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Amino Acid Neurotransmitter Levels in the Cerebral Cortex of Mice Receiving Imipenem/Cilastatin -Lack of Excitotoxicity in the Central Nervous System

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Abstract: Imipenem, a very potent carbapenem derivative beta-lactam antibiotic, has recently found a major place in the treatment of antibiotic-resistant nosocomial infections. However, a convulsive side effect is seen in 0.2-3 percent of patients. Although it is suggested that this effect is due to the inhibition of gamma-aminobutyric acid (GABA) mediated inhibitory transmission, no study has been reported so far showing its effect on the cerebral cortex free inhibitory and excitatory amino acid levels.

Twenty-one male TO albino mice were divided into three equal groups and given therapeutic (40 mg/kg/day) or excessive (500 mg/kg/day) doses of Imipenem/cilastatine (I/C) or saline solution intraperitoneally for 7 days. All animals in the excessive dose group showed seizure-like acivity with ataxia and loss of gait. However, no differences in aspartate, glumatate, glycine or GABA levels were seen on gas chromatographic evaluation of the cerebral cortexes of all three groups of animals, which were dispatched on the 7th day. Therefore it is suggested that imipenem exerts its convulsive effect without causing any change in neurotansmitter levels of barin, possibly by effecting the neuronal receptors directly.

Key Words: Amino acid, imipenem, mice, neurotoxicity.

Introduction

Several classes of pharmacological agents have been implicated in cases of drug-induced seizures, such as antidepressants, neuroleptics, antihistamines, central nervous system (CNS) stimulants, general and local anaesthetic, and antimicrobial agents (1-3). The epileptogenic properties of beta-lactams are well known. Carbapenems are broad-spectrum beta-lactam antibiotics that cover an absolute majority of all bacterial pathogens that possess a cell wall. Imipenem, the first carbapenem antibiotic introduced in the clinical field, is a derivative of thienamycin containing a nuclear carbapenem ring combined with cilastain, which inhibits the enzymatic breakdown of imipenem in the kidney (4, 5). Seizures related to imipenem have been described in both clinical studies and experimental models. An incidence of seizure of 0.2 to 3 percent has been noted among patients treated with imipenem (6-8). It is reported that the average time of onset for seizures in patients receiving imipenem was seven days after the start of therapy (6). De Sarro et al. (9) revealed that there was no significant difference in the onset, duration and severity of seizures with imipenem alone or in combination with cilastatin, and that cilastatin by itself did not have convulsive effects.

Physiological and anatomical evidence suggets that amino acid transmitters carry the major excitatory/inhibitory commands in the CNS and that other types of CNS transmitters play more diffuse modulatory roles. Gamma-aminobutyric acid (GABA) and glycine are the principal inhibitory tranmitters, whereas glutamate and aspartate are presumed to be the excitatory transmitters (10). It was suggested that the induction of beta-lactam-related convulsions was caused predominantly by the inhibition of GABA-mediated inhibitory transmission (11-13). However, no research has yet been reported on the free cerebral amino acid levels. Therefore, this study was planned in order to determine the free excitatory and inhibitory amino acid levels in the cerebral cortex of mice reveiving therapeutic or excessive doses of imipenem.

Materials and Methods

Animals: The study group consisted of twenty-one

Table 1. Excitatory and inhibitory free amino acid levels in mouse brain cortex receiving therapeutic or excessive doses imipenem/cilastatine (I/C).

	Free amino acid levels (µmol/g brain tissue)			
	Aspartate	Glutamate	Glycine	GABA*
Group I	0.08±0.03	0.21±0.09	0.11±0.06	0.24±0.08
Group II	0.04±0.02	0.27±0.23	0.16±0.03	0.18±0.04
Group III	0.06±0.02	0.38±0.24	0.26±0.16	0.17±0.07
p	>0.05	>0.05	>0.05	>0.05

Results expressed as mean±SD

*GABA: gamma-aminobutyric acid

Group I: Animals receiving therapeutic dose (40 mg/kg/day)

Group II: Animals receiving high dose (500 mg/kg/day)

Group III: Animals receiving saline only (Control group)

male TO albino mice (Ege University Veterinary Research Institute) weighing 20-28 grams. They were housed in an air-conditioned room and fed a standard diet and water.

Drug and drug administration:

Imipenem/cilastatine (I/C) was obtained from Merck Sharp & Dohme, İstanbul, Turkey. The drug was dissolved in sterile water. Group I consisted of seven mice given (I/C) 20 mg/kg/dose every 12 hours intraperitoneally for 7 days. Group II consisted of mice given I/C 250 mg/kg/dose every 12 hours intraperitoneally for 7 days. Group III, the control group, consisted of seven mice given physiologic saline (0.9% NaCl) intraperitoneally for 7 days.

Behavioural observations:

Animals were placed in their cages and observed for 60 minutes after intraperitoneal administration of I/C and behavioural events were recorded.

Preparation of brain tissue for gas chromatographic separation

On the 7th day, all mice were killed by cervical dislocation and decapitation. The brain was removed and the cerebral cortex was separated, weighed and placed in saturated picric acid (1/10) and 1ml of 2.5mM norleucine was added as an internal standard. The tissue was homogenized at 1400rpm using a glass-teflon homogenizer and centrifuged at 3000 x g for 15 minutes. The supernatant was eluted through glass columns (0.9 cm x 7 cm) containing Dowex 50 W x 12 and washed with bidistilled water. The amino acid fraction was collected by passing 7 N NH_4OH through the column with a flow rate of 3 ml/minute and then lyophilized. The lyophilizate was dissolved in 0.1 N HCl and transferred to acetylation tubes with teflon-lined screw caps. Esterification occurred in 15 minutes at 100°C with n-butanol 3N HCl, and acetylation in 5 minutes at 150°C with trifluoroacetic acid anhydryde. The amino acid trifluoro acetyl n-butyl derivatives obtained were brought to dryness under nitrogen and stored at -20°C in chloroform until gas chromatographic separation occurred (14).

Amino acid analysis

A Pye-Unicam 104 gas chromatograph was used for separation. The amino acids were separated in 2mm x 180cm glass columns containing 0.65% EGA chromosorb W. NAW using a 6°C/ min program between 60-120°C. The areas under the amino acid peaks were calculated using an integrator (Spectra Physics Minigrator) and the actual amino acid levels were determined (14).

Statistics

All values were expressed as mean±SD. Biochemical data were analyzed by ANOVA (one way). Statistical significance was defined at p values of less than 0.05.

Results

No seizure activity was observed in groups I and III. However, all animals in group II receiving excessive doses of I/C showed seizure-like activity (on days 3-7, average 5.7±1.3) within 15 minutes of receiving the injection and lasted approximately 4 hours. Seizure-like activity (ataxia) was characterized by loss of coordination of the legs and was easily detected by the sudden cessation of walking or running. Inhibitory and excitatory amino acid levels in the cerebral cortex are summarized in Table 1. The administration of therapeutic or excessive doses of I/C did not alter neurotransmitter amino acid levels in the

cerebral cortex, as all three groups had similar values ($p>0.05$).

Discussion

Data obtained from both animals and humans support the hypothesis that imipenem is a more potent inducer of seizure activity than other beta-lactam antibiotics (9, 11, 14-16). The risk of neurotoxicity with imipenem has become a factor impeding the use of high doses. Factors known to increase the risk of seizure are excessive doses, decreased renal functions, damage to the blood-brain barrier, preexisting CNS diseases (stroke, tumours, trauma), old age and concurrent use of drugs that are nephrotoxic (e.g. cyclosporin) or that may lower the seizure threshold (e.g. theophylline) (6, 7, 11).

In animal models, neurotoxic reactions have frequently appeared when beta-lactam antibiotics were applied to the brain surface or in the cerebral cisterns. Epileptogenic reactions have also been observed following the administration of very high systemic doses and it has been revealed that neurotoxicity seemed to be related to antibiotic concentration in brain tissue rather than to cerebrospinal fluid concentrations (11, 15, 17). In our study, seizure-like activity was observed in the excessive dose group.

Imipenem CNS pharmacokinetics may play an important role in inducing seizures because imipenem is transported through the blood-brain barrier principally by passive diffusion and its efflux from the CNS through the blood-cerebrospinal fluid barrier is slow (18). Although the main mechanism for the seizure activity of imipenem cannot be easily determined, a possible pathogenic mechanism is excitation of the CNS neurons by blocking the synaptic activity of GABA, possibly at its receptor level (11, 12). Williams et al. (13) showed that imipenem was able to inhibit the binding of ^{3}H-labelled GABA to synaptic membranes from rat brains, suggesting an interaction with GABA receptors.

GABA is the dominant inhibitory neurotransmitter in CNS and acts on at least two types of receptors, $GABA_A$ and $GABA_B$. Glycine classically has been considered to be an inhibitory transmitter, like GABA, but with a more limited distribution (especially in the hindbrain and spinal cord) (10). Glutamate and aspartate have powerful excitatory effects on neurons, and their interactions with specific membrane receptors are responsible for many neurological functions. They have four main receptor subtypes: N-methyl-D-aspartate (NMDA), quisqualic acid, kainic acid, and L-aminophosphonobutyric acid (10). Excessive activation of glutamate receptors may mediate neuronal injury and death. Excitotoxicity has been suggested, a final common pathway for neuronal injury due to diseases with diverse pathophysiological processes, such as cerebral ischemia, anoxia, stroke, hypoglisemia, trauma and epilepsy (19). Large amounts of glutamate stored in presynaptic terminals and other intracellular locations normally have rapid extracellular accumulation in impaired cellular uptake during cerebral ischemia, causing neuronal injury. Therefore, NMDA receptor antagonists have been used in several experimental trials in order to prevent cerebral injury after ischemia (19, 20).

De-Sarro et al. (21) have suggested an alternative hypothesis on the involvement of other neurotransmitter systems on imipenem-induced seizures. They demonstrated that MK-801, a strong NMDA receptor antagonist, showed prominent anticonvulsant activity after both intraperitoneal and intracerebroventricular I/C administration. Although imipenem-induced seizures cannot be easily determined, potential interactions with the receptors of the excitatory amino acid neurotransmitters exist. In fact, antagonists of excitatory amino acids are able to prevent seizures by increasing their threshold. In addition, De-Sarro et al. (21) showed that muscimol, a selective $GABA_A$ agonist, was able to protect against seizures induced by imipenem following intracerebroventricular I/C administration. However, in our study no difference was found between the test groups and the control group in excitatory and inhibitory free amino acid levels in the cerebral cortex.

To our knowledge there has been no previous study evaluating the free excitatory and inhibitory amino acid levels in animals receiving imipenem. This is the first and only study showing that no significant change occurs in these levels in animals receiving therapeutic or high doses of imipenem even though the latter group also showed clinical seizure. Therefore, our data suggest that imipenem-related neurotoxicity was neither due to increased excitotoxicity nor to diminished inhibitory amino acid levels in the cerebral cortex. The epileptogenic properties of beta-lactams and imipenem may be related to the inhibition of the GABA binding to its receptor sides.

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