

Full Paper

Epileptogenic Activity Induced by Teicoplanin and Effects of Some Antiepileptics in Mice

Kenshi Takechi¹, Takashi Ishikawa¹, and Chiaki Kamei^{1,*}¹Department of Medicinal Pharmacology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8530, Japan

Received May 1, 2008; Accepted June 17, 2008

Abstract. The present study was undertaken to clarify the epileptogenic activity induced by intracerebroventricular injection (i.c.v.) of methicillin-resistant *Staphylococcus aureus* (MRSA) antibiotics in mice. Teicoplanin (200 µg, i.c.v.) caused dose-related behavioral seizures such as head twitch and forelimb clonus. At the same time, the drug caused electroencephalographic (EEG) seizures characterized by spike-and-wave complex and a continuous spike with high amplitude. At a high dose (500 µg, i.c.v.), the drug caused a severe clonic convulsion followed by continuous spike and spike-and-wave complex on EEG. On the other hand, vancomycin caused no or almost no epileptogenic activity in both behavior and on EEG. Diazepam and sodium valproate dose-dependently antagonized epileptic seizures in behavior and on EEG induced by teicoplanin (500 µg, i.c.v.). In contrast, carbamazepine and ethosuximide caused no significant changes in both behavioral and EEG seizures induced by teicoplanin. From these findings, it can be concluded that teicoplanin may cause potent epileptogenic activity different from vancomycin when used clinically at extremely high doses. In addition, it may be that teicoplanin-induced seizure is closely related with the γ -amino butyric acid (GABA)-ergic mechanism.

Keywords: teicoplanin, vancomycin, electroencephalographic, antiepileptic

Introduction

It is well known that some strains of *Staphylococcus aureus* have developed resistance to methicillin, and methicillin-resistant *Staphylococcus aureus* (MRSA) is becoming an important public-health problem (1). Drago et al. (2) also reported that MRSA is an increasingly common cause of hospital infections, causing severe mortality worldwide, and accounting in some hospitals for more than 50% of all *Staphylococcus aureus* disease; therefore, an effective and safe antibiotic to treat the infections caused by MRSA is eagerly anticipated. At present, some MRSA antibiotics, including teicoplanin and vancomycin, have been used in clinical practice. We have demonstrated that a large number of cephem antibiotics caused potent epileptogenic activity not only intracerebroventricularly but also

by intravenous injection in rats (3, 4). On the other hand, Grondahl and Langmoen (5) reported that vancomycin caused no epileptogenic activity in rats. In addition, little work has been done to study the epileptogenic activity of teicoplanin, except our preliminary paper (6).

Therefore, the present study was undertaken to examine in detail whether teicoplanin and vancomycin cause epileptogenic activity in mice when injected intracerebroventricularly. In addition, the effects of some antiepileptics on teicoplanin-induced seizures were studied in order to investigate their mechanism of action. In this study, we used mice with chronically implanted electrodes for electroencephalogram recording and a guide cannula for injection of antibiotics.

Materials and Methods

Animals

Male ICR mice, 5 – 6-week-old and weighing 25 – 30 g, were used (Nippon SLC, Shizuoka). All animals were maintained in an air-conditioned room with controlled

*Corresponding author. kamei@pheasant.pharm.okayama-u.ac.jp
Published online in J-STAGE on August 2, 2008 (in advance)
doi: 10.1254/jphs.08111FP

temperature ($24 \pm 2^\circ\text{C}$) and humidity ($55 \pm 15\%$). They were group housed as 5 mice each in plastic cages with sawdust and kept under a light/dark cycle with lights on from 7:00 to 19:00. The animals were given food and water ad libitum. All procedures involving the animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Centers, and all procedures were licensed by the Animal Research Control Committee of Okayama University. Drug tests were repeated 3 times in the same animal at intervals of at least 7 days. Groups of 8 mice were used for each dose of the test drugs.

Surgery

Under sodium pentobarbital anesthesia (Nembutal®, 60 mg/kg, i.p.; Abbot Laboratories, North Chicago, IL, USA), the mice were fixed to a stereotaxic apparatus (SR-5; Narishige, Tokyo), and monopolar electrodes were implanted into the frontal cortex (A: 0.4, L: -1.2), hippocampus (A: -2.3, L: -1.5, H: -1.8), and amygdala (A: -1.8, L: -3.0, H: -4.5) according to the atlas of Franklin and Paxinos (7). A reference electrode was implanted into the occipital part of the cranium. The cortical electrodes were made of stainless steel screws. The subcortical electrode was monopolar stainless steel wire, 200 μm in diameter. All electrodes were connected to a miniature receptacle. A guide cannula (26-gauge needle) was implanted into the right lateral ventricle (A: -0.5, L: 1.0, H: -2.0). The electrodes and guide cannula

were then embedded in the skull with dental cement. At least 1 week were allowed for recovery from the surgery. After the experiments, the animals were killed, and the localization of the electrodes in the brain was verified histologically. In this experiment, localization of the electrodes in the brain was checked under the microscope and reconstructed according to the atlas of Franklin and Paxinos (7).

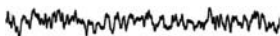
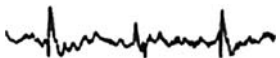

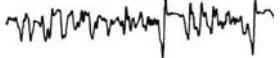




Drugs

The antibiotics used were vancomycin hydrochloride (Sigma, St. Louis, MO, USA) and teicoplanin (Targocid®; Astellas Co., Tokyo). All antibiotics were dissolved in saline. Intracerebroventricular injection was performed through an injection cannula, which was fitted inside the guide cannula, and for all antibiotics, 4 μl per mouse was given within 4 min. Sodium valproate (Depakene®; Kyowa Co., Tokyo), diazepam (Sigma), ethosuximide (Sigma), and carbamazepine (Tegretol®; Novartis Pharma, Tokyo) were used as antiepileptic drugs. They were suspended in 0.5% carboxymethyl cellulose (CMC) solution and orally administered 1.0 h before the antibiotics injection at a volume of 5 ml/kg of body weight. Doses of the drug were expressed in terms of the free base.

Measurement of behavior and EEG

The animals were placed in a plastic cage ($20 \times 35 \times 25$ cm). Monopolar electroencephalograms (EEG) were

Table 1. Scoring system used for the estimation of seizure intensity

Score	Behavior	EEG
0	No change	No change 
1	Head twitch	Spike or Sharp wave  
2	Forelimb clonus	Wave discharge or Polyspike  
3	Clonic convulsion	Continuous spike (within 15 s) or Spike-and-wave complex  
4	Generalized convulsion (more than 15 s)	Continuous spike with high amplitude (more than 15 s) 

100 μV
5 s

amplified with a bioelectric amplifier (AB-651J, JB-101J; Nihon Kohden, Tokyo) and recorded with a thermal arraycorder (WR-300; Graphtec, Tokyo). After the mice were habituated to the experimental and recording conditions, the animals were continuously observed for alterations in behavior and EEG for 60 min following intracerebroventricular injection. The intensity of the epileptogenic symptoms induced by each antibiotic was evaluated using the scoring system shown in Table 1.

Statistical analyses

All values are expressed as the mean \pm S.E.M. The Steel test was used to assess significant dose effects on both behavioral and EEG scores. A difference of $P < 0.05$ was regarded as significant.

Results

Characteristics of the behavioral and EEG seizures induced by antibiotics

Although, teicoplanin at a dose of 100 μg caused no obvious behavioral or EEG changes, the drug at a dose

of 200 μg induced head twitching and forelimb clonus (score 2), together with high voltage polyspike in the frontal cortex, hippocampus, and amygdala 20–40 min after injection (Fig. 1). At a dose of 500 μg , teicoplanin showed clonic convulsion (score 3) and generalized convulsion (score 4), together with a high frequency continuous spike in the frontal cortex, hippocampus and amygdala 20–60 min after injection (Fig. 1). On the other hand, vancomycin caused no significant behavioral and EEG seizures at doses of 100 and 200 μg (Fig. 2), but vancomycin at a dose of 500 μg sometimes caused head twitching (score 1), together with an EEG spike in the frontal cortex, hippocampus, and amygdala 20–60 min after injection (Fig. 2). Quantitative results of behavioral and EEG seizures induced by antibiotics are shown in Fig. 3. Teicoplanin caused both behavioral and EEG seizures dose-dependently, and significant differences were observed at doses of 200 and 500 μg (Fig. 3). On the other hand, vancomycin caused no significant changes to either behavioral or EEG seizures (Fig. 3).

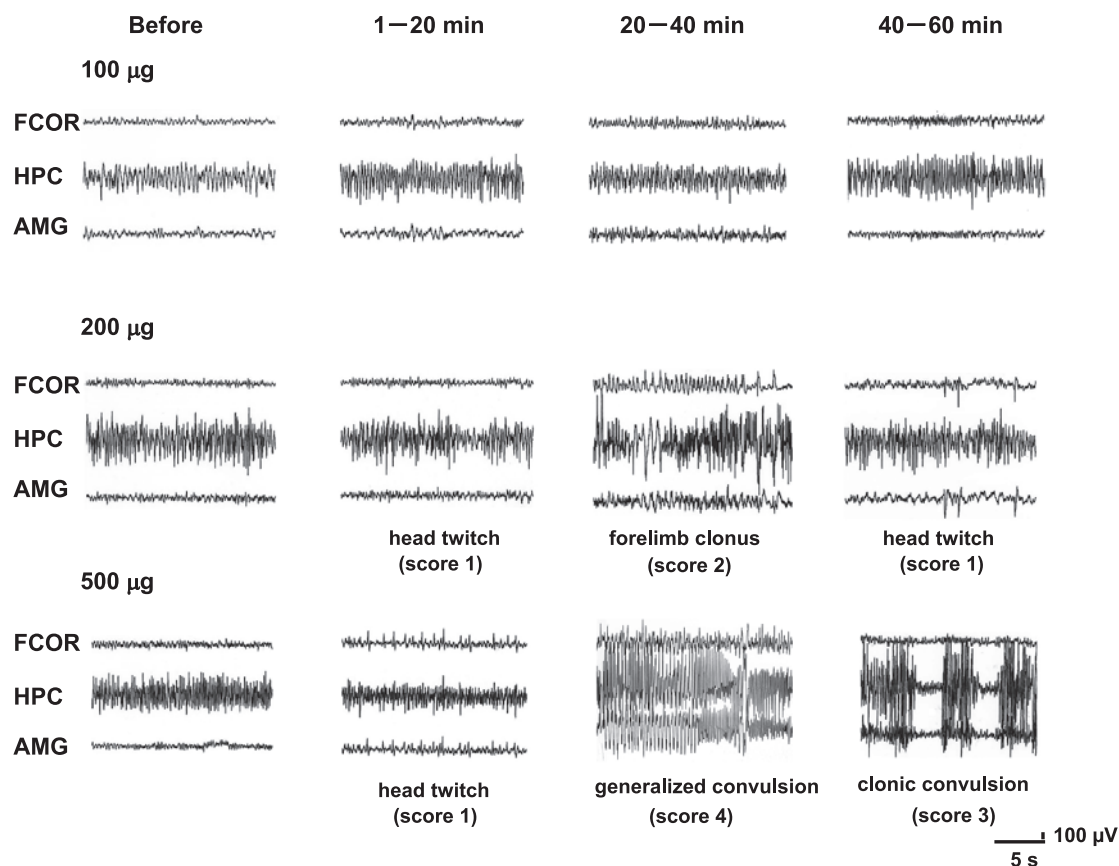


Fig. 1. Representative example of behavioral and EEG seizure patterns induced by teicoplanin (100, 200, and 500 μg , i.c.v.). FCOR, frontal cortex; HPC, hippocampus; AMG, amygdala.

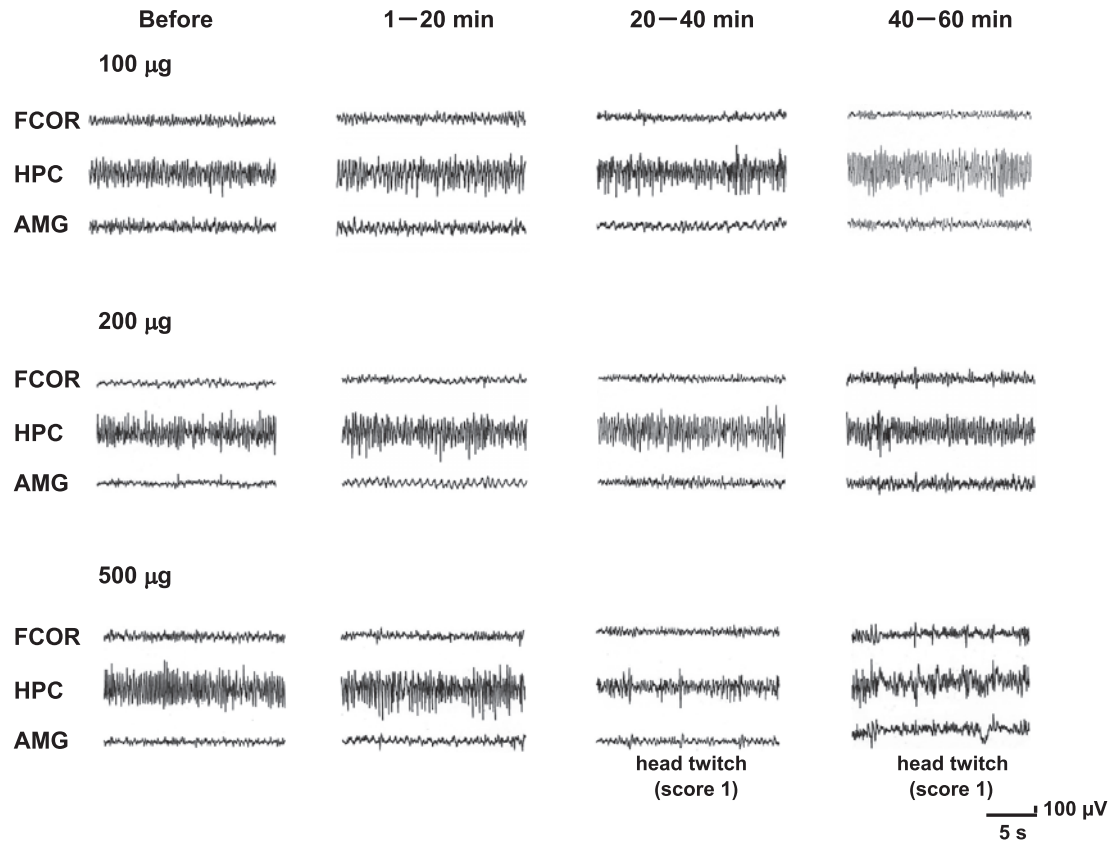


Fig. 2. Representative example of behavioral and EEG seizure patterns induced by vancomycin (100, 200, and 500 µg, i.c.v.). FCOR, frontal cortex; HPC, hippocampus; AMG, amygdala.

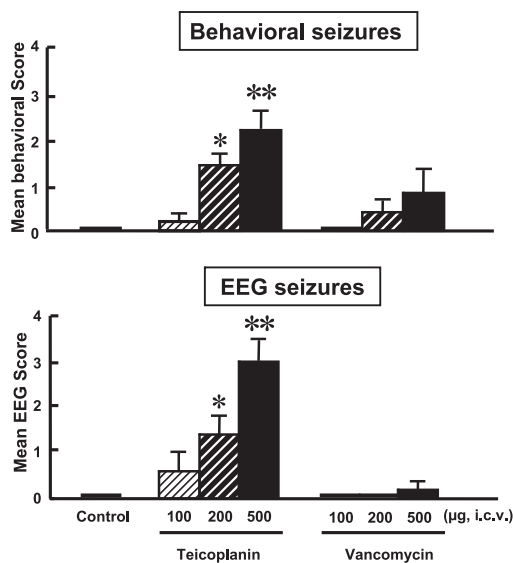


Fig. 3. Comparison of epileptiform activity induced by intracerebroventricular injection of antibiotics in mice. Behavioral and EEG seizures were scored at 60 min after antibiotics administration. Each value represents the mean \pm S.E.M. of 8 mice. *, **: Significantly different from the control group with $P < 0.05$ and $P < 0.01$, respectively.

Effects of some antiepileptics on teicoplanin-induced seizures in mice

Diazepam at a dose of 5 mg/kg inhibited behavioral seizures induced by teicoplanin (500 µg, i.c.v.) (Fig. 4). EEG seizures were also inhibited significantly by diazepam at doses of 1, 2, and 5 mg/kg (Fig. 4). The oral administration of sodium valproate also caused the dose-dependent inhibition of both behavioral and EEG seizures induced by teicoplanin (Fig. 4). At doses of 200 and 500 mg/kg, sodium valproate caused a significant inhibition of both behavioral and EEG seizures (Fig. 4). On the other hand, carbamazepine showed no significant effects on either behavioral or EEG seizures induced by teicoplanin at a dose of 100 mg/kg. Ethosuximide also caused no significant changes in either behavioral or EEG seizures induced by teicoplanin, even at a dose of 1000 mg/kg.

Discussion

In this study, we used the scoring system shown in Table 1 to estimate seizure intensity. Epileptic seizure by teicoplanin took the form of head twitching, forelimb clonus, clonic convulsion, and generalized convulsion.

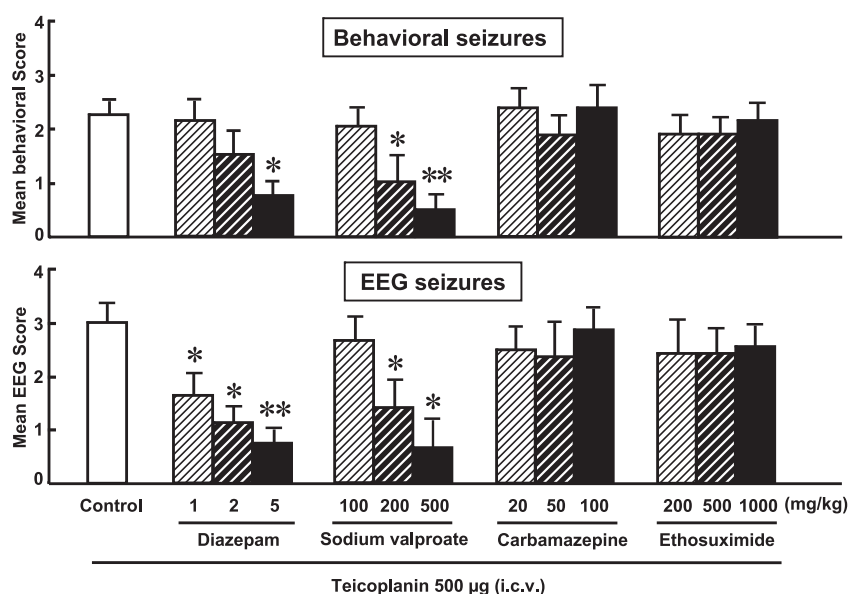


Fig. 4. Effects of diazepam, valproate, carbamazepine, and ethosuximide on teicoplanin-induced behavioral and EEG seizures in mice. Diazepam, valproate, carbamazepine, or ethosuximide was injected 60 min before teicoplanin (500 µg, i.c.v.) injection. EEG and behavioral seizures were scored at 60 min after antibiotics administration. Each value represents the mean \pm S.E.M. of 8 mice. *, **: Significantly different from the control group with $P < 0.05$ and $P < 0.01$, respectively.

On the other hand, epileptic seizure in EEG showed diversity. For instance, teicoplanin at a dose of 200 µg caused forelimb clonus, but at the same time, wave discharge or polyspike in EEG was observed; therefore, the epileptogenic activity of teicoplanin was studied in detail using our epochal scoring system.

In clinical practice, teicoplanin is widely used as an intravenous injection. Teicoplanin and vancomycin are classified as glycopeptide antibiotics, and glycopeptide antibiotics are high molecular weight (MW: 1485–1893). When teicoplanin was injected intravenously in rats, the lowest concentration was observed in the brain (8). However, in MRSA-infected patients suffering from meningitis, teicoplanin passes into the brain by blood brain barrier (BBB) disruption. That is, glycopeptide antibiotics was reported to penetrate in the cerebrospinal fluid (CSF) in patients with meningitis (9). In the working party of the British Society for Antimicrobial Chemotherapy, intracerebroventricular injection of glycopeptide antibiotics has been recommended in the field of neurosurgery (10, 11). Thus, if teicoplanin is injected intravenously in MRSA-infected patients suffering from meningitis, this antibiotic may cause epileptogenic activity by crossing the BBB.

In the present study, it was found that teicoplanin caused potent epileptogenic activity in behavior and on EEG, whereas vancomycin showed no or almost no epileptogenic activity. As for vancomycin, Grondahl and Langmoen (5) reported that vancomycin induced no epileptogenic activity in humans, almost the same finding as in our present mouse study. It is well known that both teicoplanin and vancomycin are classified as glycopeptide antibiotics; therefore, it was thought at the

time that a glycopeptide ring is unlikely to show epileptogenic activity. In fact, we have as yet little information as to whether the glycopeptide ring caused epileptogenic activity. Previously, we reported that the heterocyclic ring is responsible for epileptogenic activity of cephem antibiotics (12); however, teicoplanin has no heterocyclic ring or similar type of ring in its chemical structure. On the other hand, it has been reported that vancomycin is readily soluble in water. In contrast, Bambeke (13) demonstrated that teicoplanin had a lipophilic chain in its chemical structure and showed high penetration to the cerebrospinal fluid. The epileptogenic activity of teicoplanin may be due to its ability to get into the cerebrospinal fluid. The peak time of epileptogenic activity by teicoplanin was about 20 min. Therefore, it was thought at the time, epileptogenic activity of teicoplanin was exerted through some neurotransmitter. That is, both behavioral and EEG seizures induced by teicoplanin was inhibited specifically by diazepam. However, the chemical structure of teicoplanin has no similarity to any γ -amino butyric acid (GABA)-receptor antagonist. Therefore, we suppose that GABA-ergic neurons may be responsible for the epileptogenic activity induced by teicoplanin. This is the reason why the epileptogenic activity induced by teicoplanin occurred 20–40 min after i.c.v. injection.

Teicoplanin-induced seizures could be inhibited by pretreatment with diazepam and sodium valproate. It is well recognized that diazepam exerts antiepileptic activity by facilitating the enhancement of GABA_A receptor-mediated inhibition (14). Eghbali et al. (15) also reported that GABA_A-channel conductance was increased by diazepam in cultured hippocampal neurons.

Sodium valproate was reported to increase GABA contents in the whole brain and nerve terminals of rats, although its mechanisms of action are still unclear (16, 17). Neither carbamazepine nor ethosuximide could inhibit the epileptic seizures induced by teicoplanin. It is well known that carbamazepine has effective anti-epileptic properties through the blockade of Na⁺ channels and the inhibition of N-methyl-D-aspartate (NMDA)-induced elevation of intracellular Ca²⁺ concentration (18–21). On the other hand, ethosuximide is considered to affect prototypical absence seizure by inhibiting T-type Ca²⁺ channels in isolated rat thalamic neurons (22, 23).

From the above findings, it is concluded that teicoplanin-induced epileptogenic seizure is closely related with the GABA-ergic mechanism, but a mechanism through Na⁺ channels, NMDA, and Ca²⁺ channels may not contribute to teicoplanin-induced epileptogenic seizure.

References

- 1 Zetola N, Francis JS, Nuermberger EL, Bishai WR. Community-acquired methicillin-resistant *Staphylococcus aureus*: an emerging threat. *Lancet Infect Dis*. 2005;5:275–286.
- 2 Drago L, De Vecchi E, Nicola L, Gismondo MR. In vitro evaluation of antibiotics' combinations for empirical therapy of suspected methicillin resistant *Staphylococcus aureus* severe respiratory infections. *BMC Infect Dis*. 2007;7:111.
- 3 Kamei C, Sunami A, Tasaka K. Effects of certain antiepileptics on cephaloridine- and cefazolin-induced seizures in rats. *Arch Int Pharmacodyn Ther*. 1983;264:220–231.
- 4 Yu QH, Kitazumi K, Kamei C, Tasaka K. Epileptogenic activity induced by intravenous injection of certain cephalosporins in rats. *J Pharmacobiodyn*. 1984;7:586–592.
- 5 Grondahl TO, Langmoen IA. Epileptogenic effect of antibiotic drugs. *J Neurosurg*. 1993;78:938–943.
- 6 Rahman A, Ago J, Matsumoto N, Ishikawa T, Kamei C. Epileptogenic activity of methicillin-resistant *Staphylococcus aureus* (MRSA) antibiotics in rats. *Biol Pharm Bull*. 2006;29:2035–2040.
- 7 Franklin KBJ, Paxinos G. The mouse brain in stereotaxic coordinates. London: Academic Press; 1996.
- 8 Bernareggi A, Cavenaghi L, Assandri A. Pharmacokinetics of [¹⁴C]teicoplanin in male rats after single intravenous dose. *Antimicrob Agents Chemother*. 1986;30:733–738.
- 9 Matzke GR, Zhanel GG, Guay DR. Clinical pharmacokinetics of vancomycin. *Clin Pharmacokinet*. 1986;11:257–282.
- 10 Moellering RC Jr, Krogstad DJ, Greenblatt DJ. Pharmacokinetics of vancomycin in normal subjects and in patients with reduced renal function. *Rev Infect Dis*. 1981;3:230–235.
- 11 Infection in Neurosurgery Working Party of the British Society for Antimicrobial Chemotherapy. The management of neurosurgical patients with postoperative bacterial or aseptic meningitis or external ventricular drain-associated ventriculitis. *Br J Neurosurg*. 2000;14:7–12.
- 12 Kamei C, Sunami A, Tasaka K. Epileptogenic activity of cephalosporins in rats and their structure-activity relationship. *Epilepsia*. 1983;24:431–439.
- 13 Bambeke FV. Glycopeptides in clinical development: pharmacological profile and clinical perspectives. *Curr Opin Pharmacol*. 2004;4:471–478.
- 14 Macdonald RL, Kapur J. Acute cellular alterations in the hippocampus after status epilepticus. *Epilepsia*. 1999;40:9–22.
- 15 Eghbali M, Curmi JP, Birnir B, Gage PW. Hippocampal GABA(A) channel conductance increased by diazepam. *Nature*. 1997;388:71–75.
- 16 Löscher W. New visions in the pharmacology of anticonvulsion. *Eur J Pharmacol*. 1998;342:1–13.
- 17 Löscher W. Basic pharmacology of valproate: a review after 35 years of clinical use for the treatment of epilepsy. *CNS Drugs*. 2002;16:669–694.
- 18 Macdonald RL, Kelly KM. Mechanisms of action of currently prescribed and newly developed antiepileptic drugs. *Epilepsia*. 1994;35:41–50.
- 19 Lampe H, Bigalke H. Carbamazepine blocks NMDA-activated currents in cultured spinal cord neurons. *Neuroreport*. 1990;1:26–28.
- 20 Cai Z, McCaslin PP. Amitriptyline, desipramine, cyproheptadine and carbamazepine, in concentrations used therapeutically, reduce kainate- and N-methyl-D-aspartate-induced intracellular Ca²⁺ levels in neuronal culture. *Eur J Pharmacol*. 1992;219:53–57.
- 21 Hough CJ, Irwin RP, Gao XM, Rogawski MA, Chuang DM. Carbamazepine inhibition of N-methyl-D-aspartate-evoked calcium influx in rat cerebellar granule cells. *J Pharmacol Exp Ther*. 1996;276:143–149.
- 22 Gomora JC, Daud AN, Weiergraber M, Perez-Reyes E. Block of cloned human T-type calcium channels by succinimide antiepileptic drugs. *Mol Pharmacol*. 2001;60:1121–1132.
- 23 Coulter DA. Characterization of ethosuximide reduction of low-threshold calcium current in thalamic neurons. *Ann Neurol*. 1989;25:582–593.