J. Pharm. Pharmacol. 1995, 47: 292–296
Received May 4, 1994
Accepted December 5, 1994

Comparative Convulsant Potencies of Two Carbapenem Derivatives in C57 and DBA/2 Mice

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Abstract

The behavioural and convulsant effects of imipenem and meropenem were studied after intraperitoneal administration in DBA/2 mice, a strain genetically susceptible to sound-induced seizure, and in C57 mice, a strain not prone to seizure.

DBA/2 mice were more susceptible than C57 mice to seizures induced by imipenem–cilastatin or meropenem. Imipenem was also 1·9 times more potent than meropenem in inducing clonus in DBA/2 mice.

To investigate the possibility that the seizure-inducing activity of imipenem might be due to a probenecid-like effect of cilastatin, animals were treated with imipenem alone.

No significant differences were observed between imipenem–cilastatin and imipenem-treated animals. Thus, it is reasonable to exclude a probenecid-like effect of cilastatin. Although the main mechanism for seizure-like activity of imipenem cannot be easily determined, we believe that several mechanisms may be involved. An increased excitation of the central nervous system (CNS) by inhibition of GABA binding to receptors and a slow clearance of imipenem from the CNS may be postulated. Cilastatin did not induce seizures. In addition, meropenem, a compound structurally related to imipenem, showed weak or no convulsant effects.

New β-lactam antibiotics introduced into clinical practice have included carbapenems. The prototype of this class is imipenem (*N*-formimidoyl thienamycin) which possesses the broadest antibacterial spectrum among the β-lactams. Since imipenem penetrates well into the cerebrospinal fluid (CSF) after systemic administration (Barza 1985), the antibiotic is effective in the treatment of bacterial meningitis (Patamasucon & McCracken 1982; Kim 1985). Because of its inactivation by the renal enzyme dehydropeptidase (DHP-I), imipenem has usually been co-administered with cilastatin, a specific DHP-I inhibitor. Meropenem is a new carbapenem which is highly resistant to hydrolysis by human DHP-I and can be used alone, without any DHP-I inhibitor. Many experimental studies and several pieces of clinical evidence exist on the convulsive effects of β-lactam antibiotics (De Sarro et al 1983; De Sarro et al 1989). Imipenem with cilastatin has been reported to induce a seizure-like activity (Williams et al 1988; Eng et al 1989; Semel & Allen 1991); nevertheless such imipenem-induced seizure activity is poorly documented (Tse et al 1987). In fact, β-lactam antibiotics can induce convulsions in various animal species when administered in high doses (Nisticò et al 1980a, b; De Sarro et al 1983, De Sarro et al 1989) and their convulsant action has been attributed to the inhibition of the GABA system (Antoniadis et al 1980; De Boer et al 1980; Hori et al 1985). In our previous study we described a different pattern of responsiveness to cefazolin in two different strains of mice, the genetically epilepsy-prone mouse or Dilute Brown Agouti DBA/2J (DBA/2) mice and Swiss mice (De Sarro et al 1993). The intention of the present study was to investigate the possible seizure-like activity of meropenem as compared with the convulsant activity induced by imipenem in DBA/2 and C57 mice.

The DBA/2 mouse has been known since 1947 to be susceptible to audiogenic seizures (Hall 1947). DBA/2 mice undergo an age-dependent sequence of convulsions, when exposed to a loud mixed-frequency sound (12–16 kHz; 90–120 db) such as a door bell (Chapman et al 1984). DBA/2 mice, 16 to 30 days old, show in response to a loud tone, an ill-co-ordinated locomotion consisting of wild running followed by rhythmic clonic jerking, with the animal lying on one side, followed by tonic flexion and tonic extension of trunk, limbs and tail. The latter phase may terminate with respiratory arrest and death. In addition, it has been reported that mature DBA/2 mice, with maturity-developed resistance to sound-induced seizures (Chapman et al 1984), still have an increased seizure susceptibility to a variety of non-audiogenic convulsant treatments including chemical and physical stimuli (Chapman et al 1984, 1987; Engstrom & Woodbury 1988; De Sarro et al 1993). The nature of audiogenic seizures in DBA/2 mice was extensively studied and neurochemical abnormalities have been described by various authors (Chapman & Meldrum 1987). Thus, this mouse strain has been considered an excellent animal model for the study of certain kinds of human epilepsy and for testing convulsant or anticonvulsant drugs (Chapman et al 1984, 1987; Seyfried & Glaser 1985).

Since we also postulated that meropenem might be able to elicit seizure, the first aim of the present study was to compare the neurological effects of these two carbapenems in DBA/2 mice. In addition, since imipenem is co-administered with cilastatin, we considered the possibility

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that cilastatin might affect the incidence and the intensity of the seizures induced by imipenem.

Materials and Methods

Testing of anticonvulsant activity

DBA/2 (16–24 g, 42–48 days old) and C57 mice (18–28 g, 42–48 days old) were purchased from Charles River (Calco, Como, Italy). Mice of either strain were injected with imipenem-cilastatin (0·79–2·63 mmol kg^{-1}, i.p.) or meropenem (1·31–2·63 mmol kg^{-1}, i.p.) dissolved in sterile saline (0·1 mL/10 g body weight). Animals were placed in a Plexiglas box (40 × 40 × 30 cm) and observed for 120 min after intraperitoneal administration of imipenem-cilastatin or meropenem. For each dose of carbapenem derivative 8–10 animals of each strain were used. The intensity of seizure response was scored on the following scale: 0 = no response, 1 = wild running, 2 = clonus, 3 = tonus, 4 = respiratory arrest, which is identical to that observed after auditory stimulation (De Sarro et al 1987). A second group of animals (DBA/2 only) was treated with equimolar doses of imipenem alone or cilastatin alone (1·05–2·63 mmol kg^{-1}, i.p.). These two experimental groups were also observed and the intensity of seizure response was scored according to the scale shown above.

For ethical reasons, at the end of experiments the animals which showed seizures were killed by ether anaesthesia.

Electrocortical analysis

Electrocortical activity was recorded (8-channel machine OTE Biomedica, Florence, Italy) through four chronically-implanted steel screw electrodes, inserted bilaterally onto the frontoparietal area. At least three mice, treated with the largest dose of meropenem and imipenem, were studied for changes in electrocortical activity.

Statistical analysis

Statistical comparisons among groups of control and drug-treated animals were made using Fisher's exact probability test (incidence of the seizure phases) or Mann-Whitney U-test (median seizure score ± interquartile range). The percentage incidence of each phase of the seizure was determined for each dose of imipenem–cilastatin, imipenem or meropenem administered and log dose–response curves were fitted using linear regression analysis of probit-transformed percentage response. CD50 values (with 95% confidence limits) for each compound and each phase of seizure response were estimated using the method of probit analysis (Finney 1978); the relative convulsant activities were determined by comparison of respective CD50 values.

Drugs

Imipenem monohydrate-sodium cilastatin, imipenem monohydrate and sodium cilastatin were obtained from Merck, Sharp & Dohme, Roma, Italy; meropenem trihydrate was obtained from ICI-Pharma SpA, Milano, Italy. The drugs were dissolved in isotonic sterile saline.

Results

Comparative convulsant activity of imipenem in C57 mice and in audiogenic seizure-prone DBA/2 mice

Table 1 shows that the intensities of the seizures were significantly different in the two strains of mice studied

Table 1. The incidence of seizure induced by imipenem–cilastatin or imipenem alone in DBA/2 and C57 mice.

Strain of mice	Drugs	Dose (mmol kg^{-1})	Incidence (%)			Median seizure score ± interquartile range
			Clonic seizures	Tonic seizures	Death	
DBA/2	Imipenem + cilastatin	Vehicle	0	0	0	0 ± 0
		0·79	0	0	0	0 ± 0
		0·91	20	0	0	0 ± 0
		1·03	40	20	0	1 ± 1
		1·31	50	30	0	2 ± 1
		1·58	70	40	0	3 ± 1
		1·84	80	60	50	3 ± 1
		2·10	100	80	60	4 ± 1
DBA/2	Imipenem alone	Vehicle	0	0	0	0 ± 0
		0·91	10	0	0	1 ± 1
		1·03	40	20	0	1 ± 1
		1·31	60	40	0	2 ± 1
		1·58	70	40	10	3 ± 1
		1·84	80	60	40	3 ± 1
		2·10	100	70	60	4 ± 1
C57	Imipenem + cilastatin	Vehicle	0	0	0	0 ± 0
		1·58	0**	0	0	0 ± 0+
		1·84	20**	10*	0	1 ± 1+
		2·10	40**	20**	10	2 ± 1+
		2·37	50	30	20	2 ± 2
		2·63	60	40	40	3 ± 1

Groups of DBA/2 or C57 mice were randomly assigned to experimental groups of 10 animals and injected intraperitoneally with the stated doses of imipenem–cilastatin or imipenem alone (DBA/2 only). The animals were observed for 120 min after drug injection. Incidence of each seizure phase is expressed as the percentage of mice in each group displaying that phase. Significant differences in the incidence of seizure phases between C57 and DBA/2 mice are denoted by $*P < 0·05$ and $**P < 0·01$ using Fisher's exact probability test. Significant differences in median seizure score between DBA/2 and C57 mice are denoted by $^{+}P < 0·05$ using Mann Whitney U-test.

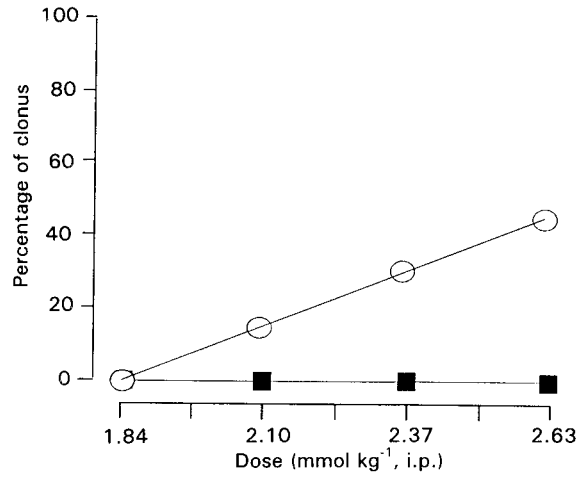

FIG. 1. Dose-response curves of meropenem in DBA/2 and C57 mice. ○ DBA/2; ■ C57 mice. Ten mice were used for the determination of each point.

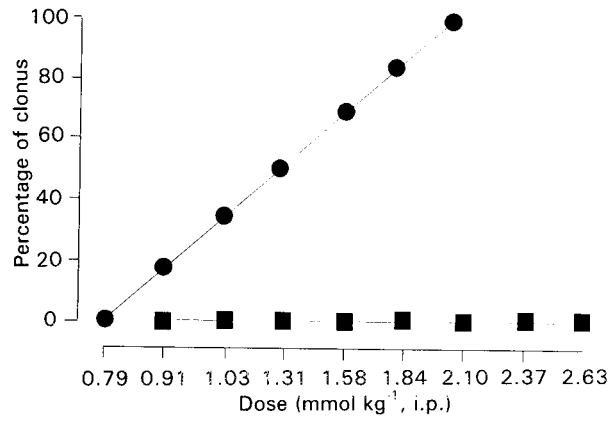

FIG. 2. Dose-response curves of imipenem-cilastatin and cilastatin alone in DBA/2 mice. ● Imipenem; ■ cilastatin. Ten mice were used for the determination of each point.

after intraperitoneal administration of imipenem–cilastatin. In particular, DBA/2 mice were more susceptible to convulsant doses of imipenem than C57 mice. Higher doses of imipenem were necessary in C57 mice to induce a similar incidence of the clonic phase of the seizures, whilst the tonic phase of the seizures was rarely observed (Table 1). No significant changes in the onset, duration and occurrence of seizures in DBA/2 mice receiving imipenem alone or imipenem + cilastatin were observed (Table 1). The CD50 value of imipenem–cilastatin for inducing clonus in DBA/2 mice was 1·44 (1·22–1·69 mmol kg^{-1}, i.p.), whilst that for triggering tonus was 1·83 (1·31–2·63 mmol kg^{-1}, i.p.). The CD50 value of imipenem–cilastatin for inducing clonus in C57 mice was 2·38 (2·05–2·75 mmol kg^{-1}, i.p.), whilst that for triggering tonus was 2·86 (2·40–3·42 mmol kg^{-1}, i.p.).

Effects of meropenem administration in C57 mice and in audiogenic seizure-prone DBA/2 mice

Neither DBA/2 nor C57 mouse strains showed behavioural epileptic changes following meropenem doses of 1·31–2·10 mmol kg^{-1} (Fig. 1). However, rare myoclonic jerks were observed only following meropenem doses of 2·37 and 2·63 mmol kg^{-1} in 2 out of 10, and 4 out of 10 DBA/2 mice, respectively; no tonic seizures were observed. Conversely, no behavioural epileptic signs were observed in C57 mice administered meropenem doses of 2·37 or 2·63 mmol kg^{-1}. The CD50 value of meropenem for triggering clonus in DBA/2 mice was 2·68 (2·41–2·99 mmol kg^{-1}, i.p.).

Effects of cilastatin administration to audiogenic seizure-prone DBA/2 mice

No behavioural epileptic effects were observed following intraperitoneal cilastatin doses of 1·05–2·63 mmol kg^{-1} (Fig. 2).

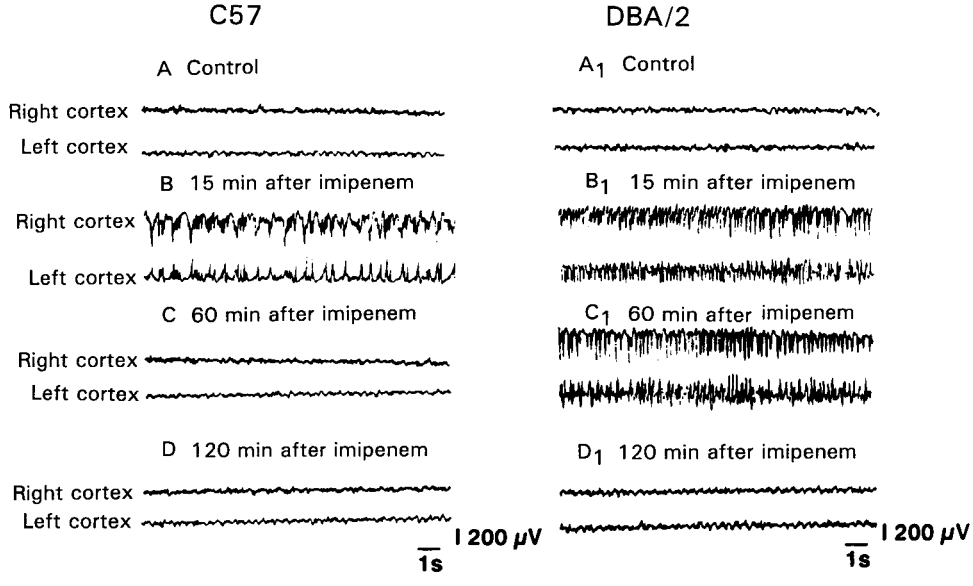

FIG. 3. Electrocortical patterns following imipenem + cilastatin (2·1 mmol kg^{-1}, i.p.) administration in C57 and DBA/2 mice. Electrocorticographic patterns from right and left fronto–parietal cortex, illustrating the effects of imipenem + cilastatin in both C57 and DBA/2 mice. B and B_1; C and C_1; D and D_1: electrocorticographic recordings 15, 60 and 120 min, respectively, following the injection of imipenem + cilastatin. A and A_1: control.

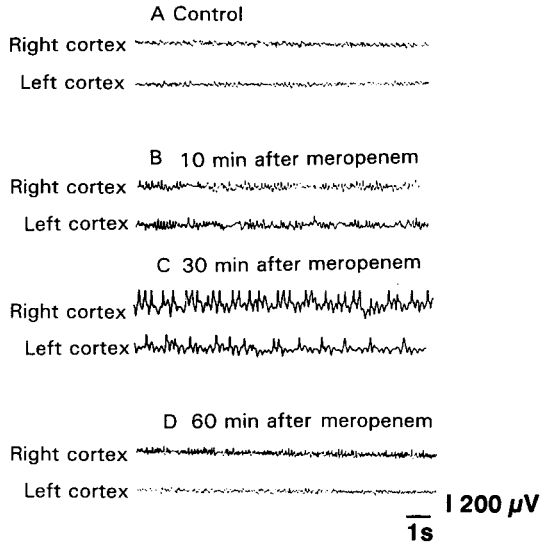

FIG. 4. Electrocorticographic patterns following meropenem (2·1 mmol kg^{-1}, i.p.) administration in DBA/2 mice. Electrographic patterns from right and left fronto–parietal cortex, illustrating the effects of a single administration of meropenem. A; control. B, C and D: electrocorticographic recordings 10, 30 and 60 min, respectively, following the injection of meropenem.

Electrocortical activity

Electrocorticographic epileptic discharges appeared more rapidly in mice treated with imipenem than in those receiving meropenem. In addition, these epileptic discharges did not disappear more rapidly in animals treated with imipenem alone than in those receiving imipenem–cilastatin. The electrocorticographic epileptic pattern was similar in DBA/2 mice which received imipenem–cilastatin or imipenem alone (Fig. 3). The animals treated with meropenem showed an occurrence of electrocorticographic epileptic discharges which was less intense and shorter than that of mice receiving imipenem (Fig. 4).

Discussion

The present study confirms that C57 and DBA/2 mice show different susceptibility to seizures induced by convulsants such as imipenem. In particular, seizure latency was consistently shorter in DBA/2 than in C57 mice; the seizure intensity was also different in the two mouse strains. In C57 mice an increased latency to clonic phase was observed following imipenem administration; this phase as well as the tonic phase of seizure failed to occur following meropenem administration. These data suggest that the neural mechanisms which delay or prevent the onset of clonic seizures are not yet present in 42–48 day-old DBA/2 mice. These findings are in agreement with previous studies which showed that DBA/2 mice have an increased seizure susceptibility to a variety of non-audiogenic convulsant treatments (Chapman et al 1984, 1987; Engstrom & Woodbury 1988; De Sarro et al 1993). We considered the possibility that the seizure-like activity induced by association with imipenem may be related to increased cerebrospinal fluid and serum levels of imipenem, which may occur during the concomitant administration with cilastatin. However, the experiments carried out with imipenem alone or in combination with cilastatin showed no significant difference in the onset, duration and severity of seizures. In addition, cilastatin by itself did not induce convulsive effects. Thus, it is reasonable to exclude a probenecid-like effect of cilastatin. Dudley (1986) suggested that the neurotoxicity of imipenem may have been caused by an accumulated open β-lactam metabolite of imipenem, and not by cilastatin. This open β-lactam metabolite is formed during β-lactam ring cleavage of imipenem and has a seizure potential similar to that of cephazolin (Tse et al 1987). In our previous report we suggested that cephazolin is a tetrazol derivative which shows a marked similarity with the pentylentetrazol, a well known convulsant drug which impairs GABA-ergic transmission (De Sarro et al 1989).

Imipenem pharmacokinetics also deserve to be considered. An interesting study carried out on the transport of imipenem in CNS of the rat showed that imipenem is transported through the blood-brain barrier principally via passive (simple) diffusion, and its efflux from the CNS through the blood-cerebrospinal fluid barrier is slow. In fact, imipenem has minimal affinity for the choroid plexus active efflux system, resulting in the slow elimination of this drug from the CNS (Suzuki et al 1989). Thus, we suggest that imipenem CNS pharmacokinetics may play a determinant role in inducing seizures with the inhibition of GABA neurotransmission (Antoniadis et al 1980; De Boer et al 1980; Hori et al 1985). In contrast, meropenem exhibited a weak seizure activity and was only half as potent as imipenem in inducing clonic seizures. Although meropenem is chemically related to imipenem, there are differences in the structure of these two agents which may account for the observed differences in precipitation of convulsions.

These differences suggest that pharmacokinetic factors may not allow meropenem to reach the site of action necessary to induce convulsions; however, this is unlikely. Because meropenem is readily hydrolysed by DHP-I from mice, rabbits and monkeys (Fukasawa et al 1992), we suggest that the facile hydrolysis of meropenem by mouse DHP-I might be responsible for the weak convulsant activity observed in DBA/2 mice and for the lack of seizures in C57 mice. Another possibility is that meropenem, as for other β-lactam derivatives which show a weak epileptogenic activity (De Sarro et al unpublished), may possess a low affinity to the binding sites at which many β-lactam antibiotics exert their convulsant effects. The convulsant action of β-lactam derivatives has been related to the reduction of GABA release from nerve terminals or to the inhibition of the GABA binding to its receptor sites (Antoniadis et al 1980; De Boer et al 1980; Hori et al 1985; Williams et al 1988; Eng et al 1989). Although the previous authors believe that the convulsant effects of β-lactam derivatives are mainly related to inhibition of the GABA system, we may consider an alternative hypothesis, i.e. the involvement of other neurotransmitter systems. We have recently demonstrated that some amino acid excitatory antagonists are able to antagonize the seizures induced by imipenem in mice (De Sarro et al unpublished).

In conclusion, we may emphasize that β-lactam derivatives may bind different sites in the brain. This suggestion, if

confirmed, might lead to development of new β-lactam derivatives without convulsant effects.

Acknowledgements

Financial support from the Italian Ministry of University and Scientific and Technological Research and the Italian Council for Research is gratefully acknowledged. Our thanks to Mr Antonino Giacopello for his skilful technical assistance.

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