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Synergistic interaction of gabapentin and oxcarbazepine in the mouse maximal electroshock seizure model—an isobolographic analysis

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Abstract

The anticonvulsant effects produced by mixtures of oxcarbazepine and gabapentin (two second-generation antiepileptic drugs) in numerous fixed-ratio combinations of 1:1, 1:2, 1:5, 1:10, 1:15, and 1:20 were examined isobolographically in the mouse maximal electroshock seizure model. Results displayed that mixtures of both drugs at the fixed-ratios of 1:2, 1:5, 1:10, 1:15, and 1:20 exerted supra-additive (synergistic) interactions against electroconvulsions. Only a fixed-ratio of 1:1 was indifferent with isobolography, although the combination displayed the trend towards supra-additivity. Furthermore, the combinations of oxcarbazepine with gabapentin, administered at their median effective doses ($ED_{50\ mix}$s), did not alter motor performance of animals challenged with the chimney test. Additionally, neither gabapentin nor oxcarbazepine affected total brain concentrations of co-administered drug, indicating a pharmacodynamic nature of interaction between these antiepileptics. Finally, based on preclinical data presented here the combination of oxcarbazepine and gabapentin is of particular importance for further therapy in patients with refractory partial seizures.

Keywords: Oxcarbazepine; Gabapentin; Electroshock maximal; Isobolographic analysis; Pharmacodynamic interaction; Seizure

1. Introduction

Overwhelming evidence indicates that oxcarbazepine and gabapentin, two second-generation antiepileptic drugs, are of clinical importance in controlling partial convulsions (gabapentin and oxcarbazepine) and/or tonic-clonic seizures with or without secondary generalization (oxcarbazepine) in epileptic patients (Brodie and Schachter, 2001; French et al., 2004a,b). Generally, both antiepileptics are used separately in adults and children with newly diagnosed and/or refractory epilepsy as adjunctive therapy with conventional antiepileptic drugs. Additionally, oxcarbazepine can be successfully used in monotherapy in patients with newly diagnosed partial seizures (French et al., 2004b).

In spite of progress in our understanding of the modes of antiepileptic drug action and of the pathophysiology of seizure initiation and propagation, as well as 10 second-generation antiepileptic drugs licensed for clinical use during the last decade, there are still approximately 25–30% of patients with epilepsy, who are refractory to the monotherapeutic use of first-line antiepileptic drugs (Kwan and Brodie, 2000a,b). In consequence, to provide these patients with a state of seizure-freedom two or more antiepileptic drugs (polytherapy) are prescribed in an attempt to enhance seizure control. Indeed, the addition of a second or a third antiepileptic drug may provide the enhanced seizure control in ~14% of these patients (Kwan and Brodie, 2000a,b). In the clinical setting, it has been documented that some antiepileptic drug combinations are able to provide the patients with intractable epilepsy with a state of seizure-freedom over 1 year (Stephen and Brodie, 2002). Among them, the combination of gabapentin with carbamazepine occurred to be advantageous in patients with refractory epilepsy (Stephen and Brodie, 2002). On the other hand, compelling clinical evidence suggests that oxcarbazepine applied in patients with epilepsy is more effective, safe, and tolerable than carbamazepine (French et

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al., 2004a,b; Schmidt and Elger, 2004). Moreover, the advanced electrochemical and neurophysiological studies have revealed that, despite their structural and chemical similarities, oxcarbazepine and carbamazepine are considered as different drugs (Schmidt and Elger, 2004).

In in vivo experimental models of epilepsy, gabapentin and oxcarbazepine reduce the seizure severity and afterdischarge duration in amygdala kindled rats (Dalby and Nielsen, 1997; Schmutz et al., 1994), as well as suppress the tonic-clonic seizures in mice induced by various chemical convulsants (Baltzer and Schmutz, 1978; Mares and Haugvicova, 1997; Taylor, 2002). In contrast, gabapentin is virtually ineffective in the mouse maximal electroshock seizure model (Bartoszyk et al., 1986; Dalby and Nielsen, 1997), although the drug significantly elevated the threshold for electroconvulsions in mice (Borowicz et al., 2002; Luszczki and Czuczwar, 2004b). In case of oxcarbazepine, the drug protects rodents against maximal electroshock-induced seizures dose-dependently (Schmutz et al., 1994).

Considering the anticonvulsant activities of oxcarbazepine and gabapentin in both clinical conditions and preclinical experiments, one can theoretically presume that both antiepileptic drugs in combination should interact synergistically with respect to seizure suppression in patients with partial refractory epilepsy. Though the direct evaluation of antiseizure efficacy and tolerability of new antiepileptic drug combinations in clinical practice is impossible, because of methodological and ethical difficulties, preclinical studies in animals can help to preselect the most promising combinations offering supra-additive (synergistic) interactions in terms of seizure protection without producing side effects that adversely affect the patients' quality of lives (Deckers et al., 2000; Luszczki and Czuczwar, 2004a). No doubt exists that seizure models in laboratory animals usually provide an invaluable means by which to identify potentially useful antiepileptic drug polytherapy regimens which could subsequently be evaluated in patients, unsatisfactorily medicated with available antiepileptic drugs in monotherapy (Deckers et al., 2000; Löscher, 2002; Löscher and Schmidt, 2004).

Up-to-date, neither clinical nor experimental evidence exists describing the anticonvulsant profile of combination of oxcarbazepine with gabapentin. We sought, therefore, to characterize the type of interaction between these antiepileptic drugs against maximal electroshock-induced seizures in mice using the isobolographic analysis for numerous fixed-ratio combinations. It is widely accepted that the maximal electroshock-induced seizure test is considered as experimental model of generalized tonic-clonic seizures and, to a certain extent, of partial convulsions with or without secondary generalization in humans (Löscher et al., 1991). Therefore, it was considered appropriate to use this test for assessing the exact characteristic of interaction between oxcarbazepine and gabapentin in our study. Additionally, the adverse-effect profiles for all the fixed-ratio combinations between these antiepileptic drugs were investigated in relation to motor coordination impairment in the chimney test. Finally, to ascertain whether the observed effects were consequent to a pharmacodynamic and/or a pharmacokinetic interaction, total brain antiepileptic drug concentrations were measured in this study.

2. Materials and methods

2.1. Animals and experimental conditions

All experiments were performed on adult male albino Swiss mice weighing 22–26 g. The mice were kept in colony cages with free access to food and tap water ad libitum, under standardized housing conditions (natural light–dark cycle, ambient temperature of 23 °C, relative humidity 50–60%). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups consisting of eight mice. Each mouse was used only once. Additionally, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The experimental protocols and procedures listed were approved by the Local Ethics Committee at the Medical University of Lublin (License nr.: 368/2002/349/02) and conformed to the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Drugs

The following antiepileptic drugs were used in this study: gabapentin ([1-(aminomethyl)-cyclohexane acetic acid]; NEURONTIN; Parke-Davis, Freiburg, Germany) and oxcarbazepine ([10,11-dihydro-10-oxo-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide]; TRILEPTAL; Novartis, Basel, Switzerland). Both drugs were suspended in a 1% solution of Tween 80 (Sigma) in saline and administered i.p., as two separate injections, in a volume of 5 ml/kg body weight. The control animals received adequate amounts of vehicle (1% solution of Tween 80 in saline). Fresh drug solutions were prepared ex tempore on each day of experimentation and administered as follows: oxcarbazepine 30 min and gabapentin 60 min prior to electroconvulsions, chimney test and brain sampling for the measurement of antiepileptic drug concentrations. These pretreatment times before testing of the antiepileptic drugs were based on information about their biological activity from the literature and our previous experiments (Borowicz et al., 2002; Luszczki and Czuczwar, 2004a,b).

2.3. Maximal electroshock-induced seizure test

Electroconvulsions were produced by means of an alternating current (0.2 s stimulus duration at a frequency of 50 Hz) delivered via ear-clip electrodes by a generator (Rodent Shocker, Type 221, Hugo Sachs, Freiburg, Germany). The criterion for the occurrence of seizure activity was the tonic hindlimb extension (i.e., the hind limbs of

animals outstretched 180° to the plane of the body axis). Protective activities of oxcarbazepine alone or combined with gabapentin were evaluated as their median effective doses (ED_{50}s in mg/kg) against maximal electroconvulsions (fixed current intensity of 25 mA, maximum stimulation voltage of 500 V). The animals were administered with different drug doses so as to obtain a variable percentage of protection against maximal electroshock-induced seizures, allowing the construction of a dose–effect line for every drug combination. Subsequently, the ED_{50}s with their 95% confidence limits were calculated according to Litchfield and Wilcoxon (1949). The anticonvulsant activities of mixtures of oxcarbazepine with gabapentin (at various fixed-ratio combinations) were evaluated and expressed as $ED_{50\ mix}$s, corresponding to doses of both antiepileptic drugs in mixture necessary to protect 50% of mice against tonic hindlimb extension in the maximal electroshock-induced seizure test. The experimental procedure has been described in more detail in our earlier studies (Luszczki and Czuczwar, 2003, 2004a; Luszczki et al., 2003a).

2.4. Isobolographic analysis

The isobolographic analysis allows the evaluation of exact types of interactions among drugs, comprehensively classifying the nature of pharmacological interactions as: supra-additive (synergistic), sub-additive (antagonistic), indifferent or pure additive (Berenbaum, 1989; Greco et al., 1995; Luszczki and Czuczwar, 2003; Tallarida, 2000).

To perform the isobolographic analysis of the interaction between oxcarbazepine and gabapentin (as regards their anticonvulsant activities against maximal electroshock-induced seizures) both drugs in numerous fixed drug-dose ratio combinations (1:1, 1:2, 1:5, 1:10, 1:15, and 1:20) were administered to animals. Subsequently, the experimentally derived $ED_{50\ mix}$s (± S.E.M.) for these mixtures were determined using log-prohibit method described by Litchfield and Wilcoxon (1949). Moreover, theoretically additive $ED_{50\ add}$s (± S.E.M.) were calculated from the equation presented by Porreca et al. (1990), as follows: $ED_{50\ add} = ED_{50\ drug\ 1}/P_1$; where P_1 is a proportion of the first drug, fully effective against maximal electroconvulsions (oxcarbazepine) in the total amount of the mixture. Noticeably, for two-drug mixture the equation presented above is true if: $P_1 + P_2 = 1$; where P_2 is the proportion of the second drug, virtually ineffective against maximal electroconvulsions (gabapentin). It is worthwhile to mention that proportions of antiepileptic drugs in mixture are based on mass quantity of both drugs. For example, the fixed-ratio combination of 1:1 comprised equal amounts of both antiepileptic drugs, whilst the mixture at the fixed-ratio combination of 1:20 consisted of drug-doses, in which the dose of gabapentin prevailed 20-fold the dose of oxcarbazepine. In our study, the $ED_{50\ mix}$ for the fixed-ratio combination of 1:20 was 91.5 mg/kg and consisted of oxcarbazepine at the dose of 4.4 mg/kg and gabapentin at 87.1 mg/kg. Analogously, the $ED_{50\ mix}$ value for the fixed-ratio of 1:1 was 18.6 mg/kg and comprised of equal doses of both antiepileptic drugs at 9.3 mg/kg (for more information see Table 1).

This particular kind of isobolographic analysis (so-called type II) allows the acceptance of mass quantity of drugs in mixture as a basis to construct the notation of fixed-ratio combinations (Berenbaum, 1989). A more detailed description and the theoretical background concerning the isobolographic analysis followed by equations showing how to calculate $ED_{50\ add}$ values and their S.E.M. (in a case if one of the investigated AEDs is virtually ineffective against maximal electroshock) have been presented in our previous studies (Borowicz et al., 2002; Luszczki et al., 2003a).

To visualize the types of interactions between gabapentin and oxcarbazepine, the isoboles were drawn by plotting the points reflecting the respective doses of gabapentin on the *X*-axis, and doses of oxcarbazepine on the *Y*-axis. The straight line, parallel to the *X*-axis, represents the theoretical line of additivity. When the experimentally derived points

Table 1
Isobolographic analysis of interactions between oxcarbazepine and gabapentin in the mouse maximal electroshock seizure model

FR	Drug_1	+	Drug_2	=	$ED_{50\ mix}$ (mg/kg)	N'_{mix}	$ED_{50\ add}$ (mg/kg)	=	Drug_1[a]	+	Drug_2	N'_{add}
1:1	9.30		9.30		18.60±1.46	16	21.54±1.89		10.77		10.77	14
1:2	8.02		16.04		24.06±1.92[b]	16	32.31±2.84		10.77		21.54	14
1:5	6.62		33.09		39.71±5.75[c]	16	64.62±5.67		10.77		53.85	14
1:10	5.42		54.24		59.66±8.20[d]	16	118.47±10.40		10.77		107.7	14
1:15	4.93		73.99		78.92±6.86[d]	24	172.32±15.12		10.77		161.55	14
1:20	4.36		87.11		91.47±8.14[d]	24	226.17±19.85		10.77		215.4	14

Results are presented as median effective doses (ED_{50}s±S.E.M. in mg/kg) for drug mixtures, determined either experimentally ($ED_{50\ mix}$) or theoretically calculated ($ED_{50\ add}$) from the equation of additivity. FR—fixed drug-dose ratio combination based on mass quantity; Drug_1—oxcarbazepine; Drug_2—gabapentin; N'_{mix}—total number of animals used at those doses whose expected anticonvulsant effects were ranged between 16% and 84% (i.e., 4 and 6 probits) for the experimental mixture; N'_{add}—total number of animals calculated for the additive mixture of the drugs examined. Statistical evaluation of data was performed using the unpaired Student's *t*-test according to Porreca et al. (1990) and Tallarida (2000).

[a] The dose of oxcarbazepine in purely additive mixture is constant for all fixed-ratio combinations.

[b] $P<0.05$ vs. the respective $ED_{50\ add}$s.

[c] $P<0.01$ vs. the respective $ED_{50\ add}$s.

[d] $P<0.001$ vs. the respective $ED_{50\ add}$s.

reflecting combinations of various fixed-ratios fall significantly below this line, the two-component drugs act supra-additively (synergistic).

2.5. Measurement of total brain antiepileptic drug concentrations

The animals were given oxcarbazepine + vehicle, gabapentin + vehicle or a combination of both drugs. The fixed-ratio combination for estimating brain concentrations of oxcarbazepine and gabapentin was chosen as 1:2 (oxcarbazepine/gabapentin). Mice were killed by decapitation at times chosen to coincide with that scheduled for the maximal electroshock-induced seizure test. The whole brains of mice were removed from skulls, weighed, and homogenized using distilled water (2:1 vol/weight) in an Ultra-Turrax T8 homogenizer (IKA-WERKE, Staufen, Germany). The homogenates were centrifuged at 10,000 × *g* for 10 min and brain supernatants were transferred into the high pressure liquid chromatography (HPLC) technique of drug detection.

Details concerning the technique of estimation of oxcarbazepine and gabapentin concentrations with HPLC have been presented in our earlier studies (Luszczki and Czuczwar, 2004b; Luszczki et al., 2003b).

All antiepileptic drug concentrations are expressed in μg/ml of supernatants as means ± S.D. of at least eight separate brain preparations.

2.6. Chimney test

The effects of oxcarbazepine and gabapentin in combinations (at doses corresponding to the $ED_{50\ mix}$s for all fixed-ratios tested) on motor performance impairment were quantified with the chimney test of Boissier et al. (1960). In this test, animals had to climb backwards up a plastic tube (3 cm inner diameter, 25 cm length), and motor impairment was indicated by the inability of the animals to climb backward up the transparent tube within 60 s.

2.7. Statistics

Median effective doses (ED_{50}s) with their respective 95% confidence limits were calculated by computer log-probit analysis according to Litchfield and Wilcoxon (1949). The obtained 95% confidence limits were transformed to S.E.M. as described previously (Borowicz et al., 2002; Luszczki et al., 2003a). In isobolography, the experimentally derived $ED_{50\ mix}$ values for the mixture of oxcarbazepine and gabapentin were statistically compared with their corresponding, theoretically additive $ED_{50\ add}$s by the use of unpaired Student's *t*-test, according to the method presented by Porreca et al. (1990) and Tallarida (2000). Total brain antiepileptic drug concentrations were statistically analyzed using the unpaired Student's *t*-test. Quantitative variables from the chimney test were compared with Fisher's exact probability test.

3. Results

3.1. Effects of oxcarbazepine and gabapentin administered singly on maximal electroconvulsions in mice

In our study, oxcarbazepine exerted a clear-cut anticonvulsant activity against maximal electroconvulsions and its ED_{50} value was 10.77 (9.07–12.79) mg/kg. In case of

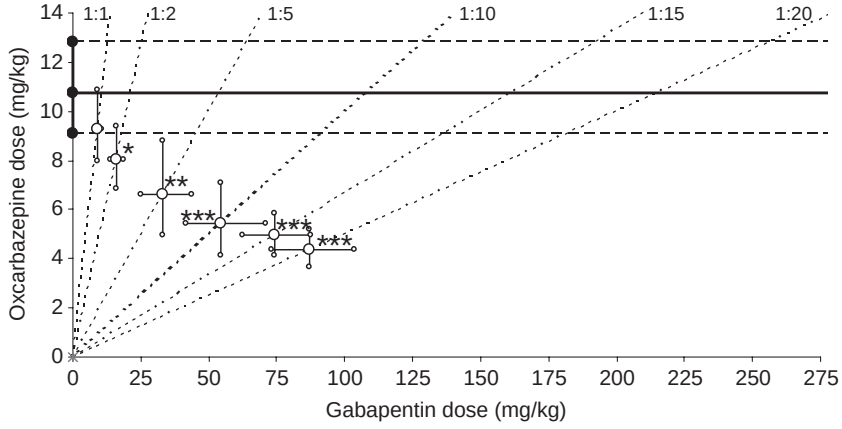

Fig. 1. Isobologram showing interactions between gabapentin and oxcarbazepine against maximal electroconvulsions in mice. Doses of gabapentin and oxcarbazepine are shown plotted graphically on the *X*- and *Y*-axes, respectively. The heavy line is parallel to the *X*-axis, representing the ED_{50} value for oxcarbazepine, and defines the theoretical dose-additive line for a continuum of different fixed dose ratios. The dashed lines represent 95% confidence limits for ED_{50} of oxcarbazepine, whereas the dotted lines correspond to the fixed drug-dose ratio combinations. The open points (○) depict the experimentally derived $ED_{50\ mix}$s for total doses of mixtures expressed as proportions of gabapentin and oxcarbazepine that produced median anticonvulsant effects. All 95% confidence limits of the experimentally determined $ED_{50\ mix}$ values are presented horizontally and vertically in the form of crosses. The $ED_{50\ mix}$s for the mixture of oxcarbazepine with gabapentin at the fixed-ratios of 1:2, 1:5, 1:10, 1:15, and 1:20 are significantly below the theoretical line of additivity, indicating supra-additive (synergistic) interactions (*$P<0.05$, **$P<0.01$, and ***$P<0.001$). In contrast, the $ED_{50\ mix}$ at the fixed-ratio of 1:1 is close to the line of additivity displaying indifferent interaction in the maximal electroshock-induced seizure test.

gabapentin, the drug administered at a dose of 100 mg/kg exerted no anticonvulsant effect against maximal electroconvulsions because none of the examined animals were protected against the maximal electroshock-induced seizures. In contrast, gabapentin at 200 mg/kg produced only a moderate antiseizure activity, since 2 out of 8 mice were protected against maximal electroconvulsions.

3.2. Isobolographic characterization of interactions between oxcarbazepine and gabapentin in the maximal electroshock-induced seizure test in mice

Isobolographic analysis of the protective effects offered by mixtures of oxcarbazepine and gabapentin, at six fixed-ratio combinations against maximal electroconvulsions, revealed that all the fixed-ratios tested (except for 1:1) were supra-additive (synergistic; Table 1; Fig. 1). The experimentally derived $ED_{50\ mix}$ values for the fixed-ratios of 1:2, 1:5, 1:10, 1:15, and 1:20 were significantly lower than the theoretically calculated $ED_{50\ add}$s, and thus, indicating supra-additive interactions between oxcarbazepine and gabapentin (Table 1; Fig. 1). Noticeably, in all the examined combinations a dose of gabapentin in mixture did not exceed 120 mg/kg (Fig. 1).

3.3. Brain antiepileptic drug concentrations

The brain concentrations of oxcarbazepine administered singly at 8 mg/kg were 1.57±0.15 μg/ml and did not differ significantly from the brain oxcarbazepine concentrations evaluated for the mixture of both antiepileptic drugs (1.72±0.14 μg/ml). Similarly, oxcarbazepine (8 mg/kg) co-administered with gabapentin (16 mg/kg) did not affect the brain concentrations of the latter drug. In this case, the brain concentrations of gabapentin (injected singly at 16 mg/kg) were 5.52±0.67 μg/ml, whereas the brain gabapentin concentrations for the two-drug mixture amounted to 6.09±0.71 μg/ml.

3.4. Effect of the combinations of oxcarbazepine and gabapentin on motor coordination of animals in the chimney test

The combinations of oxcarbazepine with gabapentin at doses corresponding to their $ED_{50\ mix}$s for all the fixed-ratios studied (i.e., 1:1, 1:2, 1:5, 1:10, 1:15, and 1:20) did not affect motor performance of animals in the chimney test (results not shown).

4. Discussion

Results presented herein indicate that gabapentin interacted synergistically with oxcarbazepine against electroconvulsions in all the fixed-ratios examined, except for 1:1, which was indifferent (although this fixed-ratio combination displayed the trend towards supra-additivity; Fig. 1). As already mentioned, gabapentin is virtually ineffective in the maximal electroshock-induced seizures in mice, despite it considerably increases the threshold for electroconvulsions in mice. Previously, it has been reported that gabapentin at doses of 75 and 100 mg/kg significantly elevated the threshold for electrocovulsions in mice (Luszczki et al., 2003b). Noteworthy, gabapentin administered separately at a dose of 100 mg/kg exerted no anticonvulsant effect against maximal electroconvulsions and only, the drug at 200 mg/kg was able to produce a moderate antiseizure activity, protecting 2 out of 8 mice (25%) against maximal electroconvulsions.

Previously, it has been shown isobolographically that oxcarbazepine interacted synergistically with topiramate and felbamate (Luszczki and Czuczwar, 2004b), and antagonistically with phenytoin and lamotrigine in the maximal electroshock-induced seizure test in mice (Luszczki and Czuczwar, 2003, 2004b). Moreover, when combined with clonazepam, oxcarbazepine exerted biphasic (antagonistic and synergistic) interactions depending on proportions of tested antiepileptic drugs (Luszczki and Czuczwar, 2003). Similarly, gabapentin combined with phenytoin, carbamazepine, phenobarbital, valproate, lamotrigine, and talampanel exerted synergistic interactions at various fixed-ratios tested isobolographically in the maximal electroshock-induced seizure test in mice (Borowicz et al., 2002). Isobolographic studies have also revealed that gabapentin interacted synergistically with tiagabine against electroconvulsions and the clonic phase of pentylenetetrazole-induced seizures in mice (Luszczki and Czuczwar, 2004a; Luszczki et al., 2003b).

Considering theoretically molecular mechanisms of action of oxcarbazepine and gabapentin, with respect to their anticonvulsant potentials, one can ascertain that both antiepileptic drugs in combination should cooperate and potentiate their antiseizure effects resulting in supra-additive interaction. Electrophysiological and neurochemical studies have indicated that oxcarbazepine and its rapidly formed 10-monohydroxy derivative, at therapeutically relevant concentrations, reduce high-frequency, repetitive firing of neurons by an action on voltage-dependent Na^+ channels, decrease the frequency of penicillin-induced epileptiform spike discharges in neurons suggesting an effect on K^+ currents, and inhibit high-voltage-activated N-type Ca^{2+} channels (Schmutz et al., 1994; Stefani et al., 1995). In case of gabapentin, the exact mechanisms of action of the drug are still unknown; however, several potential mechanisms may account largely for its anticonvulsant activity. At therapeutically relevant concentrations, gabapentin inhibits Ca^{2+} voltage-gated channels through interaction with the alpha2delta subunit (Gee et al., 1996). The drug interacts also with several enzymes of the inextricably linked metabolic pathways of gamma-aminobutyric acid (GABA) and glutamate (Goldlust et al., 1995; Taylor et al., 1998). Moreover, gabapentin increases the conductance of hyper-

polarization-activated cation currents (I_h) protecting neurons against excessive synaptic or intrinsic activity and stabilizing neuronal network within the hippocampus (Surges et al., 2003). More recently, it has been found that gabapentin selectively activates presynaptic $GABA_B$ heteroreceptors (but not $GABA_B$ autoreceptors) decreasing neurotransmitter release by reducing Ca^{2+} conductance in neurons of the CNS (Parker et al., 2004).

In light of the above-mentioned facts, the enhancement of antiseizure effects produced by mixture of oxcarbazepine and gabapentin seems to be closely related with complementary mechanisms of action of both antiepileptic drugs. So, one can presume that the combination of oxcarbazepine with gabapentin should protect the patients against partial convulsions, producing simultaneously no side effects. In fact, the combination of oxcarbazepine with gabapentin, administered at various fixed-ratios, exerted no acute neurotoxic effects with respect to motor coordination impairment in the chimney test in mice. Noteworthy, the combined treatment with antiepileptic drugs, which synergistically suppress the seizures, allows the reduction of drug-doses comprising the mixture (Schmidt, 1996; Deckers et al., 2000). Undoubtedly, the rational decrease in drug-doses in mixture may substantially minimize the risk of side effect appearance, closely associated with polytherapy in clinical practice. Generally, the lower doses of antiepileptic drugs are administered in mixture, the lower risk of potential adverse effects in the clinical setting.

Furthermore, no significant changes in total brain antiepileptic drug concentrations were identified in our study, demonstrating the lack of pharmacokinetic interaction between gabapentin and oxcarbazepine. Noticeably, the bi-directional pharmacokinetic evaluation of brain antiepileptic drug concentrations was performed exclusively for the mixture at the fixed-ratio of 1:2, when oxcarbazepine was administered at the highest dose producing supra-additivity with gabapentin. Since the other fixed-ratio concentrations were not verified pharmacokinetically in the brain homogenates we cannot unequivocally state that the observed interactions are pharmacodynamic in nature for all fixed-ratio tested. However, the mixtures of oxcarbazepine and gabapentin for the fixed-ratios of 1:5, 1:10, 1:15, and 1:20 comprised of oxcarbazepine, administered at doses lower than that for the fixed-ratio of 1:2 (Table 1). In such a case, it is improbable that gabapentin (although administered at increasing doses) would be able to change significantly the brain concentrations of oxcarbazepine and interact pharmacokinetically with the latter drug administered at lower doses. Moreover, it is noteworthy that gabapentin neither binds to nor displaces other drugs from plasma proteins and the drug is not metabolized in liver, but excreted unchanged in urine (Rambeck et al., 1996; Perucca, 1999; Vollmer et al., 1986). Gabapentin has a minimal propensity for drug–drug interactions since it neither induces nor inhibits hepatic microsomal enzymes (Vollmer et al., 1986; Riva et al., 1996). In the clinical setting, gabapentin has no effect on plasma concentrations of concomitantly administered antiepileptic drugs, and inversely, other antiepileptics have no impact on gabapentin pharmacokinetics (Vajda, 2002). In contrast, oxcarbazepine and its rapidly formed monohydroxy derivative bind to plasma protein in 40%, undergo either glucuronidation or hydrolisation to a dihydroxy derivative in the liver, and are renally excreted (May et al., 2003; Rambeck et al., 1996). Additionally, oxcarbazepine and its monohydroxy derivative induce specific isoenzymes of cytochrome *P*450 (CYP 3A4 and CYP 3A5; May et al., 2003). Considering the above-mentioned facts, it may be concluded that gabapentin has an ideal pharmacokinetic profile (May et al., 2003; Schütz et al., 1986; Vollmer et al., 1986) and any pharmacokinetic interactions between gabapentin and oxcarbazepine are improbable to appear either in humans or in rodents. So, based on our pharmacokinetic study and theoretical considerations about gabapentin pharmacokinetic characteristics, one can accept that the isobolographically denoted interactions between gabapentin and oxcarbazepine are pharmacodynamic in nature.

Summing up, synergistic cooperation of both antiepileptic drugs in suppressing the maximal electroshock-induced seizures, lack of potentially acute neurotoxic (adverse) effects and no pharmacokinetic interaction between these antiepileptic drugs, make the combination of oxcarbazepine and gabapentin of some importance for patients with refractory partial epilepsy inadequately medicated with current front-line antiepileptic drugs. Nevertheless, only clinical trials in refractory patients would be able to verify and confirm the efficacy of combined treatment with gabapentin and oxcarbazepine in humans, providing reliable evidence about its efficacy in epileptic patients.

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