

## PRO- AND ANTIAPOPTOTIC EFFECTS OF ANTICONVULSANT DRUGS

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According to a commonly accepted view, neuronal death may have a necrotic or an apoptotic form. Apoptosis is the main form of neuronal loss during development of the central nervous system, i.e. during a period characterized by the prevalence of excitatory transmission.

Recently, it has been shown that agents with inhibitory effects on neurons, such as antagonists of NMDA receptors (phencyclidine, ketamine, ethanol), agonists of GABA A receptors (barbiturates, benzodiazepines, ethanol) and general anesthetics (nitrous oxide, isoflurane, propofol, halothane) can enhance apoptosis during neuronal development [Olney et al., 2000]. Since a majority of anticonvulsants suppress excitatory transmission, the above hypothesis raises a serious concern about safety of anticonvulsant use pre- and postnatally. Indeed, some experimental data indicate that anticonvulsants induce apoptotic processes in both neuronal and non-neuronal cells.

In *in vitro* experiments, carbamazepine, oxcarbamazepine and phenytoin induce apoptosis in hippocampal and/or cerebellar cells [Ambrosio et al., 2000; Nonaka et al., 1998; Yan et al., 1995; Gao et al., 1995]. Under *in vivo* conditions, vigabatrin induces apoptosis of glia cells [Sidhu et al., 1997], while sodium valproate triggers apoptosis of neuroblastoma grafts [Cinatl et al., 1997] and phenytoin exerts similar effect in cerebellum [Ohmori et al.,

1999]. Apart from the central nervous system, phenobarbital induces apoptosis of hepatocytes and thyroid gland [Osonai et al., 1997; Lee et al., 2000; Kolaja et al., 1998], whereas phenytoin and valproate induce that process in lymphocytes and hepatoblastoma cells, respectively [Villasenor-Garcia et al., 2000; Neuman et al., 2001]. On the other hand, some reports suggest that under certain conditions anticonvulsants may have antiapoptotic properties. Apoptotic changes in the hippocampus evoked by kainate-induced seizures are blocked by diazepam [Pollard et al., 1994]. Furthermore, felbamate inhibits apoptosis evoked by ischemia [Wasterlain et al., 1996] and topiramate prevents apoptotic changes after hypoxia [Koh et al., 2001]. Some data from *in vitro* studies support antiapoptotic properties of certain anticonvulsants. Valproate protects cultured cerebellar cells against low  $K^+$  (5 mM)-induced apoptosis *via* the mechanism involving IP3/PKB pathway [Mora et al., 1999], whereas clonazepam, acting as an inhibitor of the mitochondrial Na/Ca exchanger, inhibits apoptotic processes induced by a low calcium concentration in neuroblastoma cells [Zhu et al., 2000]. Thus, the above observations suggest that anticonvulsants possess both pro- and antiapoptotic properties. However, since only few anticonvulsants have been tested with regard to their involvement in apoptotic processes, the final conclusion must await the outcome of future studies.

## MECHANISMS OF NEURODEGENERATION

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Destruction of neurons [Meldrum, 1983] and glia cells [Yoshioka et al., 1996] may result from both acute (such as traumatic brain injury, ische-

mia/hypoxia, seizures) and chronic (in the course of classical neurodegenerative diseases) pathologic processes in the central nervous system. Hunting-

ton's disease is caused by structural degeneration with cell loss in the caudate nucleus, putamen and cerebral cortex [Clarke and Lowry, 2001]. The chorea-like movements may be related to a deficiency in gamma-aminobutyric acid in basal ganglia [Kolchinsky, 2001]. Parkinsonism syndrome results primarily from a defect in the dopaminergic pathway which connects the substantia nigra to the corpus striatum, caudate and lenticular nuclei [Emerich, 2001; Naarding et al., 2001]. A patient with Alzheimer's disease suffers a severe loss of hippocampal and cortical neurons with no loss of brain parenchyma [Vickers et al., 2001]. Although all the above-described disorders are conditions involving multiple genetic, environmental and pathological factors, they lead to similar neuropathologic changes in neuronal tissues. In general, the mechanisms of neurodegeneration involve free oxygen radicals production [Vickers et al., 2001], increased

excitotoxic transmission through ionotropic (NMDA, AMPA/kainate) and metabotropic glutamate receptors [Choi, 1992], excessive  $\text{Ca}^{2+}$  inward currents and activation of several  $\text{Ca}^{2+}$ -sensitive intracellular proteins [Choi, 1992], increased nitric oxide synthesis interfering with oxidative metabolism, inflammatory reactions [Arvin et al., 1996] and activation of gelsolin which leads to dismantling of neuronal cytoskeleton [Choi, 1992]. All the changes can result in progressive reorganization of the neural network [Babb, 1999] and necrotic or apoptotic cell death [Roy et al., 1999].

Better understanding of pathomechanism of neurodegeneration may be the key to identifying the correct neuroprotective approach useful for delaying or slowing the progress of either epilepsy or classical neurodegenerative diseases. It should also improve the quality of life of both patients and caregivers.

## INTERACTION OF BENZODIAZEPINES WITH ANTIEPILEPTIC DRUGS AND AGENTS AFFECTING VARIOUS NEUROTRANSMITTER SYSTEMS

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Benzodiazepines (diazepam, clonazepam and closely related other drugs) are known to possess a very broad spectrum of anticonvulsant activity against experimental seizures. Interestingly, they may even antagonize N-methyl-D,L-aspartate-induced convulsions which is not possible to achieve by a variety of conventional antiepileptics, such as carbamazepine, diphenylhydantoin, ethosuximide or phenobarbital [Czuczwar et al., 1984].

A very potent interaction was found between diazepam (clonazepam) and diphenylhydantoin. The latter is completely ineffective against pentetrazole-induced convulsions in rodents and yet it potentiated the protective activity of diazepam and clonazepam against this convulsant [Czuczwar et al., 1981; 1982]. Also, diphenylhydantoin enhanced the protection offered by diazepam or clonazepam against electroconvulsions. These effects were in-

terpreted in terms of diphenylhydantoin-induced increase in the number of benzodiazepine receptors [Czuczwar et al., 1981; 1983].

Methylxanthines, aminophylline (theophylline-ethylenediamine) and caffeine reduce the protective potential of conventional antiepileptics, including diazepam [Czuczwar et al., 1985; 1986]. This may explain some therapeutic failures in epileptic patients who are cured with methylxanthines for other than epilepsy reasons. The problem may get even worse considering that methylxanthines do not evoke any tolerance and actually their effect is increased over time [Wlaź et al., 1994; Gašior et al., 1996]. Thus, it is probably essential for the epileptic patients to avoid methylxanthines. One cannot exclude a possibility that some cases of drug-resistant epilepsy might be dependent on methylxanthines.

Finally, the anticonvulsant activity of benzodiazepines against electroconvulsions and amygdala-kindled seizures was potentiated by some excitatory amino acid antagonists and adenosine agonists [Czuczwar et al., 1990; Żarnowski et al., 1993; Borowicz et al., 2000]. In particular, the AMPA/kainate receptor antagonist, LY 300164, very efficiently enhanced the protective effects of diazepam and clonazepam against amygdala-kindled seizures in rats [Borowicz et al., 1999; 2000]. Also, a ligand for metabotropic glutamate receptors, LY 354740,

potentiated the anticonvulsant potency of diazepam against pentetrazole-induced seizures [Kłodzińska et al., 2000]. All these data may pave the way for new, more efficient attempts to control epilepsy in man. Contrary to glutamate receptor antagonists, N-methyl-D-aspartate reduced the anticonvulsant activity of diazepam against electroconvulsions, pointing to an important role of excitatory amino acid-produced events in the protective efficacy of benzodiazepines [Urbańska et al., 1999].

## INFLUENCE OF MOLSIDOMINE ON THE ANTICONVULSIVE ACTIVITY OF CONVENTIONAL ANTIEPILEPTIC DRUGS IN PENTETRAZOLE-INDUCED SEIZURES IN MICE

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The compounds influencing L-arginine-nitric oxide (NO) pathway have been recently tested as potential anticonvulsive agents [Smith et al., 1996; Tutka et al., 1996; Alexander et al., 1998] and have been found to enhance the antiseizure efficacy of certain antiepileptic drugs [Deutsch et al., 1995; Borowicz et al., 2000]. Most attention has been focused on inhibitors of NO synthase, the compounds not used in clinical practice. In contrast to them, molsidomine (MOL), a donor of NO, is widely used for the treatment of different forms of coronary artery disease [Anderson et al., 1994]. There is no available detailed data concerning the influence of MOL on the antiseizure efficacy of antiepileptic drugs. It prompted us to study the effects of MOL on the protection provided by clonazepam, ethosuximide, phenobarbital, and valproate in clonic pentetrazole (PTZ)-induced seizures in male Swiss mice.

MOL, administered intraperitoneally (*ip*) at a subthreshold dose of 25 mgkg<sup>-1</sup>, enhanced the protective activity of valproate against PTZ-induced seizures, significantly reducing the ED<sub>50</sub> of valproate from 123.5 to 78 mgkg<sup>-1</sup>. MOL was found to be ineffective in changing the protective action of clonazepam, ethosuximide, and phenobarbital. Neither MOL *per se* (at a dose enhancing

the anticonvulsive activity of valproate) nor its combination with valproate disturbed motor performance in the chimney and rotarod tests or long-term memory in the passive avoidance task.

Since N<sup>G</sup>-nitro-L-arginine (40 mgkg<sup>-1</sup>; *ip*), an inhibitor of NO synthase, failed to reverse the effect of MOL on valproate, an involvement of NO in the mechanism of the anticonvulsive activity of valproate in PTZ-induced seizures does not seem to be likely. Interestingly, MOL (25 mgkg<sup>-1</sup>) significantly elevated the free plasma level of valproate from 33.8 to 46.2 µgml<sup>-1</sup>. Therefore, we conclude that the interaction of MOL with valproate is at the pharmacokinetic level.

The potential therapeutic combination of valproate with MOL appears beneficial because it is devoid of adverse effects, in terms of motor impairment and long-term memory deficit. Furthermore, our findings seem to have an important practical implication, namely, they suggest that the dosage of valproate in the patients with coronary artery disease treated with MOL should be decreased. It would allow us to reduce adverse effects of valproate.

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## EFFECT OF $\beta$ -ADRENOCEPTOR ANTAGONISTS AND ANTIEPILEPTIC DRUGS ON AMINOPHYLLINE-INDUCED CONVULSIONS AND LETHALITY IN MICE

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The convulsive activity of methylxanthines has been known for many years. Clinical data indicate that treatment with aminophylline may induce repetitive generalized seizures in the patients with obstructive lung diseases. Some cases of aminophylline-induced seizures may be even fatal [Schwartz and Scott, 1974; Yarnell and Chu, 1975; Zwillich et al., 1975]. Conventional antiepileptic drugs very poorly control this type of convulsions and are practically ineffective in lowering aminophylline-induced mortality [Chu, 1981; Czuczwar et al., 1987]. We evaluated the protective activity of phenobarbital, and valproate combined with propranolol, atenolol, labetalol and pindolol against aminophylline-induced convulsions and mortality. The experiments were carried out on male Swiss mice weighing 20–25 g. Chemical seizures were induced by intraperitoneal (*ip*) injections of aminophylline and defined as clonus of the whole body with an accompanying loss of righting reflex lasting for over 3 s.

Phenobarbital (up to 75 mg/kg) and valproate (up to 300 mg/kg) reduced the incidence of convulsions, but were not effective in preventing mortality. Propranolol (up to 10 mg/kg), atenolol (up to 25 mg/kg), labetalol (up to 10 mg/kg) and pindolol (up to 15 mg/kg) were ineffective in protecting against convulsions and lethality. Phenobarbital and valproate combined with atenolol and propranolol led to a significant protection against aminophylline-induced convulsions and mortality. On the contrary, antiepileptics combined with labetalol and pindolol did not protect against aminophylline-induced seizures and mortality.

The obtained results point to a novel method sufficiently reducing convulsion and lethal effects of aminophylline overdose.

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## INTERACTIONS OF LAMOTRIGINE WITH SOME ANTIEPILEPTIC DRUGS: AN ISOBOLOGRAPHIC ANALYSIS

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Despite several years of clinical experiences in the domain of the treatment of epilepsy and avail-

ability of the newest, more efficacious antiepileptic drugs (AEDs) lately introduced into the therapy,

there have been still many cases of seizures, refractory to the applied standard medication. Therefore, it is widely accepted that addition of a second AED to the established monotherapy often protects the patients from seizure attacks [Brodie M.J., 2001]. So, the combined AEDs regimen, based on the theoretical and experimental evidence, seems to be the best rational choice for the patients suffering from unsuccessfully treated seizure episodes.

Isobolographic analysis is applied in experimental studies to determine the interaction between two drugs, co-administered in several varying fixed-ratio combinations. In fact, the isobolography facilitates the choice of only these combinations of AEDs, which could be effective and used for further treatment of diverse convulsions. Theoretically, the isobolographic analysis distinguishes 3 most important types of interactions, and among them the most accepted are: pure additivity, supra-additivity and sub-additivity [Berenbaum M.C., 1989; Gessner P.K., 1995; Tallarida R.J., 1992]. From theoretical point of view in the isobolographic analysis, the most advantageous interaction would involve the situation when two drugs synergistically cooperate with each other in terms of their therapeutic activity and concomitantly, when they are antagonists their side effects are concerned. By establishing the protective index for all tested combinations in isobolography, one can discover the most beneficial combination, fulfilling all theoretical presumptions and worth recommendation in clinical practice.

It has recently been shown that lamotrigine (LTG), the drug commonly used in generalized and partial seizure disorders, possesses multiple actions within the central nervous system. LTG blocks Na<sup>+</sup> channel, acting similarly to diphenylhydantoin and carbamazepine [Lang D.G. et al., 1993].

The present study was aimed at determining the exact type of interactions between LTG and some AEDs (diphenylhydantoin, carbamazepine, valproate magnesium, phenobarbital, felbamate and topiramate) in the maximal electroshock seizure (MES) test in mice. The MES test is an experimental animal model of generalized tonic-clonic seizures and, to a certain extent, of partial convulsions in humans [Fisher R.S., 1989].

The experiments were performed on adult male Swiss mice weighing 22–27 g. The isobolographic protocols were based on the method developed by

[Tallarida et al., 1997], in which the activity of two-drug mixture, applied at 3 fixed dose ratio combinations, was estimated and expressed as the ED<sub>50</sub> values of these drugs in the MES test in mice. ED<sub>50</sub> corresponds to the dose of an AED protecting 50% of animals against MES-induced seizures in mice. Moreover, the adverse effects evoked by respective combinations of LTG with AEDs applied at the same fixed-ratio combinations, were determined in the chimney test and presented as the TD<sub>50</sub> (toxic dose 50) values. TD<sub>50</sub> refers to the dose of an AED, which caused impairment of motor coordination in the chimney test in 50% of animals. Afterwards, protective indices were calculated as TD<sub>50</sub>/ED<sub>50</sub>.

The results obtained in our study clearly demonstrated that interactions between LTG and topiramate exactly fulfilled the criterion of the best combination in the isobolographic analysis. These drugs exhibited supra-additive (synergistic) interaction as regards their therapeutic activity in the MES test and simultaneously demonstrated a sub-additivity (antagonism) with respect to the evoked side effects in the chimney test. Therefore, in the adjunctive therapy, particularly the co-administration of LTG with topiramate, might be beneficial and worth recommendations in clinical practice. Furthermore, the data confirmed the supra-additivity between LTG and phenobarbital at all examined fixed-ratio combinations in the MES test in mice. Interestingly, solely for the 1:1 fixed-ratio combination, the interaction of LTG and phenobarbital at the chimney test was synergistic, which proved that both drugs intensified their own adverse effects. Combinations of LTG and phenobarbital showing the distinct isobolographic synergism might be applied in the patients during the AEDs management of seizures. Nonetheless, the special attention must be paid to the careful dosage of these drugs in order to avoid some unexpected effects, resulting from their potential to enhance the adverse effects. Isobolography revealed also the antagonistic interaction between LTG and carbamazepine in the MES test, which strongly argues against the use of these drug combinations in clinical practice. In contrast, interactions between LTG and diphenylhydantoin showed a pure additivity in both MES and chimney tests. Moreover, the isobolographic protocol designed to determine the interaction between LTG and felbamate proved a pure

additivity in the MES test in mice and sub-additivity in the chimney test, whilst all interactions between LTG and valproate showed merely a pure additivity in both used tests. Finally, we can conclude that LTG might interact specifically with other AEDs extending the abilities of their combinations

to suppress convulsions in the patients with different seizure attacks.

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