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Animal Model for Evaluating the Convulsive Liability of β-Lactam Antibiotics

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The β-lactam antibiotics imipenem-cilastatin, BMY-26225, and cefazolin significantly lowered the convulsive threshold of pentylenetetrazole in mice. In addition, imipenem-cilastatin and cefazolin were found to inhibit ^{3}H-labeled γ-aminobutyric acid binding to synaptic membranes from rat brains. Our results suggest that the pentylenetetrazole convulsive model may be useful in evaluating the proconvulsive liabilities of new carbapenems and other β-lactam antibiotics and that the mechanism of imipenem-cilastatin and cefazolin toxicity may involve interaction with γ-aminobutyric acid receptors.

The convulsive liabilities of β-lactam antibiotics, such as penicillin and cefazolin, in humans (2, 14, 17, 18) and experimental animals (4, 7, 10, 14, 16) following massive doses or direct cerebral exposure to the antibiotics were previously reported. More recently, this liability has been extended to include a new class of β-lactams, the carbapenems. The prototype of the carbapenem class, imipenem, administered in combination with the renal dipeptidase inhibitor cilastatin, has been reported to induce seizures in humans at clinical doses (1, 3, 6, 13). Based upon concerns for the convulsive liabilities of the new carbapenems, it would be useful to test potential candidates in a model predictive of this toxicity. An animal model validated for the purpose of identifying anticonvulsant agents was, therefore, evaluated for its potential to predict the proconvulsive activity of imipenem-cilastatin (16). In addition, convulsive behavior has been shown to involve modulation of neurotransmitters, such as γ-aminobutyric acid (GABA) (8). More recently, Hori et al. (9) demonstrated that cefazolin inhibits GABA binding to synaptic membranes and proposed that the interaction is mechanistically linked to the convulsive liabilities of cephalosporins. Thus, the ability of imipenem-cilastatin to compete for GABA binding to synaptic membranes was also investigated.

Male Swiss-Webster mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.), 4.5 to 6 weeks old, were tested by the pentylenetetrazole (PTZ) method (15). The convulsive activity of PTZ administered by the intraperitoneal route was determined alone and 5 min after the intravenous injection of subtoxic doses of β-lactam antibiotics. Imipenem and two other investigational carbapenems, BMY-26225 ($C_{17}H_{20}N_2O_4S$) and BMY-25174 ($C_{16}H_{18}N_2O_4S$), were obtained from Bristol-Myers Company (Pharmaceutical Product Development); imipenem-cilastatin was obtained commercially (Merck Sharp & Dohme Laboratories). The carbapenems were prepared at a concentration of 15 mg/ml in 0.2% $NaHCO_3$ (imipenem and BMY-25174) or 0.9% NaCl (BMY-26225 and imipenem-cilastatin). Cefazolin was obtained commercially (Kefzol; Eli Lilly & Co.) and prepared at a concentration of 40 mg/ml in sterile water. PTZ was obtained from Sigma Chemical Co., St. Louis, Mo., and prepared at a concentration of 2 mg/ml in sterile water.

For the [^{3}H]GABA assay, crude synaptic membranes were prepared from rat cerebellar tissue by the method of Zukin et al. (19). Specific binding of 32 nM [^{3}H]GABA was measured alone or in the presence of antibiotics for 15 min at 4°C. Specific binding was determined in the presence of 1 mM unlabeled GABA.

As shown in Fig. 1, PTZ induced dose-dependent convulsive behavior in mice (50% convulsive dose, approximately 53 mg/kg [body weight]). Pretreatment with carbapenem antibiotics significantly lowered the convulsive threshold of PTZ in the following rank order of proconvulsive potency: imipenem-cilastatin > imipenem > BMY-26225 > BMY-25174. Hypothermia was not a factor in the observation of proconvulsive activity, since core body temperature was unaffected by carbapenems (data not shown). In addition, when administered alone, these carbapenems did not produce signs of toxicity or convulsive behavior until lethal levels were approached at doses of approximately 1,000 to 2,000 mg/kg (data not shown). For more-rapid screening purposes, a single convulsive dose of 50 mg of PTZ per kg was found to be useful, since results similar to those obtained after generating a more extensive PTZ dose response were observed (Table 1). Cefazolin at 700 mg/kg was significantly less proconvulsive than a 400-mg/kg dose of imipenem or imipenem-cilastatin, as determined by Fisher's exact test (11). The proconvulsive behavior of cefazolin was evidenced at a dose twofold that of the carbapenems. Cilastatin alone did not exhibit proconvulsive behavior when tested at intravenous doses of 400 mg/kg (data not shown).

[^{3}H]GABA-binding assays confirmed the inhibitory behavior of cefazolin reported by Hori et al. (9) and revealed marked inhibition resulting from imipenem with or without cilastatin (Table 2). BMY-26225 and BMY-25174, on the other hand, did not inhibit [^{3}H]GABA binding and in fact produced slight stimulatory activity. Thus, although the proconvulsive behavior of imipenem may involve GABA antagonism, the lack of effect with BMY-26225 and BMY-25174 suggests that other mechanisms may be operating in carbapenem-induced effects on the central nervous system (CNS).

The apparent increase in the proconvulsive behavior of imipenem in combination with cilastatin was an interesting observation which may relate to enhanced systemic expo-

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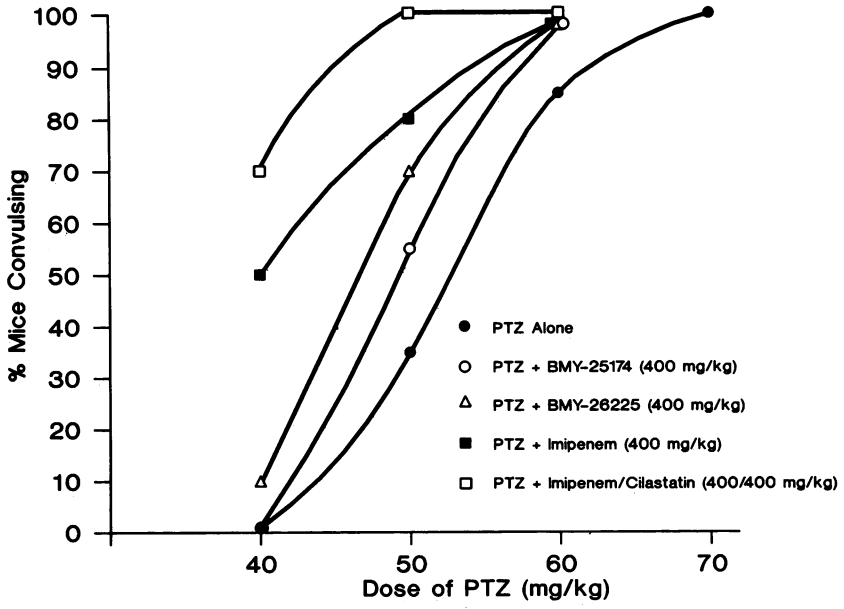

FIG. 1. Effects of carbapenem antibiotics on convulsive threshold of PTZ. Ten mice per group were tested, as described in the text.

sure to imipenem in the presence of cilastatin. Cilastatin has been reported to inhibit the renal and extrarenal metabolism of imipenem in rodents (R. Hajdu, K. Hayase, J. Sundelof, K. Hara, H. Kropp, and F. Kahan, Proc. 14th Int. Congr. Chemother., p. 1211–1212, 1985). However, in humans, extrarenal metabolism of imipenem is insensitive to cilastatin (Hajdu et al., 14th Int. Congr. Chemother.). Alternately, increased serum levels of imipenem in the presence of cilastatin, presumably through inhibition of renal tubular secretion of imipenem (1; Hajdu et al., 14th Int. Congr. Chemother.), could be a factor in enhanced systemic exposure and subsequent CNS toxicity. Since imipenem is always administered with cilastatin to humans, the clinical relevance of these observations cannot be directly determined. Nonetheless, the contribution of cilastatin to the CNS effects of imipenem-cilastatin cannot be ruled out.

In summary, our results demonstrate that imipenem and other β-lactam antibiotics are proconvulsive in the mouse PTZ model. In light of the reported convulsive effects of β-lactams in humans (1–3, 14, 18), this model may be predictive of the CNS liabilities of these antibiotics. Although further studies are required to confirm the correlation between these results and clinical effects, this model may provide insights into the mechanism(s) of antibiotic toxicity, as well as a means of screening new carbapenems and β-lactams for potential CNS side effects. Furthermore, this model may be useful in assessing the convulsive liabilities of

TABLE 1. Effects of β-lactam antibiotics on PTZ-induced convulsions in mice

Treatment[a] (mg/kg)	% of mice convulsing
PTZ alone (50)	30
PTZ plus:	
BMY-25174 (400)	60[b]
BMY-26225 (400)	80[b]
Imipenem (400)	100[b]
Imipenem-cilastatin (400/400)	100[b]
Cefazolin (800)	80[b]
Cefazolin (700)	30

[a] Groups of 10 mice each were injected with 50 mg of PTZ (intraperitoneally) per kg, and convulsions were recorded for 60 min. Pretreatment with β-lactams was performed by the intravenous route 5 min before PTZ injection.
[b] $P < 0.05$ compared with PTZ alone by Fisher's exact test (11).

TABLE 2. Effects of β-lactam antibiotics on GABA binding to rat brains[a]

Compound and concn (mM)	Specific GABA binding[b] (pmol/mg of protein; mean ± SE)	% Inhibition
None	0.203 ± 0.031 (12)	
Cefazolin		
10	0.136 ± 0.010 (3)	32.9[c]
5	0.152 ± 0.020 (12)	24.9
1	0.197 ± 0.009 (3)	2.9
BMY-25174		
10	0.276 ± 0.051 (3)	0
5	0.183 ± 0.042 (12)	9.8
1	0.251 ± 0.021 (3)	0
BMY-26225		
10	0.245 ± 0.044 (3)	0
5	0.195 ± 0.060 (12)	4.0
1	0.239 ± 0.023 (3)	0
Imipenem		
10	0.081 ± 0.007 (3)	59.8[c]
5	0.155 ± 0.024 (12)	23.6
1	0.233 ± 0.034 (3)	0
Imipenem-cilastatin		
10	0.027 ± 0.002 (3)	86.5[c]
5	0.110 ± 0.027 (12)	45.8[c]
1	0.180 ± 0.019 (3)	11.3

[a] Crude synaptic membranes were prepared from rat cerebellar tissue.
[b] Binding of 32 nM [^{3}H]GABA was measured in the presence of 1 mM unlabeled GABA (15 min, 4°C). Protein was measured as described by Lowry et al. (12). The number of experiments is shown in parentheses.
[c] $P < 0.05$ compared with control binding by Dunnett's procedure for multiple comparisons (5).

other drugs and chemicals. Lastly, our data suggest that the proconvulsive activity of imipenem may result in part from GABA antagonism in the CNS and that the presence of cilastatin potentiates the convulsive liability.

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