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Safety Evaluation of LB10522, a New Cephalosporin Antibiotic

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Abstract—All the pharmacological studies of LB10522 described here were carried out with high doses (fifteen to sixty times of the therapeutic dose) to determine an indication of potential side effects in clinical use in terms of the acute clinical signs, cardiovascular and central nervous system. LB10522 does not produce any observable clinical signs except for the symptoms such as moist eye, skin rash, slight salivation, vomitting, and slightly reduced activity. The effects of LB10522 on the hemodynamics and cardiac function of anesthetized beagle dogs are as follows; heart rates and mean arterial blood pressure had a tendency to increase mildly, which is a normal finding in anesthetized dogs. All the animals except for one showed relatively stable respiratory rates throughout the observation period. Each animal treated with LB10522 showed slight increase in the left cardiac work and left ventricular stroke work which are mainly related to corresponding increases in cardiac output. Femoral blood flow were shown to be increased in some animals treated with LB10522. The epileptogenic activities of various cephalosporins were assessed by a direct intracerebral injection of appropriate concentration of test articles. The CD_{50} values (nmol) obtained from the analysis of the dose-response data are as follows; 78.2, 175.3, 156.3, and 53.5 for cefazolin, cephaloridine, ceftazidime, and LB 10522, respectively. LB10522 seems to be equipotent with cefazolin or to be three times more potent than cephaloridine and ceftazidime in causing adverse CNS stimulation. Taken into consideration all the information obtained, LB10522 is not supposed to induce much changes in the functions examined in these studies in man at therapeutic doses.

Keywords □ cephalosporin, LB10522, epileptogenecity, hemodynamics, clinical sign

The cephalosporins have been reported to be safe class of antibiotics compared with other antibiotics. The most common type of adverse effects are allergic reactions such as skin rash, fever, or late onset urticaria (Parry, M. F., 1984). Incidence of gastrointestinal disturbances has also been reported (Smith, C. R., 1981). Hematologic effects, including agranulucytosis, thrombocytopenia, and hypoprothrombinemia have been observed with a wide variety of cephalosporins (Rey, D., *et al.*, 1989; Haubenstock, A., *et al.*, 1983). All cephalosporins are thought to be potentially nephrotoxic at high doses, and the usual site of damage is the renal tubule (Quin, J. D., 1989). It has been shown that several cephalosporins with higher doses induced epileptogenic activities both in electroencephalogram and behavior in experimental animals (Rotiroti, D., *et al.* 1983; Yu, Q. H., *et al.*, 1984).

New catecholic cephalosporins which are expected to have excellent broad spectrum antibacterial activities and improved pharmacokinetic profiles have been synthesized. Among them, LB10522, {7-[(Z)-2-(2-aminothiazol-4yl)-2-(S)-(a-carboxyl-3,4-dihydroxy benzyl oxyimino)acetamide]-3-[(E)-3-(4-amino-1 pyrimidino)-

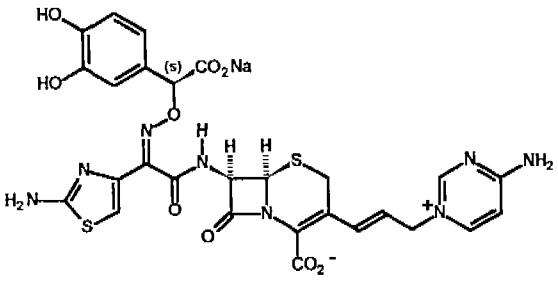

Fig. 1. Chemical structure of LB10522.

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1-propen-1-yl]-3-cephem-4-carboxylate}(Fig. 1), has been chosen for the development. It was shown to be effective in the treatment of infectious diseases in mice caused by gram-positive and gram-negative bacteria, including *Pseudomonas aeruginosa* (Oh *et al.*, 1995; Kim *et al.*, 1995). As part of the preclinical evaluation of LB10522, a series of general pharmacological studies was carried out with high dose to determine an indication of potential side effects in clinical use in terms of the clinical signs, cardiovascular, and central nervous systems.

Materials and Methods

Drugs and Animals

In order to prepare test article for administration, LB10522 (mw. 680, test article) was dissolved in 0.9% isotonic sterile saline (vehicle) at the highest concentration. Commercially available cephalosporins, such as cefazolin (mw. 454.5, Sigma), cephaloridine (mw. 415.5, Sigma), and ceftazidime (mw. 546.6, Glaxo) have been utilized as reference drugs in some experiments. Male ICR mice (23~26 g, LG Chem animal facility) and male beagle dogs (Charles River) were used for the following experiments. All the animals were housed and feeded with a standard commercial food at th LG Chem animal facility of which environment was well controlled (temperature; 20~22℃, 12 hours light and 12 hours dark).

Clinical signs

Two to three male beagle dogs weighing 10~12 kg were used in each group. Clinical signs such as skin rash, salivation, defecation, moist eye, vomitting, activity, tremor, heart rate, respiration, and vocalization were observed for 10 mins after intravenous administration of the test article (LB10522). Total scores (refer to scoring system in Table I) were used as indices reflecting overall clinical symptoms.

Hemodynamics

The effects of LB10522 on the hemodynamics and cardiac function of anesthetized beagle dogs (7~9 kg, 2 male/group) following a single intravenous dose were assessed as follows (Fegler, 1954; Evonuk *et al.*, 1961). On the day of treatment, dogs were anesthetized by inhalation of isoflurane. Using sterile surgical technique, an inguinal cut down was performed on both left and right sides. A flow directed catheter with thermodilution cardiac output capability was inserted into the left femoral vein and advanced into the pulmonary artery. In addition, a non invasive flow meter was placed around the right femoral artery to measure the femoral blood flow and a catheter was placed in the left femoral artery in order to provide one arterial line for blood pressure monitoring. Finally, a catheter was also placed in the cephalic vein for test article administration. The test article (100, 1,000 mg/kg) was administered by a single bolus intravenous injection into the cephalic vein. Animals treated with 100 mg/kg were designated as 100A, 100B and those treated with 1,000 mg/kg were named as 1,000A, 1,000B. The dose volume of all animal was 5 ml/kg administered at a rate of approximately 6 ml/min. The parameters, such as cardiac output, systolic and diastolic and mean arterial pressure, pulse rate, respiratory rate, left cardiac work, left ventricular stroke work, and femoral blood flow, were examined immediately prior to and following treatment (at −10, 0, 15, 30, 45, 60 and 120 minutes). A volumetric test was done on a test animal in order to evaluate the effects of the volume injected without the test article on the different hemodynamic parameters measured in the present study.

Table I. Clinical signs induced by i.v. administration of LB 10522

Sings	Scores	Vehicle	LB10522 (mg/kg)	
			100	500
Skin rash	−1,0,1,2,3	0	0	2
Salivation	0,1,2,3	0	0	1
Defecation	0,1,2,3	0	0	0
Moist eye	0,1	0	1	0
Vomiting	0,1,2,3	0	0	3
Activity	−2,−1,0,1,2	0	0	−0.5
Tremor	0,1,2,3	0	0	0
Heart rate	0,1,2,3	0	0	0
Respiration	−1,0,1,2	0	0	0
Vocalization	0,1	0	0	0
Total Scores	24	0	1	6.5

The clinical signs were observed for 10 min. after intravenous injection of the test articles. The scoring systems are follows; skin rash, −1: pale, 0: no change, 1: rash immediately after injection and disappear within 10 min., 2: duration of rash for more than 10 min., 3: systemic rash, salivation, 0: no change, 1: salivation around mouth, 2: runny mouth, 3: lots of salivation; defaecation, 0: not observed, 1: 1~2 times, 2: 3~4 times, 3: more than 5 times; moist eye, 0: not observed, 1: observed; vomitting, 0: not observed, 1: 1~2 times, 2: 3~4 times, 3: more than 5 times; activity, −2: no activity, −1: sluggish activity, 0: no change, 1: increased activity, 2: excited condition, tremor, 0: not observed, 1: duration for 1 min., 2: duration for 1~3 min., 3: duration for more than 3 min.; heart rate (change), 0: less than 20%, 1: 21~40%, 2: 41~60%, 3: more than 60%, respiration, −1: slow down, 0: no change, 1: enhanced, 2: very enhanced; vocalization, 0: not observed, 1: observed.

Epileptogenecity by the direct intracerebral injection

Convulsions induced by the direct intracerebral inje-

ction of test articles were assessed as follows (Brittain and Handley, 1967; Haley and McCormic, 1957; Shimada, *et al.*, 1992). Five to seven male ICR mice weighing 24-26 g were used in each group. The needle (26-gauge, 3 mm in length) was inserted perpendicularly through the skull (within 1 mm of midline and on a line joining the anterior bases of the ears) into the brain and five microliters of test articles were injected. Immediately after injection, the symptoms such as convulsion, tremor, writhing, and death were observed for 2 h. The CD_{50} (dose eliciting convulsion in 50% of animals in a group) was calculated by Probit method (Bliss, C. I., 1985). Five microliter of crystal blue (1/10 diluent) was injected intracerebrally in separate animals and the third and forth ventricles were shown to be stained through histological examination.

Results and Discussions

Clinical signs

As for the clinical sign test using dogs, moist eye was observed at 100 mg/kg of LB10522 treatment. However, LB10522 at 500 mg/kg dose caused skin rash after intravenous administration and it was prolonged over 10 min. Mild degree of salivation, more than five times of vomitting, and slightly reduced activity were also observed with 500 mg/kg dose treatment (Table I). LB10522 produced no significant effects upon measures of any other clinical signs described under "Materials and Methods"

Hemodynamics

As shown in Fig. 2, heart rates had a tendency to increase mildly, especially between time 60 min. and time 120 min. The increase in heart rate is a normal finding in anesthetized dogs and may represent a physiological response to maintain cardiac output. As for systolic blood pressure, animals 100B and 1000A showed 27% and 29% increases at time 30 min. and 15 min., respectively, and values remained higher than baseline for the rest of the observation period. Rest of the animals showed minimal variations (Fig.3). As shown in Fig. 4. animals 100A and 1000B showed minimal variations in diastolic blood pressure. 100B and 1000A showed 34% and 44% increases at time 15 min., respectively and values remained higher than baseline for the rest of the observation period. As shown in Fig. 5, animals 100B and 1000A showed 30% and 39% increase at 30 min. and 15 min., respectively, in mean arterial blood pressure. Values decrease slightly up until at 45 min. but remained higher than at time −10 min. in all animals by the end of observation period, except for animals, 100A and 1000B which showed minimal variations throughout the observation period. All the animals, except 100A, showed relatively stable respiratory rates throughout the observation period (Fig. 6). Marked variations in 100A could only be explained with variations in depth of anesthesia based on the available data. As shown in Fig. 7, each animals showed increase (100A, 44% at 15 min.; 100B, 83% at 30 min.; 1000A, 114% at 15 min.; 1000B, 85% at 15 min.) in left cardiac work. Values decreased gradually

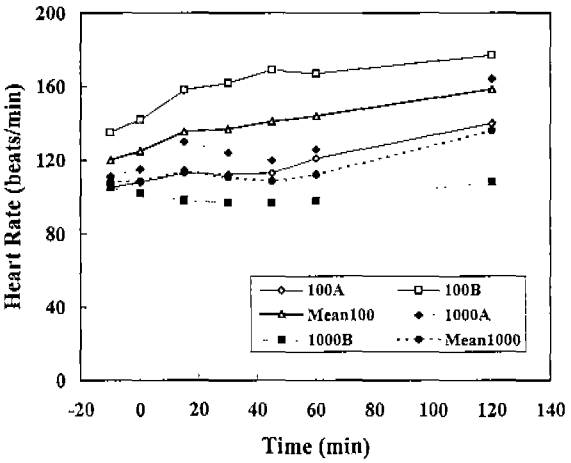

Fig. 2. Effects of LB10522 on the heart rate after a single bolus intravenous injection in beagle dogs. Animals treated with 100 mg/kg were designated as 100A and 100B (Mean 100 stands for the mean value of 100A and 100B) and those treated with 1,000 mg/kg were named as 1,000 A and 1,000B (Mean 1,000 stands for the mean value of 1,000A and 1,000B).

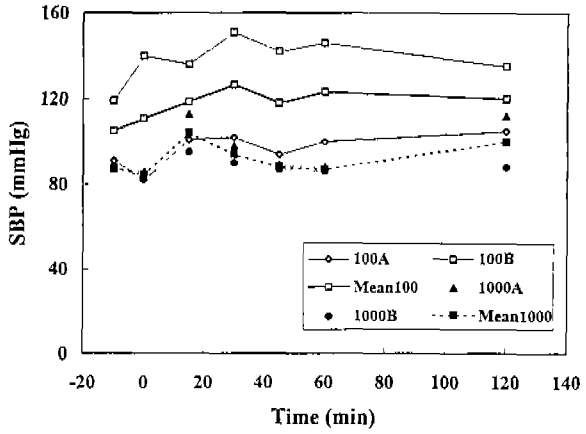

Fig. 3. Effects of LB10522 on the systolic blood pressure after a single bolus intravenous injection in beagle dogs. Animals treated with 100 mg/kg were designated as 100A and 100B (Mean 100 stands for the mean value of 100A and 100B) and those treated with 1,000 mg/kg were named as 1,000A and 1,000B (Mean 1,000 stands for the mean value of 1,000A and 1,000B).

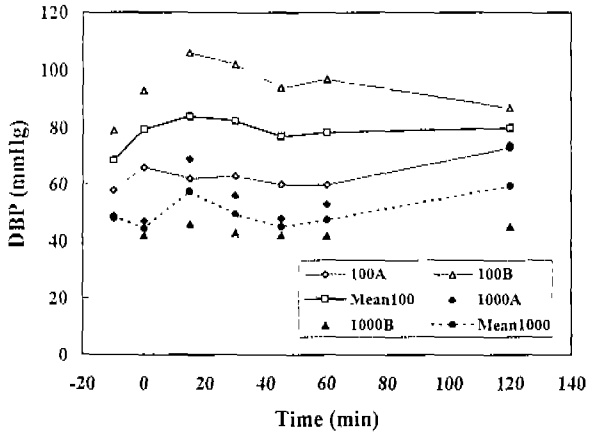

Fig. 4. Effects of LB10522 on the diastolic blood pressure after a single bolus intravenous injection in beagle dogs. Animals treated with 100 mg/kg were designated as 100A and 100B (Mean 100 stands for the mean value of 100A and 100B) and those treated with 1,000 mg/kg were named as 1,000A and 1,000B (Mean 1,000 stands for the mean value of 1,000A and 1,000B).

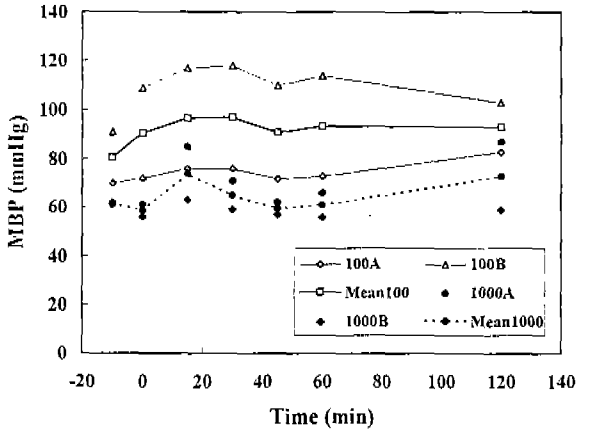

Fig. 5. Effects of LB10522 on the mean arterial blood pressure after a single bolus intravenous injection in beagle dogs. Animals treated with 100 mg/kg were designated as 100A and 100B (Mean 100 stands for the mean value of 100A and 100B) and those treated with 1,000 mg/kg were named as 1,000A and 1,000B (Mean 1,000 stands for the mean value of 1,000A and 1,000B).

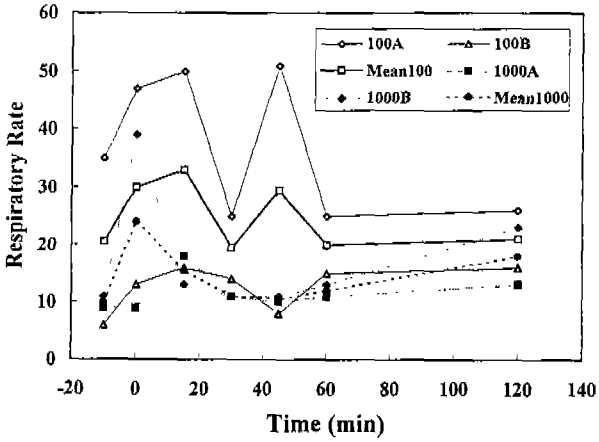

Fig. 6. Effects of LB10522 on the respiratory rate after a single bolus intravenous injection in beagle dogs. Animals treated with 100 mg/kg were designated as 100A and 100B (Mean 100 stands for the mean value of 100A and 100B) and those treated with 1,000 mg/kg were named as 1,000A and 1,000B (Mean 1,000 stands for the means value of 1,000A and 1,000B).

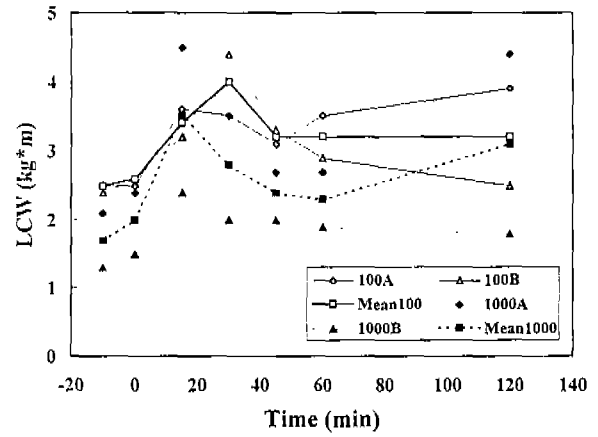

Fig. 7. Effects of LB10522 on the left cardiac work after a single bolus intravenous injection in beagle dogs. Animals treated with 100 mg/kg were designated as 100A and 100B (Mean 100 stands for the mean value of 100A and 100B) and those treated with 1,000 mg/kg were named as 1,000A and 1,000B(Mean 1,000 stands for the mean value of 1,000A and 1,000B).

thereafter until 45min. but remained higher than the baseline at 120 min., except for animals 100B and 1000 B. The increases in these animals returned to values close to −10 min. The increases in left cardiac work are mainly related to corresponding increases in cardiac output. As for left ventricular stroke work, values follow the same pattern as for left cardiac work (Fig. 8). As shown in Fig. 9, each animals showed increase (100A, 34% at 15 min.; 100B, 43% at 30 min.; 1000A, 56% at 15 min.; 1000B, 76% at 15 min.) in cardiac output. Values remain higher than −10 min. in all animals for the rest of the observation period, except in animal 100B. The values for this animal returned to base line values. Increases in cardiac output are related to increases in stroke volume and to a lesser degree to increases in heart rates. As shown in Fig. 10, each animals, except for 100B, showed increases (100A, 84% at 60 min; 1000A, 94% at 15 min; 1000B, 85% at 15 min) in femoral blood flow. Values decreased thereafter for animals 1000A and 1000B until 45 min. but remained above t= −10 min at time 120 min. Animal 100A remained higher than the baseline value

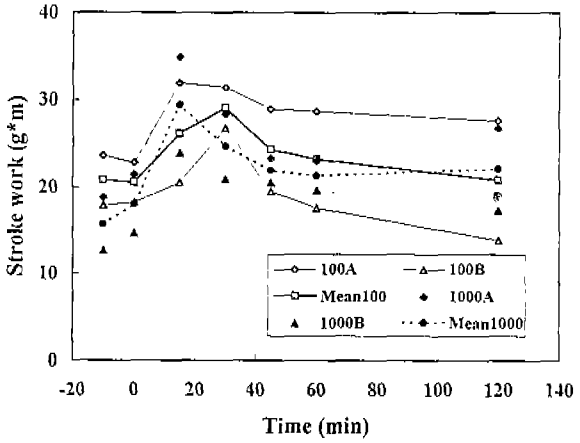

Fig. 8. Effects of LB10522 on the left ventricular stroke work after a single bolus intravenous injection in beagle dogs. Animals treated with 100 mg/kg were designated as 100A and 100B (Mean 100 stands for the mean value of 100A and 100B) and those treated with 1,000 mg/kg were named as 1,000A and 1,000B (Mean 1,000 stands for the mean value of 1,000A and 1,000B).

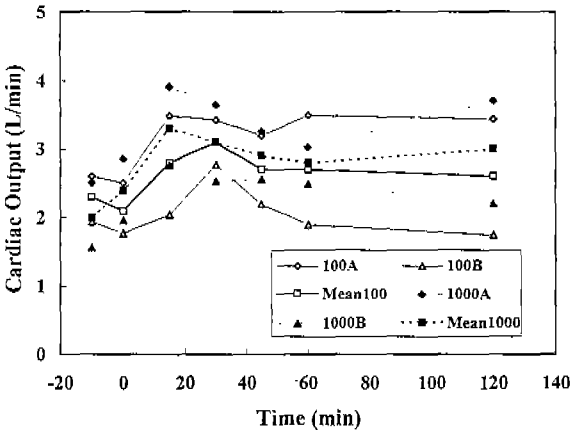

Fig. 9. Effects of LB10522 on the cardiac output after a single bolus intravenous injection in beagle dogs. Animals treated with 100 mg/kg were designated as 100A and 100B (Mean 100 stands for the mean value of 100A and 100B) and those treated with 1,000 mg/kg were named as 1,000A and 1,000B (Mean 1,000 stands for the mean value of 1,000A and 1,000B).

after the displayed increase mentioned above. The lack of correspondence between femoral blood flow and cardiac output in all animals could be associated with local arterial constriction. A volumetric test was done on a test animal in order to evaluate the effects of the volume injected without the test article on the different hemodynamic parameters measured in the present study. Both cardiac output and stroke volume increased at time zero min. and reached a maximum value at 45 min (32% increase in cardiac output and 40% increase in stroke volume) and remained higher

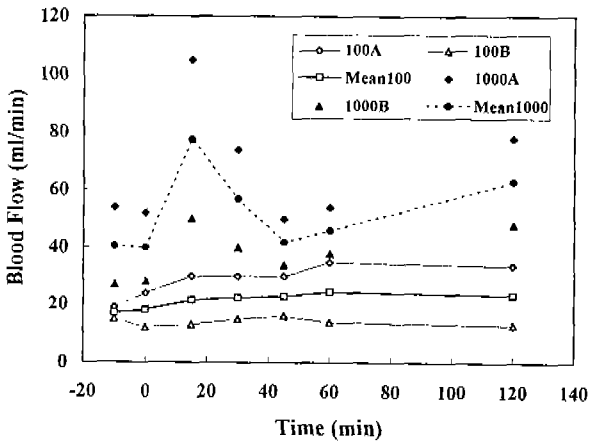

Fig. 10. Effects of LB10522 on the femoral blood flow after a single bolus intravenous injection in beagle dogs. Animals treated with 100 mg/kg were designated as 100A and 100B (Mean 100 stands for the mean value of 100A and 100B) and those treated with 1,000 mg/kg were named as 1,000A and 1,000B (Mean 1,000 stands for the mean value of 1,000A and 1,000B).

than baseline for the rest of the observation period (120 min). All other parameters which have been discussed showed minimal normal variations.

Central nervous system

It has been reported that cephalosporins induce convulsions when they are administered in massive doses or applied directly to the cerebral cortex of experimental animals. For example, intraventricular application of cefazolin and carbapenems and β-lactam antibiotics into the cerebral ventricles of experimental animals and man has been shown to induce a focal or generalized epileptic state (Yoshioka *et al.*, 1975; Yost *et al.*, 1977; Bechtel *et al.*, 1980; Kamei *et al.*, 1983). The inhibition by β-lactams of the receptor binding of γ-aminobutyric acid, an inhibitory neurotransmitter in the mammalian central nervous sytem seems to be the underlying mechanism (Hori *et al.*, 1985). The central action of test articles can be evaluated more properly by direct intracerebral injection into the brain, which eliminates the penetration process through the blood-brain barrier (BBB). As shown in Table II, the epileptogenic activities of various cephalosporins were assessed by a direct intracerebral injection of 5 μl of appropriate concentration of test articles. The CD_{50} values (nmol) obtained from the analysis of the dose-response data are as follows; 78.2, 175.3, 156.3, and 53.5 for cefazolin, cephaloridine, ceftazidime, and LB10522, respectively. LB10522 seems to be equipotent with cefazolin or three times more potent than cephaloridine and ceftazidime in causing adverse CNS stimulation.

Table II. Epileptogenetic activity of various cephalosporins

Cephalosporins	CD_{50} (nmol)
Cefazolin	78.1643
Cephaloridine	175.3194
Ceftazidime	156.3285
LB10522	53.5468

*CD_{50}: the dose which induced convulsion of 50% of animals in a group, Five to seven animals were used per each group, Data shown are mean values of a representative experiment.

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