A systematic review and meta-analysis concluded that using involves blockage of gamma-aminobutyric acid A (GABA\textsubscript{A}) receptor mechanism underlying cefepime-induced status epilepticus in neurotoxic low oral seizures (11). It is commonly caused by repeated application of pentylenetetrazol (PTZ) (10) or corneal stimulation using low-stimulus currents. Intraparenchymal injection of cefepime and meropenem at 250 or 500 mg/kg of body weight had no effect on PTZ-induced convulsions in normal mice. However, in convulsions induced by electroconvulsive shock at low-stimulus currents, mean seizure stage following cefepime administration at 500 mg/kg was significantly higher than that following saline injection. Additionally, EEG spikes were recorded for mice that were given cefepime (500 mg/kg). In corneal kindled mice following cefepime injection, mean seizure stage was significantly higher than that following meropenem injection. The convulsive liability of cefepime is significantly higher than that of meropenem in normal and corneal kindled mice. In patients with low seizure thresholds, convulsive liability of cefepime may be assumed.

Cefepime is a broad-spectrum cephalosporin that is now recommended in treatment guidelines for febrile neutropenia (1). A systematic review and meta-analysis concluded that cefepime use is associated with increased mortality risk (2) due to neurotoxic effects, including status epilepticus. Moreover, while most reported mortalities involve patients with renal failure (3–5), cases of status epilepticus in patients with normal renal function do occur (6, 7). Central nervous system toxicity is believed to be the cause of status epilepticus, and animal studies show that the mechanism underlying cefepime-induced status epilepticus involves blockage of gamma-aminobutyric acid A (GABA\textsubscript{A}) receptors (8). This study examined the convulsive effects of cefepime using both in vivo intracerebroventricular administration in mice and in vitro models. However, compared with the intravenous (i.v.) route, intracerebroventricular administration has not achieved the same level of use in clinical practice.

Cefepime and meropenem are the only two \beta-lactam antibiotics approved for empirical treatment of febrile neutropenia in Japan, and we have reported that convulsions are significantly higher in patients treated with cefepime than in those treated with meropenem. Furthermore, cefepime- and meropenem-associated convulsions occur only in patients with brain disorders (e.g., nonherpetic acute limbic encephalitis or encephaloma) and normal renal function (9). Thus, patients with brain disorders may have low seizure thresholds.

Here, we examined the acute effects of cefepime and meropenem on the susceptibility of mice to seizures induced by either pentylenetetrazol (PTZ) (10) or corneal stimulation using low-current electroconvulsive shocks (11). The most popular and widely used animal seizure model is the traditional PTZ test. PTZ is an inhibitor of GABAergic neurotransmission, and i.v. infusion of PTZ is the most sensitive model for determining seizure threshold. Kindling, an experimental model for temporal lobe epilepsy, is defined by progressive increases in electrographic and behavioral seizures (11). It is commonly caused by repeated application of subconvulsive electrical stimulation via corneal electrodes.

Corneal kindled mice are a highly sensitive and efficient screening model for antiepileptic drug discovery because they exhibit lower seizure thresholds than normal mice and display a pharmacological profile consistent with human partial epilepsy (12). Here, we compared convulsive liability of intravenously administered cefepime and meropenem in normal and corneal kindled mice.

**MATERIALS AND METHODS**

**Animals.** Male 6-week-old Institute of Cancer Research (ICR) mice were purchased from Japan SLC (Shizuoka, Japan). All animals were housed in groups of five per plastic cage (18 by 44 by 27 cm) in a room maintained at 22 ± 2°C under 12/12 h light/dark cycles, with lights on at 7:00 a.m. The mice were allowed free access to food and water except during experiments. The experimental protocol was conducted according to the guidelines of the Ethics Review Committee for Animal Experimentation of Ehime University Medical School.

**Drugs.** The antibiotics used were cefepime (Maxipime; Bristol-Myers Squibb, Tokyo, Japan) and meropenem (Meropen; Dainippon Sumitomo Pharma Co., Osaka, Japan). Both antibiotics were dissolved in saline. PTZ (Sigma, St. Louis, MO, USA) was dissolved in 10 ml of saline and administered at a volume of 40 mg/kg of body weight. Tetracaine hydrochloride (0.5%) (Sigma, St. Louis, MO, USA) was dissolved in saline. Control groups received only saline. Dosages of cefepime and meropenem (250 mg/kg and 500 mg/kg, respectively) were chosen based on those used by Horisuchi et al. (13). They administered meropenem and cefazolin at 250 or 500 mg/kg in a proconvulsive test following PTZ-induced convulsions. Additionally, cefepime at 1,000 mg/kg induced hypothermia in mice (14). Therefore, we used dosages of 250 and 500 mg/kg for meropenem and cefepime.
Proconvulsive test following PTZ-induced convulsions in mice. Groups (control, cefepime, meropenem) of 10 ICR mice, weighing 31 to 37.5 g, were injected with antibiotics by i.v. bolus into the tail vein. One minute after antibiotic injection, PTZ was intraperitoneally administered. Immediately after PTZ injection, each animal was placed in a plastic cage (32 by 18 by 24 cm) and occurrences of clonic convulsions were observed for 15 min. Convulsive behavior was assessed according to the scale of Takechi et al. (15) as follows: stage 0, no response; stage 1, ear and facial twitching; stage 2, myoclonic body jerks; stage 3, forelimb clonus and rearing; stage 4, clonic convulsions and turned on the side; stage 5, generalized clonic convulsions and turned on the back.

Proconvulsive test following electroconvulsive shock-induced convulsions in mice. Groups (control, cefepime, and meropenem) of 10 ICR mice, weighing 32 to 38 g, were injected with antibiotics by i.v. bolus into the tail vein. One minute after antibiotic injection, the cornea was stimulated with currents of 5.6 mA (60 Hz) for 3 s. Before each stimulation, saline drops containing 0.5% tetracaine hydrochloride were administered to the eyes. Each animal was placed in a plastic cage immediately after stimulation, and occurrences of clonic convulsions were observed for 15 min. Convulsive behavior was assessed according to the modified scale of Takechi et al. (15) (stage 6, status epilepticus; stage 7, loss of life).

Electroencephalogram recordings. Screw electrodes were placed on the dura matter over the frontal and parietal cortices. Each electrode was then fused to a connector and fixed on the skull with quick, self-curing acrylic resin. Animals were allowed at least 7 days of recovery from surgery before the testing day. One minute after antibiotic injection and current stimulation at 5.6 mA (60 Hz) for 3 s, electroencephalograms (EEGs) were recorded for approximately 15 min using a bioelectric amplifier (AB-651 and JB-101J; Nihon Kohden, Tokyo, Japan) and a thermal Array-corder (WR-300; Graphtec, Tokyo, Japan) in two groups (cefepime and meropenem) of ICR mice weighing 34 to 36.5 g (3 mice for each antibiotic group).

Corneal kindling in mice. A new batch of 12 mice was kindled according to the modified protocol defined by Rowley and White (11). Briefly, corneal current stimulation at 5.6 mA (60 Hz) for 3 s was performed twice daily for 10 days. Each day, stimulations were separated by at least 6-h intervals. Mice were considered kindled when they were classified as at least stage 3 according to the modified scale.

Proconvulsive test following electroconvulsive shock-induced convulsions in corneal kindled mice. Behavioral tests were performed 24 h after the last corneal stimulation using low-stimulus currents (4.0 mA). Twenty-four hours before behavioral testing, we confirmed that with corneal stimulations of 4.0 mA (60 Hz) for 3 s, kindled mice did not exceed stage 1 according to the modified scale. On the day of behavioral testing, groups (cefepime and meropenem) of 10 kindled mice were injected with antibiotics by i.v. bolus into the tail vein. One minute after antibiotic injection, mice were stimulated with corneal stimulations of 4.0 mA (60 Hz) for 3 s.

Data analysis. Statistical significance tests for the proconvulsive test in the electroconvulsive shock-induced model were performed by Steel’s test. The Wilcoxon signed-rank test was used for comparing the seizure stage between cefepime and meropenem. The significance level was set at 0.05. Statistical analysis was performed using the SPSS 16 software package (SPSS, Inc., Chicago, IL, USA).

RESULTS
Effect of cefepime and meropenem on PTZ-induced convulsions in mice. In the control group, the mean (range) seizure stage was 0.2 (0 to 2). Following cefepime administration (500 mg/kg), the mean seizure stage was also 0.2 (0 to 2). Lower doses of cefepime (250 mg/kg) and meropenem (250 and 500 mg/kg) administration did not induce convulsions (Fig. 1).

Effect of cefepime and meropenem on electroconvulsive shock-induced convulsions in mice. In the control group, the mean seizure stage was 0.3 (0 to 2). Following cefepime administration at 250 and 500 mg/kg, the mean seizure stages were 2.0 (0 to 5) and 3.3 (0 to 7), respectively. The mean seizure stage with cefepime at 500 mg/kg was significantly higher (P < 0.05) than with saline. Following meropenem administration at 250 and 500 mg/kg, the mean (range) seizure stages were 0.3 (0 to 2) and 1.3 (0 to 4), respectively (Fig. 2).

In the EEG study, cefepime administration (500 mg/kg) induced convulsions in 2 of 3 mice (scores, 2 and 5). The score 5 mouse produced EEG spikes in the parietal cortex. The other mice did not exhibit any changes in EEG. In contrast, meropenem (500 mg/kg) did not affect EEG recordings in any of the three mice (Fig. 3).

Characteristics of kindling in mice. Kindling developed in mice following corneal stimulation (5.6 mA at 60 Hz for 3 s, twice per day) (Fig. 4). On day 10, the mean seizure score in corneal kindled mice was 2.6 (0 to 5).

Effect of cefepime and meropenem on electroconvulsive shock-induced convulsions in corneal kindled mice. In corneal kindled mice, cefepime (500 mg/kg) resulted in significantly

FIG 1 Effect of cefepime and meropenem on PTZ-induced convulsions in mice. Each value represents mean seizure stage ± standard error of the mean (SEM) in 10 mice. CFP, cefepime; MEPM, meropenem.

FIG 2 Effect of cefepime and meropenem on electroconvulsive shock-induced convulsions in mice. Each value represents the mean seizure stage ± SEM in 10 mice. CFP, cefepime; MEPM, meropenem. *, P < 0.05 versus control group (Steel’s test).
higher seizure stages than meropenem (500 mg/kg) (cefepime: mean, 4.0 [range, 0 to 7]; meropenem: mean, 0.6 [range, 0 to 5]; $P < 0.01$) (Fig. 5).

**DISCUSSION**

In this study, in a proconvulsive test following PTZ-induced convulsions, cefepime and meropenem did not induce convulsions. However, in the low-current electroconvulsive shock-induced convulsion model, mean seizure stage resulting from cefepime administration at 500 mg/kg was significantly higher than that resulting from saline administration. Additionally, cefepime at 500 mg/kg produced EEG spikes in a score-5 mouse.

$\beta$-Lactam antibiotics (e.g., cephalosporins and carbapenems) are known to induce convulsions in experimental animals and patients (2, 16, 17). Convulsions induced by $\beta$-lactam antibiotics are associated with inhibition of GABA receptor binding (13, 18). Cefepime also induces convulsions in mice when administered intracerebroventricularly (8). PTZ is a potent proconvulsant that acts as an indirect GABAA antagonist at the picrotoxin binding site of the GABA$\alpha$ complex (13). PTZ antagonizes GABA-activated currents, reducing chloride channel opening in a dose-dependent manner. The PTZ-induced seizure model is considered a useful screening method for validating the proconvulsive liability of $\beta$-lactam antibiotics (19). Here, i.v. injection of cefepime and meropenem at 250 and 500 mg/kg had no effect on PTZ-induced convulsions in mice. Horizuchi et al. (13) reported that i.v. injection of meropenem and cefazolin at 500 mg/kg did not potentiate PTZ-induced convulsions. Brain cefepime concentrations after i.v. administration are likely to be much lower than those following intracerebroventricular administration, and indeed, cefepime concentrations in cerebrospinal fluid are 9% of the corresponding peak plasma drug concentrations (20). The corrected 50% cytotoxic dose (CD$_{50}$) value (calculated to normalize based on protein binding [29%]) for cefepime-induced convulsions was 51.3 $\mu$g/animal for *in vivo* intracerebroventricular administration (8).

Therefore, convulsive liability of antibiotics may be attributable to penetration through the blood-brain barrier, rather than intrinsic central nervous system effects.

Sáez-Llorens et al. reported that seizure patterns for laboratory animals and humans are similar, except for the time scale (21).

The pharmacological profile of the corneal-stimulation electroconvulsive-shock model differs from that of PTZ tests (11). The mechanism by which PTZ induces convulsions is still unclear. The pharmacological effects of PTZ are at least partly mediated by interactions with the chloride ion channel of the GABA$\alpha$ receptor (22). The PTZ test is considered an appropriate model of petit mal epilepsy, while the electroconvulsive shock test is an appropriate model of grand mal epilepsy. Electroconvulsive shock is largely the result of brain stem activation (23). Cefepime-induced convulsions are particularly sensitive to drugs blocking sodium channels, e.g., phenytoin (24), and therefore a type of general convulsion.

We investigated convulsive liability of cefepime in corneal kindled mice and found that in corneal kindled mice, mean seizure stage following cefepime administration was significantly higher.

**FIG 3** Typical EEG patterns in mice following intravenous (i.v.) injection of cefepime (A) and meropenem (B). One minute after antibiotic injection, mice were stimulated with currents of 5.6 mA (60 Hz) for 3 s, and EEGs were recorded for 15 min. CFPM, cefepime; MEPM, meropenem.

**FIG 4** Development of seizure stage during electroconvulsive shock-induced convulsions in corneal kindled mice. Each value represents the mean seizure stage ± SEM in 12 mice.

**FIG 5** Effect of cefepime and meropenem on electroconvulsive shock-induced convulsions in corneal kindled mice. Each value represents mean seizure stage ± SEM in 10 mice. CFPM, cefepime; MEPM, meropenem. ***, $P < 0.01$ versus MEPM group (Wilcoxon signed-rank test).
than that following meropenem administration. One distinct disadvantage of the corneal kindled mouse model is that in these mice, unlike the electrically kindled rat, EEG recordings are not possible as electrodes are not implanted. As such, effects of drugs on focal seizure activity can only be inferred from behavioral seizure data. However, we did confirm that cefepime at 500 mg/kg produced spikes in normal mice. Cefepime toxicity may thus be postulated in patients with low seizure thresholds.

In conclusion, with low-current electroconvulsive shock-induced convulsions, cefepime administration at 500 mg/kg significantly increases mean seizure stage. Additionally, EEG spikes were observed in cefepime-administered mice at 500 mg/kg. Compared with meropenem, cefepime resulted in significantly higher seizure stages in corneal kindled mice. Thus, the convulsive liability of cefepime is significantly higher than that of meropenem in normal and corneal kindled mice, and cefepime toxicity warrants further attention in patients with low seizure thresholds.

REFERENCES


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