European Journal of Pharmacology, 222 (1992) 193–203

EJP 53728

ucb L059, a novel anti-convulsant drug: pharmacological profile in animals

Alma J. Gower, Michel Noyer, René Verloes, Jean Gobert and Ernst Wülfert

UCB Pharmaceutical Sector, Chemin du Foriest, 1420 Braine l'Alleud, Belgium

Received 14 May 1992, revised MS received 2 July 1992, accepted 11 August 1992

The anticonvulsant activity of ucb L059 ((S)-α-ethyl-2-oxo-pyrrolidine acetamide) was evaluated in a range of animal models. ucb L059 was active after oral and intraperitoneal administration in both rats and mice, with a unique profile of action incorporating features in common with several different types of antiepileptic drugs. The compound was active, with ED_{50} values generally within the range of 5.0–30.0 mg/kg, in inhibiting audiogenic seizures, electrically induced convulsions and convulsions induced chemically by pentylenetetrazole (PTZ), bicuculline, picrotoxin and N-methyl-D-aspartate (NMDA). ucb L059 retarded the development of PTZ-induced kindling in mice and reduced PTZ-induced EEG spike wave discharge in rats. The R enantiomer, ucb L060, had low intrinsic anticonvulsant activity, showing the stereospecificity of action of the molecule although the actual mechanism of action remains unknown. Neurotoxicity, evaluated with an Irwin-type observation test, the rotarod test and open-field exploration, was minimal, with only mild sedation being observed, even at doses 50–100 times higher than the anticonvulsant doses; at pharmacologically active doses, the animals appeared calm but slightly more active. ucb L059 thus presents as an orally active, safe, broad-spectrum anticonvulsant agent, with potential antiepileptogenic and anti-absence actions.

ucb L059; Convulsions; Epileptogenesis; Petit-mal; Epilepsy

1. Introduction

The term epilepsy refers to a wide range of neurological disorders characterised by an abnormal discharge of cerebral neurones. The prevalence of epilepsy is estimated at between 3 and 6 per 1000 (Griffin and Wyles, 1991; Sander and Shorvon, 1987). There is a general consensus for the need for new, improved drugs to treat epilepsies (Löscher and Schmidt, 1988; Porter, 1986). Although epilepsy is adequately controlled in the majority of patients, there remains a significant number of sufferers who are untreated or respond only partially to drug treatment. In addition, existing drugs, even when effective, are not free from adverse side-effects and are a continuous cause of concern to clinicians, particularly in view of the chronic nature of drug treatment (Griffin and Wyles, 1991; Porter, 1986).

ucb L059 ((S)-α-ethyl-2-oxo-pyrrolidine acetamide) is the S enantiomer of the ethyl analogue of piracetam, a drug widely used on account of its purported beneficial effects on cognition in the elderly. Routine screening of ucb L059 in the audiogenic seizure-prone mouse showed potent anticonvulsant activity. The effects of ucb L059 were consequently determined in a wide range of anticonvulsant tests in rats and mice. The tests, selected with reference to recommended programmes for evaluating potential antiepileptic drugs (Fisher, 1989; Kupferberg, 1989; Löscher and Schmidt, 1988; Meldrum, 1986), included genetic animal models, models involving electrical and chemical seizure induction, as well as chemical kindling and chemically induced EEG spike-and-wave discharge as a model of absence epilepsy. Reference anticonvulsant compounds, including clinically used drugs, were tested in parallel for comparative purposes. In addition, the effects of the R enantiomer, ucb L060, were evaluated in a limited range of tests. Finally, the neurotoxic effects of ucb L059 were assessed on the basis of direct observation, using an Irwin-type evaluation, locomotor activity in an open-field test and rotarod performance. The results confirm that ucb L059 has a broad spectrum of potent anticonvulsant activity with a very wide safety margin between pharmacologically active doses and those causing neurotoxicity or adverse side-effects.

Correspondence to: A.J. Gower, CNS Department, UCB Pharmaceutical Sector, Chemin du Foriest, 1420 Braine l'Alleud, Belgium.

2. Materials and methods

2.1. Animals

The experiments described below involved both mice and rats. Two strains of mice were used, both bred in the Animal Husbandry Unit at UCB, Belgium. Female DBA-derived mice aged 4–5 weeks, weighing 14–22 g and genetically sound-sensitive were used for the audiogenic seizure tests. NMRI mice, either male or female depending on the test, aged 5–6 weeks with a body weight of 22–28 g were used for all other mouse studies. Male Sprague-Dawley rats, bought from IFFA-CREDO, Belgium, aged 6–7 weeks with a body weight of 190 ± 20 g were used for the bicuculline and picrotoxin tests and rats aged 3 months with a body weight of 270–300 g at the time of implantation of EEG electrodes were used for the pentylenetetrazole (PTZ)/EEG test.

Prior to testing, the animals were housed in animal holding rooms maintained at 20–21°C, under a 12-h light-dark cycle, with lights on at 6:00 h, and allowed ad lib access to standard cube diet and water. The mice were housed in groups of 20 per cage (38 × 26 × 14 cm) containing a bedding layer of sawdust. Rats were housed in groups of four animals in wire cages (21 × 20 × 44 cm) except for the rats implanted with EEG electrodes, which were housed individually in wire cages (20 × 19 × 55 cm). To reduce possible problems caused by prolonged isolation, the EEG rats were regrouped at least once a week for approximately 30 min. Apart from the EEG rats, all animals were used once only.

2.2. Anticonvulsant testing

2.2.1. Audiogenic seizures in mice

The day before the experiment, the mice were subjected to a preselection test and only mice in which a tonic convulsion was provoked by an acoustic stimulus were retained; approximately 10% of the population did not meet this criterion. For drug testing, the mice were injected orally (p.o.) with ucb L059 or the reference drugs and 60 min later placed in individual cages in a sound-attenuated cabinet. After 30 s to allow for orientation, a 90-dB, 10- to 20-Hz acoustic stimulus was delivered for 30 s via loud speakers positioned directly above each cage. During the 30-s sound delivery, the presence of wild running, and clonic and tonic convulsions was noted for each mouse.

For each of these three separate parameters, the % protection afforded by each dose of drug was calculated using the formula cited in the Statistics section. From this data, the ED_{50} value, defined as the effective

2.2.2. Maximal electroshock (MES) seizures in mice

MES seizures were induced in male mice by the method of Swinyard et al. (1973). The animals were subjected to a 50-mA ac current (250 cps) for 0.2 s delivered via corneal electrodes, 60 min after oral administration of ucb L059 or reference drugs. This electrical stimulus induced tonic convulsions in 80% or more of control mice. The number of mice exhibiting tonic convulsions was noted per group and the % protection per dose was calculated using the formula given in the Statistics section. From this data, the ED_{50} value, being the effective dose affording 50% protection, was computed for each drug.

2.2.3. Chemically induced seizures in mice

The effects of ucb L059 were examined on seizure activity induced by a range of chemicals, including PTZ, bicuculline, picrotoxin and N-methyl-D-aspartate (NMDA). Where appropriate, reference drugs were included for comparison.

2.2.3.1. PTZ The effects of ucb L059 and reference drugs, administered orally 60 min prior to PTZ (100 mg/kg i.p.), were determined in female mice. The dose of PTZ (100 mg/kg) was ascertained in preliminary experiments as being the maximal dose causing clonic convulsions in 100% and tonic convulsions in 75% or more of control mice. In a separate experiment, the ability of a range of doses of ucb L059 p.o. to displace the dose-response curve for PTZ (60–120 mg/kg i.p.) was assessed.

2.2.3.2. Bicuculline The protective effects of ucb L059 and reference drugs against convulsions induced by bicuculline (4.0 mg/kg i.p.) were determined in female mice after oral administration 60 min before the test. The dose of bicuculline was ascertained in preliminary experiments as being one which caused clonic convulsions in 100% and tonic convulsions in 75% or more of naïve mice. In addition, the ability of a range of doses of ucb L059 p.o. to displace the dose-response curve for bicuculline (2.75-4.5 mg/kg i.p.) was examined.

2.2.3.3. NMDA Female NMRI mice were pretreated with ucb L059 i.p. 60 min prior to intracerebral (i.c.v.) injection into the right lateral ventricle of 4 μl (1 nmol) of NMDA. The i.c.v. injections were given to non-anaesthetised mice, using a free-hand method based on that described by Clark et al. (1968), in which the head of the mouse is positioned in a specially constructed mould. The presence of tonic and clonic convulsions was noted. A similar experiment was carried out with MK-801.

2.2.3.4. PTZ-induced kindling in mice Male mice were injected i.p. once daily for 11 consecutive days with ucb L059 (5.4, 17.0 or 54.0 mg/kg) or saline, 60

were placed in individual cages and observed for tonic and clonic convulsions.

2.2.4. Chemically induced seizures in rats

The effects of ucb L059 were determined against bicuculline- and picrotoxin-induced seizures in rats.

2.2.4.1. Bicuculline Rats were pretreated with ucb L059 or selected reference antiepileptic drugs either p.o. 60 min or i.p. 30 min prior to administration of bicuculline 0.6 mg/kg injected i.v. via the tail vein. The incidences of tonic and clonic convulsions and mortality were noted. The severity of the clonic convulsions was rated subjectively as follows: 0 = absent, 1 = mild, limited to forelimbs, 2 = moderate, short-lived clonus of both fore- and hindlimbs without loss of the righting reflex and 3 = severe, prolonged clonus of both fore- and hindlimbs with loss of the righting reflex. In addition, the ability of ucb L059 and reference drugs to displace the dose-response curve for bicuculline was determined.

2.2.4.2. Picrotoxin Rats were pretreated with ucb L059 p.o. 60 min prior to i.v. administration of picrotoxin (2.75, 3.0 or 3.25 mg/kg).

The incidence of tonic and clonic convulsions and mortality was recorded. The severity of convulsions was also rated, using the scale noted above.

2.2.5. PTZ-induced spike-and-wave discharge (SWD) in rats

Rats implanted with cortical surface EEG electrodes were used. The experiment had a cross-over design in which each rat received vehicle or ucb L059 (5.4 or 17.0 mg/kg i.p.) at weekly intervals. The order of treatment was arranged according to a random latin-square design. Twenty minutes after i.p. injection of ucb L059 or saline, each rat was injected with PTZ 25 mg/kg i.p. During EEG testing, the rats were placed in individual cages located in a sound-attenuated chamber. The EEG was monitored from 20 min before injection of ucb L059 up to 2 h after injection of PTZ. The cumulative duration of SWD and number of SWD episodes per 20-min epoch of testing were determined by direct measurements from the EEG paper trace.

The criteria for SWD were as follows: repetitive frequency of the spikes between 6 and 11 Hz, spike amplitude between 200 and 600 μV with simultaneous activity on both the left and right cortical EEG. A parallel experiment was carried out with clonazepam (0.1 and 0.3 mg/kg i.p.).

2.3. Neurotoxicity testing

2.3.1. Neurotoxicity testing in mice

separate groups of mice. The tests were the Irwin test, measurement of locomotor activity and measurement of rotarod performance.

The Irwin test, based on that described by Irwin (1968), relies on the subjective scoring of a wide spectrum of behavioural and CNS parameters, by direct observation of spontaneous behaviour or after manipulation according to a standard protocol.

Locomotor activity was measured in an open field, 31-cm square with 15-cm high walls, equipped with infrared photocells located in the walls 1.5 cm above a metal grid floor. The photocells were spaced 3 cm apart, measured from centre to centre, in triads, with 4.5 cm between the triads. Activity was expressed as the distance moved, calculated on the basis of the number of interruptions of the photobeams, during a 20-min period.

Rotarod performance was assessed in terms of the number of mice per dose-group able to remain for at least 60 s on a 3-cm diameter rod rotating at a constant speed of 6 rpm. The mice were pretrained the day before drug-testing and only mice able to reach the 60 s criterion within three consecutive trials were retained.

2.3.2. Neurotoxicity testing in rats

Both the Irwin test and measurement of locomotor activity were used to assess the potential neurotoxicity of ucb L059 in rats. Each test was carried out separately, using different groups of rats.

The Irwin test was similar to that used for mice, but adapted where necessary to be applicable to rats.

As with mice locomotor activity was measured in an open field. The open field consisted of a 1-m square arena, with 40-cm high walls surrounding a grid floor. Two horizontal rows of infrared photocells, spaced 6 cm apart, were located in the walls, one row at 2 cm and the other row at 10 cm above the grid floor. Locomotor activity was recorded automatically in terms of distance, calculated on the basis of the number of photobeams interrupted. Rearing was also counted, in terms of the interruptions of the upper row of photobeams.

2.4. Drugs and injections

ucb L059 ((S)-α-ethyl-2-oxo-pyrrolidine acetamide) and the R enantiomer, ucb L060, were synthesised in the research chemical laboratories of UCB. Each compound is a white crystalline powder which dissolves readily in saline or water giving solutions of about pH 6.0. The reference drugs included phenytoin (Vel, Belgium); carbamazepine (Sigma); clonazepam and diazepam (Hoffman - La Roche, Switzerland), sodium

5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine); Research Biochemicals Incorporated). Phenytoin, carbamazepine, clonazepam and diazepam were suspended in 1% aqueous Tween solution for injection into mice and in 5% gum arabic solution for injection into rats. Sodium valproate was dissolved in saline; valproic acid was converted to sodium valproate by dissolving it in NaOH and then neutralising the solution. MK-801 was dissolved in saline.

The convulsant agents used were pentylenetetrazol (PTZ; Cardiozol; Janssen Chimica); bicuculline and picrotoxin (Fluka) and NMDA (N-methyl-D-aspartate; Sigma). Bicuculline was dissolved in a minimal quantity of glacial acetic acid for administration to mice, and in a minimal quantity of hydrochloric acid for administration to rats; in both cases the pH was adjusted to 5–6 with NaOH and the solutions were diluted to volume with saline. PTZ, picrotoxin and NMDA were dissolved in saline.

For i.p. and p.o. administration, a dose-volume of 10 ml/kg body weight was used for mice and 5 ml/kg body weight was used for rats. A dose-volume of 1 ml/kg body weight was used for i.v. administration to rats.

2.5. *Statistics*

In all experiments except the SWD/EEG study, the number of animals responding in each group was determined for each parameter measured. The statistical significances of differences from the control group were calculated using the Fisher test. The number of mice responding per group was used to calculate the % protection (P%) for each parameter, by applying the following formula:

$$P\% = \left(1 - \frac{nt/Nt}{nc/Nc}\right) \times 100,$$

where nt = the number of animals responding in the test group, with Nt = the number of animals tested and nc = the number of animals responding in the control group, with Nc = the number of animals tested. The effective dose of drug affording 50% protection (ED_{50}) was then computed using a probit analysis (Finney, 1971), or, in the case of the bicuculline rat test, the method of Berkson (1953).

In the SWD/EEG study, as each rat served as its own control, the control and test data were compared using a Wilcoxon signed ranks matched pairs test (Siegel and Castellan, 1988).

3. Results

3.1. *Audiogenic seizures in mice*

The ED_{50} values for ucb L059 and the reference drugs, administered p.o., are given in table 1. ucb L059 dose dependently protected against audiogenic seizures elicited in mice, being approximately equiactive against tonic and clonic convulsions (ED_{50} values 7.0 and 9.7 mg/kg, respectively) but requiring 3–4 times higher doses to prevent wild running. This profile of ucb L059 resembled that of clonazepam and sodium valproate, which were both equiactive against tonic and clonic seizures but less effective against wild running.

In contrast, phenytoin and carbamazepine were both preferentially active against tonic convulsions, with approximately 10 times higher doses required to block clonic than tonic convulsions. In terms of the ED_{50} values for protection against tonic convulsions, the order of potency of the drugs tested was as follows: clonazepam ≫ phenytoin = ucb L059 = carbamazepine ≫ sodium valproate.

When ucb L059 was administered i.p., the ED_{50} value with 95% confidence limits for protection against tonic seizures was 8.6 (6.2–11.2) mg/kg.

TABLE 1

Inhibition by ucb L059 and reference antiepileptic drugs of convulsions induced in mice.

WR = wild running; CC = clonic convulsion; TC = tonic convulsion.

Convulsant test	Parameter	ED_{50} mg/kg p.o. (95% confidence limits)				
		ucb L059	Phenytoin	Carbamazepine	Clonazepam	Na Valproate
Audiogenic seizures	WR	31.7 (24.7–44.5)	≥ 80.6	≥ 75.5	0.058 [b]	> 259
	CC	9.7 (7.5–11.9)	38.6 (29.5–53.2)	70.0 [b]	0.038 [b]	> 217 (193 –270)
	TC	7.0 (4.4– 9.1)	4.7 (4.2– 5.3)	7.0 (6.3– 7.9)	0.029 (0.024–0.036)	177 (157 –226)
Maximal electroshock	TC	23.5 (10.1–56.4)	2.4 (1.9– 2.8)	4.1 (2.7– 5.2)	0.3 (0.15 –0.59)	73.8 (28.3–120.4)
Bicuculline	CC	> 170	80.6 [a]	132.3 [a]	0.05 [b]	339.0 (254 –626)
4 mg/kg i.p.	TC	29.5 (21.9–42.6)	14.0 (12.5–15.4)	19.3 (16.3–21.7)	0.029 (0.007–0.12)	222.0 (158 –494)
Pentylenetetrazole	CC	1700 [a]	25.2 [a]	75.5 [a]	0.078 (0.06 –0.11)	250 (221 –287)
100 mg/kg i.p.	TC	5.4–1700	7.7 (6.1– 9.0)	17.5 (14.9–20.0)	0.037 (0.020–0.051)	160 (124 –229)

3.2. Maximal electroshock-induced convulsions in mice

ucb L059 p.o. produced dose-dependent protection against tonic convulsions induced by maximal electroshock in mice, with an ED_{50} value of 23.5 mg/kg (table 1). This value was approximately 5–10 times higher than the corresponding values for phenytoin and carbamazepine. Neither ucb L059 nor any of the reference drugs protected against clonic convulsions.

3.3. Chemically induced convulsions in mice

ucb L059 p.o. at doses of 5.4 mg/kg and higher inhibited tonic convulsions induced by a maximal dose of PTZ (100 mg/kg) by 25–30% (fig 1, table 1). This effect was not dose-dependent and no inhibition of clonic convulsions was obtained.

The reference drugs, phenytoin, carbamazepine, clonazepam and sodium valproate, all dose dependently protected against tonic convulsions although only the latter two were effective against clonic convulsions.

In contrast, at submaximal doses of PTZ (fig. 1), ucb L059 was able to antagonise tonic convulsions in a dose-dependent manner. According to this data, the ED_{50} values of ucb L059 for inhibition of tonic convulsions were 68 mg/kg against PTZ 90 mg/kg and 28 mg/kg against PTZ 80 mg/kg.

ucb L059 p.o. dose dependently reduced bicuculline-induced tonic convulsions in mice (table 1). The resulting ED_{50} valuc, 29.5 mg/kg, approached that of phenytoin and of carbamazepine. ucb L059 (17–170 mg/kg) also reduced clonic convulsions but the effect was not dose-dependent and varied between 30–60%; an ED_{50} vlaue could not be determined.

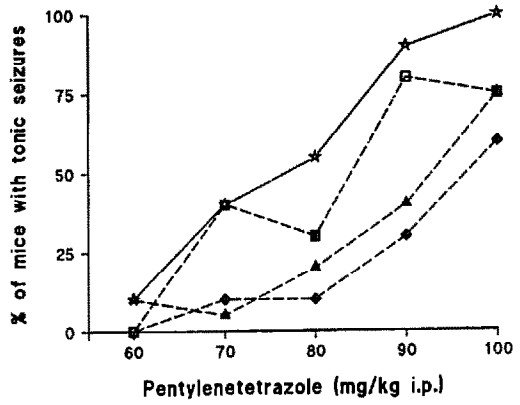

Fig. 1. Effect of ucb L059 on tonic convulsions induced by pentylenetetrazole in mice. Each value given is the percentage (%) of mice responding per group of 20. The mice received either vehicle (☆), ucb L059 17 mg/kg (□), ucb L059 54 mg/kg (△) or ucb L059 170 mg/kg (◇) p.o. 60 min before pentylenetetrazole was injected

TABLE 2

Inhibition by ucb L059 or MK-801 i.p. of convulsions induced by NMDA (1 nmol i.c.v.) in mice

N = number of mice per drug and control group.

Drug treatment (i.p. mg/kg)	N	Percentage of mice responding			
		Tonic convulsion		Clonic convulsion	
		Control	Drug	Control	Drug
ucb L059					
5.4	20	40.0	15.0	75.0	55.0
17.0	30	32.5	6.7 [b]	73.3	53.3
54.0	50	52.0	22.0 [a]	78.0	70.0
170.0	40	55.0	0 [b]	77.5	32.5 [b]
MK-801					
0.034	10	60.0	60.0	70.0	70.0
0.108	20	60.0	15.0 [a]	75.0	50.0
0.340	20	60.0	0 [b]	75.0	0 [b]

[a] P = 0.01; [b] P = 0.001; Fisher test versus control.

NMDA (1 nmol i.c.v.) caused tonic convulsions in up to 60% and clonic convulsions in up to 80% of the control mice. Tonic convulsions were reduced by ucb L059 (5.4–170 mg/kg i.p.), although clonic convulsions were significantly reduced only at 170 mg/kg. The effects were not dose-related, unlike those of MK-801 (0.034–0.34 mg/kg) (table 2).

3.4. PTZ-induced kindling in mice

Single daily injections of PTZ (55 mg/kg i.p.) produced a progressive increase in the number of control mice with clonic convulsions, increasing from less than 25% on day 1 to 90% on day 11. Pretreatment with ucb L059 (5.4–54.0 mg/kg i.p.) caused dose-dependent re-

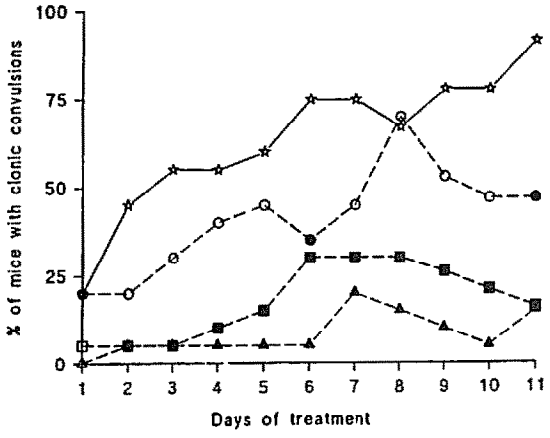

Fig. 2. Effect of ucb L059 on the development of pentylenetetrazole-induced kindling in mice. Groups of 20 mice were injected once daily with either vehicle control (☆), ucb L059 5.4 mg/kg (○), ucb L059 17 mg/kg (□) or ucb L059 54 mg/kg (△) i.p. 60 min before pentylenetetrazole was administered (55 mg/kg i.p.). Each value is the percentage (%) of mice per group with clonic convulsions. Solid

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.