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Lacosamide, a novel anti-convulsant drug, shows efficacy with a wide safety margin in rodent models for epilepsy

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Summary This paper comprises a series of experiments in rodent models of partial and generalized epilepsy which were designed to describe the anti-convulsant profile of the functionalized amino acid lacosamide.

Lacosamide was effective against sound-induced seizures in the genetically susceptible Frings mouse, against maximal electroshock test (MES)-induced seizures in rats and mice, in the rat hippocampal kindling model of partial seizures, and in the 6 Hz model of psychomotor seizures in mice. The activity in the MES test in both mice (4.5 mg/kg i.p.) and rats (3.9 mg/kg p.o.) fell within the ranges previously reported for most clinically available anti-epileptic drugs. At both the median effective dose for MES protection, as well as the median toxic dose for rotorod impairment, lacosamide elevated the seizure threshold in the i.v. pentylenetetrazol seizure test, suggesting that it is unlikely to be pro-convulsant at high doses. Lacosamide was inactive against clonic seizures induced by subcutaneous administration of the chemoconvulsants

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pentylenetetrazol, bicuculline, and picrotoxin, but it did inhibit NMDA-induced seizures in mice and showed full efficacy in the homocysteine model of epilepsy.

In summary, the overall anti-convulsant profile of lacosamide appeared to be unique, and the drug displayed a good margin of safety in those tests in which it was effective. These results suggest that lacosamide may have the potential to be clinically useful for at least the treatment of generalized tonic—clonic and partial-onset epilepsies, and support ongoing clinical trials in these indications.

Introduction

Epilepsy is a common disorder, and each year more than a 100,000 new cases are diagnosed in the US (Browne and Holmes, 2001). Unfortunately, universally effective pharmacotherapy is lacking. Although, approximately 70% of patients will become seizure-free using a single anti-epileptic drug (AED), the remaining 30% may develop a chronic form of epilepsy that is often refractory to all available pharmacological treatments. These patients may suffer both recurrent seizures and intolerable side effects (Schmidt, 2002; LaRoche and Helmers, 2004).

Traditional AEDs such as carbamazepine, phenytoin, phenobarbital, primidone, valproic acid, and ethosuximide are effective against seizures, but their utility can be limited by clinically significant cognitive and behavioral side effects which adversely affect quality of life for many patients (Diaz-Arrastia and Agostini, 2002). Furthermore, their utility has also been limited by their pharmacokinetic profile and propensity for drug—drug interactions. Since 1993, nine new anti-epileptic drugs have been developed for the treatment of partial epilepsy. While these newly developed AEDs have not necessarily shown improved effectiveness over older drugs in the proportion of patients who experience significant seizure reduction, they have provided physicians additional treatment options. Moreover, several of the newer agents appear to have improved efficacy in refractory partial-onset seizures, appear to be better tolerated, and possess more favorable pharmacokinetic profiles (Diaz-Arrastia and Agostini, 2002; LaRoche and Helmers, 2004; Schmidt, 2002). Despite these advances, there remains a need for new drugs that modify the course of epilepsy in patients, particularly those who are refractory to current medications, as well as those who are not necessarily good candidates for surgery or vagal nerve implants.

Lacosamide ((*R*)-2-acetamido-*N*-benzyl-3-methoxypropionamide, also formerly called ADD 234037, SPM 927, or harkoseride) is a functionalized amino acid synthesized as an anti-convulsant drug candidate for the potential treatment of epilepsy and neuropathic pain (Choi et al., 1996). Only the (*R*)-isomer of this class of chemicals (propionamides) is pharmacologically active (Kohn et al., 1988). Lacosamide displays amphiphillic properties which allow it to be sufficiently water soluble to formulate a parenteral product and sufficiently lipophilic to pass the blood—brain barrier (Hovinga, 2003). Pharmacokinetic analysis indicates a high oral bioavailability which is not affected by food (Bialer et al., 2002). Preliminary human data indicate it is excreted in the urine (30—40% as unchanged lacosamide, and about 30% as the *O*-desmethyl-metabolite) (Bialer et al., 2002). The precise mode of action of lacosamide is still uncertain. Radioligand binding experiments on more than 100 receptors, ion channels and enzymes at a concentration of 10 μM have not detected significant binding, suggesting that it acts via a unique mechanism (Bialer et al., 2002). This report presents results from a series of pre-clinical experiments designed to further characterize the anti-convulsant properties of lacosamide in models of partial and generalized epilepsy in mice and rats.

Methods

To evaluate the anti-convulsant profile and potency of lacosamide, a series of experiments was carried out using a range of electrical, chemical and audiogenic seizure models. These models are widely used for screening and differentiating new anti-convulsant drug candidates, in both rats and mice (White et al., 1998; White, 2004). In addition, parameters of efficacy and safety, such as median effective dose (ED_{50}), the dose causing motor impairment (minimal motor impairment {MMI} in rats or TD_{50} in mice) and the protective index (MMI or TD_{50}/ED_{50}) were determined. Lacosamide was also evaluated in several potential models of epileptogenesis and status epilepticus. All animal experiments were carried out in accordance with the respective ethical guidelines and were formally approved by the animal subjects institutional review boards. All efforts were made to minimize the number of animals used and their suffering.

Animals

For this series of tests, the following animals were used: male albino CF No. 1 mice (18—25 g, Charles River, Wilmington, MA); male albino Sprague—Dawley rats (100—150 g and 200 g, Simonsen, Gilroy, CA); male and female Frings audiogenic seizure (AGS)-susceptible mice (18—25 g, Anti-convulsant Drug Development Program, University of Utah, Salt Lake City, UT); male Rj:NMRI mice (21—27 g, Elevage Janvier, Le Genest-Saint Isle, France). All animals were maintained in temperature and humidity controlled AAALAC (University of Utah) approved quarters with a 12 h on—12 h off light cycle and permitted access to food and water ad libitum except when removed from their cages for testing.

Groups of at least eight mice or rats were tested with various doses of lacosamide until at least two points were established between the limits of 0% and 100% protection.

Determination of efficacy and safety parameters

Minimal neurotoxicity

Minimal motor impairment (MMI) following treatment with lacosamide was evaluated in mice using the rotorod procedure (White et al., 1998). Inability of a treated mouse to maintain equilibrium for 1 min on a slowly rotating (6 rpm) rod in at least one of three trials was used as the endpoint indicating motor impairment.

In rats, MMI was evidenced by the presence of ataxia, abnormal gait and stance following administration of lacosamide.

Initial anti-convulsant screening tests

MES test

The MES test involves induction of a seizure with a supramaximal current. It is demonstrated to have predictive value when assessing efficacy of drugs in treating partial seizures (Schmidt, 2002), and is also believed useful for identifying drugs likely to limit spread to generalized tonic—clonic seizures (White et al., 1998; Barton et al., 2001).

A drop of anesthetic electrolyte solution (0.5% tetracaine hydrochloride in 0.9% saline) was applied to the eyes of each animal (male albino CF No. 1 mice, and male albino Sprague—Dawley rats) prior to placement of corneal electrodes (Woodbury and Davenport, 1952). The electrical stimulus employed in this test was 50 mA, 60 Hz for mice and 150 mA, 60 Hz for rats, delivered for 0.2 s. Lacosamide was administered in 0.5% methylcellulose in a volume of 0.01 ml/g body weight, i.p., for mice, or 0.04 ml/10 g body weight, p.o., for rats 30 min prior to the application of current. Protection was defined as the ability of lacosamide to prevent hind leg tonic extensor component of the seizure.

Subcutaneous pentylenetetrazol test

The convulsant drug pentylenetetrazol produces a behavioral seizure which is markedly different from that induced during the MES test (White et al., 1998). Pentylenetetrazol was dissolved in 0.9% saline and was administered subcutaneously (s.c.) at doses of 85 mg/kg and 70 mg/kg, to male albino CF No. 1 mice and male albino Sprague—Dawley rats, respectively. The injection volume was 0.01 ml/g body weight in mice and 0.02 ml/10 g body weight in rats. Lacosamide was administered according to the protocol in the MES test, except it was given 15 min prior to the administration of pentylenetetrazol. Protection was defined as the ability of lacosamide to prevent a 3-s clonic episode during the 30 min following pentylenetetrazol administration.

Anti-convulsant differentiation tests

6 Hz psychomotor seizure test

Low frequency, long-duration electrical stimulation of the brain produces psychomotor seizures in mice which resemble those seen in patients with partial epilepsy (Barton et al., 2001; Kupferberg, 2001). It is thought that the ability to protect against these seizures is an indicator that a drug, particularly one that has been found to be inactive in the MES test and the s.c. pentylenetetrazol test, may be effective for therapy of pharmacoresistant partial seizures (Barton et al., 2001).

Groups of eight male albino CF No. 1 mice were pre-treated i.p., with lacosamide. At 30 min after treatment, they were administered sufficient current (32 mA at 6 Hz for 3 s delivered through corneal electrodes) to elicit a psychomotor seizure. Typically, these seizures are characterized by a minimal clonic phase followed by stereotyped automatistic behaviors originally described as being similar to the aura of human patients with partial seizures (Brown et al., 1953; Barton et al., 2001). Animals not displaying this behavior were considered protected.

Subcutaneous bicuculline and picrotoxin tests

The GABA-A receptor antagonist bicuculline (BIC) and the chloride-channel blocker picrotoxin (PIC) are potent inducers of chemoconvulsant seizures and were used to further characterize the anti-convulsant profile of lacosamide. Both BIC and PIC were dissolved in 0.9% saline and injected s.c. in a volume of 0.01 ml/g body weight in male albino CF No. 1 mice at doses of 2.7 mg/kg and 2.5 mg/kg for BIC and PIC, respectively. Lacosamide was given 1 h prior to the administration of BIC or PIC. Animals that received BIC were observed for at least 30 min, and those that received PIC were observed for 45—60 min. Absence of a 3-s clonic episode during the period of observation was used as the endpoint indicating protection.

NMDA-induced convulsions test

Activation of the *N*-methyl-D-aspartate (NMDA) receptor in the CNS contributes to many aspects of neuronal signaling and excitability. Indeed, NMDA receptor antagonists have been shown to block or delay seizure activity in rats and mice, suggesting a role for NMDA receptor activation in epileptogenesis (Rice and DeLorenzo, 1998).

The NMDA seizure test was performed according to a previously described method which involved intracerebroventricular (i.c.v.) injection of NMDA (Wilmot, 1989; Lipman and Spencer, 1980). Male Rj:NMRI mice received either saline vehicle, a dose of lacosamide, or the NMDA antagonist MK-801 maleate (0.125, 0.25, or 0.5 mg/kg) administered i.p., 30 min before receiving either NMDA (6 μg/mouse) or saline administered i.c.v. Twelve mice were used per group. The dose of NMDA was chosen on the basis of pilot experiments which established a threshold for clear but not excessive convulsive activity. All experiments were conducted blind to treatment. To ensure that all animals were treated similarly, animals received both an i.p. and i.c.v. injection. Therefore, when NMDA injection was not indicated the mice received saline i.c.v.

Intravenous pentylenetetrazol test

The i.v. pentylenetetrazol test is used to determine if drugs affect seizure threshold (White et al., 1998). A 0.5% solution of pentylenetetrazol in heparinized saline was infused intravenously at a rate of 0.34 ml/min in mice and lacosamide was administered i.p. to mice in doses ranging from its ED_{50} to TD_{50} (i.e. the dose at which 50% of animals experience either efficacy in the maximal electroshock test or toxic effects, respectively) 30 min before the start of the pentylenetetrazol infusion. The endpoints were the time to first twitch of the whole body, and time to sustained clonus of the forelimbs.

Audiogenic seizure test

Ability to protect genetically bred mice against audiogenic seizures is a feature of many AEDs active in the CNS (White et al., 1998). This test is used primarily to further differentiate the anti-convulsant profile of a drug.

Audiogenic seizure (AGS)-susceptible male and female Frings mice are individually placed into a plexiglass cylinder (diameter 15 cm; height 18 cm) fitted with an autotransducer (Model AS-ZC; FET Research and Development, Salt Lake City, UT) and exposed to a sound stimulus of 110 dB (11 kHz) delivered for 20 s at 30 min after drug administration. Sound-induced seizures are characterized by wild running followed by generalized tonic—clonic seizures (GTCS) with loss of righting reflex accompanied by forelimb and hindlimb extension; mice not displaying hindlimb tonic extension are considered protected.

Assessment of potential to treat partial seizures

Although, there are many AEDs that are effective anti-convulsants, there remains a need for drugs that interfere with the pathophysiological processes involved in the development of epilepsy, and pharmacoresistant epilepsy in particular (Walker et al., 2002). The electrical kindling model was used to evaluate the potential for lacosamide to alter the underlying epileptogenic process.

Kindled rat model of focal seizures

The kindled seizure model is a suitable system for evaluating the anti-epileptic (versus anti-convulsant) potential of a drug (White et al., 1998). In this model, seizures are stimulated by an initially sub-convulsive electrical or chemical stimulus that culminates in a generalized seizure. Reduction of seizure score from 5 (maximum) to 3, without any effect on afterdischarge duration is indicative of a drug that may be useful against secondary generalized seizures but not against focal seizures; greater decreases in seizure score (e.g., 5 to <1) accompanied by reductions in afterdischarge suggest the potential for efficacy against focal seizures (White et al., 1998).

A previously described rat rapid hippocampal kindling model was used in this study (Lothman and Williamson, 1994). A bipolar electrode was stereotaxically placed into the ventral hippocampus (−3.6, ML −4.9, VD −5.0 from dura, incisor bar +5.0) of adult male Sprague–Dawley rats (275–300 g body weight) under ketamine–xylazine anesthesia. After a 1-week recovery period, animals were kindled to stage 5 behavioral seizure using a stimulus consisting of a 50 Hz, 10 s train of 1 ms biphasic 200 μA pulses, delivered every 30 min for 6 h (12 stimulations per day) on alternating days, for a total of 60 stimulations. Testing began after a 1-week stimulus-free period. On each day of a trial, rats received two to three suprathreshold stimulations, delivered every 30 min prior to lacosamide treatment, to assess the stability of the behavioral seizure stage and afterdischarge duration. Lacosamide was administered i.p. at 7 mg/kg, 13 mg/kg, 19 mg/kg, and 25 mg/kg 15 min prior to seizure induction. After 15 min, each rat was stimulated every 30 min for 3–4 h. The mean seizure score and afterdischarge duration was calculated. Animals were used for multiple tests, with four or five drug- and stimulus-free days between tests.

Evaluation in the cobalt/homocysteine model for status epilepticus

Focal motor seizures that will undergo secondary generalization can be induced in rats via brain lesions produced with cobalt, and suppression of seizures in these models can identify drugs that are likely to have clinical efficacy for the acute treatment of status epilepticus (Walton and Treiman, 1988). Epileptogenic lesions of the motor cortex were created in adult, male Sprague–Dawley rats via surgical application of cobalt powder (25 mg) on the left frontal cortical surface. Four epidural electrodes were arranged in a square grid (5.0 mm) with the left frontal electrode over the cobalt lesions, and the EEG was monitored daily beginning 4–5 days after surgery. Focal cobalt is epileptogenic and causes seizures on average 7–9 days following cobalt exposure (Walton and Treiman, 1988, 1992; Walton et al., 1994). As soon as the presence of focal motor seizures were observed accompanied by appropriate epileptiform EEG activity as described earlier (Walton and Treiman, 1988) status epilepticus was induced by injection of 5.5 mmol/kg of homocysteine thiolactone i.p. Two independent experiments were performed in different groups of rats.

Treatment of status epilepticus

Lacosamide was prepared in five different concentrations (1.25 mg/ml, 2.5 mg/ml, 5.0 mg/ml, 10.0 mg/ml and 12.5 mg/ml) by dissolving in normal saline with 0.5% benzyl alcohol as a bacteriostat. Each vial was labeled in a blinded manner and assigned by a predetermined random schedule. Treatment was given at 8.0 ml/kg body weight i.p. immediately after the second GTCS following homocysteine injection, with eight rats tested at each dose. Rats were observed for 30 min after treatment, and all evidence of seizure activity was documented.

Diazepam as adjunct therapy in status epilepticus

The efficacy of low dose lacosamide in combination with low dose diazepam was studied for synergism. In one experiment, diazepam (0.75 mg/ml in 40% propylene glycol, 10% ethanol, 50% normal saline) was administered i.p. at a dose of 0.75 mg/kg 5 min after receiving 0, 1.25, 2.5, 5 or 10 mg/kg lacosamide. Ten rats were treated in each group, and each was observed for 120 min or until it had two additional GTCS.

In a second experiment, lacosamide was prepared in a concentration of 12.5 mg/ml in normal saline containing 0.5% benzylalcohol. Diazepam was prepared in concentrations of 0.25 mg/kg, 0.5 mg/kg, and 1.0 mg/kg in 40% propyleneglycol, 10% ethanol, and 50% normal saline. The vials were labeled in a blinded manner and treatment was administered at 1.0 ml/kg body weight in random order. Ten rats were treated in each group and each also received 12.5 mg/kg lacosamide. As previously described, treatment with lacosamide began immediately after the second GTCS following homocysteine injection. Rats were observed for 90 min or until the occurrence of additional GTCS, with continuous EEG recording during the observation period.

Statistical analysis

For quantitative tests with continuous variables, mean values, standard error (S.E.M.) and 95% confidence intervals (CI) were calculated; statistical significance and *P*-values were determined using Student's *t* or Dunnett's test. ED_{50} or TD_{50}, the 95% CI, the slope of the regression line, and the standard error of the slope were calculated using probit analysis (Finney, 1971). Significant differences in behavioral seizure score data from control and treated groups in the kindling paradigms were determined using the non-parametric Mann–Whitney *U* test. For the NMDA-induced convulsions test, Fisher's exact probability test was used for determining *P*-values. Differences were considered statistically significant at $P < 0.05$.

Results

Minimal motor impairment

Rotorod performance in mice was impaired after i.p. administration of lacosamide starting from a dose of 26.8 mg/kg. After oral administration to rats, doses up to 500 mg/kg did not affect motor behavior (Table 1).

Initial anti-convulsant screening and differentiation tests

Lacosamide was effective in the MES test, with a time of peak effect of 30 min, suppressing tonic extension seizures in mice after i.p. administration (ED_{50} 4.46 mg/kg, 95% CI, 3.72–5.46 mg/kg) and in rats after p.o. administration (ED_{50} 3.9 mg/kg, 95% CI, 2.58–6.20 mg/kg). However, it was ineffective against s.c. pentylenetetrazol-induced clonic seizures in both mice and rats, following administration of a dose 5 and 64 times greater than the MES ED_{50}, in mice and rats, respectively (Table 1). However, in the more discriminating intravenous pentylenetetrazol test, lacosamide administered i.p. to mice at a dose equivalent to the MES ED_{50} (4.5 mg/kg) significantly increased the threshold for both first twitch and clonus induced by timed intravenous infusion of pentylenetetrazol ($P < 0.05$, Dunnett's test, Table 2). A higher dose of lacosamide equivalent to the lowest dose causing MMI (27 mg/kg) did not result in further elevation above that already achieved at the MES ED_{50} level.

Lacosamide was ineffective in mice against threshold clonic seizures induced by s.c. BIC and PIC, with

Table 1 Summary of anti-convulsant profile of lacosamide in initial screening and differentiation tests in mice and rats

Species and route of lacosamide	Test[a]	Time of test (h)	ED_{50} (mg/kg)	TD_{50} (mg/kg)	95% CI (mg/kg)	Protective index (TD_{50}/ED_{50})[b]
Mice, i.p.	MES	0.5	4.46	–	3.72–5.46	6.0
	Frings AGS	0.5	0.63	–	0.37–0.99	43.0
	6 Hz	0.5	9.99	–	7.73–12.78	2.7
	sc PTZ	0.25	>25	–		n.a.
	sc BIC	1	>50	–		n.a.
	sc PIC	1	>30	–		n.a.
	Rotorod	0.25	–	26.8	25.50–28.00	–
Rats, i.p.	Kindling	0.25	13.5	–	9.11–17.8	n.a.
Rats, p.o.	MES	0.5	3.90	–	2.58–6.20	>128
	sc PTZ	0.5	>250	–	–	
	Minimal motor impairment	0.25–24	–	>500	–	–

[a] At least eight animals were used per treatment group in each test.
[b] PI calculated with TD_{50} obtained in CF#1 mice and ED_{50} in Frings mice.

ED_{50}s > 50 mg/kg and >30 mg/kg, for BIC and PIC, respectively. However, lacosamide was protective in the psychomotor seizure (6 Hz) test, with an ED_{50} of 9.99 mg/kg (95% CI, 7.73–12.78 mg/kg), and was also effective against audiogenic seizures in Frings mice at very low doses (ED_{50} 0.63, 95% CI, 0.37–0.99 mg/kg; PI 43.0).

In the NMDA-induced convulsion test in mice (Table 3), lacosamide administered alone had no pro-convulsant effects whereas NMDA produced clonic convulsions (83%), tonic convulsions (42%), and deaths (58%). Lacosamide prevented NMDA-induced seizures and deaths. In this test, lacosamide completely prevented tonic convulsions from 20 mg/kg, and at a dose of 50 mg/kg (the highest tested) it additionally provided complete protection against death and partial (50%) protection of clonic convulsions. The NMDA antagonist MK-801 completely antagonized tonic convulsions from 0.25 mg/kg, and markedly prevented deaths (71–84%) but had no effects on clonic convulsions.

Assessment of efficacy against focal seizures

Hippocampal kindled rat model

In the hippocampal kindled rat model, lacosamide produced a dose-dependent reduction in seizure score and afterdischarge duration following i.p. administration. For doses of 7 mg/kg, 13 mg/kg, 19 mg/kg, and 25 mg/kg, mean seizure scores were 3.9, 2.7, 1.0, and 0.8, respectively, with doses of 19 mg/kg and 25 mg/kg being significantly different from the pre-drug control period 15 min prior to lacosamide administration ($P < 0.05$, t-test). The respective mean afterdischarge durations (seconds ± S.E.M.) were 64.5 ± 4.5 (7 mg/kg), 34.6 ± 8.3 (13 mg/kg), 13.4 ± 10.1 (19 mg/kg), and 11.0 ± 10.2 (25 mg/kg). Lacosamide at doses of 13 mg/kg, 19 mg/kg and 25 mg/kg significantly reduced afterdischarge durations when compared to afterdischarge durations during the pre-treatment period ($P < 0.05$, t-test). The ED_{50} was 13.5 mg/kg (95% CI, 9.11–17.8).

Table 2 Lacosamide displays efficacy in CF-1 mice on the threshold for minimal seizures induced by the timed intravenous infusion of PTZ

Lacosamide dose (mg/kg)	PTZ (mg/kg ± S.E.M.)	
	First twitch	Clonus
Vehicle	31.6 ± 0.7	38.9 ± 1.6
4.5	35.9 ± 1.2*	43.0 ± 1.7
27	34.7 ± 1.2	44.3 ± 2.0

* Significantly different from control, $p < 0.05$, Dunnett's test.

Assessment in the cobalt/homocysteine status epilepticus model

Treatment of status epilepticus

Homocysteine was administered on average 7.5 ± 2.7 (mean ± S.D.) days post-operatively after occurrence of seizure activity has been confirmed in each individual animal by visual observation and EEG recordings. Status epilepticus was induced with a latency of 17.5 ± 12.3 min from the time of homocysteine injection. The interval between the onset of first and second GTCS was 7.0 ± 4.2 min. ANOVA indicated no significant differences among the different treatment groups for any of these parameters. In this test, lacosamide dose-dependently protected against GTCS (ED50: 45.4 mg/kg, Table 4). In the 80 mg/kg group, the interval from treatment to next GTCS significantly differed from the other treatment groups and from the mean interval between onset of the two GTCS prior to treatment. There were no statistically significant differences in the number of GTCS after treatment among the dosage groups. All of the rats continued to experience other types of seizures following treatment, including focal motor seizures similar to those seen at baseline and brief tonic seizures lasting 2–6 s. Epileptiform activity continued on EEG, although, no electrographic seizures similar to GTCS were observed unless the rats also had motor seizure symptoms. There was no evidence of sedation or gross ataxia.

Table 3 Effects of lacosamide and MK-801 on NMDA-induced convulsions and deaths in mice

Treatment i.p. (30 min)	Treatment i.c.v.	Clonic convulsions[a]		Tonic convulsions[a]		Deaths[a]	
		# mice	% reversal	# mice	% reversal	# mice	% reversal
Lacosamide							
10 mg/kg	Saline	0/12	—	0/12	—	0/12	—
20 mg/kg	Saline	0/12	—	0/12	—	0/12	—
50 mg/kg	Saline	0/12	—	0/12	—	0/12	—
Saline	NMDA[b]	10/12	—	5/12	—	7/12	—
Lacosamide							
10 mg/kg	NMDA	8/12	20%	1/12	80%	4/12	43%
20 mg/kg	NMDA	9/12	10%	0/12*	100%	2/12	71%
50 mg/kg	NMDA	5/12	50%	0/12*	100%	0/12**	100%
MK-801							
0.125 mg/kg	NMDA	11/12	20%	1/12	80%	2/12	71%
0.25 mg/kg	NMDA	11/11	−20%	0/11*	100%	1/11*	84%
0.50 mg/kg	NMDA	11/12	−10%	0/12*	100%	2/12	71%

*$P < 0.05$; **$P < 0.01$ Fisher's exact probability test. *Note*: 12 mice were used per group except $n = 11$ mice for MK-801 (0.25) due to false injection. A negative percent antagonism indicates potentiation of seizures.

[a] Number observed in 60 min.

[b] NMDA given at 6 μg/mouse.

Diazepam as adjunct therapy in status epilepticus

A dose of 0.75 mg/kg diazepam produced control of GTCS in 30% of rats not receiving lacosamide (data not shown). Lacosamide was protective against GTCS, and this effect was markedly potentiated by diazepam (Fig. 1). The ED_{50} for lacosamide in the presence of a low dose (0.75 mg/kg) diazepam was 3.85 mg/kg (95% CI, 2.54—5.84 mg/kg). This was 91.5% less than the ED_{50} when lacosamide was used as monotherapy (ED50: 45.4; 95% CI, 28.3—73.0; $p < 0.01$ *t*-test). GTCS recurrence in these rats occurred 5.0—86.8 min after the last GTCS prior to injection, with no clear relationship between dose and time to recurrence. Other types of motor seizures occurred in 90% of rats receiving 0 or 2.5 mg/kg lacosamide, and 70% of rats receiving 5—20 mg/kg.

When varying doses of diazepam were administered concomitantly with a dose of 12.5 mg/kg of lacosamide, the ED_{50} for diazepam (0.48 mg/kg, 95% CI, 0.32—0.72 mg/kg) was decreased by 64% compared to the ED_{50} for diazepam monotherapy (1.34 mg/kg; not significant, *t*-test). Sedation was the only observed side effect and tended to be more severe as the dose of diazepam increased. However, none of these animals remained obtunded longer than 45 min.

Discussion

Anti-convulsant activity and safety

Lacosamide was effective against MES-induced seizures in mice and rats, suggesting potential efficacy for preventing seizure spread in humans. At both the ED_{50} for MES protection (4.46 mg/kg) and the TD_{50} for rotorod impairment (26.8 mg/kg), lacosamide elevated the seizure threshold in the intravenous pentylenetetrazol test, indicating it does not possess any seizure lowering or pro-convulsant tenden-

Table 4 Effects of lacosamide on generalized tonic—clonic seizures (GTCS) in the cobalt homocysteine model of self-sustaining status epilepticus in rats

Dose of lacosamide (mg/kg)	Number of rats with GTCS[a]	Mean # GTCS[b]	Time to first GTCS (min)[c]
10	8/8	4.8	6.6
20	7/8	2.7	8.9
40	4/8	3.8	5.6
80	3/8	2.7	14.0*
100	0/8	N/A	N/A

*$P < 0.05$ (*t*-test) compared to other treatment groups. Generalized tonic—clonic seizures (GTCS) were induced by homocysteine and treatment with either vehicle or lacosamide administered immediately following the second GTCS. Eight rats were tested in each treatment group and observed for 30 min following treatment.

[a] Number of rats experiencing GTCS following with either vehicle or lacosamide.

[b] Mean number of GTCS following treatment with either vehicle or lacosamide.

[c] Mean interval from treatment until onset of next GTCS.

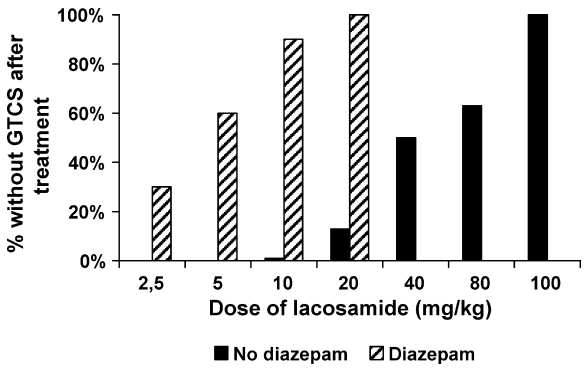

Figure 1 Lacosamide protected against GTCS in the cobalt/homocysteine model of sustained status epilepticus in rats, and this effect was potentiated by concurrent administration of diazepam, 0.75 mg/kg. Percentage protection from cobalt-induced status epilepticus is shown for various doses of lacosamide with concurrent administration of diazepam (▨, striped bars) or without diazepam (■, black bars). Ten rats per group were injected with lacosamide immediately following the second GTCS and 5 min later with diazepam (0.75 mg/kg) or vehicle. In the presence of diazepam, the ED_{50} for lacosamide was reduced from 45.4 mg/kg to 3.9 mg/kg.

cies, even at higher doses. Lacosamide also showed efficacy in the 6 Hz model of psychomotor seizures, and was particularly potent in the genetically susceptible Frings mouse against sound-induced seizures. Lacosamide was inactive, however, against clonic seizures induced by s.c. administration of pentylenetetrazol, bicuculline, and picrotoxin.

The PI values in the MES test for rats (>128) were improved in magnitude compared to those reported for traditional and the newer generation AEDs (traditional AEDs: carbamazepine 101, phenytoin >22, valproate 2.2; new AEDs: felbamate >63, lamotrigine 101, gabapentin 5.7) (White et al., unpublished data from the anti-epileptic drug screening program). Lacosamide was approximately seven times more potent against sound-induced seizures (Frings mouse model) than against MES-induced seizures. As can be seen from the PIs, a 42.5- and 6-fold separation between anti-convulsant activity and neurotoxicity (rotorod impairment) was observed in mice against sound- and MES-induced seizures, respectively, suggesting a wide margin of safety.

The 6 Hz model of psychomotor seizures is considered a model for treatment resistant seizures (Barton et al., 2001). Lacosamide displayed a higher potency in this model when compared to that of newer generation anti-epileptic drugs such as lamotrigine, topiramate, felbamate and levetiracetam (Barton et al., 2001). Since up to 40% of patients can be regarded pharmacoresistant (Kwan and Brodie, 2004), this finding highlights the potential impact of lacosamide as a therapy for pharmacoresistant seizures. Nonetheless, any firm conclusion regarding efficacy against refractory epilepsy will necessitate the completion of appropriately designed clinical trials.

Activity against complex partial seizures

Lacosamide showed efficacy in several rodent models believed to mimic temporal lobe epilepsy, the most common type in adult humans (Stables et al., 2002; Löscher, 2002). The most striking finding was that lacosamide showed efficacy at a non-toxic dose (ED_{50} 13.5 mg/kg) in the kindled rat model. Furthermore, in that model, the decrease in seizure score was accompanied by a marked reduction in the afterdischarge durations, suggesting that lacosamide might be effective in human complex partial seizures.

Activity against status epilepticus

In the homocysteine model of self-sustaining status epilepticus, lacosamide was less potent against GTCS (ED_{50} 45.5 mg/kg) than in the kindled rat model. However, even at the maximum dose tested (100 mg/kg) which was also 100% effective in suppressing GTCS, there was no sedation or ataxia. Low doses of diazepam (0.75 mg/kg) markedly increased the potency for lacosamide (ED_{50} 3.85 mg/kg) in this model, and conversely, a similar synergistic enhancement was observed for the ED_{50} of diazepam when combined with lacosamide.

Mechanism of action

Of the many molecular mechanisms through which anti-convulsant drugs exert their effect, the majority of currently available drugs primarily work by antagonizing sodium or calcium channels, inhibiting glutamate receptors, or facilitating GABAergic inhibition (White, 1999). A series of experiments by Errington and coworkers examined the effects of lacosamide at all established anti-convulsant drug targets (Errington et al., 2006). In contrast to the sodium channel blocker phenytoin lacosamide failed to block sustained repetitive firing. No effect was observed on voltage-clamped Ca^{2+} channels (T-, L-, N- or P-type). Delayed-rectifier or A-type potassium currents were not modulated by lacosamide. Lacosamide did not mimic the effects of diazepam as an allosteric modulator of GABA-A receptor currents, nor did it significantly modulate evoked excitatory neurotransmission mediated by NMDA or AMPA receptors. The lacosamide-mediated antagonism of NMDA-induced seizures suggested that it may bind to the NMDA receptor in the CNS. However, subsequent studies (Errington et al., 2006) could not establish any interaction with the NMDA receptor, suggesting that the seizure inhibition occurred via a different mechanism. Evidently lacosamide does not act via a high-affinity interaction with an acknowledged recognition site on a target for existing anti-epileptic drugs. The anti-convulsant effects of lacosamide are highly stereospecific in the sense that the active (*R*)-enantiomer was at least 10-fold more active than the (*S*)-enantiomer (Lees et al., 2006) suggesting the presence of a yet unknown but specific lacosamide binding site.

Conclusion

The efficacy of lacosamide in antagonizing seizures in the MES test, 6 Hz psychomotor test, in AGS mice, and in the kindled rat model, coupled with the lack of antagonism of seizures induced by the application of sc pentylenetetrazol, BIC, and PIC in mice, provides evidence that the overall

anti-convulsant and mechanistic profiles of lacosamide may be unique when compared to those reported for established anti-epileptic drugs. Furthermore, these results suggest that lacosamide has the potential to be clinically useful for generalized tonic—clonic seizures as well as for pharmaco resistant partial epilepsies and status epilepticus. Lacosamide has a high potency, is active orally and displays a good margin of safety in those tests in which it is effective. Results of phase I studies in over 400 healthy volunteers and early phase II studies in over 500 patients with epilepsy suggest that lacosamide is well tolerated and reduces the occurrence of partial seizures (Doty et al., 2007). The results obtained from these studies support phase III clinical trials currently being undertaken.

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