a cumulative dose of 90 μg/kg (three 30 μg/kg injections, each given over 30 s at 30 min intervals), the maximum serum concentration (71.8 nM) was recorded 5 min after the third injection. Allopregnanolone distribution and elimination half-lives were 44 and 261 min, respectively, its volume of distribution at steady state (V_{ss}) was 7.3 L/kg and its clearance was 33 mL/min/kg. The authors concluded that allopregnanolone could safely be administered i.v. at low doses to women (Timby et al., 2006). Another study utilizing the same dosing paradigm described in Timby et al.

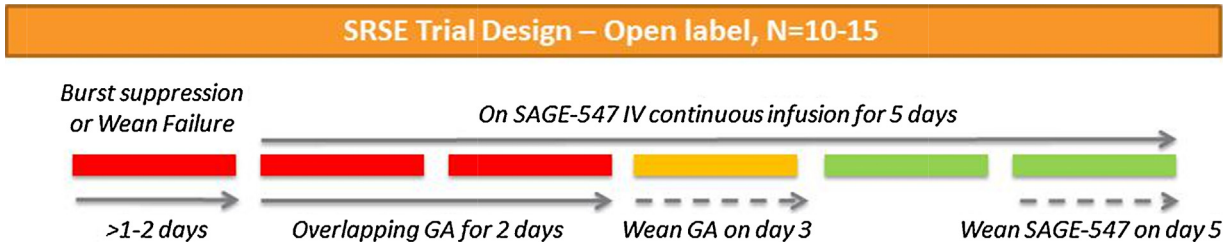


Figure 1 Trial design of SAGE-547 in super-refractory status epilepticus (SRSE).

(2006), characterized the pharmacokinetics of i.v. administered allopregnanolone to 9 men and in 9 women who were taking oral contraceptives (van Broekhoven et al., 2007). Maximum serum concentrations were observed within 10 min after the third injection and were higher than those reported in the earlier study, with a mean maximum serum concentration of 149 nM in men and 100 nM in women. The difference between men and women was statistically significant.

Drug interactions

The potential for SAGE-547 (0.3, 0.5, 3, 5, 30, and 50 μM) to induce human CYP1A2, CYP2B6, and CYP3A4/5 was assessed in primary cryopreserved human hepatocytes from 3 different donors. A mild concentration-dependent increase in metabolic activity was recorded for CYP2B6, as measured by an increase in bupropion hydroxylation, but it only reached 2-fold at the two highest concentrations tested (30 and 50 μM). A concentration-dependent increase in CYP2B6 mRNA was also observed in these samples, but was only >2-fold in one of the three donors. Although SAGE-547 shows the potential to induce CYP2B6, the increase was small at the highest concentration tested. Clinical exposures are targeted at 150 nM, which are well below the 30—50 μM required for induction of the enzyme.

The potential for SAGE-547 (1 and 10 μM) to inhibit human CYP1A2, CYP2C9, CYP 2C19, CYP2D6, and CYP3A4 was investigated *in vitro* utilizing human liver microsomes incubated in the presence of clinically relevant marker substrates. No inhibition was observed at 1 μM of SAGE-547. At 10 μM, no inhibition was observed for any of the CYPs, except for CYP2C9, which showed 47% inhibition. This result was followed up by determining half maximal inhibitory concentration (IC_{50}) and K_i for CYP2C9 utilizing human liver microsomes and measuring formation of 4′-hydroxy-diclofenac from diclofenac. IC_{50} was 0.41 μM and K_i was 0.256 μM. There was no evidence of time-dependent or metabolism-dependent inhibition based on monitoring IC_{50}-shift after pre-incubation. SAGE-547 has the potential to alter the metabolism of CYP2C9 substrates when administered concomitantly.

Tolerability and adverse effect profile

With systemic exposures up to 150 nM, no drug-related serious TEAEs have been reported. Drug related TEAEs reported with i.v. allopregnanolone are generally mild, the most frequently reported (>5% patients) being feelings of intoxication, sedation, and vertigo. One patient experienced an anxiety attack, which is potentially a withdrawal effect. Clinical observations in the i.v. studies included decreased saccadic eye movements, reduced episodic memory, as well as reduced plasma levels of LH and FSH (Timby et al., 2006, 2011; van Broekhoven et al., 2007; Kask et al., 2008, 2009).

Efficacy data and ongoing study

The first patient with SRSE to receive allopregnanolone was a 23-year-old male, in whom discontinuation of pentobarbital anesthesia was achieved during the allopregnanolone infusion (Vaitkevicius et al., 2013). A similar experience was obtained in 2 children with SRSE (Broomall et al., 2014).

An open-label Phase I/II clinical trial was initiated to study the safety, tolerability and efficacy of SAGE-547 in at least 10 adult patients with SRSE.

This trial is enrolling patients who have been in SE for at least 24 h despite treatment with first and second line SE treatments and have either failed to have SE controlled after 24 h on a third-line general anesthetic (GA) agent or if SE was controlled by a GA have failed at least 1 wean attempt. A patient is excluded from the trial if pregnant or lactating, or if the patient has a known allergy to progesterone or allopregnanolone, has clinically significant ECG abnormalities, has a significant medical or surgical condition that may compromise vital organ systems, has been exposed to another experimental treatment within 30 days, is receiving a continuous i.v. AED (third-line agent) for seizure suppression or burst-suppression that will require greater than 24 h to wean, has enrolled in this trial previously, or if the SRSE was due to anoxic/hypoxic encephalopathy. Fig. 1 demonstrates the design of the screening and treatment periods of this trial. Following the treatment period, there will be an acute 2 days follow-up period and an extended 3-week follow-up period.

The primary objective of this trial are to evaluate the safety and tolerability of SAGE-547 injection in SRSE patients as assessed by monitoring treatment-emergent adverse events (TEAE), EEG, physical and neurological examinations, vital signs, clinical laboratory measures, ECGs and concomitant medication usage. The secondary objective of this trial is to assess the efficacy of SAGE-547 in SRSE, a assessed by the need to place the patient back into general anesthesia for seizure control during administration of SAGE-547 prior to taper. Other secondary objectives include duration of response, as well as changes in behavior as measured by rating scales of agitation, depth of coma, and survival. This trial is ongoing. To date, 4 patients have completed the full 5 days administration of SAGE-547 at a target plasma

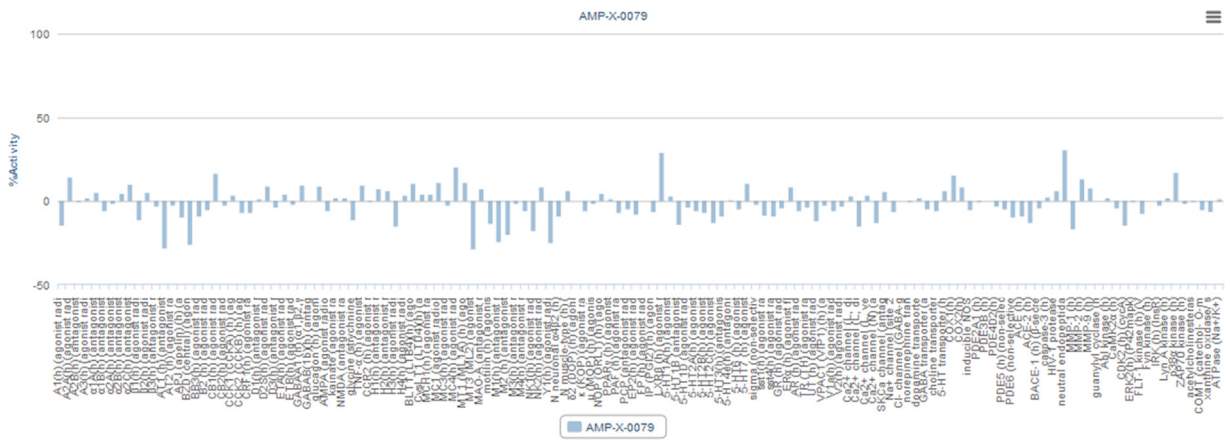


Figure 2 Histogram for AMP-X-0079 (Binding Assays and Enzyme and Uptake Assays).

exposure of 150 nM and 30-day follow-up period. All have met the key efficacy endpoint of SE control during SAGE-547 administration. To date there have been no drug-related serious TEAEs in these patients.

Planned studies

Depending upon the outcome of the Phase I/II trial, the plan is to follow-up with a larger scale controlled trial in a similar population of SRSE patients.

Acknowledgement

IND rights and the active pharmaceutical ingredient in SAGE-547 were contributed under agreement by the University of California, Davis and the Regents of the University of California.

AMP-X-0079

A. Pesyan[1], R.J. Porter[2]

[1] AurimMed Pharma, Park City, Utah, USA
[2] University of Pennsylvania, Philadelphia, PA, USA, and USUHS, Bethesda, MD, USA

Introduction and rationale for development

AurimMed Pharma, Inc. is developing innovative prescription drugs using the Privileged Structure Platform™ (PSP) strategy to generate lead compounds. The PSP approach ensures that the process of drug design proceeds exclusively from a highly enriched pool of pharmaceutically and medicinally relevant chemical structures, greatly shortening product development times while enhancing clinical success (Muller, 2003; DeSimone et al., 2004; Costantino and Barlocco, 2006; Duarte et al., 2007). The PSP strategy has been used in the development of novel anti-seizure drug candidates with potential superior efficacy and greatly diminished adverse effects.

Pharmacology

Anticonvulsant profile (animal models/electrophysiology) Based on studies in animal models, AurimMed Pharma's most advanced AED candidate, AMP-X-0079, has a very broad spectrum, with the potential to become a superb anti-seizure drug for a variety of seizure types, including absence/petit mal, tonic–clonic and myoclonic seizures as well as *status epilepticus*. In addition to the compound's broad spectrum of activities across a number of animal models (Tables 1 and 2), it has (in rodent models) good oral bioavailability, CNS penetration, long duration of action, good neurological safety margins (Protective Indices, PIs), and substantial potency.

AMP-X-0079 anticonvulsant data included in Tables 1 and 2 were largely derived from the Anticonvulsant Screening Program of NINDS, NIH. As one can see, AMP-X-0079 is active in all the models tested, including the new semi-chronic mesial temporal lobe epilepsy model. Protective Index (PI) = TD_{50}/ED_{50} (''Toxicity'' is defined here as rotarod motor impairment in mice and a battery of tests to determine neurological deficit as indicated by ataxia or other minimal motor impairment (MMI) in rats).

In studies conducted by the NIH and the US Department of Defense (DOD), AMP-X-0079 is active in models of chemically induced *status epilepticus* (SE), both when administered at the time of the chemical insult (time zero) as well as 30 min after the appearance of seizures; the compound is therefore effective in both the prevention and the treatment of SE. Moreover, in a pilocarpine-induced model of SE, followed by extensive, 9 days, evaluation in a Morris water maze task, AMP-X-0079 preserved cognition, spatial learning and memory. The compound also demonstrated substantial *in vivo* neuroprotective effects in the dentate gyrus, CA1, and CA3 hippocampal neurons in the brains of a vast majority (87%) of the treated animals (results confirmed histologically). In the pilocarpine insult and SE, 100% of the AMP-X-0079-treated animals survived compared to 54% of controls. In addition, the compound is active against non-convulsive seizures in benzodiazepine-resistant electrographic *status epilepticus* (ESE), confirmed by EEG studies (Lehmkuhle et al., 2009).

AMP-X-0079 has been shown in *in vivo* studies to be orally bioavailable, fast-acting (rapid oral absorption within

Table 1 Anticonvulsant profile of investigational AEDs in mouse models.

Compound	Route	Time of test (min)	ED_{50} (mg/kg)							TD_{50} (mg/kg)	*In vivo* activity in other model systems
			MES	s.c. PTZ	6 Hz 22 mA	6 Hz 32 mA	6 Hz 44 mA	Corneal kindled mouse	Audiogenic seizures		
Adenosine-releasing Silk	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT in mice yet, because current implant design is too big for mice.
Allopregnanalone (SAGE-547)	i.p.	30	Inactive <300 mg/kg	13.7 mg/kg	NT	14.2 mg/kg	NT	NT	In *Fmr1* KO mice, minimal effective dose = 3 mg/kg	Hypoactivity in open field observed with TD_{50} = 43 mg/kg	Significantly reduces audiogenic seizures in *Fmr1* KO mice after repeated dosing (10 mg/kg, i.p., bid for 5 days)
AMP-X-00791	i.p.	30—60	48	51	NT	20	58	34	16	128	At dose of 76 mg/kg (*single dose*) affects both acute and inflammatory pain s.c. Bic ED_{50} = 115 mg/kg s.c. Pic ED_{50} = 65 mg/kg MLTE ED_{50} = 66 mg/kg

Table 1 (*Continued*)

Compound	Route	Time of test (min)	ED_{50} (mg/kg)							TD_{50} (mg/kg)	*In vivo* activity in other model systems
			MES	s.c. PTZ	6 Hz 22 mA	6 Hz 32 mA	6 Hz 44 mA	Corneal kindled mouse	Audiogenic seizures		
Brivaracetam	i.p.	30	113	30	NT	NT	4.4	1.2	2.4	55 (kindled mice)	Active in corneal kindled mouse (ED_{50}: 1.2 mg/kg), and on development of corneal kindling; active in phenytoin-resistant amygdala-kindled rat
Cannabidiol	i.p.	60	40 (est)	NT	NT	NT	NT	NT	45		
Cannabidivarin	i.p.	60	30 (est)	NT	NT	NT	NT		25		
2-Deoxy-D-glucose	i.p.	15—120	NT	NT	79.7	NT	NT		206	NA	
Everolimus		NT	NT	NT	NT	NT	NT	NT	NT	NT	Everolimus is an analog of rapamycin, that was not protective in the 6 Hz or PTZ seizure tests

Ganaxolone	i.p.	30	30	4.3	NT	6.3	NT	NT	33.4	Active against bicuculline- and aminophylline-induced seizures (ED50's: 4.6 and 11.5 mg/kg, respectively). In a murine model of PTSD (social isolation), 3.75—30 mg/kg ganaxolone s.c. decreased aggressive and anxiety-like behaviors and increased retention of fear extinction learning.
Huperzine A	i.p.	1 h	1.69	>1	0.28	0.34	0.78	ND	0.21 pmol/5 1-L injection volume, with a TD_{50} of 36.15 pmol, indicating protective index of 172 $PI = TD_{50}/ED_{50}$	84% protected at 0.5 mg/kg and 100% protection at 1 mg/kg in SCN1A model of Dravet Syndrome

Table 1 (*Continued*)

Compound	Route	Time of test (min)	ED_{50} (mg/kg)							TD_{50} (mg/kg)	*In vivo* activity in other model systems
			MES	s.c. PTZ	6 Hz 22 mA	6 Hz 32 mA	6 Hz 44 mA	Corneal kindled mouse	Audiogenic seizures		
Imepitoin (AWD 131–138 or ELB 138)	i.p.	30	94	17	NT	NT	NT		2.6 (DBA); 5.0 (Frings)	176	Active in mouse and rat models of anxiety including innate fear and fear induced by open and high areas and light stimulation (*e.g.*, elevated maze, light–dark chamber, social interaction test) at doses of 3 mg/kg, i.p. and p.o.
Minocycline	i.p.	15–240	Ineffective	NT	NT	NT	NT	170	NT	250	Active in mouse model of ischemia, spinal cord injury, Parkinson's multiple sclerosis, ALS, and Alzheimer's disease
NAX 810-2		60	>20	NT	NT	2.5	5.9	7.4	N/A	>32	Active in mouse formalin and carrageenan models of inflammatory pain
	p.o.								N/A		

	i.v.	30	NT	NT	NT	1.1	NT	1.8	N/A	NT	NT
Pitolisant (tripolisant)	i.p.			NT	NT	NT	NT	NT	NT	N/A	Kainate hippocampal seizures (10 and 20 mg/kg); promnesiant effects (object recognition in mice at 15 mg/kg); enhanced wakefulness in ''narcolepsy'' mice model
	p.o.	60	100% (at 100 mg/kg)								Reduction (5 and 10 mg/kg) of neocortical slow activity and spindles and increase in power density (EEG in Cats)
SAGE-217	i.p.	30	NT	Minimal effective dose = 0.3 mg/kg	NT	1.9	>10	NT	NT	Hypoactivity in open field: TD_{50} = 3.8 mg/kg	After repeated dosing of SAGE-217 (10 mpk, IP, BID for 9 days), motor effects in rotarod but not anticonvulsant effects in PTZ assay tolerated out.

Table 1 (*Continued*)

Compound	Route	Time of test (min)	ED_{50} (mg/kg)							TD_{50} (mg/kg)	*In vivo* activity in other model systems
			MES	s.c. PTZ	6 Hz 22 mA	6 Hz 32 mA	6 Hz 44 mA	Corneal kindled mouse	Audiogenic seizures		
sec-Butyl-propylacetamide (SPD) racemate	i.p.	15	71	62	NT	27	NT		20 (Frings)	88	Blocks minimal clonic seizures induced by s.c. picrotoxin and bicuculline and secondarily generalized seizures in the corneal kindled mouse with $ED_{50's}$ of 17, 94, and 39 mg/kg, respectively.
(2R,3S)-SPD	i.p.	15	95	93	NT	29	<100	48	NT	67	
(2S,3S)-SPD	i.p.	15	75	69	NT	27	59	>65	NT	126	
(2R,3R)-SPD	i.p.	15	70	53	NT	38	52	57	NT	111	
(2S,3R)-SPD	i.p.	15	69	44	NT	27	48	49	NT	81	
Valnoctamide (VCD) racemate	i.p.	15	58	32		37	67			77	
(2S,3S)-VCD	i.p.	15	132	69	25	33	80	>65		128	
(2R,3S)-VCD	i.p.	15	119	67	19	48	67	28		127	
(2S,3R)-VCD	i.p.	15	144	79	NT	40	NT	45	NT	170	
(2R,3R)-VCD	i.p.	15	105	71	NT	59	64	44	NT	143	
VLB-01	p.o.	60	26.4	360						1000	Active in bicuculline, strychnine and the pain formalin paw tests with ED_{50} values of 130, 155 and 100 mg/kg, respectively
YKP 3089	i.p.	15	9.8	28.5	11	17.9	16.5	NT	NT	58	Anxiety; HI-induced lethality

ED_{50}, median effective dose in mg/kg; TD_{50}, median toxic dose in mg/kg; HI, Hypoxia Ischemia; i.p., intraperitoneal; i.v., intravenous; p.o., per os; MED, median effective dose; NT, not tested; MES, maximal electroshock seizure; sc, subcutaneous; PTZ, pentylenetetrazol; SPD, *sec*-butyl-propylacetamide; VCD, valnoctamide.

Table 2 Anticonvulsant profile of investigational AEDs in rat models.

Compound	Route	Time of test (min)	ED_{50} (mg/kg)			TD_{50} (mg/kg)		*In vivo* activity in other disease model systems actions
			MES	s.c. PTZ	Kindled rat (*i.e.*, corneal, amygdala, hippocampal)	Spike-wave seizure model (lethargic mouse, GAERS, GHB rat, WAG/Ri rat)	Behavioral toxicity (*e.g.*, rotarod, observational)	
Adenosine-releasing silk	i.c.v.	Up to 10 days	NT	NT	Active against seizures in rat hippocampal kindling model (2 μg/kg/d) Prevention of epileptogenesis in rat hippocampal kindling model (2 μg/kg/d for 10 days)	NT	>10 μg/kg/d	Prevention of epileptogenesis in rat systemic kainic acid model (2 μg/kg/d for 10 days)
Allopregnanolone	s.c.			2.14	1—10 mg/kg, i.p.			
AMP-X-0079	i.p.	60	25	31	16	39	84	Pilocarpine-induced SE (t-30 min) ED_{50} = 155
	p.o.	240	42	74			178	Soman-induced SE (t = 20 min) ED_{50} = 63
Brivaracetam	i.p.	60	N/A	N/A	44 mg/kg (amygdala)	2.6 mg/kg (GAERS)	163 mg/kg (kindled rats) 177 mg/kg (GAERS)	Self-sustaining status epilepticus model (perforant path stimulation); diabetic and CCI models of neuropathic pain; and post-hypoxic myoclonus
	p.o.	60	N/A	N/A	45 mg/kg (amygdala)	N/A	N/A	
Cannabidiol	i.p.	60	10 (est)				>200	Pilocarpine-induced SE-ED_{50} = 75
Cannabidivarin	i.p.	60	100 (est)				>200	
2-Deoxy-D-glucose	p.o.	15—240	No effect at doses up to 200 mg/kg	Partial protection at doses up to 400 mg/kg, p.o.	NT	NT	NT	Delays the acquisition of kindling induced by perforant path or olfactory bulb stimulation at doses of 37.5—50 mg/kg; neuroprotective in the CCI model of TBI.
Everolimus								Everolimus is an analog of rapamycin, that was not protective in the 6 Hz or PTZ seizure tests

Table 2 (*Continued*)

Compound	Route	Time of test (min)	ED_{50} (mg/kg)			TD_{50} (mg/kg)		*In vivo* activity in other disease model systems actions
			MES	s.c. PTZ	Kindled rat (*i.e.*, corneal, amygdala, hippocampal)	Spike-wave seizure model (lethargic mouse, GAERS, GHB rat, WAG/Ri rat)	Behavioral toxicity (*e.g.*, rotarod, observational)	
Ganaxolone	i.p.	30	58	7.8	4.5 (corneal)	NT	14.2	
Huperzine A	i.p.	1 h	50% protection at 1 mg/kg	>4 mg/kg in scMET	>2 mg/kg	NT	88% had acute tremors at 1 mg/kg	
	p.o.	1 h	25% protection at 1 mg/kg	50% protection at 1 mg/kg in scMET	NT	NT	0% toxicity at 1 mg/kg	
Imepition	p.o.		21	16	Increases after discharge threshold at doses as low as 1 mg/kg; completely inhibits secondary generalization at 20 mg/kg, i.p.	Effective in WAG rat model of generalized absence at 3 mg/kg, p.o. (98% suppression of SWD observed at 30 mg/kg p.o.)	>400	Blocks SWD induced by 25 mg/kg, i.p. dose of PTZ at 3 mg/kg, p.o.; increases PTZ seizure threshold in dogs by 33—59% after repeated oral administration of 5 mg/kg; prolongs kindling acquisition in amygdala kindled rat when administered prior to kindling stimulation.
Minocycline	i.p.	60	NT	NT	Amygdala kindling 50 mg/kg reduced after discharge duration (ADD), stage 4 latency (S4L), stage 4 duration (S5D) and seizure duration (SD), 25 mg/kg decreased ADD and S5D while 12.5 mg/kg decreased S5D. Daily injection of 25 mg/kg $\times$ 10 days significantly decreased ADD, S5D and SD and retarded kindling acquisition.	NT	50 mg/kg, but not 25 mg/kg caused decreased the length of time on the rotarod.	Protection against traumatic brain damages and beta-amyloid peptide-induced cell death

NAX 810-2	i.p.	0.25—24 h	NT	NT	NT	NT	>4.0 mg/kg	Elevates threshold for mechanical allodynia in rat sciatic nerve ligation model of neuropathic pain at 4.0 mg/kg
Pitolisant (tripolisant)	i.p.	20, 40, 60				25% after 5 mg/kg; 77% after 20 mg/kg		
sec-Butyl-propylacetamide (SPD)	i.p.	15	20	62	19 mg/kg fully expressed hippocampal kindled rat secondarily generalized seizures SNL-ED_{50} = 49 mg/kg	NT	49	Pilocarpine-induced SE (30 min) ED_{50} = 84 Soman-induced SE (20 min) ED_{50} = 68
	p.o.	60	29	18 (30 min) 82 (60 min)		NT	154	
(2R,3S)-SPD	i.p.	15	33	15	NT	NT	41	Pilocarpine-induced SE (30 min) ED_{50} = 135 Soman-induced SE (20 min) ED_{50} = 56
	p.o.	60	72	50	NT	NT	79	
(2S,3S)-SPD	i.p.	15	31	14	NT	NT	34	Pilocarpine-induced SE (30 min) ED_{50} = 94 Soman-induced SE (20 min) ED_{50} = 70
	p.o.	60	48	20	NT	NT	124	
(2R,3R)-SPD	i.p.	15	36	15	NT	NT	34	Pilocarpine-induced SE (30 min) ED_{50} = 98 Soman-induced SE (20 min) ED_{50} = 40
	p.o.	60	79	18	NT	NT	102 (30 min)	
(2S,3R)-SPD	i.p.	15	81	17	NT	NT	42	Pilocarpine-induced SE (30 min) ED_{50} > 130 Soman-induced SE (20 min) ED_{50} = 50

Table 2 (*Continued*)

Compound	Route	Time of test (min)	ED_{50} (mg/kg)			TD_{50} (mg/kg)		*In vivo* activity in other disease model systems actions
			MES	s.c. PTZ	Kindled rat (*i.e.*, corneal, amygdala, hippocampal)	Spike-wave seizure model (lethargic mouse, GAERS, GHB rat, WAG/Ri rat)	Behavioral toxicity (*e.g.*, rotarod, observational)	
	p.o.	60	106	25	NT	NT	105	
Valnoctamide (VCD)	i.p.	15	67	17	NT	NT	72	Pilocarpine-induced SE (0 min) ED_{50} = 40 Soman-induced SE (20 and 40 min) ED_{50} = 60 and 62, respectively
	p.o.	60	29	54	SNL-ED_{50} = 52	NT	>100	Racemate and isomers of VCD were found to be active against tactile allodynia in Cheung model of neuropathic pain
(2R,3S)-VCD	i.p.	15	43	13			71	Pilocarpine-induced SE (0 min) ED_{50} = 39
	p.o.	60	34	11	SNL-ED_{50} = 61		123	
(2S,3S)-VCD	i.p.	15	55	20			80	Pilocarpine-induced SE (0 min) ED_{50}.65
	p.o.	60	64	33	SNL-ED_{50} = 39		194	
(2S,3R)-VCD	i.p.	15	73	17			80	Pilocarpine-induced SE (0 min) ED_{50} = 82
	p.o.	60	95	27			92	
(2R,3R)-VCD	i.p.	15	43	12			70	Pilocarpine-induced SE (0 min) ED_{50} = 86
	p.o.	60	48	41			75	
VLB-01	p.o.	60	55	60				
YKP-3098	i.p.	150—240	2.9	13.6	16.4 (hippocampal)	NT	38.9	Bennett and Chung models of chronic pain; Lithium-pilocarpine-induced SE
	p.o.	60	1.9	8.3	NT	NT	50.7	

i.p., intraperitoneal; i.v., intravenous; p.o., per os; MED, minimum effective dose; ED_{50}, median effective dose in mg/kg; TD_{50}, median toxic dose in mg/kg; MES, maximal electroshock seizure; sc, subcutaneous; PTZ, pentylenetetrazol; GAERS, genetic absence epileptic rat of Strasbourg; N/A, not available; NT, not tested; CCI, controlled cortical impact; SPD, *sec*-butyl-propylacetamide; VCD, valnoctamide.

Table 3 Proposed mechanisms of action of investigational AEDs currently in development.

Compound	Proposed mechanism(s) of action
Adenosine-releasing silk	Adenosine A1 receptor agonist; inhibits adenylyl cyclase activity, activates potassium channels, blocks transient calcium channels and increases intracellular calcium and inositol-1,4,5-trisphosphate levels by activating phospholipase C. Adenosine receptor independent epigenetic effect: inhibits DNA methylation through biochemical interference with transmethylation pathway.
Allopregnanolone (SAGE 547)	Positive allosteric modulator of synaptic and extrasynaptic $GABA_A$ receptors
Brivaracetam	Selective and high affinity binding to SV2A (IC_{50} = 0.08 μM)
Bumetanide	Blocks NKCC co-transporters of GABAergic neurons which is inhibitory in neonatal neurons and excitatory in adult neurons.
Cannabidiol	Precise mechanism unknown; however, it is a potent inhibitor of adenosine uptake, an effect that is thought to contribute to inhibition of TNFα production by retinal microglia and suggests an inflammatory potential. CBD is able to modulate intracellular calcium mobilisation and has activity at a number of TRP channels.
Cannabidivarin	Not known, see text for discussion.
2-Deoxy-D-glucose	Inhibits glycolysis; suppresses *in vitro* burst discharges induced by high potassium, 4-aminopyridine, bicuculline, and the mGluR1 agonist DHPG; blocks kindling acquisition by repressing expression of BDNF and TrKB
Everolimus	Binds to the intracellular protein FKBP-12, forming a complex that inhibits mTOR complex-1 (mTORC1) activity
Ganaxolone	Positive allosteric modulator of synaptic and extrasynaptic $GABA_A$ receptors
Huperzine A	Acetylcholinesterase inhibition
Imepitoin (AWD 131—138 or ELB 138)	Low-affinity partial agonist at the benzodiazepine (BZD) recognition site of the $GABA_A$ receptor
Minocycline	Immunomodulatory action of minocycline is attributed to inhibition of the activity of matrix metalloproteinase (MMPs), inducible nitric oxide synthase (iNOS), and cyclooxygenases-2 (COX-2)
NAX 810-2	Enhances galanin receptor neurotransmission by preferential activation of GalR2 receptors
Pitolisant (tripolisant)	Histamine 3 receptor antagonist
PRX-0023	5HT1A receptor agonist
SAGE 217	Positive allosteric modulator of synaptic and extrasynaptic $GABA_A$ receptors
sec-Butyl-propylacetamide (SPD)	Unknown
Valnoctamide (VCD)	Inhibits *myo*-inositol-1-phosphate synthase, unknown anticonvulsant and antiallodynic mechanism of action
VLB-01	Melatonin receptor 3 agonist that selectively binds to the melatonin receptor 3/ribosyldihydronicotinamide dehydrogenase
YKP 3089	Preferentially blocks persistent sodium current (INaP) through an action at the inactivated state of the sodium channel. Increases inhibitory synaptic transmission by facilitating presynaptic GABA release

SV2A, synaptic vesicle protein 2A; IC_{50}, inhibitory concentration to produce 50% inhibition; μM, micromolar; mGluR1, metabotropic glutamate receptor subtype 1; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; BDNF, brain-derived neurotrophic factor; TrKB, tyrosine kinase B (high-affinity catalytic receptor for several neurotrophins including BDNF and neurotrophin 3 and 4); Nav, sodium channel isoform; $GABA_A$, γ-aminobutyric acid receptor; N-type, neuronal voltage-sensitive calcium channel; P/Q-type, Purkinje/Q-type voltage-sensitive calcium channel; L-type, long-lasting type of voltage-sensitive calcium channel; SPD, *sec*-butyl-propylacetamide; VCD, valnoctamide.

15 min), with a reasonably long pharmacological half-life in animal models. The compound passes the blood-brain barrier, thereby manifesting its pharmacological effects directly in the central nervous system.

Because of its unique pharmacological profile and significant suppression of ESE, the lead candidate (AMP-X-0079) was tested against a nerve agent (*i.e.*, soman) by the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) of the DOD. AMP-X-0079 is one of the few drugs capable of terminating ongoing *status epilepticus* seizures due to this nerve agent. It controls seizure very rapidly (typically <15 min) and has an ED_{50} of 63 mg/kg.

Additional testing performed by the NIH has shown that AMP-X-0079 has significant analgesic activity in models of both neuropathic and inflammatory pain, *i.e.*, in the sciatic nerve-ligation and formalin pain models, respectively.

Mechanism(s) of action

To investigate the compound's mode of action, AMP-X-0079 was subjected to a Cerep® Full-BioPrint study consisting of

more than 140 commonly known molecular targets (70% of which are human). The results indicate that the compound has no appreciable activity at any of the commonly known targets (Fig. 2). Moreover, the Cerep Full-BioPrint profile, together with the broad spectrum of *in vivo* CNS activity exhibited by AMP-X-0079, strongly suggests that it is working through a novel mechanism of action.

Toxicology

AMP-X-0079 performed very well in the rotorod tests of the ASP, with good protective indices, as noted above. In addition, the compound's lack of cytotoxicity has been demonstrated in the Cerep *in vitro* cell-viability test.

Drug interactions

In the Cerep® Full-BioPrint study, AMP-X-0079 was metabolized by a number of CYP isozymes (primarily CYP2C19) and did not inhibit P-glycoprotein (PgP) at a concentration of 10 μM. These results suggest that AMP-X-0079 has little predisposition for drug—drug interactions.

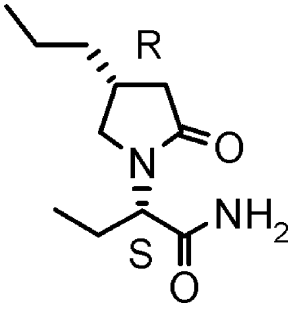
Planned studies

AMP-X-0079 will be evaluated in *in vitro* and *in vivo* ADMET and PK/PD tests, including hERG, cardiovascular, and respiratory toxicity in dogs and rats, as well as 28-day toxicological studies. If preclinical testing and the IND are successful, the company then intends to oversee the clinical testing of AMP-X-0079 up through the end of Phase III human clinical trials, FDA registration, and approval, which are to be conducted and completed by a pharmaceutical partner with experience in clinical development and a commercialization infrastructure already in place.

Brivaracetam (UCB 34714)

J. D'Souza[1], H. Klitgaard[2], A. Stockis[3], J. Whitesides[4]

[1]UCB Pharma, Smyrna, GA, USA
[2]UCB Pharma, Brussels, Belgium; [3]UCB Pharma, Braine-L'Alleud, Belgium; [4]UCB Pharma, Raleigh, NC, USA



Introduction and rationale for development

Brivaracetam (UCB 34714) is a rationally designed ligand with high selectivity and approximately 20-fold higher affinity for synaptic vesicle protein 2A (SV2A) than levetiracetam (Gillard et al., 2011; Kenda et al., 2004; Lynch et al., 2004). Brivaracetam is currently in Phase III development for epilepsy.

Pharmacology

Brivaracetam has higher brain permeability than levetiracetam, resulting in rapid onset of action after acute dosing in audiogenic mice. Physiology-based pharmacokinetic modeling suggests this fast onset of action extends to humans, highlighting the potential for rapid acute intervention against status epilepticus or cluster seizures (Nicolas et al., 2014). *In vitro* binding experiments in animal and human brain tissue confirmed the increased potency for SV2A and rapid efficacy of brivaracetam *versus* levetiracetam, as have efficacy studies in audiogenic mice (Gillard et al., 2011).

Preclinical studies in a range of focal and generalized epilepsy models (Table 1) have shown evidence for broad spectrum therapeutic activity (Matagne et al., 2008). In mouse kindling models of focal drug-resistant epilepsy and rat genetic absence epilepsy, brivaracetam induced more potent and complete seizure suppression than levetiracetam. Further, unlike levetiracetam, brivaracetam exerted protection at relatively high doses against seizures induced in normal animals by maximal electroshock or maximal bolus dose of pentylenetetrazol (Matagne et al., 2008). Experiments in corneal kindled mice have shown a potent and persistent ability to inhibit kindling acquisition, suggesting potential antiepileptogenic properties (Matagne et al., 2008).

Safety testing revealed that brivaracetam had a high and promising therapeutic index in fully amygdala kindled rats between doses producing seizure suppression and acute motor effects (Matagne et al., 2008). Brivaracetam had no negative impact on cognitive performance in both normal and kindled animal models and on long-term potentiation in hippocampal slices, suggesting hippocampal-dependent cognitive function is not adversely affected (Detrait et al., 2010).

Toxicology

Brivaracetam showed low acute toxicity in mice, rats, and dogs; data have been summarized previously (Bialer et al., 2009).

Pharmacokinetics and metabolic profile

Brivaracetam pharmacokinetics was extensively evaluated in healthy subjects, showing evidence for a linear and predictable pharmacokinetic profile. Brivaracetam is rapidly and completely absorbed after oral administration, and absorption is unaffected by food (Rolan et al., 2008; Sargentini-Maier et al., 2007, 2008). Brivaracetam is weakly bound to plasma proteins (~17.5%) (Sargentini-Maier et al., 2008), and its half-life is about 9 h (UCB, data on file). Brivaracetam is renally excreted following extensive metabolism, primarily by hydrolysis and to a lesser extent by CYP-dependent hydroxylation; the main isoenzyme responsible for hydroxylation is CYP2C19 (Nicolas et al., 2012;

Stockis et al., 2014b). Consistent with this, only 5—8% unchanged brivaracetam is excreted in urine (Rolan et al., 2008), along with pharmacologically inactive metabolites (von Rosenstiel and Perucca, 2009).

CYP2C19 poor metabolizers are rare in Western populations; however, they occur in up to 20% of Asian populations. Pharmacokinetics of brivaracetam in healthy Japanese subjects genotyped for common mutations *2 and *3 of CYP2C19 (with no enzyme activity) showed that, although hydroxy metabolite formation was reduced 10-fold, there was only a 29% reduction in brivaracetam clearance, which was considered to be of small clinical relevance. Therefore, dose adjustment is not required in CYP2C19 poor metabolizers (Stockis et al., 2014b).

In subjects with severe renal impairment (creatinine clearance <30 mL/min/1.73 m^2), exposure to brivaracetam increased by 21% while exposure to its three metabolites (acid, hydroxy, and hydroxyacid) notably increased. However, no safety issues were considered to be associated with increased exposure, as the metabolites are pharmacologically inactive and of low toxicity, suggesting that dose adjustment in patients with renal dysfunction is not necessary (Sargentini-Maier et al., 2012). Hepatic impairment, however, may alter brivaracetam pharmacokinetics. Metabolic clearance of brivaracetam was reduced in subjects with hepatic impairment, resulting in increased exposure to brivaracetam (50—60%), irrespective of impairment severity. These data suggest that the maximum daily dose of brivaracetam could be reduced by one-third in patients with impaired liver function (Stockis et al., 2013).

Drug interactions

In clinical trials conducted to date, brivaracetam administered as adjunctive therapy did not affect the plasma concentrations of concomitantly administered AEDs, although the plasma concentrations of carbamazepine-10,11-epoxide were approximately doubled at the highest dose of 200 mg/day. Consequently, no dose adjustment is required when brivaracetam is added to most AEDs. The clinical relevance of the increase in plasma concentration of carbamazepine-10,11-epoxide remains to be established.

Potential interactions between brivaracetam and oral contraceptive have been investigated in healthy women (Stockis et al., 2014a; Stockis and Rolan, 2013). For brivaracetam 100 mg/day, no pharmacokinetic interaction with oral contraceptive was found, and the oral contraceptive did not affect brivaracetam plasma concentrations (Stockis et al., 2014a). At the supratherapeutic dose of 400 mg/day, brivaracetam somewhat decreased the plasma concentrations of the contraceptive's estrogen and progestin components, but the levels of endogenous hormones LH, FSH, estradiol, and progesterone were similar and normal during brivaracetam and control cycles (Stockis and Rolan, 2013).

Efficacy data

Phase IIb studies

Two double-blind, placebo-controlled, dose-ranging Phase IIb studies have been conducted to assess the safety and efficacy of adjunctive brivaracetam in adults patients with focal epilepsy and inadequate response to 1—2 concomitant AEDs (N01193; NCT00175825 and N01114; NCT00175929) (French et al., 2010; van Paesschen et al., 2013). In the N01193 study, the primary efficacy endpoint, a statistically significant reduction in baseline-adjusted focal seizure frequency/week over placebo, was achieved at the 50 mg/day dose. The ≥50% responder rate was significantly greater than with placebo for brivaracetam 5, 20, and 50 mg/day (French et al., 2010). In contrast, study N01114 (50 and 150 mg/day brivaracetam) did not achieve its primary efficacy end-point, although numerically greater reductions in baseline-adjusted focal seizure frequency/week over placebo were seen at both doses and several secondary efficacy outcomes attained significance (van Paesschen et al., 2013).

Phase III studies

Two fixed-dose (N01253; NCT00464269 and N01252; NCT00490035) (Biton et al., 2014; Ryvlin et al., 2014), and one flexible dose (N01254; NCT00504881) (Kwan et al., 2014), prospective, multicenter, randomized, double-blind, placebo-controlled, parallel-group Phase III trials have been completed.

Both fixed-dose studies were conducted in adults with uncontrolled focal epilepsy despite treatment with 1—2 concomitant AEDs. Patients received 12 weeks of adjunctive brivaracetam (N01253: 5, 20, or 50 mg/day; N01252: 20, 50, or 100 mg/day) or placebo, without titration for 12 weeks. The primary efficacy endpoint was reduction in baseline-adjusted focal seizure frequency/week over placebo.

In study N01253, 361/396 (91.2%) patients completed the study. The primary efficacy endpoint was achieved at the 50 mg/day dose (12.8% reduction; $p = 0.025$). Brivaracetam 50 mg/day also achieved statistical significance *versus* placebo for secondary endpoints, including ≥50% responder rate (32.7% *versus* 16.7%, respectively; $p = 0.008$) (Biton et al., 2014).

In study N01252, 367/398 (92.2%) patients completed the study. The primary efficacy endpoint for brivaracetam 50 mg/day was not achieved. However, the 100 mg/day dose reduced baseline-adjusted focal seizure frequency/week over placebo (11.7%, $p = 0.037$). Further, there was a numerical advantage for brivaracetam over placebo for multiple secondary endpoints, including ≥50% responder rate at 100 mg/day (36.0% *versus* 20.0%, respectively; $p = 0.023$) (Ryvlin et al., 2014).

In a *post hoc* analysis of data pooled from both fixed-dose studies (Biton et al., 2014; Ryvlin et al., 2014), in patients without prior levetiracetam use, ≥50% responder rates were significantly higher on brivaracetam 50 or 100 mg/day (37.2%, $p = 0.0008$ and 43.1%, $p = 0.0004$, respectively) than placebo (17.4%). In patients who had previously used levetiracetam, ≥50% responder rates were numerically higher on brivaracetam (50 mg/day: 27.1%; 100 mg/day: 36.4%) than placebo (20.4%). In the approximately 20% of patients who received concomitant levetiracetam, no additional benefit in responder rates was seen (brivaracetam 50 mg/day: 12.8%, 100 mg/day: 15.0%, and placebo: 18.9%) (UCB, data on file).

Study N01254 (Kwan et al., 2014) enrolled adult patients with focal ($n = 431$) and generalized ($n = 49$) epilepsy, with uncontrolled seizures despite treatment with 1—3 AEDs. Treatment (brivaracetam:placebo, 3:1) comprised an 8-week dose-finding period (dosing increased as required to maximum 150 mg/day), followed by an 8-week maintenance period at the determined dose. Completion rates were 90% for brivaracetam-treated and 91.7% for placebo-treated patients. This was primarily a safety study; efficacy was a secondary endpoint. In patients with focal seizures, baseline-adjusted percent reduction in seizure frequency/week for brivaracetam over placebo was 7.3% ($p = 0.125$), and responder rates (≥50%) for brivaracetam-treated *versus* placebo-treated patients were 30.3% *versus* 16.7%, respectively ($p = 0.006$). Potential efficacy of brivaracetam was apparent in patients with generalized seizures; reduction in number of seizure days per week was 0.79 in brivaracetam-treated *versus* 0.21 in placebo-treated patients, and ≥50% responder rates were higher in brivaracetam- *versus* placebo-treated patients (44.4% *versus* 15.4%, respectively).

Tolerability and adverse effect profile

Exposure to brivaracetam in clinical trials to date is approximately 6000 patient years, with some patients followed for ≥8 years (UCB, data on file). Adjunctive brivaracetam demonstrated a favorable safety and tolerability profile in adults with uncontrolled focal epilepsy in the two Phase IIb (Brodsky et al., 2007) and the three Phase III trials (Biton et al., 2014; Ryvlin et al., 2014; Kwan et al., 2014); ≥1 treatment emergent adverse event (TEAE) was reported by 61.6% patients on brivaracetam and 59.4% on placebo. TEAEs leading to permanent discontinuation were infrequent (brivaracetam 5.4%, placebo 3.5%) (UCB, data on file).

In the Phase III flexible-dose safety study, proportions of patients reporting ≥1 TEAE and TEAEs leading to treatment discontinuation were similar for brivaracetam *versus* placebo (66% *versus* 65.3% and 6.1% *versus* 5.0%, respectively). Incidence of TEAEs was highest during the dose-finding period, decreasing during the maintenance period (brivaracetam: 56.0—36.8%, placebo: 55.4—40.9%) (Kwan et al., 2014).

In the two Phase II and three Phase III trials, the majority of TEAEs were of mild-to-moderate severity. The most commonly reported TEAEs were headache (brivaracetam 10.8%, placebo 12.0%), somnolence (8.2%, 5.2%), dizziness (6.7%, 5.4%), and fatigue (4.8%, 2.8%) (UCB, data on file).

An open label, randomized, parallel-group, Phase III study investigated the safety and tolerability of intravenous (i.v.) brivaracetam (200 mg/day, bolus over 2 min or infusion over 15 min) as an alternative to oral therapy (N01258; NCT01405508). Adult patients ($n = 105$) with focal or generalized epilepsy uncontrolled on 1—2 concomitant AEDs were randomized to receive brivaracetam i.v. bolus or infusion following a run-in period with either brivaracetam or placebo tablets (1:1:1:1). Similar incidences of TEAEs were reported for brivaracetam i.v. (bolus + infusion) following run-in oral brivaracetam *versus* placebo (66.0% *versus* 70.6%). No new safety concerns were raised; 98% of patients completed the study. Brivaracetam 200 mg/day i.v. bolus and infusion were similarly well tolerated; most frequent i.v.-related TEAEs were injury site erythema (3.8%) and pain (3.8%). The most common TEAEs were somnolence (22.6% *versus* 21.6%) and dizziness (3.8 *versus* 11.8%) for run-in oral brivaracetam *versus* placebo, respectively (Klein et al., 2014).

Adjunctive brivaracetam oral solution was investigated in a Phase IIa, open-label study (N01263; NCT00422422) in infants and children with epilepsy, taking 1—3 concomitant AEDs (excluding levetiracetam). Dosing levels were based on physiologically based pharmacokinetic predictions (0.4—1.6 mg/kg bid for patients ≥8 years and 0.5—2.0 mg/kg bid for patients <8 years) to ensure similar plasma exposures as in adults receiving 25—100 mg bid. Brivaracetam was generally well tolerated; 90/99 (90.0%) patients completed the study. At least one TEAE was reported in 66.7% of patients; 32.3% reported ≥1 drug-related TEAE. The most common drug-related TEAEs were somnolence (7.1%) and decreased appetite (6.1%). The incidence of TEAEs was higher in patients aged ≥1 month to □2 years (80.0%) *versus* patients aged ≥2 to ≤12 years (60.8%) or ≥12 to ≤16 years (61.1%), but the incidence of drug-related TEAEs was lower (16.7%, 41.2%, and 33.3%, respectively). Serious TEAEs were reported in 8.1% of patients, most commonly convulsion (Liu et al., 2014).

Ongoing clinical studies

An additional large three-arm (placebo, brivaracetam 100 mg/day, or 200 mg/day) adjunctive-therapy Phase III trial (N01358; NCT01261325) has recently been completed, the results from which may further confirm the efficacy of adjunctive brivaracetam in patients with focal epilepsy. There are several long-term, open-label, follow-up trials of adjunctive brivaracetam in progress.

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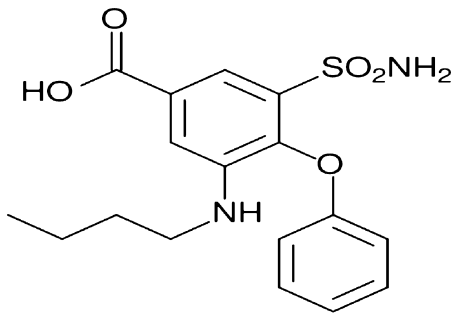
Bumetanide

R.M. Pressler

Section of Clinical Neurosciences Unit, Institute of Child Health, University College London, UK



Bumetanide

Introduction and rationale for development

Bumetanide (3-(butylamino)-4-phenoxy-5-sulfamoylbenzoic acid) is a potent loop diuretic with a rapid onset and short duration of action. It has been used routinely for the treatment of oedema associated with congestive heart failure, hepatic, pulmonary and renal diseases, both in adults and children including term and preterm infants for the last 30 years. Pharmacologically, bumetanide is about 40-fold more potent than furosemide (frusemide), with the exception of its effects on urinary potassium excretion where its potency is lower. Its diuretic properties are due to blocking the renal Na^+ K^+ $2Cl^-$ (isoforms NKCC1 and NLCC2) co-transporter and thus inhibiting sodium reabsorption in the ascending limb of the loop of Henle.

More recently, several animal studies have shown that due to the ubiquitous distribution of the isoform NKCC1, bumetanide also has antiepileptic properties by influencing abnormal $GABA_A$.

Pharmacology

Due to overexpression of neuronal NKCC1 and low expression of KCC2 (K^+ Cl^- co-transporter isoform 2), immature neuron have a high intracellular Cl^- concentration, rendering GABAa receptor-mediated Cl^- currents depolarising (excitatory state) instead of hyperpolarising (inhibitory state) as in mature neurons (Dzhala et al., 2005; Rheims et al., 2008; Ben-Ari et al., 2012). This may at least partially explain the high incidence of seizures and poor response to conventional AEDs in the newborn infants. The switch from excitatory to inhibitory function is assumed to happen around birth but varies between species and between different brain regions.

Recurrent seizures and other traumatic insults can lead to down-regulation of KCC2 and to a re-establishment of NKCC1-dependent depolarizing GABAergic signalling (Löscher et al., 2013).

Several *in vitro* and *in vivo* studies suggest that bumetanide can switch the GABA equilibrium potential of immature neurons or abnormal mature neurons from depolarizing to hyperpolarizing, resulting in a reduced neuronal firing (Dzhala et al., 2005; Rheims et al., 2008; Ben-Ari 2012; Löscher et al., 2013) (see Fig. 3 and Table 3).

Furthermore, bumetanide can augment phenobarbital anticonvulsive action in different *in vitro* rodent models including a hypoxic rat model. This suggests that the combination of phenobarbital and bumetanide may provide a clinically feasible antiepileptic therapy for neonatal seizures with the possible additional benefit of augmenting the neuroprotective efficacy of therapeutic hypothermia in asphyxiated neonates (Liu et al., 2012).

There is also some evidence that bumetanide has antiictal action by blocking glial NKCC1 leading to expansion of the extracellular space together with a net efflux of water across the blood-brain barrier (BBB) (Hochman, 2012; Löscher et al., 2013).

Toxicology

Toxicity testing in rats, rabbits, dogs, and baboons indicate that bumetanide is well tolerated in various species.

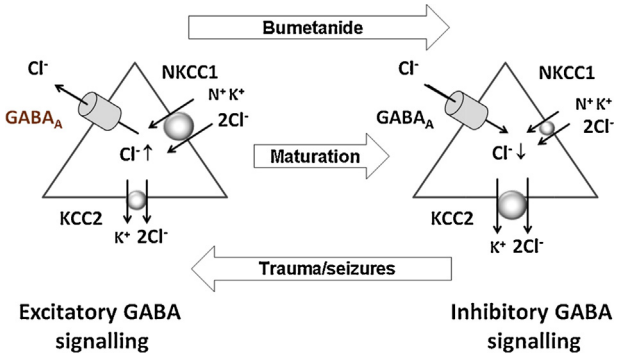


Figure 3 Mode of action: in immature neurons (left) $GABA_A$ receptor-mediated Cl currents are depolarizing (excitatory) due to an overexpression of Na^+ K^+ $2Cl^-$ co-transporter isoform 1 (NKCC1) and under expression of K^+ $2Cl^-$ co-transporter isoform 2 (KCC2) resulting in high intracellular chloride. During neuronal maturation a shift from depolarizing to hyperpolarizing $GABA_A$ receptor-mediated Cl^- currents takes place *via* down regulation of NLCC1 and up regulation of KCC2; an opposite effect is often seen following epilepsy and trauma. Bumetanide blocks neuronal NKCC co-transporters rendering GABA inhibitory.

The oral LD_{50} is 4.6 g/kg (mouse) — 6 g/kg (rat) and the intraperitoneal LD_{50} is 519 mg/kg (mouse) — 891 mg/kg (rat). Toxicity observed mostly the direct consequence of its potent diuretic activity. Bumetanide was fetotoxic at maternal toxic doses in the rabbit, but no evidence of teratogenic effects was observed in rabbits, rats, mice, or hamsters. The compound was devoid of mutagenic activity by the Ames test and was found to be non-carcinogenic in long-term rat studies (McClain and Dammers, 1981).

Wang and Kriegstein (2008) have shown that treatment with bumetanide during the perinatal period can disrupt excitatory synapse formation in the cortex. Conversely, treatment of mice dams and pups used in these studies was for a longer period of time and at a much earlier stage in development (intrauterine and in first few days of life) which corresponds to a more premature developmental stage than in the human. Thus, this study was not comparable to treatment of newborn babies where the switch of GABA from excitation to inhibition occurs physiologically. Furthermore, this study investigated the effect of bumetanide on healthy mice pups which means that issues of risk-benefit ratio (adverse effects of seizures *versus* adverse effect of drugs) are not taken into account.

Pharmacokinetics and metabolic profile

Pharmacokinetic studies of bumetanide have concentrated on treatment of fluid overload in cardiopulmonary disease both in adults and children, including critically ill term and preterm babies (Pacifici, 2012; Pressler and Mangum, 2013). Bumetanide has a well-characterized pharmacokinetic profile, including a fast absorption rate, almost complete bioavailability, and first-order clearance.

Bumetanide is extensively (72—95%) bound to plasma proteins in adults. The degree of protein binding in cord sera from healthy neonates was approximately 97%, suggesting the potential for bilirubin displacement (Turmen et al.,

1982). A study using pooled sera from critically ill neonates found that bumetanide at concentrations of 0.5—50 μg/mL, but not 0.25 μg/mL, caused a linear increase in unbound bilirubin concentrations (Walker and Shankaran, 1988).

The half-life of bumetanide is 0.8—1.5 h in adults, but in infants it is significantly longer, with a mean value of approximately 6 h and a range of up to 15 h (Pacifici et al., 2012). Studies in adult patients have demonstrated about 50—70% recovery of unchanged bumetanide in the urine over 24—48 h after i.v. administration. The elimination of bumetanide appears to be considerably slower in babies compared with adults, possibly because of immature renal and hepatobiliary functions. Bumetanide administered i.v. in preterm and full-term infants with respiratory disorders is reported to have a serum clearance ranging from 0.2 to 1.1 mL/min/kg (Pacifici, 2012). In infants approximately 40% is cleared *via* the renal system and the reminder *via* non-renal elimination pathways which includes CYP-mediated metabolism (Sullivan et al., 1996).

As bumetanide is highly bound to plasma protein and highly ionized at physiological pH, it penetrates poorly into the brain and thus it is of limited use in chronic epilepsy. Although most clinical conditions associated with neonatal seizures such as hypoxia and cerebrovascular insults are likely to result in increased BBB permeability, which may allow better penetration, it is unclear whether the intracerebral concentrations of bumetanide in these patients is sufficient to inhibit the NKCC1 co-transporter.

Drug interactions

A number of drug interactions involving bumetanide have been reported (Halstenson and Matzke, 1983). Non-steroidal anti-inflammatory drugs can decrease the effects of bumetanide. Salicylates can impair diuretic response to bumetanide in patients with cirrhosis and ascites. Thiazide diuretics can have synergistic effects that may result in profound diuresis and serious electrolyte abnormalities. Bumetanide-induced electrolyte disturbances may predispose to digitalis-induced arrhythmias. The combination of bumetanide with aminoglycosides and cisplatin may lead to increased ototoxicity. Likewise, bumetanide can increase the plasma levels and the toxicity of lithium.

Considerations for dose-finding

In vitro animal studies initially suggested 0.1—0.2 mg/kg as the optimal dose of bumetanide required to block the NKCC1 co-transporter, which is at the upper range of the dose used as a diuretic (0.05—0.1 mg/kg). A more recent *in vivo* study evaluating the effect of bumetanide on the neuroprotective properties of phenobarbital in rodents (Liu et al., 2012) used much higher doses, up to 10 mg/kg. This is far more than the recommended dose used for diuresis, and the potential risk of ototoxicity and other side effects is unclear. However, studies in infants have shown that the diuretic effect is maximal at doses of around 0.04 mg/kg and any further dose increase results in an increase of drug excretion but no further increase in urine output or electrolyte excretion. Because the metabolism of bumetanide is different in rodents compared to humans, the optimal dose to be used as an anticonvulsant in humans is unknown.

Efficacy data

Despite the large number of preclinical studies, there is little clinical evidence for bumetanide's efficacy as an anti-seizure agent apart from a couple of case studies. In adults, bumetanide at total daily dose of 2 mg was associated with a reduction of seizure frequency and EEG discharges in three patients with temporal lobe epilepsy over a 4-month period (Eftekhari et al., 2013). However, it is not specified how the dose was divided over the day and how the drug was administered. Considering the half-life in adults, that dose appears to be rather small. In infants, Kahle and colleagues (2009) published a single case study of a 6-week old infant with seizures due to meningitis, showing that a single dose of 0.1 mg/kg i.v. bumetanide was associated with a reduction of electrographic seizures by less than 50% (36 ± 7 seizures per hour in a 2-h window before treatment compared to 21 ± 7 seizures in the 2-h window following the administration of bumetanide).

Tolerability and adverse effect profile

The expected adverse effects of bumetanide are linked to its diuretic effect and include fluid loss, dehydration, hypotension, tachycardia, and changes in electrolytes, including hypochloraemia, hypokalaemia, hyponatraemia, hypophosphataemia, and hypocalcaemia as well as hyperglycaemia (Halstenson and Matzke, 1983). Other adverse effects include pruritus, muscle cramps, weakness and ototoxicity.

Although there are no specific studies on the safety of bumetanide in a neonatal population, pharmacokinetic studies have showed that it is well tolerated at diuretic doses, *e.g.*, up to 0.05—0.1 mg/kg (both with single and multiple dose administration) in term and preterm infants, including critically ill babies (Ward and Heel, 1984; Lopez-Samblas et al., 1997; Clark et al., 2006). Specifically, single doses of bumetanide up to 0.1 mg/kg were well tolerated in infants younger than 6 months of age including newborn babies without significant effects on electrolytes, bilirubin, renal, and cardiovascular function (Sullivan et al., 1996). At repeated high doses, hypokalaemia and hypotension was reported requiring replacement therapy (Marshall et al., 1998). However, it is unclear whether hearing was systematically tested in these babies.

Hyperbilirubinaemia has been described in the adult population (Halstenson et al., 1983) and is a concern for term and preterm infants who are at risk of hyperbilirubinaemia.

Similar to other loop diuretics, bumetanide carries a potential risk of ototoxicity. When the ototoxicity of bumetanide and furosemide was compared in guinea pigs pre-treated with kanamycin, both bumetanide and furosemide produced permanent alterations of cochlear activity at high doses near the LD_{50} (Taylor et al., 2008). Although the ototoxic effect of bumetanide is five times that of furosemide on a milligram-for-milligram basis, at equipotent doses the relative ototoxicity of bumetanide is lower (diuretic potency of 1 mg bumetanide = diuretic potency of 40 mg furosemide). In adults, drug-related hearing loss was reported in 2 of 179 bumetanide-treated patients as compared to 4 of 62 furosemide-treated patients (Tuzel, 1981).

There is, however, some evidence that the susceptibility to drug-induced hearing loss is age-dependent, with younger animals being more susceptible.

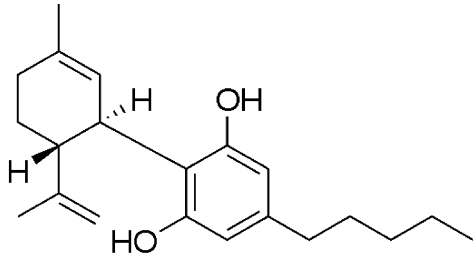
Planned studies

There are currently two trials with bumetanide registered with *clinicaltrials.gov*. NEMO1 (treatment of Neonatal Seizures using Medication Off-patent) is a Phase I/II dose finding and feasibility trial of bumetanide for the treatment of neonatal seizures (http://www.clinicaltrials.gov/ct2/show/NCT01434225). The aim of this open label exploratory dose finding and pharmacokinetic trial was to estimate the optimal dose of bumetanide for the treatment of neonatal seizures not responding to an initial loading dose of phenobarbital. This trial has been terminated early due to safety concerns, and results will be published shortly. The other clinical dose-finding study of bumetanide as an anti-seizure treatment, which is currently still ongoing, is a U.S. pilot study in neonatal seizures (http://www.clinicaltrials.gov/ct2/show/NCT00830531). This is a randomized, double-blind, controlled, dose escalation study of bumetanide as add-on therapy for refractory seizures comparing standard phenobarbital therapy *versus* standard phenobarbital therapy combined with 0.1 mg/kg, 0.2 mg/kg, or 0.3 mg/kg bumetanide. The primary outcome is the assessment of the pharmacokinetics and safety of bumetanide in newborns with refractory seizures, while the secondary outcome is to determine the feasibility of a novel study design to test AEDs to treat neonatal seizures.

Cannabidiol

S. Wright[1], K. Sommerville[1+], N.A. Jones[1], B.J. Whalley[2]

[1] GW Pharma GW Research Limited, Porton Down Science Park, Salisbury, Wiltshire SP4 0JQ, UK
[1+] GW Pharmaceuticals and Duke University Medical School, UK
[2] Reading School of Pharmacy, University of Reading, Whiteknights, Reading, Berkshire RG6 6AP, UK



Cannabidiol

Introduction and rationale for development

There has been speculation for many years that cannabis (marijuana), or some components of the cannabis plant may have antiepileptic properties. Cannabidiol (CBD) was identified as a potential candidate cannabinoid responsible for these proposed antiepileptic properties, and has been subjected to a number of non-clinical evaluations, which have generally confirmed that it has antiepileptic properties in pre-clinical models of epilepsy.

More recently, CBD has also been shown to have neuroprotective and anti-inflammatory effects and has been confirmed to possess anticonvulsant properties. A systematic pharmacological evaluation of its antiepileptic potential has been carried out, using both well-established and novel methodologies. In addition, progress has been made in identifying putative mechanisms of action.

Clinical evaluations of cannabidiol as a potential treatment for epilepsy have involved small numbers of patients, variable treatment periods and dosing regimens, and have produced conflicting results. These have been reviewed in a recent Cochrane review, which concluded that ''no reliable conclusions can be drawn at present regarding the efficacy of cannabinoids as a treatment for epilepsy'', and went on to suggest that ''there would need to be a series of properly designed, high quality, and adequately powered trials'' (Gloss and Vickrey, 2012).

More recently, cannabidiol has become especially newsworthy because of a series of anecdotal reports that CBD-rich marijuana is highly effective in treating children with drug-resistant epilepsy (Gedde and Maa, 2013). Cannabidiol is present in the therapeutic product Sativex (USAN = nabiximols), which is an approved medicine throughout most of Europe and in other countries (*e.g.*, Canada, Switzerland, Australia, New Zealand, Kuwait). Because of this, there is an extensive database of information available with regard to its metabolism, toxicology and safety.

Pharmacology

Anticonvulsant profile (animal models/electrophysiology)
A number of researchers have reported over the last two decades or more that CBD has anti-seizure activity in a variety of animal models. This literature has been extensively reviewed elsewhere, and the reader is referred there for a comprehensive review of the historical data (Devinsky et al., 2014).

More recently, we have reported significant antiepileptiform and anticonvulsant activity for CBD using a variety of *in vitro* and *in vivo* models (Jones et al., 2010, 2012). Using *in vitro* models, CBD ($\geq$10 nM) exerted significant antiepileptiform effects on both 4-aminopyridine and Mg^{2+}-free epileptiform local field potentials in a concentration-related and region-dependent manner; findings that were comparable with clinically-used AEDs. Subsequently, we examined the anticonvulsant activity of CBD (1—100 mg/kg, i.p.) in three different *in vivo* seizure models. In the PTZ-induced acute, generalised seizure model, CBD (100 mg/kg) demonstrated significant anticonvulsant activity in lowering the incidence of the most severe seizures, mortality rate and seizure severity; these anti-convulsant effects of CBD were retained when co-administered with either ethosuximide (ESM) or valproic acid (VPA). In the acute pilocarpine model of temporal lobe seizure, CBD ($\geq$1 mg/kg) exhibited modest anticonvulsant effects, significantly lowering the incidence of the most severe seizures; these anticonvulsant effects of CBD were retained when co-administered with VPA, but not

phenobarbital (PB). In the penicillin model of partial seizure, CBD demonstrated significant anticonvulsant effects, lowering the incidence of the most severe seizures (≥1 mg/kg) and caused a significant 2-fold reduction in mortality rate (≥10 mg/kg). Finally, motor function tests confirmed that the anticonvulsant effects of CBD (50—200 mg/kg) in these acute models of seizure were due to genuine anti-convulsant properties and not suppression of motor function. Whereas, acutely administered standard AEDs (VPA, PB and ESM) exerted negative effects on motor function (Jones et al., 2010).

Other pharmacological properties

CBD has a rich and varied pharmacology, which has been well described in the literature. It has anti-inflammatory properties, shown particularly in animal models of joint and intestinal inflammation (Malfait et al., 2000; De Petrocellis et al., 2011). CBD also shows neuroprotective properties in a number of established animal models of neurodegenerative disease (Walter and Stella, 2004). Both of these properties may be relevant to the treatment of epilepsy.

Mechanism(s) of action

Because of the diverse pharmacology exhibited by CBD, the precise mechanism of its action in models of epilepsy is unclear. Recent work in our laboratory suggests that CBD does not exert its anti-seizure effects through a cannabinoid (CB) receptor dependent mechanism, nor through blocking of the sodium channel. Perhaps the prime candidates are, firstly, the ability of CBD to act as a potent inhibitor of adenosine uptake, a mechanism that has been shown to be responsible for its ability to inhibit TNFα production by retinal microglia (Liou et al., 2008), an observation that demonstrates its anti-neuroinflammatory potential (Table 3). Secondly, CBD is able to modulate intracellular calcium mobilisation, possibly by modulation of expression of the Voltage Dependent Anion Channel-1 (VDAC-1), with a potential anti-glutamatergic consequence (Ryan et al., 2009), and thirdly, CBD has activity at a number of TRP channels.

Toxicology

The tolerability of CBD in animal models of epilepsy and seizures is good, and compares very well with that of established AEDs in the static beam assay. CBD has been subject to extensive preclinical safety pharmacology, genetic toxicology, reproductive toxicology and chronic, repeat dose toxicology studies as a major component of the product Sativex. Furthermore, a range of preclinical safety studies has been conducted with CBD alone, including extensive preclinical safety pharmacology (rat CNS, rat respiratory, dog telemetry studies plus hERG channel and rabbit purkinje fibre cardiac assays). In addition, genetic toxicology studies did not produce any evidence of mutagenic potential and *in vivo* studies have yielded NOAELS between 350 and 500 mg/kg CBD. A 2-week exposure in rats yielded little toxicity at 150 mg/kg/day and a 3-month dose range finding repeat dose toxicology study indicated that a dose of 50 mg/kg/day CBD (dietary administration) would be suitable for a 2 year carcinogenicity study. This single carcinogenicity study (also administered *via* the diet) produced no carcinogenic effects and little toxicity up to 50 mg/kg/day CBD. A drug discrimination study in rodents demonstrated that CBD (1, 3 and 10 mg/kg) does not have the same subjective effects as THC, and that animals can distinguish between the two. In addition, at clinically relevant doses CBD does not produce classical CB1 agonist like activity (as evidenced by testing in the tetrad assay).

Pharmacokinetics and metabolic profile

Following oral administration to healthy subjects, peak serum CBD concentrations are reached after 90—120 min. The oral bioavailability is around 10%, with the consequence that the between-subject variability in CBD pharmacokinetics is substantial. CBD elimination half-life of CBD is around 6 h, but the intense lipophilicity of CBD means that it is taken up into tissues, resulting in a late-phase half-life of about 24 h. CBD is highly plasma protein bound (>99%).

Drug interactions

Effects of CBD on the CYP system and on PgP only seem to occur at exposures/concentrations far above those that are clinically relevant (>6 μM). The major metabolites of CBD in rat, dog and human microsomes have been identified using [^{14}C]-CBD, with the major CBD metabolite in humans being 7-hydroxy-CBD. CBD is metabolised principally by CYP3A4 and CYP2C19.

CBD IC_{50} values for the various CYPs are in the micromolar range, meaning that inhibition of CYP enzymes by CBD is unlikely to occur at therapeutic doses. The effects of rifampicin (a potent CYP3A4 inducer), ketoconazole (a potent CYP3A4 inhibitor) and omeprazole (a CYP2C19 inhibitor) on the pharmacokinetics of CBD have been shown not to be of clinical significance. To our knowledge, there are no publications which describe a significant drug—drug interaction for CBD. Overall, available data suggest that significant drug—drug interactions with CBD are unlikely.

Efficacy data

As part of a programme of compassionate use, a substantial number of children with drug-resistant childhood epilepsies are being treated with CBD under a series of expanded access INDs, following approval of the protocol by the US FDA. The preparation being used is a liquid preparation (Epidiolex, GW Pharmaceuticals) available in a 25 mg/mL and 100 mg/mL concentration. Under the protocol, children from the age of 1 year, suffering from a range of childhood epilepsy syndromes, and all with a history of multiple AED use, are treated with CBD at a starting dose of 2.5 or 5 mg/kg/day, given as two divided doses. The dose is gradually increased up to a total dose of 25 mg/kg/day. Children are seen at regular intervals, and seizure type and frequency are recorded on a daily diary. Additional information being collected includes episodes of status epilepticus and

unscheduled hospitalisations. Blood is taken at regular intervals for routine haematology and clinical chemistry, and for the determination of the serum concentration of concomitant AEDs to identify potential signals of drug—drug interactions. Initial results are available for the first 62 children treated under this protocol, who include a wide range of diagnoses including Dravet syndrome ($n = 13$), ''generalized intractable epilepsy'' ($n = 6$) and tuberous sclerosis complex ($n = 5$). Overall, outcomes in terms of seizure control are promising to date, there have been fewer hospitalisations and fewer episodes of status epilepticus in the treated children compared with the pre-treatment (baseline) period (Pelliccia et al., 2005; Trembly and Sherman, 1990).

Tolerability and adverse effect profile

In human studies published to date (Ames and Cridland, 1986; Carlini and Cunha, 1981), there does not appear to be any recorded organ toxicity, and CBD has been well tolerated at doses up to 1500 mg daily. In the expanded access protocol described above, safety data are available for the first 62 children treated, with a total exposure of 122 patient months. Overall, 80% of children have reported treatment-emergent adverse events (TEAE) of which a very large majority (>80%) are mild or moderate. Five events occurred in more than 10% of children, and are therefore classified as 'common'. They include somnolence (40%), fatigue (26%), diarrhoea (16%), decreased appetite (11%), and increased appetite (10%). Seven patients have reported serious TEAEs, which in no case were deemed to be related to CBD. In no case have TEAEs resulted in the treatment being withdrawn. At baseline, the majority of the children were being treated with more than 3 concomitant AEDs, the serum levels of which are available in a number of cases. To date, there is no clear evidence of a drug—drug interaction with the two most commonly taken AEDs in this population, which are valproic acid and clobazam.

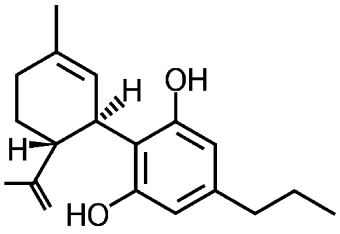
Planned studies

A structured set of randomised placebo-controlled studies, which include an assessment of the dose-related pharmacokinetics of CBD in young children with Dravet syndrome, are due to start in the second half of 2014. The studies will assess the efficacy and safety of CBD as an add-on therapy in the treatment of children with Dravet syndrome and Lennox—Gastaut syndrome. A formal drug—drug interaction study of CBD with clobazam is also being planned.

Cannabidivarin (GWP 420006)

N.A. Jones[1], B.J. Whalley[2]

[1] GW Pharma GW Pharmaceuticals plc, Porton Down Science Park, Salisbury, Wiltshire SP4 0JQ, UK
[2] Reading School of Pharmacy, University of Reading, Whiteknights, Reading, Berkshire, UK



Cannabidivarin

Introduction and rationale for development

Cannabis has been used to treat a variety of disorders for many centuries but the number of licensed cannabinoid-based medicines remains small and the clinical evidence base relatively limited (Greydanus et al., 2013). In recent years, the use of cannabis for the treatment of epilepsy has received considerable scientific and popular attention due to new preclinical study results, the initiation of human clinical trials in epilepsy and the decriminalisation of cannabis use in some US states (http://bit.ly/1f6O1f8). Whilst the former has resulted in the first formal clinical trials for cannabis components in epilepsy, the latter has triggered a surge in the use of herbal cannabis without regulation for the treatment of childhood epilepsies (Gedde and Maa, 2013).

Cannabidavarin (CBDV) was first isolated in 1969 (Vollner et al., 1969) and is the propyl analogue of the non-psychoactive plant cannabinoid (phytocannabinoid) cannabidiol (CBD). We and others have previously shown that CBD exerts significant anti-seizure effects in a variety of preclinical models and a small human clinical evidence base exists which supports these findings (Hill et al., 2012b). CBD has now entered Phase 2 human trials to establish safety and tolerability in refractory childhood epilepsy patients, while CBDV entered Phase I clinical trials in 2013.

Pharmacology

Anticonvulsant profile (animal models/electrophysiology)
CBDV exerts significant antiepileptiform effects on both 4-aminopyridine- and magnesium-free-induced epileptiform local field potentials (LFPs) in acute hippocampal slices, recorded using multi-electrode arrays (Hill et al., 2012a) in a region-dependent and model-specific manner at concentrations $\geq 1\ \mu M$. The magnitude of these effects is largely comparable to those produced by CBD (Jones et al., 2010) and the clinically used AEDs, felbamate and phenobarbital (PB) in such models (Hill et al., 2010; Sagratella, 1998).

Thereafter, the effects of CBDV were investigated in the MES (30 mA; 100 Hz; 200 ms) model of generalised seizures in ICR (CD-1) mice where it significantly inhibited the severity of tonic convulsions ($\geq$50 mg/kg, i.p.) and the incidence of both hindlimb and forelimb extension ($\geq$50 mg/kg, i.p.) (Hill et al., 2012a). CBDV's effects have also been investigated in audiogenically induced generalised seizures in DBA/2 mice where the incidence of tonic-clonic seizures was significantly reduced and the percentage of those animals remaining seizure-free was increased (Hill et al., 2012a). Using the same model, results of an isobolographic study design to examine interactions between CBDV and CBD have

also been reported where the effects of the two drugs were found to linearly additive in their ability to reduce the incidence of clonic convulsions (Hill et al., 2013).

The effects of CBDV have also been examined in generalised seizures induced *via* i.p. administration of PTZ (Hill et al., 2012a). Here CBDV (200 mg/kg, i.p.) significantly lowered the incidence of the most severe seizures, mortality rate and seizure severity, as well as increasing the latency to seizure onset and the percentage of those animals that remained seizure-free; effects that were retained when CBDV was co-administered with either ethosuximide (60—175 mg/kg, i.p.) or valproic acid (50—250 mg/kg, i.p.) (Hill et al., 2012a). CBDV was also found to be effective in this model when administered *via* the oral route (400 mg/kg, p.o.) and, in this case, the behavioural measures of antiepileptic effects were validated using molecular markers such that seizure-induced increases of Fos, Egr1, Arc, Ccl4 and Bdnf expression were suppressed in CBDV-treated animals (Amada et al., 2013).

Finally, the effects of CBDV have been examined in acute convulsions induced by administration of pilocarpine and in acute seizures induced by cerebroventricular administration of penicillin. In both cases, small but significant antiepileptic effects of CBDV were seen at the highest dose tested (200 mg/kg; i.p.) (Hill et al., 2012a).

Other pharmacological properties

Relatively little remains known about the pharmacological properties of CBDV (Izzo et al., 2009). Scutt and Williamson (Scutt and Williamson, 2007) reported that it can act *via* CB2 cannabinoid receptor-dependent mechanisms but that such actions were not a direct result of effects at the CB2 receptor. CBDV also exerts a variety of effects at transient receptor potential (TRP) channels *in vitro*, where it is an agonist at human TRPA1, TRPV1 and TRPV2 (EC_{50} values: 0.42, 3.6 and 7.3 μM, respectively) but acts as an antagonist at TRPM8 receptors (IC_{50}: 0.90 μM) (De Petrocellis et al., 2011, 2012). CBDV can also inhibit diacylglycerol lipase-α (IC_{50} 16.6 μM), the primary synthetic enzyme of the endocannabinoid, 2-arachidonoylglycerol (Bisogno et al., 2003) *in vitro* (De Petrocellis et al., 2011) although the relatively high EC_{50} value is unlikely to be achieved *in vivo* (Deiana et al., 2012).

Mechanism(s) of action

Presently, little is known about the pharmacological mechanism or mechanisms through which CBDV exerts its antiepileptic effects. It has been shown that CBDV does not exert its effects *via* interaction with the cannabinoid type 1 (CB1) receptor (Hill et al., 2013). Moreover, even if sufficiently high concentrations could be achieved *in vivo* to inhibit diacylglycerol lipase-α function, the inhibition of endocannabinoid synthesis is not consistent with an antiepileptic mechanism (Hill et al., 2012b). With regard to TRP channel targets, many are not present in brain and, in the case of TRPV1, its role in epilepsy and therapeutic relevance remain tentative (Sun et al., 2013), particularly given its propensity to rapidly desensitise upon stimulation (Vyklicky et al., 2008). Thus, since all of the proposed mechanisms by which CBDV may be acting remain to be confirmed *in vivo*, additional research into the pharmacology of this compound, particularly by comparison to CBD, is required.

Toxicology

Static beam, inclined screen and grip strength tests have been undertaken to assess the effect of CBDV on rodent motor function. CBDV (50—200 mg/kg, i.p. to Wistar-Kyoto rats) exerted no detectable effects upon any parameter measured in these assays, unlike acutely administered valproic acid, ethosuximide and phenobarbital which each produced significant adverse effects upon motor function at doses showing anti-seizure activity (EC_{25}, EC_{50} and EC_{75}) (Hill et al., 2012a).

The pre-clinical safety of CBDV has been investigated in a conventional approach, and includes cardiovascular, respiratory and CNS safety pharmacology studies, hERG channel and Purkinje fibre studies, 3 models of genetic toxicology, 14 days dose ranging repeat dose oral toxicology studies, and 3 month rat and dog repeat dose toxicology. No target organ toxicity has been observed in chronic studies, even at substantial doses.

Pharmacokinetics and metabolic profile

Data on the pharmacokinetics of CBDV in animals have been reported (Deiana et al., 2012). Human pharmacokinetic data are not available as yet.

Drug interactions

In animal studies, CBDV (200 mg/kg; i.p. Wistar-Kyoto rats) was well tolerated when co-administered with effective doses of either sodium valproate, ethosuximide or phenobarbital (Hill et al., 2012a).

Tolerability and adverse effect profile

A single- and multiple-dose Phase I study has been conducted in healthy volunteers at doses up to 800 mg daily for 1 week. No significant toxicity was reported and details of the pharmacokinetics in humans have yet to be reported.

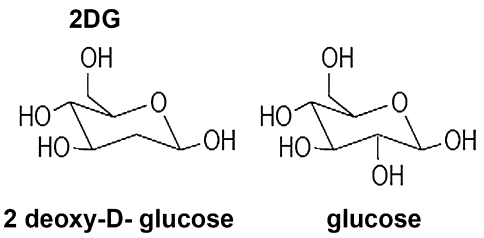
Planned studies

Proof of concept studies in adults with focal seizures, with or without secondary generalisation, are planned to start within the end of 2014.

2-Deoxy-D-glucose

T. Sutula

Department of Neurology, University of Wisconsin, Madison, WI, USA and NeuroGenomeX, Inc., Madison, WI, USA



Introduction and rationale for development

2-Deoxy-D-glucose is a glucose analogue currently in preclinical development for the treatment of epilepsy. The anticonvulsant and disease-modifying antiepileptic properties of glycolytic inhibition by 2-deoxy-D-glucose were discovered during investigations of mechanisms underlying the efficacy of the ketogenic diet. More detailed information on this compound can be found in the reports of previous Eilat conferences (Bialer et al., 2009, 2010, 2013).

Pharmacology

Glycolytic inhibition by 2-deoxy-D-glucose

After activity-dependent uptake into cells through glucose transporters, 2-deoxy-D-glucose undergoes phosphorylation to 2-deoxy-D-glucose-6-phosphate, which does undergo isomerization to fructose-6-phosphate and thereby prevents subsequent steps of glycolysis.

Anticonvulsant profile (animal models/electrophysiology)

2-Deoxy-D-glucose displays both *acute* and *chronic* mechanisms of action in experimental in the mouse *in vivo* 6 Hz model and also protected against audiogenic seizures evoked in Frings mice. 2-Deoxy-D-glucose also demonstrated some evidence of anticonvulsant activity against seizures evoked by pentylentetrazol but overall the results were not sufficient to calculate an ED_{50} or a time to peak effect. There was no protective effect of 2-deoxy-D-glucose against MES in rats. 2-Deoxy-D-glucose actions include *in vivo* chronic ''disease-modifying'' antiepileptic effects consisting of 2-fold slowing of progression of repeated seizures evoked by perforant path or olfactory bulb kindling (Garriga-Canut et al., 2006; Stafstrom et al., 2009) in response to doses of 37.5—50 mg/kg administered 30 min prior to perforant path stimulation, and also when the minimum effective dose was administered immediately after, and approximately 10 min after evoked seizures (Sutula and Franzoso, 2008). This novel activity-dependent uptake of 2-deoxy-D-glucose into those brain regions with increased energy demand as in hyperactive neural circuits during seizures represents an opportunity for ''post-seizure'' anticonvulsant administration as well as treatment of seizure clusters and status epilepticus. 2-Deoxy-D-glucose also reduces the latency to seizures evoked by pilocarpine and kainic acid (Lian et al., 2007).

Administration of 2-deoxy-D-glucose (40 mg/kg) by the i.v. route in normal human volunteers induces regional increases in cerebral blood flow in cingulate gyrus, sensorimotor cortex, superior temporal cortex, occipital cortex, basal ganglia, limbic system, and hypothalamus (Elman et al., 1999), which are likely to explain the shorter latencies of onset of behavioral seizures in response to i.v. administration of convulsants reported in some rodent studies (Gasior et al., 2010) rather than a proconvulsant effect.

Neuroprotective action in experimental traumatic brain injury (TBI)

2-Deoxy-D-glucose has neuroprotective actions against progressive structural damage in after TBI induced by controlled cortical impact (CCI) in Sprague Dawley rats and in unique strains of rats bred for susceptibility or resistance to kindling progression evoked by perforant path stimulation of the hippocampus referred to, respectively as Perforant Path Kindling Susceptible (PPKS) rats, or resistance to kindling progression referred to as Perforant Path Kindling Resistant (PPKR) rats (Hutchinson et al., 2010, 2012; Langberg et al., 2012; Cech et al., 2012). After CCI, serial *in vivo* magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) performed at 1 month and 6 months demonstrate progressively increasing ventricle volume, increasing hippocampal diffusion abnormalities, and evolving patterns of hippocampal and corpus callosum anisotropy. Brief treatment with 2-deoxy-D-glucose (40 mg/kg at the time of injury and 250 mg/kg/day for 2 weeks) did not modify the severity of the initial injury, but reduced the secondary progression of the initial structural damage assessed by MRI and DTI measures over the 1—6 month post-TBI observation period (Hutchinson et al., 2010). These structural neuroprotective effects against secondary damage progression were accompanied by therapeutic functional effects including reduction in fear conditioning learning at 1 month after CCI and preservation of fear context spatial memory at 6 months after CCI in PPKS rats (Rutecki et al., 2013). As PPKS rats but not PPKR rats develop a form of post-traumatic epilepsy with generalized spike wave discharges after CCI (Cech et al., 2012), studies are planned to evaluate the possibility that treatment with 2-deoxy-D-glucose might also have preventative effects against emergence of post-traumatic epilepsy in addition to the therapeutic preventative effects against fear conditioning learning and spatial context memory. The latter effects are potentially pertinent to cognitive and affective sequellae of severe TBI such as post-traumatic stress disorder (PTSD) and repeated mild TBI such as concussion and chronic traumatic encephalopathy (CTE).

Toxicology

Experiments in rodents using the Morris water maze test found no effects on spatial memory at doses nearly 30 times greater than the minimum dose required to reduce kindling progression (Bialer et al., 2010). Further, no effects on spatial memory at 15 min after treatment with 50 or 250 mg/kg, i.p. were observed. Dose-dependent effects in the open field tests were reported at doses of 250 mg/kg i.p., but not at doses of 50 mg/kg i.p. (Ockuly et al., 2012). Cardiac toxicity and increased incidence of pheochromocytomas were reported with chronic administration of 2-deoxy-D-glucose at doses of 0.25—0.4% of dietary intake (corresponding to

total daily exposures of ~125—200 mg/kg/day). Reduced life-span was also reported at a daily dietary intake of 0.4% (Minor et al., 2010). Cardiotoxicity was accompanied by myofibrillar vesicular alterations consistent with autophagy, and subtle evidence of myofibrillar vesicular alterations was also reported at dietary exposures of about 20—25 mg/kg/day. Toxicological studies of 2-deoxy-D-glucose in F344 rats demonstrated histological cardiac abnormalities at doses of 250 mg/kg/day administered bid by gavage, but after a 15 days recovery period, there was complete recovery in this group. Plasma concentrations of NT pro-BNP increased at earliest from day 14 and were generally associated with cardiac microscopic findings consisting of vacuolar changes. The no-observed-adverse-effect-level (NOAEL) was 50 mg/kg for 2-deoxy-D-glucose administered bid by oral gavage (Bordelon et al., 2013).

Pharmacokinetics and metabolic profile

In Phase I studies in human cancer patients, the half-life of 2-deoxy-D-glucose during 14 days of once daily oral dosing at 45 mg/kg was 7.3—8.2 h (Stein et al., 2010).

Tolerability and safety in clinical studies

2-Deoxy-D-glucose has been evaluated in Phase I/II cancer trials and reported to be well tolerated in dose escalation studies up to 64 mg/kg/day for 5—8 weeks (Raez et al., 2007, Singh et al., 2005). In a recent open label multiple dose Phase I study in patients with prostate cancer, dose-limiting reversible toxicity of grade 3 asymptomatic QTc prolongation was seen in two patients treated at a dose of 60 mg/kg, but no evidence of QTc prolongation was observed at doses of 45 mg/kg (Stein et al., 2010).

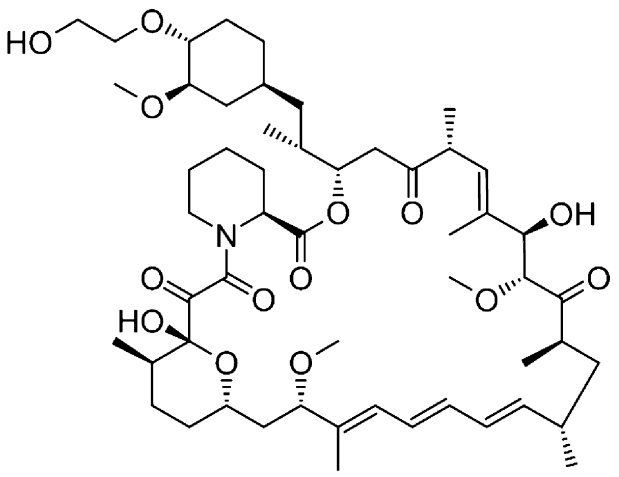
Planned studies

Further preclinical toxicology and pharmacokinetic studies are ongoing. Anticipated clinical trials of 2-deoxy-D-glucose include, in addition to conventional trial designs with scheduled oral administration, protocols for administration immediately after seizures. Potential indications include the treatment of refractory patients with frequent seizures, including patients with Lennox—Gastaut syndrome, the treatment of seizure clusters and status epilepticus, and use in combination with device therapies. Preclinical efficacy studies for use of 2-deoxy-D-glucose to prevent long-term sequelae of traumatic brain injury (TBI) including post-traumatic epilepsy, post-traumatic stress disorder (PTSD), and concussion are planned.

Everolimus

P. Curatolo

Systems Medicine Department, Child Neurology and Psychiatry Unit, Tor Vergata University Hospital of Rome, Italy



Everolimus

Introduction and rationale for development

Everolimus is a selective inhibitor of the mTOR (mechanistic/mammalian target of rapamycin) complex, specifically targeting mTORC1. Everolimus was initially developed as an antitumoral agent, but it later became evident that it could serve as a targeted drug for tuberous sclerosis complex (TSC)-related manifestations. TSC is a multisystem disease in which the hyperactivation of mTOR plays a key role in determining the characteristic pathologic lesions as well as the neurologic phenotype, including epilepsy (Napolioni et al., 2009).

Pharmacology

Anticonvulsant profile in experimental models

Preclinical research using animal models, either due to genetic modification or treatment with seizure-inducing agents, has demonstrated a reduction in seizure activity with mTOR inhibitors. A neuronal-specific conditional knockout of *TSC1* experiences spontaneous seizure activity that causes death by day 35. This could be completely blocked by treatment with the mTOR inhibitors rapamycin or RAD001 (everolimus), allowing survival to more than 100 days (Meikle et al., 2007). Disruption of *TSC1* in cerebral astroglia produced similar seizures that were reversed by administration of mTOR inhibitors (Zeng et al., 2008). The effects of mTOR inhibition in mouse models of TSC were considered to be extremely important as they are more consistent with an antiepileptogenic effect rather than only seizure suppressant (Curatolo and Moavero, 2013).

Other pharmacological properties

Everolimus inhibits mTOR which is a key serine-threonine kinase, whose activity is upregulated in a number of human cancers. Everolimus binds to the intracellular protein FKBP-12, forming a complex that inhibits mTOR complex-1 (mTORC1) activity. Inhibition of the mTORC1 signaling pathway interferes with the translation and synthesis of

proteins by reducing the activity of S6 ribosomal protein kinase (S6K1) and eukaryotic elongation factor 4E-binding protein (4EBP-1) that regulate proteins involved in the cell cycle, angiogenesis and glycolysis. Everolimus can reduce levels of vascular endothelial growth factor (VEGF). In patients with TSC treatment with everolimus increases VEGF-A and decreases VEGF-D levels. Everolimus is a potent inhibitor of the growth and proliferation of tumour cells, endothelial cells, fibroblasts and blood-vessel-associated smooth muscle cells and has been shown to reduce glycolysis in solid tumors *in vitro* and *in vivo*.

In a mouse neuronal model of TSC in which TSC1 is ablated in most neurons during cortical development, everolimus increased median survival from 33 days to more than 100 days, and also markedly improved behavioral phenotype and weight gain. There was brain penetration, with accumulation over time with repetitive treatment, and effective reduction of levels of phospho-S6, a downstream marker of mTORC1. Neurofilament abnormalities, myelination and cell enlargement were all improved by the treatment, although dysplastic neuronal features persisted, and there were only modest changes in dendritic spine density and length. Furthermore, mice treated with everolimus for 23 days only (postnatal days 7—30) displayed a persistent improvement in phenotype, with median survival of 78 days. In summary, everolimus is a highly effective therapy for this neuronal model of TSC, with benefit apparently attributable to effects on mTORC1 and Akt signaling and, consequently, cell size and myelination. Although caution is appropriate, the results suggest the possibility that everolimus may have benefit in the treatment of TSC brain disease, including infantile spasms.

Toxicology

Preclinical safety profile of everolimus was assessed in several species. It appeared to spontaneously exacerbate background disease, and it crossed the placenta being toxic to the fetus. In rats everolimus caused embryo/fetotoxicity at systemic exposure below the therapeutic level. This was manifested as mortality and reduced fetal weight. In juvenile rat toxicity studies systemic toxicity included decreased body weight gain, decreased food consumption and delayed attainment of some developmental landmarks, with full or partial recovery after cessation of dosing. Genotoxicity studies covering relevant genotoxicity endpoints showed no evidence of clastogenic or mutagenic activity.

Pharmacokinetics and metabolic profile

In patients with advanced solid tumors, peak everolimus plasma concentrations (C_{max}) are reached at a median time of 1 h after daily administration of 5 and 10 mg under fasting conditions or with a light fat-free snack (O'Donnell et al., 2008). C_{max} is dose-proportional between 5 and 10 mg. In healthy subjects, high fat meals reduced the systemic exposure to everolimus 10 mg (as measured by AUC) by 22% and the C_{max} by 54%. Light fat meals reduced AUC by 32% and C_{max} by 42%. Food, however, had no apparent effect on the post-absorption phase concentration-time profile (O'Donnell et al., 2008).

The blood-to-plasma ratio of everolimus, which is concentration-dependent over the range of 5—5000 ng/mL is 17—73% (O'Donnell et al., 2008). In cancer patients given everolimus 10 mg/day, approximately 20% of the everolimus concentration in whole blood is confined to plasma. The plasma protein binding of everolimus is approximately 74% both in healthy subjects and in patients with moderate hepatic impairment (Kirchner et al., 2004). There are no clinical data on the distribution of everolimus in the human brain. Studies in rats demonstrated distribution into the brain following administration by both the i.v. and the oral route (Dancey, 2005).

After administration of everolimus to patients with advanced solid tumors, the AUC at steady-state over a dosing interval was dose-proportional over the 5—10 mg/day dosing range. Steady-state was achieved within 2 weeks (Kirchner et al., 2004).

Following oral administration, everolimus is the main circulating component in human blood. Six main metabolites have been detected in human blood, including three monohydroxylated metabolites, two hydrolytic ring-opened products, and a phosphatidylcholine conjugate of everolimus (Kirchner et al., 2004). These metabolites were also identified in animal species used in toxicity studies, and showed approximately 100 times less activity than everolimus itself. Hence, everolimus is considered to contribute the majority of the overall pharmacological activity.

The mean oral clearance (CL/F) of everolimus after a 10 mg daily dose in patients with advanced solid tumors was 24.5 L/h (O'Donnell et al., 2008). The mean elimination half-life of everolimus is approximately 30 h. Excretion studies in transplant patients given a single dose of radiolabelled everolimus in conjunction with cyclosporine showed that 80% of the radioactivity was recovered from the feces, while 5% was excreted in urine. The parent substance was not detected in urine or feces.

Drug interactions

Everolimus is a substrate of CYP3A4 and PgP. Therefore, its absorption and elimination can be influenced by compounds that affect CYP3A4 and/or PgP, and these compounds should be preferably avoided in patients treated with everolimus (Kovarik et al., 2006). If co-administration of a moderate CYP3A4 and/or PgP inhibitor or inducer cannot be avoided, adjustments of everolimus dose may be required. Concomitant treatment with potent CYP3A4 inhibitors, such as some antifungal and antiviral agents, clarithromycin and telithromycin, results in dramatically increased blood concentrations of everolimus, and there are currently not sufficient data to allow dosing recommendations in this situation. On the other hand, drugs which are inducers of CYP3A4 or PgP, such as rifampicin, corticosteroids, carbamazepine, phenytoin and phenobarbital, may decrease everolimus blood concentrations by increasing everolimus metabolism or its efflux from intestinal cells (Kovarik et al., 2006).

In vitro, everolimus is a competitive inhibitor of CYP3A4 and a mixed inhibitor of CYP2D6. Caution should be exercised when everolimus is taken in combination with CYP3A4

substrates with a narrow therapeutic index, due to the potential for drug interactions (Kovarik et al., 2006).

Efficacy data

There are still limited data on the effect of everolimus in TSC-related epilepsy. In a single case report, a child treated with everolimus for a recurring subependymal giant cell astrocytoma (SEGA) experienced seizure freedom at 12 months follow-up after previously intractable epilepsy (Perek-Polnik et al., 2011). A prospective trial designed to evaluate the safety and efficacy of everolimus on subependymal giant cell tumors followed-up 28 patients for 6 months (Krueger et al., 2010). Among 16 patients who underwent 24-h video-EEG monitoring, seizure frequency during everolimus treatment decreased in 9, did not change in 6, and increased in one. The same group performed a Phase I/II trial to evaluate the efficacy of everolimus in medically refractory TSC-related epilepsy, and reported a >50% seizure frequency reduction in 12/20 patients (Krueger et al., 2013). Recently, two case series of patients with refractory TSC-related epilepsy treated with everolimus in Germany and Australia have been published, with an overall good response and more than 50% of patients being apparent responders (Wiegand et al., 2013).

Tolerability and side effect profile

In TSC studies, very common (≥1/10) treatment-emergent adverse events (TEAE) were upper respiratory tract infections, hypercholesterolemia, and stomatitis (Curatolo and Moavero, 2012). Common (≥1/100 to <1/10) TEAEs included other kind of infections, abnormal blood cell count and other laboratory abnormalities (including increased LDH, hyperlipidaemia, hypophosphatemia, and hypertriglyceridemia), decreased appetite, headache, hypertension, gastrointestinal disorders, and mild skin manifestations. Overall all these TEAEs appeared to be mild and self-limited, rarely causing drug discontinuation.

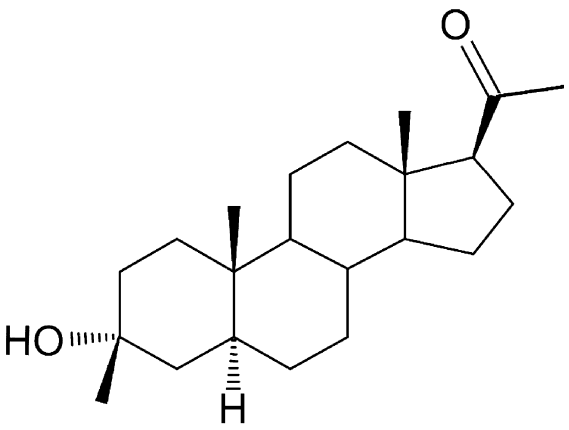
Planned studies

A placebo-controlled, three-arm, randomized, double-blind study of the efficacy and safety of 2 trough-ranges of everolimus as adjunctive therapy in 345 TSC patients with refractory focal seizures (EXIST-3, *clinicaltrials.gov*) is currently ongoing. The trial consists in an 8-week baseline phase, 6-week titration phase, and 12-week maintenance phase The primary aim is to compare the reduction in frequency of focal seizures on each of two trough ranges of everolimus (3—7 ng/mL and 9—15 ng/mL) *versus* placebo.

Ganaxolone

G. Farfel[a], J. Tsai[a], V. Nohria[b]

[a] Marinus Pharmaceuticals, Inc., New Haven, CT, USA
[b] Mercer University, Atlanta, GA, USA



Ganaxolone

Introduction and rationale for development

Ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan-20-one) is a small molecule synthetic analog of allopregnanolone, an endogenous modulator of GABA in the CNS. Ganaxolone was designed to have the same modulatory effects at $GABA_A$ receptors as allopregnanolone without activation of nuclear (classical) progesterone receptors, and seizure protection has been demonstrated with ganaxolone in a broad range of animal seizure models (Carter et al., 1997; Reddy and Rogawski, 2000, 2012). Ganaxolone is being developed as adjunctive therapy for the treatment of partial-onset seizures in adults, as well as in two orphan pediatric disorders related to gene mutations affecting neurosteroid signaling at extrasynaptic $GABA_A$ receptors: PCDH19 Female Pediatric Epilepsy, and Behaviors in Fragile X Syndrome.

Pharmacology

Neurosteroids including ganaxolone have two effects on $GABA_A$ receptors: they provide persistent inhibition of neuronal excitability, termed tonic inhibition, *via* activation of extrasynaptic $GABA_A$ receptors while also providing rapid, phasic inhibition *via* synaptic $GABA_A$ receptors activation (Schumacher et al., 2014); these actions occur at distinct sites on the receptor complex that do not correspond with the modulatory sites of benzodiazepines and barbiturates (Hosie et al., 2006). Unlike benzodiazepines that are specific for synaptic $GABA_A$ receptors, neurosteroids modulate both synaptic and extrasynaptic $GABA_A$ receptors and their modulatory action is of greater magnitude for extrasynaptic $GABA_A$ receptor isoforms that contain a δ subunit and are known to be desensitized in rodent models of temporal lobe epilepsy (Herd et al., 2007; Joshi et al., 2011). The modulatory activity of ganaxolone at $GABA_A$ receptors is comparable to that of allopregnanolone, established through a series of experiments in which ganaxolone enhanced the binding of [3H]flunitrazepam and [3H]muscimol, and inhibited the binding of [35S] *t*-butylbicyclophosphorothionate (TBPS), at the $GABA_A$ receptor complex (Carter et al., 1997). Unlike allopregnanolone and other neurosteroids, neither

ganaxolone nor its metabolites have a ketone ring at the 3-position, a prerequisite for hormonal activity. In binding studies, ganaxolone showed no appreciable affinity for estrogen or progesterone receptors nor any other unintended activity through 37 non-target CNS receptors from the steroid, monoamine, second messenger, or amino acid classes (Marinus Pharmaceuticals, data on file).

Toxicology

Preclinical safety pharmacology and toxicology testing, including reproductive toxicology, have been conducted with ganaxolone at doses relevant for chronic human administration. No evidence was found to indicate functional or anatomical adverse effects in blood, liver, kidney or gastrointestinal systems associated with either single- or multiple-dose treatment with ganaxolone from studies in rat (up to 6 months) and dog (up to 1 year). Additionally, no end organ toxicities have been identified in preclinical safety pharmacology studies, nor has evidence of any end organ toxicity been identified from human clinical trials. To date, no studies indicate potential for ganaxolone to cause cellular mutations or carcinogenicity. In reproductive toxicology studies, ganaxolone did not cause any malformations of the embryo or fetus in rats or mice and did not significantly affect the development of offspring. No changes in sperm parameters were found (Marinus Pharmaceuticals, data on file).

Pharmacokinetics and metabolic profile

Ganaxolone is a lipophilic, high clearance compound. Ganaxolone is available in oral suspension and capsule forms. Multiple dose pharmacokinetic studies using the bid oral capsule show that steady state is achieved within 48—72 h at total daily doses of 400 through 2000 mg when dosed with food. Ganaxolone (capsule) $t_{1/2}$ is 7—10 hence its mean C_{max} and $AUC_{(0-12)}$ at steady-state were close to dose-proportional, losing proportionality at the upper end of the dose range (Marinus Pharmaceuticals, data on file).

Drug interactions

Animal pharmacokinetic and *in vitro* studies show that ganaxolone is primarily metabolized by CYP3A. All *in vitro* studies have shown ganaxolone has low potential for interaction with other drugs at concentrations several folds higher than its clinically relevant plasma concentrations in humans. Interaction study in normal volunteers between ganaxolone (400 mg capsules) twice daily for 12 days and midazolam indicated that ganaxolone is not a CYP3A4 inhibitor and showed low or no potential to induce CYP3A4 metabolism (Marinus Pharmaceuticals, data on file).

Efficacy data

Prior to acquisition by Marinus Pharmaceuticals (the current sponsor), ganaxolone was studied in three open-label pediatric studies and a double-blind randomized pre-surgical study in adults with seizure disorders (Laxer et al., 2000; Kerrigan et al., 2000; Pieribone et al., 2007).

The current sponsor has also completed a double-blind, placebo-controlled study of ganaxolone (1042-0600) as adjunctive therapy in adults with drug-resistant partial onset seizures and a long term open label extension, reported at previous Eilat congresses. Briefly, subjects in the trial were US adult outpatients diagnosed with epilepsy on average 25 years prior and 75% were taking two or three AEDs to control seizures before they entered the study. Subjects were treated for 10 weeks with placebo or ganaxolone 1500 mg/day as adjunctive treatment to existing therapy and recorded their seizures daily in a diary. Mean baseline seizure frequency was 6.5 and 9.2 seizures per week in the ganaxolone and placebo groups, respectively.

Ganaxolone treatment produced an 18% decrease in mean weekly seizure frequency (WSF), compared with a 2% increase for placebo over the 10-week treatment period ($p = 0.014$). Responder rates in the ganaxolone group compared to the placebo group in the ITT population were 23.5% and 14.3% ($p = 0.192$) for the titration plus maintenance period, and 26.3% *versus* 13.0% ($p = 0.057$) for the maintenance period. No gender effect or effect of concomitant medication was observed. Of 131 completers, 94% entered a 104-week open-label extension (OLE) study. At endpoint of the OLE, 24% of subjects met responder criteria (50% improvement), 29% having originally been randomized to placebo and 22% having been randomized to ganaxolone. Subjects were dosed for a mean of 274 days (39 weeks) in the OLE, with a target dose of 1500 mg/day and ranging from 900 to 1500 mg/day.

Tolerability and adverse effect profile

Approximately 1000 subjects have received treatment with ganaxolone ranging in duration from one day to more than two years using doses from 50 to 2000 mg/day. A total of 289 healthy subjects received ganaxolone doses of 50—2000 mg/day in Phase 1 studies, for periods of up to 2 weeks. In completed Phase 2 clinical studies, 697 unique subjects have received ganaxolone including 135 pediatric subjects with epilepsy, 169 adult subjects with epilepsy, and 393 adult subjects with migraine. Ganaxolone was administered in Phase 2 studies to pediatric subjects at doses up to 54 mg/kg and to adult subjects at doses up to 1875 mg/day. No drug-related deaths occurred in any of these clinical trials, and the majority of adverse events were not medically serious and resolved upon discontinuation of therapy. In the ganaxolone safety database there are no trends of medically important changes in blood chemistry, vital signs, liver function, renal function or cardiovascular parameters in the adult or pediatric populations.

In the trial described above investigating ganaxolone and placebo in 147 adult outpatients with drug-resistant partial-onset seizures, adverse events (AEs) reported by at least 5% of subjects were dizziness, fatigue (both 16% *versus* 8% for ganaxolone and placebo, respectively) and somnolence (13% *versus* 2%). Seven percent of ganaxolone subjects and 6% of placebo subjects discontinued treatment due to AE. There were no deaths during the study, and serious AEs occurred in 5% of ganaxolone *versus* 8% of placebo subjects. No clinically

important trends were seen in laboratory parameters, ECGs, vital signs or body weight.

Ganaxolone continued to be safe and well tolerated in the OLE, and no new safety concerns were identified during extended treatment with ganaxolone for up to 104 weeks.

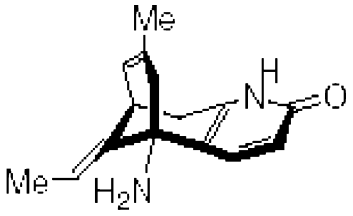
Planned studies

Proof-of-concept data for ganaxolone in the treatment of refractory pediatric seizures and as monotherapy for adult refractory focal onset seizures has been generated and supports investigation of ganaxolone for a multiple seizure indications, including genetically defined pediatric seizure syndromes. Marinus currently has Phase 2 proof-of-concept (POC) pediatric clinical trials in progress for ganaxolone as a treatment for PCDH19 female pediatric epilepsy, and for behaviors in Fragile X Syndrome, an autism spectrum disorder. Both disorders have been related to mutations affecting neurosteroid signaling at extrasynaptic $GABA_A$ receptors. An intravenous formulation of ganaxolone is in development to support inpatient use.

Huperzine A

S.D. Collins, J.T. Johnstone, S.S. Schachter

Insero Health, Inc., USA



Huperzine A

Introduction and rationale for development

Huperzine A (HupA) is a small naturally occurring alkaloid derived from the Chinese herb *Huperzia serrata* possessing potent, selective, and reversible acetylcholinesterase (AChE) inhibitory properties. It is extracted from a variety of club moss which grows at altitudes above 10,000 ft and requires 10 or more years to grow to harvesting stage. At low doses, HupA has been shown to be effective in improving cognitive dysfunction, reducing neuroinflammation, enhancing neuroprotection in various animal models of neurological disease by increasing cortical inhibition and potentially by acting as a potent mitochondrial protectant. INS-001, Insero's patented synthetic form of HupA, acts to increase GABA-ergic inhibition of abnormal CNS activity *via* pre-synaptic mechanisms, avoiding typical GABA axis side effects such as drowsiness and decreased cognition. This combination of decreasing abnormal activity while increasing cognitive abilities is extremely well suited for an anticonvulsant medication.

Pharmacology

Anticonvulsant profile

HupA was studied by the NIH Anticonvulsant Screening Program and demonstrated clear and potent anti-seizure effects in multiple models. Very significantly, in the 6 Hz model which is predictive of efficacy in refractory Complex Partial Seizures, HupA was able to deliver seizure relief even under the most refractory conditions. When administered *via* intraperitoneal route (i.p.) HupA resulted in ED_{50} values of 0.28, 0.34, and 0.78 mg/kg in the 22, 32, and 44 mA paradigms, respectively (Table 1). Of the approved anticonvulsants tested, HupA had the lowest ED_{50} at all seizure-inducing current (mA) levels measured, being 57 times more potent than levetiracetam and 301 times more potent than valproic acid at 32 mA. Importantly, the protection produced by HupA was confirmed to occur *via* an ACh-dependent mechanism, as treatment with the mAChR inhibitor atropine (30 mg/kg) was able to abolish the protective effect. Following intracerebral ventricle (i.c.v.) administration, the ED_{50} of HupA in the 6 Hz (32 mA) model was 0.21 pmol/5 1-L injection volume, with a TD_{50} of 36.15 pmol, indicating a protective index of 172 ($PI = TD_{50}/ED_{50}$) (White et al., 2005).

Studies to further characterize the mechanism of action of HupA were performed with the assistance of Dr. Alex Rotenberg (Boston's Children Hospital) using a paired-pulse transcranial magnetic stimulation (TMS) with electromyography (EMG) paradigm. In TMS with EMG, intracranial stimulation by electrical currents produced by an extracranial magnetic field results in a pair of motor-evoked potentials (MEPs). When analyzed as pairs, the second MEP produced is predictably smaller than the first due to GABAergic long interval intracortical inhibition (LICI). In order to test the hypothesis that HupA treatment results in GABA release, rats receiving 0.6 mg/kg HupA (i.p.) were subjected to TMS with EMG to assess HupA effect on intracortical inhibition. Treatment with HupA significantly reduced the paired pulse ratio, indicating an increase in paired pulse inhibition as a result of $GABA_A$ receptor activation. This finding provides evidence that the mechanism of action of HupA is to increase GABAergic signaling.

In order to assess the therapeutic potential of HupA in a preclinical animal model of Dravet Syndrome, the ability of HupA to increase seizure resistance was investigated with the assistance of Dr. Andrew Escayg (Emory University) in the *Scn1a*$^{R1648H/+}$ (RH) mouse model generated in his lab. These mice were produced by knock-in of the human SCN1A mutation (R1648H). Wild-type (WT) and RH mutants were randomized into two groups (6 mice/genotype/group) and received treatment (i.p.) with either HupA (1 mg/kg) or vehicle 1 h prior to seizure induction *via* 6 Hz stimulation (22 mA). The mice were observed for behavioral responses immediately following stimulation. Seizure score was based on a modified Racine Scale (RS): 0, no abnormal behavior; 1, staring, unresponsive for $\geq$5 s; 2, forelimb clonus; 3, rearing and falling. In the first experiment, one group from each genotype received HupA and the other group received vehicle. A second experiment was performed 1 week later in which each group received the opposite treatment. Seizures with a maximum Racine Score of 3 (RS3) were observed in 92% (11/12) of RH mutants administered vehicle. In contrast,

among the WT littermates that were administered vehicle, 7 did not respond (RS0) and 5 exhibited a mild response (RS1). HupA treatment resulted in a robust increase in seizure resistance ($p \leq 0.001$), with 84% of the mutants gaining complete seizure suppression (RS1). The experiment was repeated 1 week later using HupA at a lower dose (0.5 mg/kg). Significant protection ($p \leq 0.05$) was again observed in HupA treated RH mutants. These doses in mice would predict human doses of roughly 2 mg daily when estimated using allometric scaling techniques.

Other pharmacological properties

HupA displays potent anti-nociceptive characteristics. Mice receiving 0.5 mg/kg of HupA (i.p.) reduced flinches per minute by 97% in the acute phase (Phase I) and 100% in the inflammatory phase (Phase II), when compared to controls in the formalin model of neuropathic pain. Increasing the treatment dosage to 1 mg/kg was sufficient to eliminate flinching behavior in both phases. Additionally, HupA has been shown to be highly effective at reducing flinching behavior following intrathecal (i.t.) administration. Doses of 1, 3, and 10 μg significantly reduced flinching behavior during both the acute and inflammatory phases (Park et al., 2010). Importantly, the mechanism of action of the anti-nociceptive capabilities of HupA was confirmed to be cholinergic in nature, as pre-treatment with the muscarinic acetylcholine receptor antagonist atropine reversed the observed reduction in flinching behavior.

Four weeks following a moderate spinal cord compression, rats were administered 50, 167, or 500 μg/kg of HupA (i.p.) and underwent von Frey filament testing to assess paw withdraw threshold (Yu et al., 2013). Treatment with HupA effectively induced dose-dependent anti-hypersensitivity, which lasted for 6 h on average. Furthermore, administration of HupA i.t. *via* an osmotic pump abolished the chronic hypersensitivity induced by SCI. The chronic anti-nociceptive ability of HupA following SCI was determined to be cholinergic in nature, as treatment with atropine blocked INS-001's effect.

HupA has also been shown to display neuroprotective characteristics in multiple models of neurological disease. Rat primary cortical neurons treated with 1 μM HupA were significantly protected from apoptosis induced by serum deprivation by affecting endogenous caspase-3 signaling (Zhou and Tang, 2002). Twice daily oral administration of 0.1 mg/kg HupA following transient global ischemia attenuated memory impairment and neurodegeneration in the CA1 region of the hippocampus (Zhou et al., 2001). In addition, HupA has been shown to improve spatial memory following scopolamine-induced cognitive impairment (Ye et al., 1999).

Mechanism(s) of action

HupA is a potent AChE inhibitor. Based on the half-maximal inhibitory concentration (IC_{50}), HupA is more potent at inhibiting AChE than other known cholinesterase inhibitors, with over a 900-fold higher affinity for AChE over BuChE found outside of the CNS (Tang and Han, 2009). Furthermore, HupA displays higher AChE inhibitory activity in various brain regions at lower doses than other AChE inhibitors. In tests run by the NIH Anticonvulsant Screening Program (ASP) of currently marketed AChE compounds such as donepezil, it was determined that this class has anticonvulsant properties, but in compounds other than HupA the seizure relief only occurred at serum exposure levels that led to severe adverse effects. Our findings in animal models of epilepsy and pain, and in multiple laboratories have demonstrated that the anticonvulsant and anti-nociceptive effects of HupA are dependent upon its centrally acting cholinergic mechanism of action.

Toxicology

Low toxicity of HupA has been observed in dogs and rats. In dogs, 200 μg/kg and 1—3 mg/kg of HupA in rats have been well tolerated with little to no side effects in chronic toxicity studies that supported an IND. No histological changes were found in liver, kidney, heart, lung, or brain in rats (1.5 mg/kg p.o.) or dogs (0.3 mg/kg p.o.) after administration of HupA for >90 days.

Pharmacokinetics and metabolic profile

Both human and animal pharmacokinetic studies show a high bioavailability, rapid absorption, and wide distribution. For example, following a single 0.99 mg oral dose of an immediate release formulation of HupA in healthy volunteers, the pharmacokinetics demonstrated an absorption half-life of 12.6 min, an elimination half-life of 288.5 min, a t_{max} of 79.6 min, a C_{max} of 8.4 ng/mL, and an AUC of 4.1 ng/mL/min (Qian et al., 1995).

Drug interactions

In a dose-escalating Phase I clinical trial performed by Insero Health in adult patients with refractory epilepsy, treatment with HupA four times daily did not significantly alter the plasma concentrations of levetiracetam, lacosamide, carbamazepine, lamotrigine, topiramate, or zonisamide. There are no publications investigating drug interactions between HupA and other AEDs.

Efficacy data

There are currently no published efficacy studies of HupA in patients with refractory epilepsy.

Tolerability and side effect profile

In a recently completed Phase I trial, the only adverse events observed were dose-dependent and typical cholinergic in nature (predominantly nausea and vomiting) felt to be due to the very rapid rise in serum levels associated with the immediate release formulation. In order to address the safety of HupA, cardiovascular monitoring with digital Holter monitoring was conducted throughout the trial. QTc interval was unaltered. Some potential improvements in abnormal EKG patterns seen in patients with refractory seizures were noted with treatment. Thus, cardiac electrical activity remains normal after HupA administration. In a well-controlled study of elderly patients with Alzheimer's disease, doses up to 400 μg BID were well tolerated, with the

most common adverse events being nausea and vomiting, *i.e.*, cholinergic in nature (Rafii et al., 2011).

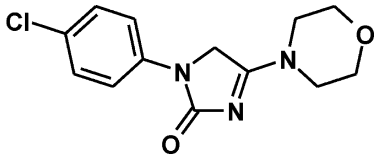
Planned studies

Insero Health owns rights to a modern synthetic method allowing for a reliable and safe supply. The method has been validated in a commercial scale-up run. Insero Health plans to develop INS-001 as an HupA extended release formulation appropriate for small children and adults. This formulation will be used in a pilot study in children with Dravet Syndrome, as well as in a phase 2 study in adult patients with refractory Complex Partial Seizures.

Imepitoin

W. Löscher

Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine, Hannover, Germany



Imepitoin (ELB138)

Introduction and rationale for development

Imepitoin (AWD 131-138 or ELB 138; 1-(4-chlorophenyl)-4-morpholino-imidazolin-2-one) is a new chemical entity that was previously presented at the EILAT IV, V, and XI conferences (Bialer et al., 1999, 2001, 2013). The development and pharmacology of imepitoin have been reviewed in detail recently (Rundfeldt and Löscher, 2014), so that imepitoin will be only shortly described here. It was developed in the 1990s from a series of imidazolinones by Asta Medica and Arzneimittelwerk Dresden (AWD; later Elbion, Radebeul, Germany). Furthermore, it was tested in the NINDS-sponsored Anticonvulsant Drug Development (ADD) program. Imepitoin was selected for further development because of its broad spectrum of anticonvulsant activity, high therapeutic index, and its efficacy in tests predictive for anxiolytic effects. It underwent Phase I clinical studies, but further clinical development for humans was suspended. However, interesting findings in dogs led Boehringer Ingelheim (Germany) to the decision to develop imepitoin as a new antiepileptic drug (AED) for canine epilepsy. Epilepsy is one of the major neurological diseases in dogs, affecting at least 1% of the dog population. In Europe, only phenobarbital and potassium bromide are approved for treatment of canine epilepsy, but both AEDs are associated with severe adverse effects. Furthermore, at least 50% of all dogs with epilepsy do not become seizure-free with these drugs, so that new AEDs are urgently needed. Imepitoin is a first-in-class low-affinity, low-efficacy partial agonist at the benzodiazepine (BZD) binding site of the $GABA_A$ receptor, which is associated with anti-seizure efficacy and a favorable safety profile.

Although traditional BZDs, such as diazepam, clonazepam or clobazam offer a wide spectrum of antiepileptic activity against diverse types of epileptic seizures, their use in the treatment of epilepsy is limited because of adverse effects, loss of efficacy (tolerance), and development of physical and psychological dependence. BZDs act as positive allosteric modulators of the inhibitory neurotransmitter GABA by binding to the BZD recognition site (''BZD receptor'') of the $GABA_A$ receptor. Traditional BZDs such as diazepam or clonazepam act as full agonists at this site, so that one strategy to resolve the disadvantages of these compounds would be development of partial agonists with lower intrinsic efficacy at the BZD site of the $GABA_A$ receptor. Several partial or $GABA_A$ receptor subtype selective compounds, including bretazenil, abecarnil or alpidem, have been developed as anxioselective anxiolytic drugs, but epilepsy was not a target indication for such compounds. More recently, the imidazolone derivatives imepitoin (ELB138) and ELB139 were shown to act as low-affinity partial agonists at the BZD site of the $GABA_A$ receptor, and imepitoin was developed for treatment of epilepsy (Rundfeldt and Löscher, 2014). In comparison to diazepam, the intrinsic efficacy of imepitoin to potentiate GABA *via* the BZD binding site of the $GABA_A$ receptor is only about 20%, which is high enough to induce anti-seizure and anxiolytic effects, whereas the adverse effects occurring at higher receptor occupancy with full agonists do not occur, explaining the favorable safety profile of imepitoin and the lack of development of tolerance and dependence upon chronic administration (Rundfeldt and Löscher, 2014).

Pharmacology

Imepitoin displayed a broad spectrum of anti-seizure effects in diverse seizure and epilepsy models at tolerable doses, and, as expected from its mechanism of action, lacked tolerance and abuse liability in rodent and primate models (*cf.*, Rundfeldt and Löscher, 2014). The anticonvulsant profile of imepitoin in numerous animal models has been described in detail in previous reviews (Bialer et al., 2013; Rundfeldt and Löscher, 2014) and will not repeated here. In addition to anti-seizure activity, imepitoin and ELB139 exerted anxiolytic effects in various rodent models. The more favorable pharmacokinetic profile of imepitoin in dogs *versus* humans led to the decision to develop imepitoin for treatment of canine epilepsy.

Efficacy data

Based on several randomized controlled trials with imepitoin that demonstrated antiepileptic efficacy and high tolerability and safety in epileptic dogs (Rundfeldt and Löscher, 2014; Tipold et al., 2014), the drug was approved for this indication by the European Medicines Agency (*EMA*) in February 2013 and is marketed by Boehringer-Ingelheim under the trade name Pexion®. Preliminary findings in epileptic dogs indicate that, in addition to

suppressing seizures, imepitoin also suppresses some of the behavioral abnormalities, including anxiety, associated with epilepsy in dogs (Rundfeldt and Löscher, 2014).

Tolerability and adverse event profile

The safety profile of imepitoin was characterized *in vitro* in genotoxicity assays, and in mice, rats, and dogs upon multiple dosing (Rundfeldt and Löscher, 2014). As expected from the safety profile observed during pharmacological characterization, single and repeated dose oral administration of imepitoin was found to be well tolerated.

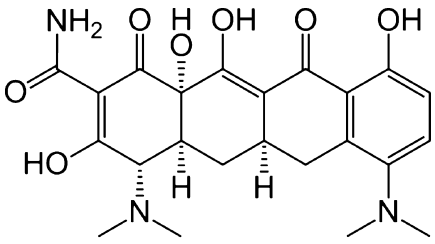
Planned studies

Further trials to obtain approval in the U.S. are ongoing. Since its approval, imepitoin has rapidly gained acceptance in the EU as a new effective and safe treatment for canine epilepsy with a much better safety profile than phenobarbital or bromides, the only other AEDs approved for canine epilepsy in the EU. Hopefully, the favourable profile of imepitoin for treatment of epilepsy in dogs will reactivate the interest in partial BZD site agonists as novel treatments for human epilepsy, too.

Minocyciline

S. Koh

Department of Pediatrics, Ann & Robert H. Lurie, Children's Hospital of Chicago, Neurobiology Program, Stanley Manne Children's Research Institute, Northwestern University, Feinberg School of Medicine, Chicago, IL 60614, USA



Minocycline

Introduction and rationale for development

Minocycline is a second generation, semi-synthetic, broad-spectrum, bacteriostatic tetracycline analogue. It is the most lipid-soluble of the tetracycline-class antibiotics, giving it the greatest penetration into the brain. Minocycline has anti-inflammatory, immunomodulatory and anti-apoptotic properties. Neuroprotective effects of Minocycline were noted in models of ischemia, spinal cord injury, infection, neuroinflammation and neurodegenerative diseases. Minocycline has merit to be considered for epilepsy therapeutics because of its ability to penetrate blood brain barrier (BBB), proven human safety record, low cost and promising actions against activated microglia. Minocycline may work synergistically with compounds targeting other pathological mechanisms of disease progression in epilepsy.

Pharmacology

Anticonvulsant profile (animal models/electrophysiology)
Anticonvulsant action of minocycline was tested *in vivo* by using the maximal electroshock (MES), 6-Hz test and subcutaneous Metrazol (scMet) models of seizures in mice (Wang et al., 2012). Minocycline showed dose-dependent anticonvulsant effects in abolishing focal seizures in the 6-Hz seizure test with ED_{50} of 170 mg/kg. Minocycline had no effects on the MES and scMet tests. Minocycline attenuated blocked the long-term epileptogenic effect of early life seizures. Likewise, minocycline retarded kindling epileptogenesis, decreased stage 5 seizure duration, reduced after discharge duration and made animals more resistant to seizure generation in amygdala kindled rats (Beheshti Nasr et al., 2013).

Other pharmacological properties
Role of activated microglia in pathogenesis of acquired epilepsies has gained increasing acceptance. Microglia, the resident macrophage in the brain parenchyma, constitutes the first line of defense against pathological changes within the CNS microenvironment. Cytokines, free radicals, and excitatory neurotransmitters released from activated microglia have potential to enhance neuronal excitability. In fact, extensive microglia activation occurs in the brain parenchyma of individuals with chronic intractable epilepsy and in animal models of acute seizures. Anti-inflammatory/immunomodulatory action of minocycline appears to be, at least in part, mediated by inhibition of excitotoxin-induced proliferation and activation of microglia. Minocycline attenuated seizure-induced microglia activation and blocked the long-term epileptogenic effects of early-life seizures (Abraham et al., 2012). Time course of microglia activation following kainic acid-induced status epilepticus (KA-SE) in Cx3cr1GFP/+ transgenic mice showed nearly a two-fold increase in microglia activation within 24 h. Significant seizure-induced activation persisted for 7 days and returned to baseline by 14 days. It appeared that early-life seizure-induced microglia activation primed the central immune response to overreact and to increase the susceptibility to a second seizure later in life. Postnatal day (P) 39 animals with prior exposure to KA-SE at P25 not only responded with greater microglial activation in response to ''second hit'' of KA, but shorter latency to express seizures. Minocycline given for 7 days after the first seizure at P25 blocked the seizure-induced inflammation and abrogated both the exaggerated microglia activation and the increased susceptibility to the second seizure later in life. Minocycline treatment post SE may be useful to block the epileptogenic process and mitigate the long-term damaging effects of early-life seizures (Abraham et al., 2012). Minocycline has also been shown to inhibit KA-SE-induced cell death *via* attenuation of pro-inflammatory cytokine through caspase-dependent and caspase-independent pathways (Heo et al., 2006).

Mechanism(s) of action

Immunomodulatory action of minocycline is attributed to inhibition of the activity of matrix metalloproteinase (MMPs), inducible nitric oxide synthase (iNOS), and cyclooxygenases-2 (COX-2). Minocycline inhibits points of convergence of signaling pathways mediating multiple distinct and interacting inflammatory signals which may influence monocyte activation, traffic and recruitment into the brain. The anti-inflammatory effects of minocycline in human monocytes include decreased NF-kappaB activation, abrogation of the LPS-stimulated LOX-1 (the lectin-like oxidized low density lipoprotein receptor-1), LITAF (LPS-induced TNF-alpha factor), Nur77 pathways, p38 MAPK (mitogen-activated protein kinase) inhibition and PI3K/Akt (phosphoinositide 3-kinase/Akt pathway) activation. Minocycline ameliorates the innate immune response by attenuation of CD4 (+) T cell activation *via* selective impairment of NFAT (nuclear factor of activated T)-mediated transcriptional activation, and by modulating microglia-T cell interaction *via* down regulation of CD40 ligand in the T cells. Mechanisms of minocycline-mediated neuroprotection include direct action of minocycline on the mitochondria to inhibit cytochrome-c release, blockade of death receptor pathways and inhibition of activated microglia. Thus, minocycline has shown efficacy in focal and global ischemia, traumatic brain injury, SIV-macaque model of HIV CNS disease, experimental autoimmune encephalomyelitis, as well as in animal models of Huntington, Parkinson and Alzheimer's disease and amyotrophic lateral sclerosis (Garrido-Mesa et al., 2013).

Toxicology and adverse effect profile

Like most lipid-soluble of the tetracycline-class antibiotics, minocycline also gives the greatest amount of central nervous system (CNS)-related side effects, such as vestibular toxicity (vertigo), dizziness, headache and intracranial hypertension. Long-term treatment with minocycline at dosages of up to 200 mg/day, the highest dosage recommended by the US FDA, is generally well-tolerated. In a prospective open-label safety pilot trial of patients with brain vascular malformations (arteriovenous malformations (AVM) or intracranial aneurysm), minocycline (200 mg/day) were given for up to 2 years, and 4/13 (31%) patients had dose-limiting intolerance (including photosensitivity, vertigo, yeast infection, GI symptoms and hyperpigmentation). A safety and tolerability study with Huntington Disease (HD) in a double-blind, randomized, placebo-controlled study of minocycline in 60 HD patients, minocycline at 100 or 200 mg/day was well tolerated and safe in HD patients over 8 weeks; tolerability and adverse event frequency were similar between treatment and placebo groups (Huntington Study Group, 2004). Similarly, an open-label add-on treatment trial of minocycline at 100 mg or 200 mg/day in fragile X syndrome ($n = 20$, ages 13—32) showed good tolerability and significant functional benefits. A phase II, placebo-controlled, randomized study of minocycline administration after acute traumatic spinal cord injury showed that neurological, functional, pharmacological and adverse event outcomes were comparable between subjects administered 7 days of intravenous minocycline (200 mg or 400 mg, $n = 27$) or placebo ($n = 25$). Functional neurological recovery at one year showed promising trends toward improvement ($p = 0.05$, in cervical injury) (Casha et al., 2012). A Phase III clinical trial of 400 mg per day of minocycline in amyotrophic lateral sclerosis (ALS) patients accelerated disease progression (Gordon et al., 2007). Most common reversible side effects of minocycline are nausea, vomiting, diarrhea, vertigo and mild dizziness. Uncommon side effects occurring in less than 10% of patients such as lupus-erythematosus-like autoimmune syndrome and irreversible pigmentation of gums or teeth and skin in sun exposed areas can occur with prolonged use of minocycline over an extended period of time beyond 6 months.

Pharmacokinetics and metabolic profile

Minocycline shows a better pharmacokinetic profile than the first generation tetracycline when used orally, being rapidly and completely absorbed, even in elderly populations, with a longer half-life and almost complete bioavailability. Once orally absorbed, the drug is widely distributed and is primarily excreted through urine. Some drug is present in bile and breast milk. Minocycline is highly lipophilic and therefore crosses easily the BBB, thereby enabling its use in the treatment of CNS diseases. Onset of antimicrobial action is 4—6 h and duration of action is 24 h, with a single oral administration of 200 mg producing a peak serum concentration of 3—5 mg/mL with a half-life of 11—13 h.

Drug interactions

Antacids (aluminum, calcium, zinc, and magnesium), iron salts, bismuth salts, cimetidine as well as some dairy products reduce absorption of minocycline and can reduce its efficacy. Minocycline can increase the effect of anticoagulants such as coumadine while combined use with methoxyflurane may enhance nephrotoxic effects of both. Minocycline can increase serum levels of digoxin leading to digoxin toxicity. Minocycline interacts with oral contraceptives, potentially resulting in breakthrough bleeding and unintended pregnancy.

Efficacy data

In a randomized, double-blind, placebo-controlled crossover study of transcranial magnetic stimulation in healthy subjects, minocycline significantly increased the duration of mean cortical silent period, a measure of intracortical inhibition (Lang et al., 2013). Thus, minocycline can exert acute inhibitory effects on cortical excitability in humans. Of note, a case was reported of successful use of minocycline as anti-seizure medication in a patient with drug-resistant epilepsy due to astrocytoma, raising a possibility of future use of minocycline in epilepsy patients (Nowak et al., 2012).

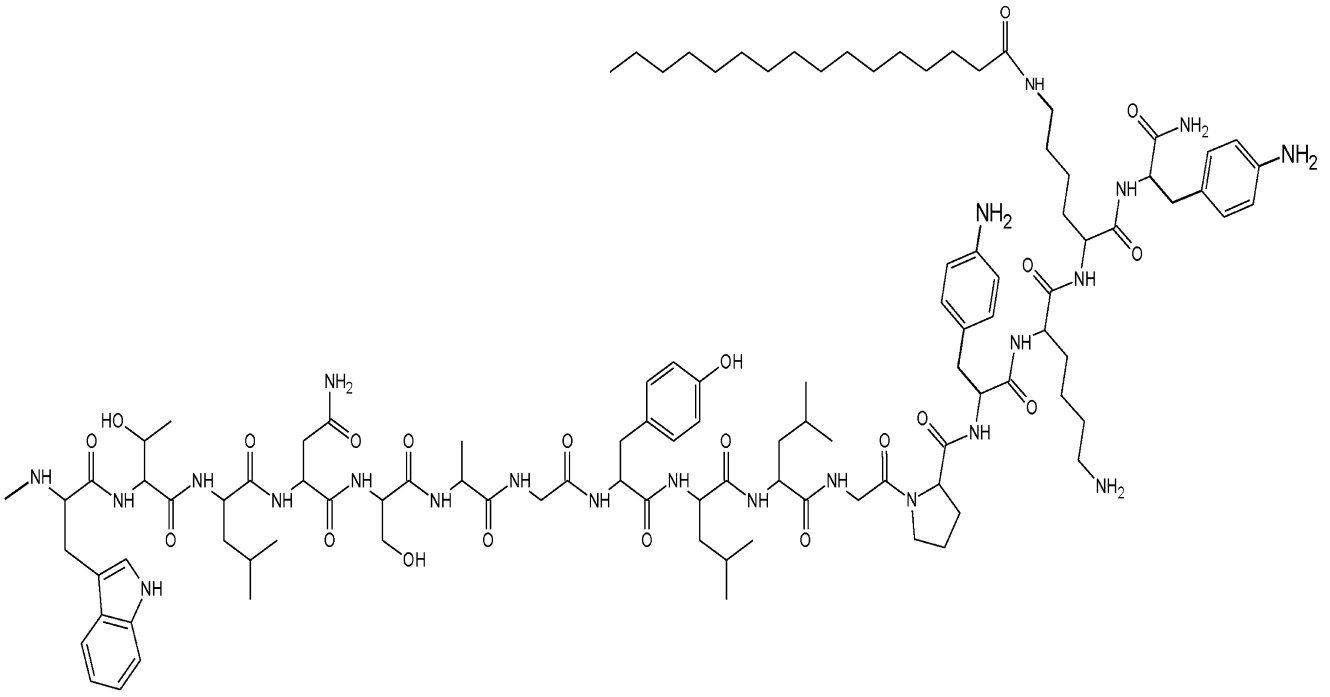
Planned studies

Extensive PubMed search as well as a search among 161 minocycline studies reported to *ClinicalTrial.gov* revealed that there was currently no clinical trial planned using minocycline in epilepsy.

NAX 810-2

H.S. White[a,c], G. Bulaj[b], B.D. Klein[a,c], C.S. Metcalf[c], D.R. McDougle[c], M.D. Smith[a], L. Zhang[b]

[a] Department of Pharmacology and Toxicology
[b] Department of Medicinal Chemistry, College of Pharmacy, University of Utah, Salt Lake City, UT, United States; [c] Neuroadjuvants, Inc., Salt Lake City, UT, United States



NAX 810-2

Introduction and rationale for development

Galanin is one of several neuropeptides with demonstrated anticonvulsant activity (Robertson et al., 2010). Both GAL_1 and GAL_2 receptor subtypes have been shown to play a role in seizures (Mazarati et al., 2004a,b; Mazarati and Lu, 2005; Saar et al., 2002). However, the development of galanin as an anticonvulsant has been hindered by poor metabolic stability and lack of blood—brain barrier penetration and GAL_1 produces insulin inhibition in several species (Lindskog and Ahrén, 1987, 1989; Lindskog et al., 1990). Activation of GAL_2 has been shown to reduce or inhibit seizure activity (Mazarati et al., 2004b; Mazarati and Lu, 2005). Since the initial characterization of the lead GAL_2-preferring analog, NAX 810-2, in a variety of animal models (Bialer et al., 2013), additional studies have been performed using an intravenous (i.v.) formulation, which is the anticipated route of administration in clinical proof-of-concept studies.

Pharmacology

NAX 810-2 is a GAL_2-preferring agonist with demonstrated anticonvulsant and analgesic efficacy. Pharmacologic characterization of NAX 810-2, including anticonvulsant activity in experimental animal models, mechanism of action, and other analgesic activity have been described previously (Bialer et al., 2013). Additional characterization of this lead analog has been conducted following i.v. administration in selected seizure models. As shown in Table 1, NAX 810-2 is potently active in the 6 Hz (32 mA) model following i.v. administration, with an ED_{50} value of 1.1 mg/kg (95% CI 0.7—1.8). Similarly, NAX 810-2 was also active in corneal kindling following i.v. administration, with an ED_{50} of 1.8 mg/kg (95% CI 1.0—3.7).

Toxicology

Galanin, acting through GAL_1 receptors in the pancreas, elevates blood glucose by inhibiting insulin secretion. To demonstrate that the preferential activity of NAX 810-2 on GAL_2 receptors does not lead to hyperglycemia arising from insulin inhibition, NAX 810-2 was studied using a glucose tolerance test in mice. Fasted mice (6 h) were administered glucose (1 g/kg, i.p.) followed by NAX 810-2 (2.5 or 8 mg/kg, i.p.; ED_{50} and ED_{97} doses, respectively, in

6 Hz 32 mA) and compared to a GAL_1-preferring analog (NAX 505-5, 0.8 mg/kg, i.p.; ED_{50} dose in 6 Hz 32 mA). In contrast to NAX 505-5, which produces sustained hyperglycemia and reduced insulin levels, NAX 810-2 did not lead to prolonged hyperglycemia and insulin levels were comparable to vehicle-treated mice.

Pharmacokinetics and metabolic profile

NAX 810-2 is highly plasma protein-bound (>99%) and possesses linear pharmacokinetics following i.v. administration to mice (single bolus) at doses between 0.375 and 1.5 mg/kg, i.v. Clearance was estimated to be 0.4 (L/h) and half-life was 1.2 h.

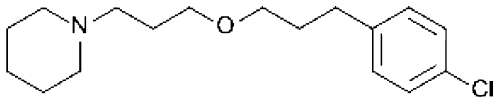
Planned studies

Upcoming studies include screening for potential off-target effects, evaluation of CYP enzymes for potential drug-drug interactions, dose-ranging toxicology studies in rats and pharmacokinetic studies in non-human primates that will further support IND-enabling studies and Phase 1 clinical studies in patients with epilepsy.

Pitolisant (Tripolisant)

D. Kasteleijn-Nolst Trenité

University of Utrecht, The Netherlands; University Sapienza, Rome, Italy



Pitolisant (Tripolisant)

Introduction and rationale for development

Pitolisant (also known as BF 2.649 and tripolisant) is the very first Histamine 3 receptor (H3R) antagonist investigated in patients with epilepsy. H3Rs are primarily located in the CNS, with the highest expression in the cerebral cortex, hippocampus, basal ganglia and hypothalamus (Martinez-Mir et al., 1990).

The pharmacology of H3Rs is determined not only by their localization, but also by different splicing, with more than 20 splice variants (isoforms) having been identified (Gemkow et al., 2009; Drutel et al., 2001). Through G-protein coupled receptors, histamine influences the action of other neurotransmitters, including dopamine, GABA, serotonin and glutamate (Ellenbroek and Ghiabi, 2014). Although H1 and H2 receptor antagonists have long been used effectively to treat allergic reactions and peptic ulcer respectively, H3R antagonists are not yet in the market, but they show promising activity on a variety of functions such as alertness, learning and memory.

Potential benefits of pitolisant, a non-imidazole inverse H3R agonist/antagonist developed by Bioproject (Paris, France), have been documented by demonstrating improved refractory daytime sleepiness in Phase II trials in patients with narcolepsy, Parkinson disease and obstructive sleep-apnea (Schwartz, 2011; Inocente et al., 2012).

Epilepsy represents another possible indication because histamine influences many neurotransmitters, and changes in histaminergic transmission may affect seizure threshold. This is also indicated by some clinical observations, such as the occurrence of seizures in some elderly patients after use of the H2 receptor antagonist cimetidine (Sonnenblick et al., 1982) and the finding of low histamine levels in the CSF of children with febrile seizures (Kiviranta et al., 1995).

Pharmacology

As shown in Tables 1 and 2, pitolisant, 1-[3-[3-(4-chlorophenyl)propoxy]propyl]-piperidine monohydrochloride, has been very effective in the following seizure models in rats and mice, predictive for generalized and partial type of epilepsies:

(a) Genetic Absence Epilepsy Rats of Strasbourg (GAERS)
(b) Maximal electroshock test in mice
(c) Kainate induced hippocampal seizures in mice

Toxicology

Rotarod tests have been done in mice to test possible interactions of pitolisant with carbamazepine, valproic acid, phenytoin, phenobarbital and diazepam. No changes in pattern of motor behavior were found.

Pharmacokinetics and metabolic profile

In healthy volunteers, serum pitolisant concentrations after single oral doses up to 120 mg were linearly related to dose. After an oral dose of 20, 40 or 60 mg, peak serum concentrations (C_{max}) occurred at 1—3 h and the half-life was 11 h (Schwartz, 2011)

Drug interactions

No information about potential interactions involving pitolisant is available.

Efficacy data

A proof-of-concept study to explore the potential anti-seizure activity of pitolisant was performed in 14 photosensitive adults (11 females, 3 males; age range 19—39 years) using the photosensitivity standard model. Five subjects were drug-naïve, 6 were on monotherapy with valproic acid, levetiracetam, topiramate or carbamazepine and 3 were on two AEDs. All subjects showed generalized photoparoxysmal responses (PPRs) and four had clinical signs (myoclonic jerks) during the PPRs (Kasteleijn-Nolst Trenité et al., 2013).

During three consecutive days PPR-range assessments (pharmacodynamic effect) were performed at times 0, 1, 2, 3, 4, 6, 8 and 10 h. Patients on AEDs continued their medications unchanged. On Day 1 and Day 3 placebo was

administered at the same time as pitolisant on Day 2, namely shortly after the time 0 PPR-range assessment (baseline). Four patients received 20 mg pitolisant orally, 4 received 40 mg and 6 received 60 mg.

After pitolisant, 9 of the 14 patients (64%) showed a statistically significant suppression of generalized epileptiform discharges (with complete abolition in 6 cases), with onset at 2 h on Day 2 and for a duration of 4 h. Some long lasting effects (up to 28 h) were seen after the 60 mg dose. The most prominent effect was seen at the highest dose (5/6 subjects at 60 mg) (Table 2). In three patients, myoclonic jerks associated with their PPR disappeared 1—2 h after intake of pitolisant.

Tolerability and adverse effect profile

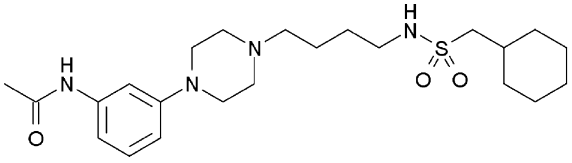
In healthy volunteer EEG studies, 36 male subjects given single doses of pitolisant (1, 5, 10, 20, 40 and 60 mg) orally showed an increase in beta activity 2 h after intake of the 40 and 60 mg doses. No effects were recorded on self-perception of sleep (LEEDS), alertness and calmness (Bond-Lader VAS), choice reaction times (CRT) or ARCI 49. A statistically significant increase in vigilance was found in the flicker-fusion thresholds after the 60 mg dose.

The proof-of-concept study in photosensitive subjects study showed no effect on the ECG and vital signs. After the 60 mg dose, two patients complained of insomnia and cognitive slowing respectively.

PRX 0023 (Nalutozan)

W.H. Theodore

Clinical Epilepsy Section, National Institutes of Health, Building 10, Room 7D-43, Bethesda, MD 20892, USA



PRX0023 (Nalutozan)

Introduction and rationale for development

Development of PRX-00023 (N-{3-[4-(4-cyclohexyl-methanesulfonylamino-butyl)-piperazin-1-yl]-phenyl}-acetamide dihydrochloride; Naluzotan) as an anti-seizure drug is based on data from animal models and human positron emission tomography (PET) imaging studies showing a role for serotonin (5HT) 1A receptors in the pathophysiology of epilepsy. In addition, many patients with epilepsy suffer from depression and anxiety, and modulation of serotoninergic neurotransmission is a standard treatment for those disorders. A $5HT_{1A}$ receptor agonist might have broad therapeutic potential in epilepsy. Some data suggest that sudden unexpected death in epilepsy (SUDEP) may be related to 5HT neuronal dysfunction (Richerson and Buchanan, 2011).

5-HT1A receptor activation shows antiseizure effects in several seizure models (Bagdy et al., 2007). The highly selective 5-HT1A antagonist WAY100635, one of the tracers, labeled with carbon-11 or fluorine-18, used for human PET studies, blocked the protective effect of 5HT infusion on pilocarpine-induced seizures (Bagdy et al., 2007). 5HT may play a role in the mechanism of action of antiepileptic drugs: Carbamazepine and valproate release 5-HT as part of their mechanism of action, while lamotrigine inhibits 5-HT re-uptake (Bagdy et al., 2007). Indirect data, and limited open clinical trials, suggest that selective serotonin reuptake inhibitors may have anti-seizure effects (Favale et al., 1995; Richerson and Buchanan, 2011).

PET studies by several groups of independent investigators have shown reduced 5HT-1A receptors in patients with temporal lobe epilepsy (Toczek et al., 2003; Savic et al., 2004; Merlet et al., 2004). Patients with both depression and epilepsy have greater reduction in $5HT_{1A}$ binding in cingulate cortex, hippocampus, insula, and midbrain raphe, consistent with PET studies of 1A receptors in major depressive disorders showing bilateral reductions of 5HT1A receptors in mesial temporal structures and cingulate cortex (Hasler et al., 2007; Drevets et al., 2007).

Pharmacology

Anticonvulsant profile (animal models/electrophysiology)
PRX-00023 has not been tested in any of the standard epilepsy models.

Other pharmacological properties
PRX-00023 stimulated dose-proportional increases in mean human prolactin levels. In the rat forced swim test, PRX-00023 showed effects comparable to fluoxetine.

Mechanism(s) of action

PRX-00023 is a high affinity 5HT1A receptor agonist. It has no significant affinity for benzodiazepine receptors and does not affect GABA binding *in vitro* or *in vivo* when tested in preclinical models. PRX-00023 has no significant binding to other serotonin receptors, $\alpha 1$ or $\alpha 2$ adrenergic receptors (Rickels et al., 2008).

Toxicology

At 30 mg/kg/day female rats had slight anemia, decrease in serum albumin, total protein levels, increased liver and spleen weights and decreased uterus weight; all changes resolved. Increased and persistent diestrus, mucification of vagina and mammary hyperplasia were observed in females. These changes were not observed in dogs, though the latter had transient inactivity and poor coordination at higher doses.

Pharmacokinetics and metabolic profile

Following single oral doses of 10—60 mg to healthy subjects PRX-00023 was rapidly absorbed, with peak serum concentrations occurring at 0.5—1.25 h, and a half-life of 4.8—10.4 h. On chronic daily dosing of 10—60 mg for 28 days the half-life ranged from 9.8 to 13.5 h. Plasma protein binding is about 90%. PRX-00233 metabolism is mainly by CYP3A4 to a hydroxylated metabolite, and partially by CYP2D6 (Iyer et al., 2007).

Drug interactions

PRX-0023 metabolism is inhibited by ketoconazole. Although no data are available, drugs affecting CYP3A4 or CYP2D6 might affect serum PRX-00023 levels. PRX-00023 itself is not a significant inducer or inhibitor of CTP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. However, it is a PgP substrate *in vitro*.

Efficacy data

In a clinical trial to assess potential efficacy in anxiety, 140 patients received PRX00023 80 mg per day or placebo. The primary efficacy measure was mean change in total HAM-A score from baseline (week 0) to study end (week 8), which showed a non-significantly greater decrease ($p = 0.116$) in the PRX00023 group. However, a significant reduction in MADRS depression score was observed (Rickels et al., 2008). In a second trial, 180 patients received up to 240 mg per day of PRX00023 or placebo. The primary efficacy measure was mean MADRS score change from baseline (week 0) to study end (week 8). The active treatment group showed a decline in MADRS score that was greater than the placebo group but the difference was not statistically significant (PRX-0023 Investigator's Brochure).

Tolerability and adverse effect profile

In clinical trials, the main treatment-emergent adverse events (TEAE) noted were headache, nausea, and dizziness. In the first anxiety trial reported above, 72.9% of subjects receiving PRX-00023 and 64.2% receiving placebo reported at least one TEAE. All AEs were rated as mild to moderate except for one subject who experienced diverticulitis that was not attributed to the study medication. Early terminations due to TEAEs included 2 subjects receiving PRX-00023 (rash; palpitations) and 4 receiving placebo (rash; infection; injury; dizziness). Headache was reported in 15.7% of subjects receiving PRX-00023 and 10.9% receiving placebo. In the second trial, TEAEs were reported by 58.3% of PRX-00023 and 54.2% of placebo subjects. The two TEAEs most frequently reported were headache, reported by 13.9% of patients on PRX-00023, compared to 7.8% on placebo, and dizziness, reported by 8.9% on PRX-00023 compared to 2.2% of placebo subjects. Nausea occurred in 8.3% of PRX-00023 subjects and 5.0% of placebo. Insomnia also occurred more frequently in PRX-00023 subjects, 5.6% compared to 1.7% for placebo. Most TEAEs were rated as mild to moderate. Only a few were considered to be severe in the PRX-00023 group: cholecystitis, increase in alanine aminotransferase, fibromyalgia, pain in extremity, headache, depression in one subject each, and insomnia in two subjects. In the placebo group, fungal infection, depression, restlessness and urine retention were rated severe in one subject each and headache in two subjects.

Smaller groups of patients have tolerated up to 320 mg per day.

Planned studies

A randomized cross-over pilot study in 24 patients with epilepsy is ongoing. The trial will have a baseline phase in which each patient will undergo physical and neurological examination, standard blood tests, neuropsychological and mood evaluation, FCWAY PET (if not already performed) and MRI. During the subsequent first treatment phase, patients will be randomized to PRX-00023 (120 mg bid) or matching placebo. After completion of the first treatment phase, patients will be crossed over to the alternate treatment arm. The outcome measures are seizure frequency counts during the 3 month placebo and active treatment phases; neuropsychological and mood measures; and safety assessments, including TEAEs, vital signs, laboratory signs and physical examination.

SAGE-217

A.J. Robichaud, J.J. Doherty, S.J. Kanes, G.J. Belfort, M.A. Ackley, E.C. Hoffman, R.S. Hammond, G. Martinez Botella, F.G. Salituro, J.M. Jonas

Sage Therapeutics, Cambridge, MA 02142, USA

Introduction and rationale for development

SAGE-217 is a second generation neuroactive steroid which acts as a $GABA_A$ receptor positive allosteric modulator. The compound exhibits robust efficacy in preclinical rodent models of seizures and SE, including a model that exhibits significant pharmacoresistance to other classes of AEDs. In contrast to both the endogenous neuroactive steroid allopregnanolone and its 3β-methyl derivative, ganaxolone, SAGE-217 possess a pharmacokinetic profile optimized for once daily oral administration and exhibits fewer off-target effects. This novel profile supports advancing its clinical development as a therapy for pharmacoresistant seizure disorders.

Pharmacology

Anticonvulsant profile (animal models/electrophysiology) SAGE-217 demonstrates anticonvulsant efficacy in a variety of preclinical rodent seizure models. Acute administration of SAGE-217 (0.3—10 mg/kg, i.p.) effectively reduces pentylenetetrazol (PTZ)-induced seizures in mice ($n = 10$/group), significantly increasing the latency to both tonic and clonic seizures, as well as reducing the number of tonic and clonic seizures. The efficacy of SAGE-217 is

dose-dependent, with improved potency (MEC_{plasma} = 85 nM) relative to either allopregnanolone (MEC_{plasma} = 452 nM) or ganaxolone (MEC_{plasma} = 327 nM). In the mouse 6 Hz electrical stimulation model (n = 10/group), SAGE-217 (0.3—3 mg/kg, i.p.) produces a dose-dependent anticonvulsant effect (MEC_{plasma} = 342 nM).

SAGE-217 (0.3—5 mg, i.v.) also effectively abolishes both behavioral and electrographic seizure activity in the rat lithium—pilocarpine model of SE when administered 60 min after induction of SE (MEC = 3125 nM), a latency that renders the ongoing seizure activity resistant to many AEDs (Morrisett et al., 1987; Pouliot et al., 2013). Neither diazepam (10 mg/kg, i.v.) nor lamotrigine (3—30 mg/kg, i.v.) blocked SE when administered following 60 min of SE. These results highlight the potential for SAGE-217 as a novel therapy for treatment-resistant seizure disorders.

SAGE-217 has also been assessed in two rodent seizure models by the Anticonvulsant Screening Project of the National Institute of Neurological Disorders & Stroke (ASP-NINDS, NIH), the 6 Hz electrical stimulation model and the maximum electric shock (MES) model. SAGE-217 demonstrates an anticonvulsant profile in both models, with greater potency in the 6 Hz electrical stimulation model than in the MES model. These results are consistent with the profiles for allopregnanolone and ganaxolone in these models (see Kaminski et al., 2004), although SAGE-217 demonstrates improved potency over both neuroactive steroids.

Other pharmacological properties

Acute treatment with SAGE-217 produces motor effects at higher plasma concentrations than were required for anticonvulsant activity (MEC_{plasma} in open field locomotor activity test = 3681 nM). Thus the therapeutic window between anticonvulsant efficacy in the PTZ model and acute motor impairment is approximately 40-fold, comparable to margins determined for either diazepam or allopregnanolone. Repeated exposure to diazepam (10 mg/kg, p.o.; BID for 7 days) produces — tolerance to motor impairing effects in the rotarod assay and to the anticonvulsive effects in the PTZ model. In contrast, although repeated exposure to SAGE-217 (10 mg/kg, p.o.; BID for 7 days) results in tolerance to the motor impairing effects, there is no significant loss of anticonvulsant efficacy.

Pharmaco-EEG studies were conducted with both intravenously and orally administered SAGE-217. Similar to other neuroactive steroids (Visser et al., 2002), systemic administration of SAGE-217 produces biphasic, concentration dependent changes in spectral EEG power that are localized predominately in the Beta-frequency (12.5—30 Hz) range. Specifically, whereas initial elevations of Beta-EEG occur at plasma concentrations that are devoid of overt sedation, a lowering of EEG-Beta is observed at highly sedative drug concentrations.

SAGE-217 exhibits extremely limited secondary pharmacology when examined across a variety of *in vitro* screening assays. In a cardiac ion channel functional *in vitro* screen, SAGE-217 has no significant effect on any of the 8 channels examined up to the highest concentration tested (30 μM). In a broad off-target receptor selectivity panel, SAGE-217 (10 μM) shows significant binding to only sigma and glycine receptors, and inhibition of the TRPV1 receptor. The ability of SAGE-217 to interact with nuclear hormone receptors signaling was assessed with a functional assay measuring both agonist and antagonist effects on a panel of NHRs. Whereas both allopregnanolone and ganaxolone show significant functional activity at several NHRs, there is no significant agonist or antagonist activity of SAGE-217 at any of the 20 NHRs examined up to the highest concentration tested (10 μM).

Mechanism(s) of action

SAGE-217 is a neuroactive steroid $GABA_A$ receptor positive allosteric modulator with nanomolar potency at synaptic $\alpha1\beta2\gamma2$ (EC_{50} = 375 nM; Emax = 473%) and extra-synaptic $\alpha4\beta3\delta$ (EC_{50} = 299 nM, Emax = 574%) receptors, as determined using *in vitro* patch clamp electrophysiology on recombinant human $GABA_A$ receptors expressed in heterologous mammalian cell lines. The ability of SAGE-217 to potentiate the $\alpha4\beta3\delta$ receptor subtype differentiates the neuroactive steroid class of $GABA_A$ receptor positive allosteric modulators from benzodiazepines, which require the presence of a γ-subunit to modulate $GABA_A$ receptor function (Belelli and Lambert, 2005). In addition to this allosteric potentiation of GABA-mediated conductance, SAGE-217 is also able to directly gate conductance through the $GABA_A$ receptor at higher (micromolar) concentrations.

Toxicology and adverse effect profile

SAGE-217 is currently in the preclinical research stage and limited toxicology studies have been completed.

Pharmacokinetics and metabolic profile

SAGE-217 has not been administered to human subjects to date, so no human pharmacokinetic data are available. Estimates of human clearance predicted from non-clinical *in vivo* and *in vitro* data are 0.25—0.53 L/h/kg, respectively. The oral bioavailability is predicted to be >50%, with a half-life from 2 to 8 h after oral administration. *In vitro* metabolic profiling was conducted in cryopreserved human, mouse, rat, and dog hepatocytes, and reveals essentially four discreet metabolites. Although structural identification of these metabolites has not been conducted to date, their molecular mass suggests that they all result from single hydroxylations. In addition, there is no evidence for the occurrence of unique human metabolites.

Drug interactions

Although no formal drug interaction studies have been conducted, *in vitro* CYP inhibition (IC_{50}) is >30 μM for all major isozymes, suggesting low potential for SAGE-217 to inhibit the metabolism of substrates for those enzymes. Induction of drug metabolizing enzymes assessed in human hepatocytes indicates low level of induction of CYP3A4 and CYP2B6, suggesting that SAGE-217 has the potential to alter the pharmacokinetics of substrates for those enzymes.

Efficacy data

To date, no human studies have been completed with SAGE-217.

Tolerability and side effect profile

To date, no human studies have been completed with SAGE-217.

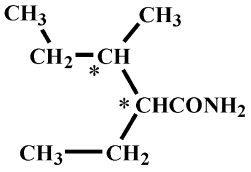
Planned studies

Currently, to support a first in man (FIM) study, a number of IND-enabling toxicology studies are planned or on-going, including 28-day general toxicology studies in rats and dogs, a genetic toxicity battery of Ames, rat micronucleus and Comet assays, and a safety pharmacology battery of rat CNS, rat respiratory and dog telemetry studies. In addition, to support later stage clinical investigations and a potential market launch, Sage Therapeutics will perform sub-chronic (3-month) and chronic (6 or 9-month) studies in rats and dogs, reproductive toxicology studies with Seg II in rats and rabbit. Rat Seg I and Seg III, a battery of abuse liability of drug dependence, drug discrimination and self administration, and carcinogenicity studies in rats and mice are also planned. Pending outcome of these trials, a Phase III clinical program will be conducted.

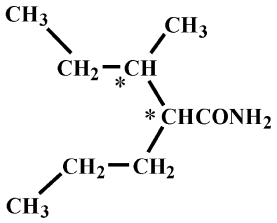
Valnoctamide and *sec*-butyl-propylacetamide (SPD): Second generation drugs to valproic acid (VPA)

M. Bialer[1], S. Kadosh[2], B. Yagen[1]

[1] Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel
[2] *StatExcellence*, Kfar Saba, Israel



Valnoctamide (VCD)



sec-Butyl-propylacetamide (SPD)

Introduction and rationale for development

Valnoctamide (VCD) is a CNS-active chiral constitutional isomer of valpromide, the corresponding amide of valproic acid (VPA). Since EILAT XI (2012), VCD underwent phase IIb clinical trial in patients with acute mania. VCD's one-carbon homologue *sec*-butyl-propylacetamide (SPD) was evaluated in a wide array of anticonvulsant models (White et al., 2012; Pouliot et al., 2013; Shekh-Ahmad et al., 2013). Both VCD and SPD possess two stereogenic centers in their chemical structure (denoted above with the asterisk *) and exhibit stereoselective pharmacokinetics (PK) in humans (VCD) and animals (Bialer and Yagen, 2007; Bialer, 2012). Consequently, the pharmacodynamics (PD; anticonvulsant activity) and PK of the four individual stereoisomers of VCD and SPD were evaluated in several rodent anticonvulsant models including: maximal electroshock (MES), 6 Hz psychomotor, subcutaneous metrazol (scMet) and the pilocarpine- and soman-induced status epilepticus (SE) (Hen et al., 2013; Shekh-Ahmad et al., 2014). The PK—PD (anticonvulsant activity) relationship of VCD and SPD stereoisomers were evaluated following i.p. administration (70 or 60 mg/kg) to rats. Induction of neural tube defects (NTDs) by VCD and SPD stereoisomers was evaluated in a mouse strain highly susceptible to teratogen-induced NTD.

Pharmacology

Anticonvulsant profile (animal models/electrophysiology)
Valnoctamide (VCD). VCD (racemate and/or its individual enantiomers) demonstrated anticonvulsant activity in a wide array of anticonvulsant models in mice and rats (MES, scMet and 6 Hz, kindling) as described in the previous Eilat (EILAT X & XI) Conference manuscripts (Bialer et al., 2010, 2013).

In mice VCD four individual stereoisomers exhibited similar anticonvulsant activity to one another as well as to racemic-VCD in the scMet and 6 Hz tests, while at the MES test (2R,3R)-VCD was more potent than its enantiomer (2S,3S)-VCD and diastereoisomer (2S,3R)-VCD. In rats (p.o.) racemic-VCD and its four individual stereoisomers exhibited similar anticonvulsant activity in the MES test while (2R,3S)-VCD was the most potent compound at the scMet test with an ED_{50} value 5-times more potent than the racemate (Shekh-Ahmad et al., 2014).

Racemic-VCD as well as (2R,3S)-VCD and (2S,3S)-VCD administered at seizure onset blocked the pilocarpine-induced SE with similar ED_{50} values of about 40 mg/kg. However, racemic-VCD and its individual strereoisomers lost this anti-SE activity when given (75—100 mg/kg) 30 min after seizure onset (Shekh-Ahmad et al., 2014).

Racemic-VCD administered with the standard medical countermeasures at treatment delays of 5 min, 20 min and 40 min after seizure onset was capable of stopping soman-induced SE seizures with ED_{50} values of 26 mg/kg, 60 mg/kg and 62 mg/kg, respectively. Following administration of VCD the average latency (sec) for electrographic seizure termination (mean ± SEM) at 5 min, 20 min and 40 min was: 115 ± 15, 497 ± 15 and 1336 ± 318, respectively. VCD is one of the few drugs effective at 40 min delay at the soman-induced SE model (Shekh-Ahmad et al., 2014).
sec-Butyl-propylacetamide (SPD). Racemic-SPD had a wide spectrum of anticonvulsant activity similar to VPA but was 4—30 times more potent than VPA in a wide array of anticonvulsant animal models (Shekh-Ahmad et al., 2013). Three SPD individual stereoisomers: (2R,3R)-SPD, (2S,3S)-SPD and (2R,3S)-SPD, exhibited anticonvulsant activity similar to racemic-SPD, while (2S,3R)-SPD exhibited less potent anticonvulsant activity at the rat (p.o.) MES model (Hen et al., 2013).

SPD stereoisomers, administered 30 min after the first observed Stage 3 motor seizure at the lithium–pilocarpine induced SE model, prevented the expression of further convulsive seizures in a dose-dependent fashion with ED_{50} values ranging between 95-135 mg/kg. (2R,3R)-SPD was the least potent SPD stereoisomer (ED_{50} > 130 mg/kg) (Hen et al., 2013).

SPD and its individual stereoisomers were capable of stopping soman-induced SE seizures with ED_{50} values ranging between 40 and 71 mg/kg. Following administration of SPD individual stereoisomers the average latency (sec) for electrographic seizure termination at the 20-min treatment delay time (mean ± SEM) was: 550 ± 149; n = 16 [(2R,3R)-SPD], 994 ± 280; n = 8 [(2R,3S)-SPD], 719 ± 216; n = 15 [(2S,3R)-SPD], 1589 ± 684; n = 13 [(2S,3S)-SPD]. All four individual SPD stereoisomers had a steep dose response curve with Hill (shape) coefficient of 4.5–8.9 that lead to a tight 95% confidence interval around their ED_{50} values. (2R,3R)-SPD was more potent than racemic-SPD as well as SPD three other individual stereoisomers (p < 0.05) and had the shortest mean latency for seizure control (Hen et al., 2013).

Other pharmacological properties

The formalin test was performed according to the method of Tjolsen et al. (1992). Racemic-SPD was effective agonist formalin-induced acute and inflammatory phases of hyperalgesia. This finding suggests that SPD may be useful in attenuating inflammatory pain.

Mechanism(s) of action

Recently Spampanato and Dudek showed that racemic-VCD acts directly on the $GABA_A$ receptors similarly to the effects of benzodiazepines (BZDs). But the effect of VCD persisted in the presence of the BZD-binding site antagonist flumazenil, and was additive to the effect of diazepam. These data suggest that VCD acts through a different binding site than that of BZDs, which likely accounts for the VCD effect on BZD-refractory SE (Spampanato and Dudek, 2014).

Pharmacokinetics and metabolic profile

The pharmacokinetics of VCD and SPD stereoisomers were studied following i.p. administration (70 and 60 mg/kg, respectively) of each individual stereoisomer as well as the racemate to rats. The dose was chosen for the PK study as the intermediate dose among the various ED_{50} values of VCD stereoisomers. VCD had a stereoselective PK with (2S,3S)-VCD exhibiting the lowest clearance and consequently, a twice-higher plasma exposure (AUC) than all other stereoisomers. The apparent volume of distribution and half-life of VCD's four individual stereoisomers were similar and ranged between 1–1.6 L/kg and 2.1–3 h, respectively

(2R,3S)-SPD and (2S,3R)-SPD have clearance values twice higher than racemic-SPD, (2S,3S)-SPD and (2R,3R)-SPD. This relatively high clearance led to lower plasma exposure (AUC < 50%) that may contribute to their lower anticonvulsant activity in the pilocarpine-induced BZD-resistant SE model. Although the relative higher clearance of (2R,3S)-SPD did not affect its anticonvulsant activity [compared to racemic-SPD, (2S,3S)-SPD or (2R,3R)-SPD] in the rat-MES and scMet models,

(2S,3S)-SPD and (2R,3R)-SPD and racemic-SPD have similar anticonvulsant activity and PK profile that is better than that of (2R,3S)-SPD and (2S,3R)-SPD. If SPD (and VCD) would exert its broad-spectrum anticonvulsant activity due to a single mechanism of action (MOA) it is likely that it would exhibit stereoselective PD. The fact that there was no significant difference between (2R,3S)-SPD and (2S,3R)-SPD and racemic-SPD in the various anticonvulsant rodent models (except the soman-induced SE) may indicate that SPD (and VCD) anticonvulsant activity is due to multiple MOA. The choice for further drug development between racemic-SPD, (2S,3S)-SPD or (2R,3R)-SPD as well as between racemic-VCD and its individual stereoisomers will be based on comparative toxicological analysis and additional anticonvulsant testing that will discriminate between the CNS-active compounds.

Efficacy data

A double-blind controlled Phase IIa clinical trial with racemic-VCD as add-on to risperidone in 32 patients with acute mania showed that in all efficacy measures VCD (n = 15) was significantly more effective than placebo (n = 17), differences between the two groups being significant from weeks 3 to 5. It is worth emphasizing that VCD could be the first potential effective mood stabilizer without significant teratogenicity (Bersudsky et al., 2010; Bialer et al., 2010).

Consequently, a phase IIb study VCD-BP-01 (funded by the Stanley Medical Research Institute – SMRI) started in Europe in 2013 (Bialer et al., 2013). The objective of study VCD-BP-01, a 3-week, double-blind, randomized, placebo-and risperidone controlled parallel group trial, was to evaluate the efficacy of racemic-VCD (monotherapy), compared to placebo in the treatment of patients with bipolar disorder in a manic or mixed episode.

A total of 313 subjects with bipolar disorder in a manic or mixed episode as confirmed by the modified version of the Structured Clinical Interview for DSM-IV (SCID) were planned to be recruited into the study. A Young Mania Rating Scale (YMRS) total score of ≥20 was required prior to randomization. Subjects with psychotic features and rapid cycling were allowed in the study. Randomization employed a block size of 5 stratified by center using the 2:2:1 assignment ratio; VCD-placebo-risperidone, respectively. The primary endpoint of the study was the change from baseline (Day 0) to week 3 in the total YMRS Score and the interim analysis was performed on the modified intentional to treat (mITT) Cohort (n = 151) and on the Completers Cohort (N = 114) (total drop-out rate 24.5%).

The drop-out rate of subjects treated with VCD (32.8%) was higher as compared to groups treated with risperidone (20.0%) or with placebo (18.3%). Reasons were lack of efficacy and withdrawal of consent. The higher drop-out rate in patients treated with VCD was reflected in the shorter follow-up duration (17.3 ± 6.1 days) as compared to groups treated with risperidone (19.4 ± 4.2 days) or with placebo (19.1 ± 5.4 days). Overall, 67.5% of the subjects were females with lower frequency at the risperidone group

(53.3%) as compared to the VCD group (68.9%) and placebo group (73.3%). All patients were Caucasians and subjects ranged in age from 23 to 67 years with a median age of 48.6 years.

Analysis of the primary study endpoint for the mITT Cohort demonstrated the superiority of the risperidone-treatment as compared to placebo ($p = 0.0446$) while treatment with VCD showed similar reduction in YMRS at week 3 as compared to placebo arm ($p = 0.9169$). In contrast, the Completers Cohort analysis demonstrated the superiority of both risperidone ($p = 0.0188$) and VCD ($p = 0.0159$) over placebo treatment in YMRS change from baseline at week 3. The difference in the VCD effect between the mITT and Completers cohorts is unclear and is in contrast to the Phase IIa results.

Futility analysis has been conducted. To this end, the conditional probability, namely the probability that the final study result (primary endpoint VCD *versus* placebo contrast) will be statistically significant ($p < 0.05$), given the data observed thus far was calculated for 3 potential scenarios about future data. For the worst case scenario, intermittent scenario and best case scenario there is a 22.4%, 45.0% and 57.1%, respectively, conditional probability of study success in achieving the primary outcome measure for the VCD *versus* placebo comparison if the study will continue to enrol the originally preplanned population ($N = 313$). Due to the mITT interim analysis results combined with the futility analysis outcome the SMRI decided to terminate the study VCD-BP-01 early.

In summary, the reasons for the conflicting outcomes of the mITT and Completers cohort results, is unclear and it posed the question whether the non-completers suffered from intractable bipolar illness. This Phase IIb study showed that patients can tolerated VCD daily doses of 1500 mg with minimal side effects and that patient who stayed on VCD may benefited from it.

Tolerability and adverse effect profile

Teratogenicity

The teratogenic potential of racemic-VCD and racemic-SPD and their four individual stereoisomers was assessed for their ability to induce gross morphological defects in the SWV/Fnn mice that are highly susceptible to VPA-induced exencephaly. VPA, at a dose of 2.7 mmol/kg was embryotoxic and teratogenic causing almost two-fold increase in the resorption rate compared to the control group (11.9% *versus* 6.3%, respectively) and NTDs in 29.1% of live fetuses.

In contrast to VPA, racemic-VCD and its four individual stereoisomers did not cause statistically significant increase of NTDs at doses of 257 or 389 mg/kg. (2S,3S)-VCD, (2R,3S)-VCD and racemic-VCD were embryotoxic and induced resorptions in 16%, 23% and 21% of conceptions respectively, when tested at the higher 2.7 mmol/kg dose. These doses are 3—12 times higher than VCD anticonvulsant-ED_{50} values (Shekh-Ahmad et al., 2014).

(2R,3R)-SPD and racemic-SPD were embryotoxic and induced resorptions in 22.9% and 13.4% of conceptions respectively, when tested at the higher 1.8 mmol/kg dose. (2R,3R)-SPD was also teratogenic at the 1.8 mmol/kg dose, causing exencephaly in 6 in 6 out of 111 fetuses (5.4%). In contrast three of the SPD stereoisomers (2S,3S)-SPD, (2R,3S)-SPD and (2S,3R)-SPD were neither embryotoxic nor teratogenic in our study on SWV mice (Hen et al., 2013). SPD (racemate) and three of its individual stereoisomers were found to be non-embryotoxic and non-teratogenic and they all failed to induce neural tube defects at doses 2—9 times [(2R,3R)-SPD] and 3—14 times [(2S,3S)-SPD] higher than their anticonvulsant-ED_{50} values (Hen et al., 2013). VCD stereoisomers (258 or 389 mg/kg) and SPD stereoisomers (141 and 283 mg/kg) did not cause NTD and thus are superior to VPA not only by their more potent anticonvulsant activity but also by their lack of teratogenicity.

Planned studies

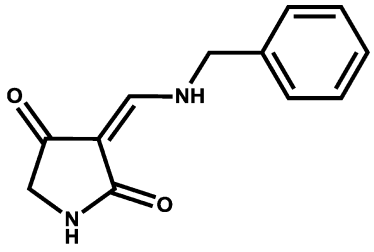
Although VPA is a frequently prescribed AED, its clinical use is restricted in women of child-bearing age and in children due to its teratogenicity and hepatotoxicity, respectively (Bialer and Yagen, 2007). The development of SPD and/or VCD (as racemate or an individual stereoisomer) as new potentially non-teratogenic and non-hepatotoxic, broad-spectrum AEDs that are more potent than VPA and benzodiazepines (in SE models), may answer unmet clinical needs in the treatment of therapy-resistant patients with epilepsy (Bialer and White, 2010).

Consequently, SPD and its individual stereoisomers are currently being evaluated (in comparison to midazolam) in paraoxon-induced SE models as well as in their potential to protect against other nerve gas exposure. In addition SPD and VCD neuroprotection properties are planned to be investigated in near future.

VLB-01 (Beprodone)

M. Toumi[1], A. Bakhutashvili[2], A. Miller[3]

[1] Faculte' de Medicine, Laboratoire De Sante' Publique, Aix Marseille Universite, Universite De La Mediterranee, Marseille, France
[2] Chief Scientific Officer, Marco Polo, Luxembourg
[3] Health Economics and Outcomes Research, Creativ Ceutical, Paris, France



VLB-01 (Beprodone)

Introduction and rationale for development

VLB-01 (Beprodone or 3-benzilaminometilen-pyrrolidine-2,4-dione) is a novel oral antiepileptic drug (AED)

in clinical development for the treatment of focal epilepsy. VLB-01 binds to the melatonin receptor-3/ribosyldihydronicotinamide dehydrogenase (NQO-2). Thus, VLB-01 differs from other AEDs by its mechanism of action, a fact which may be useful for treatment of refractory focal seizures.

VLB-01 is the first NQO-2 receptor agonist to be evaluated for its anticonvulsant and neuroprotective properties. It is supported by a large dataset, from preclinical studies, including the maximum electroshock (MES), audiogenic seizures, strychnine, bicuculline and pentylenetetrazole (PTZ) in mice and rats as well as Phase I and Phase II studies.

Pharmacology

Anticonvulsant profile (animal models/electrophysiology)
VLB-01 demonstrated a definitive anti-seizure profile in several anticonvulsant animal models.

Following oral administration to mice and rats, VLB-01 is active in the MES test with ED_{50} values of 26 mg/kg and 55 mg/kg, respectively. In the subcutaneous PTZ test, VLB-01 was found to increase the seizure threshold at doses up to 300 mg/kg (p.o.), with no impairment of beam walking performance.

VLB-01 was tested for its ability to block pilocarpine-induced seizures and did not elicit any protection at doses up to 100 mg/kg. However, at the same dose, the latency time to seizure onset was significantly increased.

In addition, VLB-01 is active in the PTZ-kindled model (ED_{50} = 360 and 60 mg/kg in mice and rats, respectively) and in the mouse-bicuculline (ED_{50} = 130 mg/kg) and strychnine (ED_{50} = 155 mg/kg) tests (Parshin et al., 2013).

Other pharmacological properties
At a dose of 100 mg/kg (i.p.), VLB-01 reduced the locomotor response to repeated administration of D-amphetamine (days 1—13) by 30% ($p < 0.001$). At dose of 50 mg/kg, significant reduction was achieved in the last days (7—13). These results suggest a possible antipsychotic/mood stabilizer activity.

Mechanism(s) of action

VLB-01 has a high affinity to the melatonin receptor ML_2 [MT_3/Quinone oxidoreductase 2 (QR_2)], with a unique receptor profile. *In vitro* studies showed that VLB-01 has no effect on benzodiazepine site of GABA-receptors and NMDA-type glutamate receptors

Toxicology

VLB-01 is considered to be a low-toxic substance according to the accepted classification of toxic substances by Hodge & Sterner (Grossel and Crowl, 1995). In mice (p.o.) VLB-01 LD_{50} values (1460—1893 mg/kg) were 56 times higher than its MES-ED_{50} (26 mg/kg). This wide protective index (PI) for VLB-01 demonstrates that it is effective at doses well below those that produce behavioral impairment. In rats, VLB-01 was well tolerated at doses of 150—300 mg/kg bid after 3 months. At the 300 mg/kg dose there was some growth retardation and reversible changes in blood biochemical parameters (*i.e.*, increased cholesterol) at the end of the 6 months treatment period.

In rabbits, no significant differences from control (vehicle)-treated rabbits were recorded at a dose of 30 mg/kg. At dose of 150 mg/kg, a 10% growth in retardation, some excitability, lowering of asparate aminotransferase (AST) and alanine transaminase (ALT) levels, unreliable increase in relative adrenal weight and reduced weight of thymus were observed.

In dogs, following repetitive oral dosing of 100 mg/kg for one month VLB-01 had no effect on hematological parameters and functional status of major organs and systems. These findings were confirmed by morphological assessment that showed no toxic damage to internal organs, as well as absence of local irritation. VLB-01 had no mutagenic activity in *in vitro* tests and in animal experiments.

Pharmacokinetics and metabolic profile

In rats VLB-01 had a oral bioavailability of 92% and linear pharmacokinetics. VLB-01 was rapidly distributed to organs and tissues and its C_{max} in the kidneys, liver and brain was observed 1 h after oral dosing.

In humans, VLB-01 pharmacokinetics was studied following oral administration of 100 mg, 250 mg or 500 mg to 36 different healthy subjects in a parallel escalating dose design. VLB-01 half-life was 8—9 h, t_{max} 1.5—2.5 h and its plasma exposure (AUC) and C_{max} increased in a dose-proportional manner. At these doses VLB-01 was well tolerated and did not cause any adverse event (Granik et al., 2013).

Efficacy data

Phase IIa study
A 9-week, phase IIa, multicentre, randomized, double blind, placebo controlled study was conducted in 60 male and female patients (age 18—65 years) with refractory focal seizures, who received VLB-01 bid, at doses of 500, 750 or 1000 mg/day for 4 weeks (maintenance phase) after titration, concomitantly with 1—3 AEDs. Patients were screened during a 4-week period to assess the baseline seizure frequency, they were then randomized to receive active or placebo. Treatment was titrated over 3 weeks, then stable dose administered for 5 weeks and tapered down over 2 weeks. Patients were randomized to three 20-patients groups. In each group, 15 received VLB-01 (500, 750 or 1000 mg/day) and 5 received placebo.

VLB-01 was safe and well tolerated in all tested doses. Minor adverse events (headache and nausea) were 7% at the 500 mg and 1000 mg treated-groups as well as the placebo and 20% (nausea) and 13% (headache) at the 750 mg treated-group. No relevant changes were observed in ECG and no weight gain was observed.

Efficacy was not the initial primary goal for this study as the sample size was not powered to show statistically significant differences in efficacy and study duration was too short

to capture efficacy. However, efficacy was assessed by computing the change in frequency of seizure during the 4 weeks maintenance *versus* the 4 weeks of the screening period prior to randomization. The analysis showed reduction of seizure frequency of 19% for the placebo arms, 40% for 750 mg arm and 34% for the 1000 mg arm. The 500 mg arm did not shown any effect. These results did not reach statistical significance.

Phase IIb study

A 24-week, randomized, double-blind, placebo-controlled, Phase IIb trial of VLB-01 (1500 mg/day) was initiated in 224 patients (age 18—65 years) with focal onset seizures secondary generalized or not. The number of seizures before randomization was mean (SD): placebo = 5.1(1.7); VLB-01 = 5.8(2.8). Subjects started the treatment with 500 mg/day, before being titrated by addition of a dose of 500 mg/day (given bid) during 2 weeks. During the 12 weeks maintenance period, patients received the target dose of 750 mg bid.

The number of seizure was recorded each visit and covered every 28 days period. Primary measured outcomes included seizure reduction and response rate, defined as proportion of patients who experienced at least a 50% reduction in seizure frequency during treatment compared to baseline. Rate of seizure-free patients was considered as a secondary criterion. Adverse events were also recorded at each visit, for safety assessment.

Interim analysis was performed on 112 patients randomized to either VLB-01 ($n = 58$) or placebo ($n = 54$). At the baseline visit, the mean and median numbers of seizures was similar in the two groups (VLB-01: mean = 5.84, median = 5.00; placebo: mean = 5.51, median = 5.00, $p = 0.45$). The median percent seizure reduction was 57.8% for VLB-01 *versus* 20.7% for placebo ($p = 0.002$). The response rate was also higher for VLB-01 (70.4% *versus* 38.5%, $p = 0.001$). The percentage of seizure-free patients during the last 28 days for the ITT population ranged from 19 to 33% for VLB-01 compared to 10—17% for placebo.

Tolerability and adverse effect profile

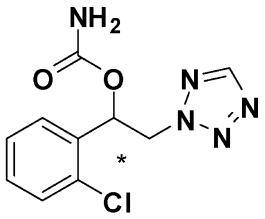
In terms of safety, at dose of 1500 mg/day, VLB-01 was very well tolerated and there were no safety issues and the objective of the interim analysis was to make decision on study continuation based only on efficacy. In conclusion, VLB-01 recorded 60% reduction in seizure frequency after 224 days as compared to 20% reduction for placebo.

Following interim analysis the recruitment was terminated and the patients already enrolled continued the final visit. A total of 168 patient s were enrolled with 85 in VLB-01 and 83 in placebo. Database was frozen following the blinded classification meeting and statistical analysis is about to start.

YKP3089

S.J. Lee, M. Kamin, P. Ilankumaran

SK Life Science Inc., Fair Lawn, NJ, USA



YKP3089

Introduction and rationale for development

YKP3089 is a novel tetrazole derived compound with one chiral center (denoted above with an asterisk). As summarized in Tables 1 and 2, YKP3089 is active in a broad range of animal models of epilepsy. It is currently in Phase II clinical development at SK Life Science.

Pharmacology

Anticonvulsant profile (animal models/electrophysiology)

The anticonvulsant profile of YKP3089 has been previously described in Bialer et al. (2013).

Mechanism(s) of action

In vitro electrophysiological studies have shown YKP3089 acts in two different ways: It is a selective blocker for the inactivated state of the sodium channel and preferentially blocks persistent sodium current (INaP). It increases inhibitory synaptic transmission by facilitating presynaptic GABA release.

Pharmacokinetics and metabolic profile

A phase I study evaluated single doses of YKP3089 in healthy volunteers from 5 to 750 mg. Median t_{max} ranged between 1.5 and 3.5 h, and C_{max} increased in a linear fashion from 0.1 μg/mL to 17 μg/mL. $t_{1/2}$ increased from 30 to 75 h over the dose range. Mean oral clearance (CL/F) values decreased from 1.42 to 0.396 L/h over the dose range. In multiple-dose studies with once-daily dosing, both C_{max} and AUC correlated linearly with the dose over the range of 100—500 mg/day. There was no significant effect of food (high-fat diet) on YKP3089 pharmacokinetics.

Efficacy data

A phase II randomized, double-blind, placebo-controlled 12 week study of YKP3089 to assess efficacy and tolerability of 200 mg/day of YKP3089 in approximately 200 patients with focal seizures has been completed. The results will be published elsewhere.

Tolerability and adverse effect profile

In Phase I healthy volunteer studies YKP3089 was well tolerated after single oral doses ranging from 5 mg to 500 mg.

At 600 mg and 750 mg doses subjects developed dizziness, somnolence and nystagmus. In a multiple ascending dose study, doses of 50 mg/day to 200 mg/day were well tolerated. In higher dose multiple ascending dose studies doses of 250—500 mg/day were associated with mild to moderate somnolence, dizziness, gait disturbances and nausea; 600 mg/day was poorly tolerated. No clinically significant changes in ECGs or laboratory parameters were observed.

Planned studies

A Phase II, multicenter, double-blind, randomized, placebo controlled dose response study to evaluate the efficacy and safety of three doses of YKP3089 as adjunctive therapy in subjects with treatment-resistant partial onset seizures (NCT01866111) is ongoing.

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