

## The effects of phenytoin and phenobarbital on seizures induced by imipenem/cilastatin in rats

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### Abstract

The effects of phenytoin (PHT) and phenobarbital (PHB) on EEG activity and behavior was studied in the model of epilepsy induced by intracerebroventricular (i.c.v.) administration of imipenem/cilastatin (Imi/Cil). Under intraperitoneal (i.p.) sodium pentobarbital anesthesia adult male Wistar albino rats were implanted with electrodes and cannulas were placed into the right lateral ventricle. Animals were divided into groups: 1) Imi/Cil (100/100 µg, i.c.v.), 2) PHT (40 mg/kg) + Imi/Cil (100/100 µg, i.c.v.), 3) PHT (80 mg/kg) + Imi/Cil (100/100 µg, i.c.v.), 4) PHT (160 mg/kg) + Imi/Cil (100/100 µg, i.c.v.), 5) PHB (50 mg/kg) + Imi/Cil (100/100 µg, i.c.v.), and 6) PHB (80 mg/kg) + Imi/Cil (100/100 µg, i.c.v.). PHT and PHB were injected intraperitoneally (i.p.) 1 h before Imi/Cil. Seizures were scored according to the scale: 0 – normal behavior, 1 – twitching, 2 – head nodding, forelimb clonus, 3 – rearing, and 4 – clonic-tonic convulsions. Imi/Cil provoked maximal seizures in all animals, and all rats died 10-18 min after the injection. Epileptiform activity preceded behavioral seizures. Clonic-tonic seizures were associated with continuous bursts of high-frequency high-amplitude spikes in the EEG. PHT and PHB suppressed Imi/Cil-induced seizures dose-dependently. PHB reduced epileptiform discharges during behavioral seizures elicited by Imi/Cil, while PHT had no effect on EEG epileptic phenomena. These results suggest that PHT acts as anticonvulsant, and PHB as anticonvulsant and antiepileptic agent in the model of epilepsy induced by Imi/Cil.

**Key words:** Imipenem/cilastatin; seizures; phenytoin; phenobarbital; EEG; rat.

### Introduction

Phenytoin (PHT) and phenobarbital (PHB) are classical antiepileptic drugs. Clinically PHT is effective mainly against focal epileptic seizures, but also against generalized tonic-clonic convulsions. PHB exhibits marked effects against generalized clonic-tonic as well as against partial seizures. PHT and PHB influence on both excitatory and inhibitory neurotransmission. The most important mechanisms of action of PHT are decrease of per-

meability of voltage-gated sodium channels and calcium channels (preferentially L-type). PHT blocks K-stimulated influx of Ca<sup>2+</sup> into neurones (Pincus and Lee, 1973), and decreases synaptic release of glutamate in vitro (Potter *et al.*, 1991). It has been shown that PHT suppresses the spread of epileptic activity from the focus (Edmonds *et al.*, 1974). PHB prolongs the time of opening of GABA-associated Cl<sup>-</sup> channels. It has been found that barbiturates block AMPA/kainate receptors in a non-competitive manner (Miljkovic and MacDonald, 1986).

PHT and PHB are effective anticonvulsants in many models of epilepsy. They suppressed audiogenic seizures in genetically epilepsy-prone rats (GEPR) (Dailey and Jobe, 1985), sound-induced convulsions in metaphit (1-[1(3-isothiocyanatophenyl)cyclohexyl]piperidine)-treated mice (Debler *et al.*, 1993), seizures elicited by electroshock and bicuculine (Clineschmidt *et al.*, 1982). They also decreased the incidence of convulsions induced by intracerebroventricular administration of L-glutamate in mice (Stone and Javid, 1983). Phenytoin did not block seizures induced by pentylenetetrazole (PTZ), strychnine, N-methyl-D-aspartate (NMDA) and kainate, while PHB was effective in these models (Clineschmidt *et al.*, 1982; Czuczwar *et al.*, 1985; Koek and Colpaert, 1990; Kubova and Mares 1991; Velisek *et al.*, 1992). None of them suppressed seizures elicited by N-methyl-D,L-aspartate (NMDLA) in mice (Czuczwar *et al.*, 1985).

The convulsant action of antibiotics is known for decades, and was described for the first time after the use of penicillin (Johnson and Walter, 1945). Imipenem is the first carbapenem antibiotic introduced in clinics. It is used in combination with cilastatin, an inhibitor of enzyme dipeptidase. Interestingly, preclinical studies did not reveal the convulsant activity of imipenem, and the seizures have been reported for the first time in patients on therapy with imipenem/cilastatin (Imi/Cil) (Barza, 1985). Seizures, mostly in the form of generalized convulsions, but also focal and myoclonic seizures,

appeared a few hours to several days after introducing the therapy with imipenem (Calandra *et al.*, 1986; Eng *et al.*, 1989). Phenytoin, diazepam and phenobarbital were effective in suppressing clinical seizures induced by imipenem/cilastatin (Eng *et al.*, 1989). According to different studies the incidence of Imi/Cil-induced seizures in patients varied from 0.9 to 22% (Barza, 1985; Calandra *et al.*, 1986; Eng *et al.*, 1989), while more recent study reported that careful use of this antibiotic is safe (Koppel *et al.*, 2001).

After the seizures have been observed in clinical practice, a series of experiments on convulsant activity of imipenem were conducted in animals. Convulsant effects of Imi/Cil depend on animal species and route of administration. Imi/Cil induced seizures after systemic (De Sarro *et al.*, 1995a) and intracerebroventricular (i.c.v.) application (Hikida *et al.*, 1993, De Sarro *et al.*, 1995a). Cilastatin itself, administered at high doses, did not provoke convulsions (De Sarro *et al.*, 1995b). In DBA/2 mice Imi/Cil provoked seizures in the form of running, clonus and tonus (De Sarro *et al.*, 1995a), while in rats seizures were of limbic type (Hikida *et al.*, 1993). Binding of [<sup>3</sup>H]GABA (Williams *et al.*, 1988), [<sup>3</sup>H]muscimol (Day *et al.*, 1995), and [<sup>3</sup>H]diazepam (Hikida *et al.*, 1993) to GABA<sub>A</sub> receptors was decreased in the presence of imipenem. Other  $\beta$ -lactam antibiotics did not reduce the binding of GABA, but like imipenem, they had proconvulsant effect on seizures induced by pentylene-tetrazole (Williams *et al.*, 1988), indicating that the convulsant effect of imipenem is due to decrease of inhibition, and due to other mechanisms. Antagonists of  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors, competitive and non-competitive NMDA receptor antagonists and muscimol blocked seizures induced by imipenem in mice (De Sarro *et al.*, 1995).

In order to evaluate the model of seizures induced by imipenem/cilastatin as a model for drug screening, and to assess the effects of antiepileptic drugs on epileptiform activity, we investigated the effects of PHT and PHB on behavioral and EEG characteristics of imipenem/cilastatin-induced seizures in rats.

### Methods and materials

Adult male Wistar albino rats, weighing about 250 g, were used in experiments (Military Medical Academy Breeding Laboratories, Belgrade, Serbia). The experiments were performed in accordance with the Declaration of Helsinki. Animals were kept individually in transparent cages (55 × 35 × 30 cm) under standard conditions (temperature 22 ± 1°C, humidity 50%, light/dark cycle 12/12 h) with food and water available ad libitum.

Under intraperitoneal (i.p.) sodium pentobarbital (40 mg/kg) anesthesia the animals were positioned

in the stereotaxic apparatus. Cannulas were placed into the right lateral ventricle (coordinates from bregma : AP = -1.3, L = 2.0, 4.5 mm deep from the skull surface) (Paxinos and Watson, 1982), and electrodes (gold-plated screws) were implanted over the frontal, parietal and occipital cortices. Cannulas 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 : 1) Imi/Cil (100/100  $\mu$ g, i.c.v.), n = 8, 2) PHT (40 mg/kg, i.p.) + Imi/Cil (100/100  $\mu$ g, i.c.v.), n = 6, 3) PHT (80 mg/kg, i.p.) + Imi/Cil (100/100  $\mu$ g, i.c.v.), n = 6, 4) PHT (160 mg/kg, i.p.) + Imi/Cil (100/100  $\mu$ g, i.c.v.), n = 6, 5) PHB (50 mg/kg, i.p.) + Imi/Cil (100/100  $\mu$ g, i.c.v.), n = 6, and 7) PHB (80 mg/kg, i.p.) + Imi/Cil (100/100  $\mu$ g, i.c.v.), n = 6.

Phenytoin and phenobarbital were injected i.p. 1 h before i.c.v. injection of Imi/Cil. Imi/Cil was freshly dissolved in physiological saline before the administration. Imi/Cil was applied by a 10  $\mu$ l Hamilton syringe. The volume of injection was 10  $\mu$ l, and the rate was 1  $\mu$ l/5 s. During the administration the rats were gently restrained by hand.

EEG activity was recorded by means of an 8-channel EEG apparatus. Analog data were digitized at a sampling rate of 128/s 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). Calculation of ED<sub>50</sub> was done according to Litchfield and Wilcoxon (1949). Statistical comparisons were carried out by means of ANOVA followed by Bonferroni correction and Fisher's exact probability test.

We measured the latency to seizures, seizure severity, lethality, and time to lethal outcome.

Seizures were scored on the descriptive rating scale : 0 – normal behavior, 1 – twitching, 2 – forelimb clonus, head nodding, 3 – rearing and 4 – clonic-tonic convulsions.

The drugs used in this study were imipenem/cilastatin (Thienam<sup>®</sup>, Merck Sharp & Dohme B. V., Haarlem, Holland), phenytoin (Phenytoin<sup>®</sup>, Elkins-Sinn, Inc., Cherry Hill, NJ, USA), and phenobarbital (Phenobarbital sodium<sup>®</sup>, Elkins-Sinn, Inc., Cherry Hill, NJ, USA).

At the end of experiments, before sacrificing the animals, 5  $\mu$ l of blue ink was injected i.c.v. Brains were examined to ensure that the dye was distributed throughout the ventricular spaces. The patency and position of cannulas were confirmed in all animals.

### Results

In the EEG there were no signs of spontaneous epileptiform activity before Imi/Cil (100/100  $\mu$ g,

Table 1

The effects of phenytoin and phenobarbital on Imi/Cil-induced seizures in rats

Group	Nc/Nt	Latency to seizures (min)	Grade	Lethality	Time to lethality (min)
Imi/Cil	8/8	1.4 ± 0.8	4.0 ± 0.0	8/8	13.8 ± 2.5
PHT (40) + Imi/Cil	5/6	4.1 ± 3.0	3.0 ± 1.6	2/6#	64.5 ± 13.4*
PHT (80) + Imi/Cil	6/6	3.0 ± 3.3	3.5 ± 1.2	5/6	18.7 ± 3.9
PHT (160) + Imi/Cil	6/6	2.1 ± 1.0	2.6 ± 1.5	2/6#	17.5 ± 2.1
PHB (50) + Imi/Cil	6/6	2.4 ± 1.2	3.5 ± 1.2	2/6#	32 ± 11*
PHB (80) + Imi/Cil	3/6	5.1 ± 2.6	1.0 ± 1.5*	0/8##	/

Values are mean ± S.D. PHT and PHB were injected i.p. 1 h before Imi/Cil (100/100 µg, i.c.v.). Doses of anticonvulsants are expressed in mg/kg. Nc/Nt – number of rats that convulsed/number of rats tested. Statistically significant difference in latency to seizures, time to lethality and seizure severity \* $p < 0.05$ , (ANOVA followed by Bonferroni correction,  $p < 0.05$ ). Statistically significant difference in number of animals which died compared to rats treated with Imi/Cil only # $p < 0.05$ , ## $p < 0.01$  (Fisher's exact probability test).

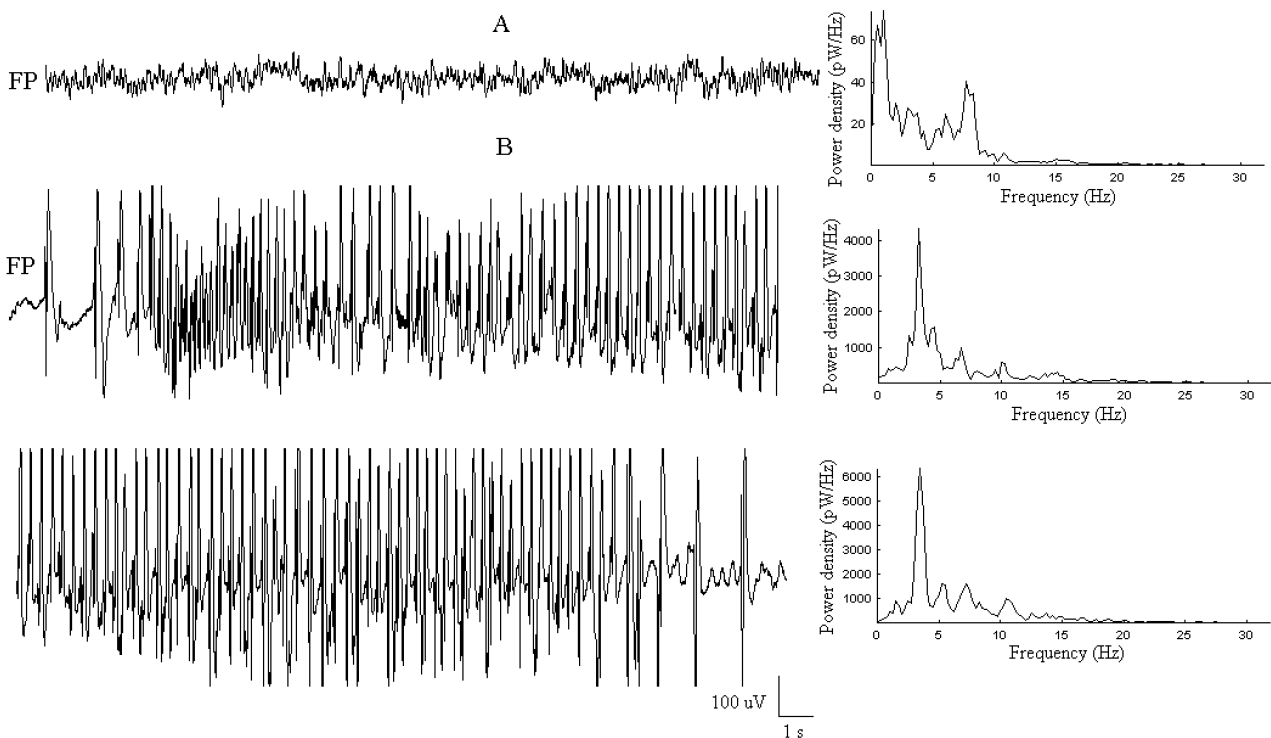


FIG. 1. — EEG record and corresponding power spectra A – baseline, B - clonic-tonic convulsions obtained 4 min after injection of Imi/Cil (100/100 µg, i.c.v.). FP – fronto-parietal cortex.

i.c.v.) injection, and all tracings had low spectral powers. All rats treated with Imi/Cil only (100/100 µg, i.c.v.) had severe clonic-tonic seizures, and died 10-18 min after the injection (Table 1). EEG epileptiform activity preceded behavioral convulsions. Spikes, polyspikes, and continuous bursts of high-frequency high-amplitude spikes were recorded in the EEG during clonic-tonic seizures (Fig. 1). Epileptiform activity was characterized by increase in power spectra.

Phenytoin caused sedation of animals dose-dependently. Phenytoin in a dose of 40 mg/kg i.p. significantly prolonged the time to lethal outcome (ANOVA followed by Bonferroni correction,  $p < 0.05$ ), and decreased the lethality (Fisher's exact

probability test,  $p < 0.05$ ) of Imi/Cil (100/100 µg, i.c.v.)-induced convulsions (Table 1). This dose of PHT was significantly more potent in prolonging the time to lethality compared to higher doses of PHT (80 and 160 mg/kg) and PHB (50 mg/kg) (ANOVA followed by Bonferroni correction,  $p < 0.05$ ). Administered in a dose of 80 mg/kg, phenytoin did not influence significantly on the parameters of Imi/Cil-induced seizures (Table 1). The dose of PHT of 160 mg/kg i.p. significantly decreased lethality (Fisher's exact probability test,  $p < 0.05$ ), while the latency to seizures, seizure severity and time to lethal outcome were not affected. Effective dose of PHT which suppressed tonic-clonic convulsions in 50% of animals ( $ED_{50}$ ) was

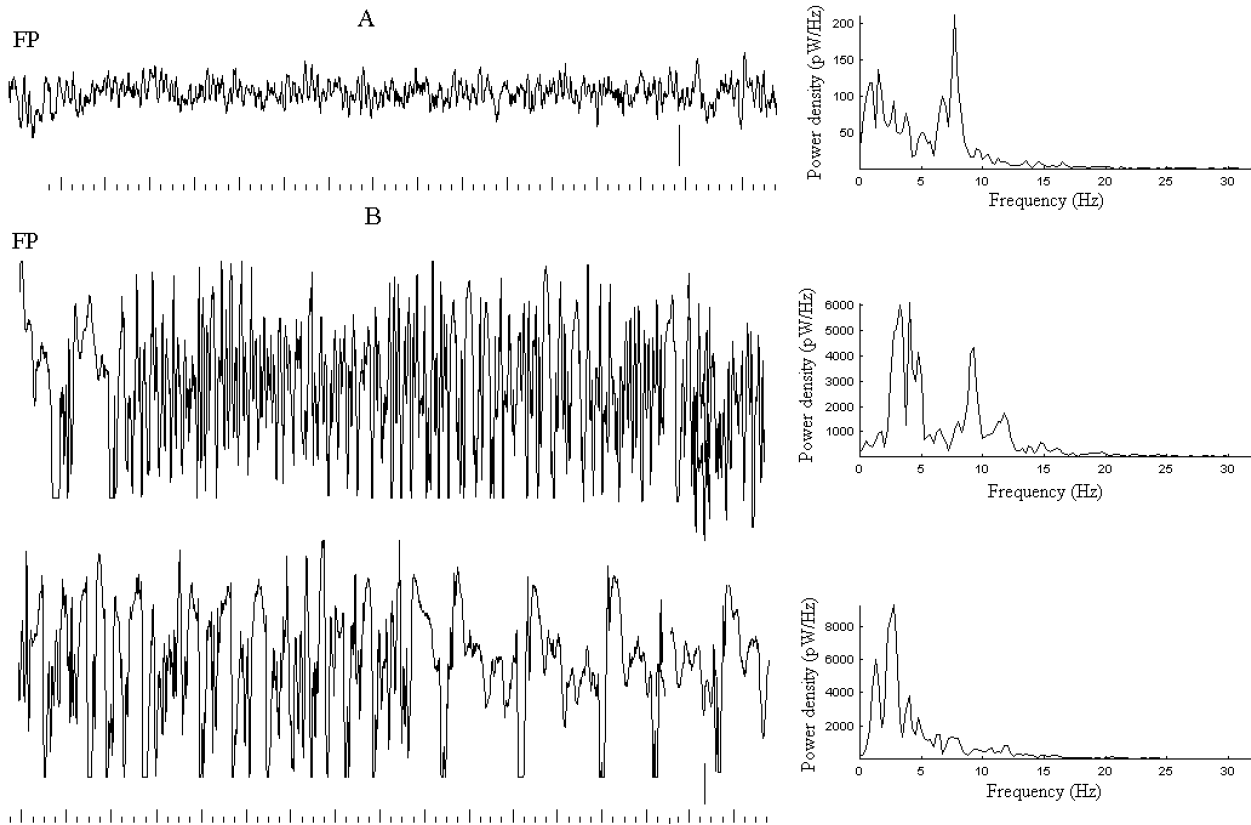


Fig. 2. — EEG correlates and power spectra A – control, B – clonic-tonic seizure 11 min after Imi/Cil (100/100  $\mu$ g, i.c.v.) and 71 min after PHT (80 mg/kg, i.p.) injection. Abbreviations as to the Fig. 1. Time calibration 1 s, voltage 100  $\mu$ V.

358 mg/kg, with 95% interval of confidence 44-2907 mg/kg (Litchfield and Wilcoxon, 1949). Phenytoin did not reduce epileptiform discharges in the EEG during behavioral convulsions elicited by Imi/Cil (100/100  $\mu$ g, i.c.v.) (Fig. 2).

Phenobarbital induced sedation, hypotonia and decreased locomotor activity dose-dependently. Administration of Imi/Cil (100/100  $\mu$ g, i.c.v.) to PHB-pretreated rats provoked increase of locomotion, extension of hind limbs and loud breathing in the first minute post-injection.

Phenobarbital in a dose of 50 mg/kg i.p. significantly decreased the number of animals which died (Fisher's exact probability test,  $p < 0.05$ ), and increased the time to lethal outcome (ANOVA followed by Bonferroni correction,  $p < 0.05$ ) (Table 1). The higher dose of PHB (80 mg/kg) significantly reduced seizure severity of Imi/Cil-induced seizures (ANOVA followed by Bonferroni correction,  $p < 0.05$ ). PHB (80 mg/kg) was significantly more potent in decreasing seizure severity compared to PHB (50 mg/kg) and PHT (80 mg/kg) (ANOVA followed by Bonferroni correction,  $p < 0.05$ ). PHB drastically altered the behavior induced by Imi/Cil. The very characteristic clonic-tonic seizure behavior was dramatically reduced, and in several cases did not appear at all in rats pretreated with PHB (80 mg/kg) 1 h before Imi/Cil injection.

None animal injected with this dose of PHB died (Fisher's exact probability test,  $p < 0.01$ ) (Table 1). The animals which survived seizures after clonic-tonic convulsions characteristically developed less severe seizures in the form of myoclonus of forelimbs, head nodding and twitching. Convulsive behavior was registered up to 2 h after administration of Imi/Cil, and after that period the behavior became normal. Phenobarbital reduced epileptiform discharges in the EEG elicited by Imi/Cil. EEG correlate of twitching was virtually identical like in rats treated with low doses of Imi/Cil only. However, EEG correlates of forelimb clonus and clonic-tonic convulsions (Fig. 3) had lower frequency, and lower increase in power spectra.

## Discussion

Results of this study extend a battery of seizure models in which PHT and PHB are effective anti-convulsants.

The lowest dose of phenytoin (40 mg/kg) in this study significantly influenced on all parameters of Imi/Cil (100/100  $\mu$ g, i.c.v.)-induced seizures, except on seizure severity. Unexpectedly, higher dose (80 mg/kg) had lower anticonvulsant effect. The highest dose of PHT (160 mg/kg) significantly reduced the lethality, but had no effect on seizure

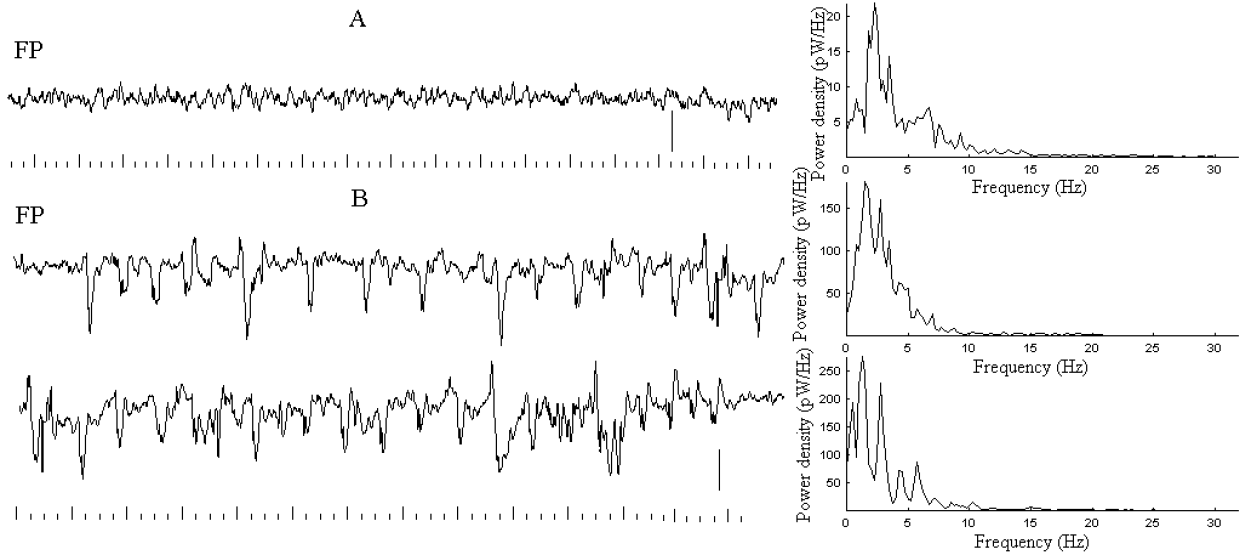


Fig. 3. — EEG correlates and power spectra A – control, B – clonic-tonic seizure 8 min after Imi/Cil (100/100 µg, i.c.v.) and 68 min after PHB (80 mg/kg, i.p.) injection. Abbreviations as to the Fig. 1. Time calibration 1 s, voltage 100 µV.

severity and latencies to seizure and lethal outcome. It seems that low doses of PHT preferentially influenced the latencies to seizure and time to lethal outcome, while leaving the severity of convulsions - most resistant parameter, unaffected. The ineffectiveness of higher doses of PHT on some parameters of Imi/Cil seizure is in agreement with the study which reported proconvulsant effect of PHT : at a dose of 60 mg/kg PHT significantly prolonged duration of afterdischarges (ADs) induced by electrical stimulation of sensorimotor cortex in rats (Kubova *et al.*, 1996).

Since PHT did not reduce epileptiform discharges during behavioral Imi/Cil (100/100 µg, i.c.v.)-induced seizures, it can be postulated that phenytoin acts as anticonvulsant rather than antiepileptic agent in this model of seizures. PHT produced sedation of animals dose-dependently, and only the dose of 160 mg/kg sedated the animals significantly.

Phenobarbital inhibited Imi/Cil-induced seizures in a dose-dependent fashion, and reduced epileptiform discharges induced by Imi/Cil. In this model of epilepsy PHB acts as antiepileptic and anticonvulsant agent.

PHT and PHB were effective in suppressing seizures in various models of epilepsy within the dose range tested in this study, while in other models were more effective than against Imi/Cil-induced seizures. The effects of PHT and PHB in various seizure models are presented in Table 2.

PHB was more potent than PHT against seizures induced by PTZ, bicuculline, (Clineschmidt *et al.*, 1982), Imi/Cil (this study), and against audiogenic seizures in GEPR-3 rats (Dailey and Jobe, 1985). They were equipotent against sound induced

seizures in GEPR-9 rats (Dailey and Jobe, 1985), and convulsions induced by glutamate in mice (Stone and Javid, 1983). Only seizures induced by electroshock were more potently blocked with PHT than PHB (Clineschmidt *et al.*, 1982 ; Czuczwar *et al.*, 1984).

It has been reported that mechanisms of convulsant action of imipenem are both decrease of inhibition (Williams *et al.*, 1988 ; De Sarro *et al.*, 1995a), and increase of excitation (De Sarro *et al.*, 1995a). Imipenem/Cilastatin-induced seizures are the model for human limbic seizures, and a convenient test tool for assessing potential anticonvulsive drugs acting both on inhibitory and excitatory neurotransmission. PHB was more potent in preventing Imi/Cil-induced seizures than PHT, and also decreased epileptiform activity in the EEG. Our results are in agreement with the study which reported a relatively minor importance of excitatory neurotransmission in the mechanism of phenytoin activity (Czuczwar *et al.*, 1984). The anticonvulsant and antiepileptic effects of PHB against Imi/Cil-elicited seizures in rats are probably due to effects on both excitatory and inhibitory neurotransmission. PHB suppresses seizures by decreasing repetitive firing and enhancing inhibition mediated by GABA, while PHT suppresses repetitive firing without influencing GABA-mediated inhibition (Macdonald *et al.*, 1985).

In conclusion, results suggest that PHB is more effective than PHT in blocking behavioral seizures elicited by Imi/Cil, and together with its effect on suppressing epileptiform activity indicate more suitable use of PHB in treatment of patients with seizures elicited after Imi/Cil treatment.

Table 2  
Effects of PHT and PHB in various seizure models

Seizure model	Species	Effect	PHT	PHB	Reference
bicuculline	rats	ED <sub>50</sub>	12.6 p.o.	2.3 p.o.	Clineschmidt <i>et al.</i> , 1982
electroshock	mice	ED <sub>50</sub>	8.3 i.p.	10.7 i.p.	Clineschmidt <i>et al.</i> , 1982
bicuculline	mice	ED <sub>50</sub>	22 p.o.	15 p.o.	Clineschmidt <i>et al.</i> , 1982
metaphit audiogenic	mice	blockade	40 i.p.	30 i.p.	Debler <i>et al.</i> , 1993
electrical stimulation of the cortex	rats	severity, shortened ADs	NE	20-40 i.p.	Kubova <i>et al.</i> , 1996
L-glutamate	mice	incidence	40 i.p.	50 i.p.	Stone and Javid, 1983
PTZ	mice	ED <sub>50</sub>	> 285 p.o.	50 p.o.	Clineschmidt <i>et al.</i> , 1982
Strychnine	mice	ED <sub>50</sub>	NE	77 p.o.	Clineschmidt <i>et al.</i> , 1982
PTZ	rats	suppression	/	10-80 i.p.	Kubova and Mares, 1991
kainic acid	rats	blockade	NE	20-80 i.p.	Velisek <i>et al.</i> , 1982
metaphit audiogenic	mice	protection	60 i.p.	100 i.p.	Debler <i>et al.</i> , 1993
NMDA	mice	latency, severity	NE	100 i.p.	Debler <i>et al.</i> , 1993
NMDLA	mice		NE	NE	Czuczwar <i>et al.</i> , 1985

The doses for PHT and PHB are expressed in mg/kg. i.p. – intraperitoneal, p.o. – peroral route of administration. NE – not effective. ADs – afterdischarges.

### Acknowledgments

This research was supported by a grant No. 1943 from the Republic Ministry of Science (Serbia).

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