Effects of manipulation of N-methyl-D-aspartate receptors on imipenem/cilastatin-induced seizures in rats

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Background & objectives: Epileptic seizures have been reported in patients on imipenem/cilastatin (Imi/Cil) therapy. To investigate contribution of N-methyl-D-aspartate (NMDA) receptors in inducing imipenem/cilastatin (Imi/Cil) seizures, the effects of competitive NMDA antagonist, APV [(±)-2-amino-5-phosphonovaleric acid], non-competitive NMDA antagonist remacemide [(±)-2-amino-N-(1-methyl-1,2-diphenylethyl)-acetamide], and glycine receptor partial agonist HA-966 [(±)-(3-amino-1-hydroxypyrrolid-2-one)] on electroencephalographic (EEG) activity and behaviour were studied in rats.

Methods: Adult male Wistar albino rats were implanted with electrodes and cannulae were placed into the right lateral ventricle. Animals were divided into five groups: (i) saline (icv)+Imi/Cil (ii) APV (0.2 µmol)+Imi/Cil, (iii) APV (0.4 µmol)+Imi/Cil, (iv) remacemide (100 mg/kg, ip)+Imi/Cil, and (v) HA-966 (200 µg, icv)+Imi/Cil. The drugs were administered 30 min before icv injection of Imi/Cil (100/100 µg), and their effects on incidence of seizures, latencies to EEG changes and convulsions, severity, lethality and time to lethal outcome were studied.

Results: Imi/Cil provoked complete seizure response in all rats and all animals died within 10-18 min after the injection. EEG epileptiform activity preceded behavioral seizures. Clonic-tonic seizures were characterized by continuous bursts of high frequency high amplitude spikes in the EEG. The dose of 0.2 µmol of APV prolonged only the latency to the first EEG changes, while 0.4 µmol dose significantly influenced all seizure parameters. HA-966 increased only the latency to Imi/Cil-induced convulsions, while remacemide had no significant effect on seizure parameters.

Interpretation & conclusion: The results suggested that excitatory neurotransmission contributed to the generation and/or propagation of Imi/Cil-induced seizures in rats, and that the effects of NMDA antagonists depended on a particular binding site within the NMDA receptor complex, and affinity to that site.

Key words APV - EEG - HA-966 - imipenem/cilastatin - NMDA - remacemide - seizures
receptor complex, acts as functional antagonist in vivo. HA-966 antagonized seizures elicited by NMDA, N-methyl-D, L-aspartate (NMDLA), sound-induced seizures in mice, and raised the threshold in amygdala kindling epilepsy in rats.

Imipenem is a broad-spectrum antibiotic, which is used for treatment of serious infections in clinics in combination with cilastatin, an inhibitor of enzyme dipeptidase. Interestingly, preclinical studies have not revealed the convulsant action of imipenem, and seizures have been reported for the first time in patients on imipenem/cilastatin (Imi/Cil) therapy. In experimental animals Imi/Cil induced seizures after systemic and icv administration. Cilastatin per se, administered at high doses, did not provoke convulsions. Convulsant effects of Imi/Cil depend on animal species and route of administration. In DBA/2 and C57 mice Imi/Cil provoked seizures in the form of running, clonus and tonus, while in rats seizures were of limbic type. The convulsant effect of imipenem in mice was due to decrease in inhibition and increase in excitation; GABA agonist muscimol, a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate antagonists, competitive and non-competitive NMDA antagonists effectively blocked Imi/Cil-induced seizures in DBA/2 mice.

Binding of [3H]GABA to GABA receptors was decreased in the presence of imipenem.

In order to investigate if NMDA receptors contribute to the development of Imi/Cil-induced epilepsy in rats, the present study was carried out to the effects of APV, remacemide and HA-966 on behavioural and EEG characteristics of Imi/Cil-induced seizures.

Material & Methods

The drugs used in this study were imipenem/cilastatin (Thienam®, Merck Sharp & Dohme B. V., Haarlem, Holland), APV (ICN Pharmaceuticals Inc, Costa Mesa, CA, USA), remacemide (Astra Charnwood, Loughborough, England), and HA-966 (Tocris, Bristol, England).

Adult male Wistar albino rats, weighing about 250 g, were used in experiments (Military Medical Academy Breeding Laboratories, Belgrade, Serbia). The study protocol was approved by the Animal Care Committee of University of Belgrade. The rats were housed individually in transparent plastic cages (50x35x30 cm) under standard conditions (temperature 22±1ºC, humidity 50%, light:dark cycle 12:12h). Food and water were continuously available.

Animals were anaesthetized with sodium pentobarbital (40 mg/kg, ip) and positioned in the stereotaxic apparatus. Cannulae were implanted into the right lateral ventricle (coordinates from bregma: AP=-1.3, L=2.0, 4.5 mm deep from the skull surface), and electrodes were fixed to the skull with dental acrylic cement.

Rats were allowed to recover from surgery one week before the experiments started. The animals were randomly assigned to the following groups: (i) saline (icv)+Imi/Cil (100/100 µg, icv, n=8), (ii) APV (0.2 µmol, icv)+Imi/Cil (100/100 µg, icv, n=6), (iii) APV (0.4 µmol, icv)+Imi/Cil (100/100 µg, icv, n=8), (iv) remacemide (100 mg/kg, ip)+Imi/Cil (100/100 µg, icv, n=8), and (v) HA-966 (200 µg, icv)+Imi/Cil (100/100 µg, icv, n=6).

The drugs were freshly dissolved in physiological saline before administrations. Remacemide was injected ip, while APV and HA-966 were administered icv 30 min before icv injection of Imi/Cil. The volume of remacemide injection was 0.1 ml. APV, HA-966 and Imi/Cil were applied by a 10 µl Hamilton syringe. APV and HA-966 were applied in a volume of 5 µl, and Imi/Cil in a volume of 10 µl. The rate of injection was 1 µl/5 sec. During the administration the rats were gently restrained by hand.

EEG activity was recorded by means of an 8-channel EEG apparatus (Alvar, France). Analog data were digitized at a sampling rate of 128/sec and after analog to digital conversion the analog EEG data were stored on hard disk. EEG tracings were analyzed visually and power spectral analysis was
provided by FFT (Fast Fourier Transformation).

The latency to seizures, latency to the first EEG changes, seizure severity, lethality and time to lethal outcome were measured.

Seizure severity grade was scored on the descriptive rating scale: 0 _ normal behavior, 1 _ twitching, 2 _ fore limb clonus, head nodding, 3 _ rearing, and 4 _ clonic-tonic convulsions.

At the end of experiments, before sacrificing the animals, 5 µl of blue ink was injected icv. Brains were examined to ensure that the dye was distributed throughout the ventricular spaces. The cannulae were found to be placed correctly in the lateral ventricle in all animals.

Data were statistically analysed using Kruskal-Wallis test and Mann-Whitney U test (for latencies to EEG changes, latencies to convulsions, seizure severity, and time to lethal outcome) and Fisher's exact probability test (for number of animals that died).

**Results**

In the EEG there were no signs of spontaneous epileptiform activity before Imi/Cil (100/100 µg, icv) injection, and all tracings had low spectral powers. All animals treated with Imi/Cil exhibited severe clonic-tonic seizures and died within 10-18 min (13.8±2.5) post-injection. EEG epileptiform activity preceded behavioral convulsions (Table). Clonic-tonic seizures were associated with continuous bursts of high-amplitude spikes occurring at 5-8 per sec, and increase in power spectra (Fig. 1).

<table>
<thead>
<tr>
<th>Group (N)</th>
<th>Latency to seizures (min)</th>
<th>Latency to EEG changes (min)</th>
<th>Grade</th>
<th>Lethality</th>
<th>Time to lethality (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline+Imi/Cil (8)</td>
<td>1.4±0.8</td>
<td>0.7±0.2</td>
<td>4.0±0.0</td>
<td>8</td>
<td>13.8±2.5</td>
</tr>
<tr>
<td>APV (0.2)+Imi/Cil (6)</td>
<td>2.2±1.0</td>
<td>2.1±0.7*</td>
<td>3.5±0.8</td>
<td>4</td>
<td>23.7±10.5</td>
</tr>
<tr>
<td>APV (0.4)+Imi/Cil (8)</td>
<td>7.1±7.0*</td>
<td>5.7±4.8*</td>
<td>2.6±1.4*</td>
<td>3#</td>
<td>26.0±12.0*</td>
</tr>
<tr>
<td>Remacemide (100)+Imi/Cil (8)</td>
<td>2.0±1.2</td>
<td>0.61±0.51</td>
<td>3.7±0.7</td>
<td>6</td>
<td>17.7±4.0</td>
</tr>
<tr>
<td>HA-966 (200)+Imi/Cil (6)</td>
<td>3.2±1.4*</td>
<td>1.33±0.65</td>
<td>3.3±1.6</td>
<td>3</td>
<td>26.9±14.9</td>
</tr>
</tbody>
</table>

* P <0.05 (Mann-Whitney U test) compared to saline+Imi/Cil group
# P <0.05 (Fisher’s exact probability test) compared to saline+Imi/Cil group
APV - (±) - 2-amino-5-phosphonovaleric acid
HA-966-(±)-(3-amino-1-hydroxypyrrolid-2-one)
Administration of APV produced hypotonia and ataxia in a dose-dependent manner, while in the EEG sharp waves and spikes were recorded (Fig. 2). The only significant effect of APV at a dose of 0.2 µmol was on latency to the first EEG discharges, which appeared later compared to rats treated with saline+Imi/Cil ($P<0.05$) (Table). The dose of 0.4 µmol of APV significantly increased the latency to the first EEG changes, latency to seizures, and time to lethal outcome ($P<0.05$). The same dose significantly decreased the lethality ($P<0.05$) and seizure severity ($P<0.05$) compared to saline group (Table). Rats, which survived clonic-tonic convulsions, characteristically developed less severe seizures in the form of fore limb clonus, head nodding and twitching. However, APV did not reduce epileptiform discharges during behavioral seizures elicited by Imi/Cil (100/100 µg, icv) (Fig. 2).

Injection of remacemide (100 mg/kg, ip) elicited sedation, hypotonia, and decreased locomotor activity in animals. The righting reflex was preserved. Remacemide (100 mg/kg, ip) did not affect significantly any parameter of Imi/Cil-induced convulsions (Table). The latency to the first spikes after Imi/Cil injection was shorter in animals pretreated with remacemide, compared to Imi/Cil group (Table). Remacemide did not suppress epileptic activity in the EEG elicited by Imi/Cil (100/100 µg, icv) (Fig. 3).

Administration of HA-966 (200 µg, icv) provoked hypotonia and occasionally twitching of head and fore limbs in 3 out of 6 animals. HA-966 significantly ($P<0.05$) increased only the latency to Imi/Cil-induced seizures (Table). The number of animals that died was decreased, and almost reached the level of significance ($P=0.054$). There was no change in epileptiform discharges elicited by Imi/Cil due to HA-966 (Fig.4).

Discussion
The anticonvulsant action of APV against Imi/Cil-induced seizures in rats was in agreement with the results of previous studies in rodent models \(^4,6,8\). APV inhibited Imi/Cil-induced seizures in a dose-

![Fig. 2. EEG correlates and their power spectra: A - control, B - 18 min after APV administration (0.4 µmol, icv), and C - clonic-tonic seizure 6 min after Imi/Cil (100/100 µg, icv) and 36 min after APV (0.4 µmol, icv) injection. Lower tracing is continuation of the upper. FP - fronto-parietal cortex. Time calibration 1 sec, voltage 100 µv.](image1)

![Fig. 3. EEG correlates and their power spectra: A - control, B - clonic-tonic seizure 2 min after Imi/Cil (100/100 µg, icv) and 32 min after remacemide (100 mg/kg, ip) administration. Lower tracing is continuation of the upper. FP - fronto-parietal cortex. Time calibration 1 sec, voltage 100 µV.](image2)
dependent fashion. High dose of APV (0.4 μmol) significantly influenced all parameters of Imi/Cil-induced seizures, but did not abolish convulsions, or lethality. Since APV had anticonvulsant effect against Imi/Cil (100/100 μg, icv)-induced seizures without affecting epileptiform activity, it might be suggested that APV acted as an anticonvulsant rather than an antiepileptic agent in this model of epilepsy. In contrast to this result, APV suppressed both myoclonic jerks and high-voltage spikes elicited by penicillin in rats. The effective dose of APV against Imi/Cil-induced seizures in rats was higher than in other epilepsy models, in which the increase of excitation in the CNS was the main factor provoking seizures. APV inhibited seizures elicited by electrical stimulation of the amygdala in a dose range tested in the present study (0.2 μmol). The different potency of APV in various models of epilepsy may be due to a different contribution of excitatory neurotransmission and NMDA receptors in seizure initiation and propagation.

HA-966 (200 μg, icv) significantly increased only the latency to Imi/Cil-induced seizures, while lower doses (10-100 μg, icv) had no significant effect on latency to NMDLA-elicited seizures in mice. HA-966 (icv) blocked NMDA-induced convulsions with ED₅₀ of 184 μg, but was not effective in antagonizing seizures elicited by kainate and quisqualate in mice. Higher doses of HA-966 were not used in the present study, since the dose of 200 μg produced proconvulsant effect, which was not reported in studies using lower doses. Generally, all categories of NMDA receptor antagonists at high doses exert proconvulsant effects.

Remacemide (100 mg/kg, ip) caused no significant change in any parameter of Imi/Cil-induced seizures in rats. In other models remacemide was effective at much lower doses (6-66 mg/kg). MK-801 (5-methyl-10, 11-dihydro-5H-dibenzo[a,d]cyclohepten-5, 10-imine), another non-competitive NMDA receptor antagonist, significantly decreased the incidence and severity of Imi/Cil-induced convulsions in DBA/2 mice after both ip and icv application. In contrast to remacemide, MK-801 has high affinity to PCP (phencyclidine) recognition site of NMDA receptor complex. It is possible that this difference in affinity, besides species difference, contributes to the
different potency of non-competitive NMDA antagonists against Imi/Cil-induced convulsions. It has been reported that remacemide decreased epileptiform discharges in absence epilepsy\textsuperscript{11}, and that normalized EEG in epileptic patients\textsuperscript{25}. Adverse behavioural effects of remacemide and HA-966 in our model were in agreement with literature\textsuperscript{14,26}. Remacemide did not suppress seizures induced by bicuculline, picrotoxine, strychnine and pentylenetetrazole\textsuperscript{10}, in which decrease of inhibition in the CNS plays a key role for generation of seizures, like for imipenem.

The results of this study, together with that of a previous study in mice\textsuperscript{18}, indicated the importance of NMDA receptors in inducing Imi/Cil seizures, but has not ruled out contribution of other mechanisms in provoking the seizures. It has been reported that GABA-ergic system influenced Imi/Cil-elicited seizures\textsuperscript{18}, while the roles of cholinergic, dopaminergic, opioid and peptidergic systems are to be determined.

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**References**


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