· Advances in Medical Sciences · Vol. 54(1) · 2009 · pp 66-74 · DOI: 10.2478/v10039-009-0004-9

Indapamide enhances the protective action of carbamazepine, phenobarbital, and valproate against maximal electroshock-induced seizures in mice

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Received 01.12.2008
Accepted 30.01.2009
Advances in Medical Sciences
Vol. 54(1) · 2009 · pp 66-74
DOI: 10.2478/v10039-009-0004-9

ABSTRACT

Purpose: To determine the influence of indapamide on the protective action of numerous conventional and second-generation antiepileptic drugs (carbamazepine, lamotrigine, oxcarbazepine, phenobarbital, topiramate and valproate) in the mouse maximal electroshock seizure model.

Material and Methods: Electroconvulsions were evoked in Albino Swiss mice by a current (sine-wave, 0.2 s stimulus duration) delivered via auricular electrodes. Adverse-effect profiles with respect to motor performance, long-term memory and skeletal muscular strength were measured along with total brain antiepileptic drug concentrations.

Results: Indapamide (up to 3 mg/kg, i.p., 120 min before the test) neither altered the threshold for maximal electroconvulsions, nor protected the animals against maximal electroshock-induced seizures in mice. Moreover, indapamide (3 mg/kg, i.p.) significantly enhanced the anticonvulsant action of carbamazepine, phenobarbital and valproate, but not that of lamotrigine, oxcarbazepine or topiramate in the maximal electroshock seizure test in mice. Indapamide (1.5 mg/kg) had no impact on the anticonvulsant action of all studied antiepileptic drugs in the maximal electroshock seizure test in mice. Estimation of total brain antiepileptic drug concentrations revealed that the observed interaction between indapamide and phenobarbital was complicated by a significant pharmacokinetic increase in total brain concentrations of phenobarbital. In contrast, indapamide had no impact on the total brain concentrations of carbamazepine and valproate in mice.

Conclusions: The selective potentiation of the anticonvulsant action of carbamazepine and valproate by indapamide and lack of any pharmacokinetic interactions between drugs, make the combinations of indapamide with carbamazepine or valproate of pivotal importance for epileptic patients taking these drugs together.

Key words: indapamide, antiepileptic drugs, maximal electroshock-induced seizures, pharmacokinetic interactions, pharmacodynamic interactions

INTRODUCTION

Indapamide [4-chloro-N-(2-methyl-1-indolinyl)-3-sulfamoylbenzamide hemihydrate] is an antihypertensive diuretic of the nonthiazide type [1], possessing also vasorelaxing [2], antiplatelet [3], antioxidant [4], and prostacyclin generation enhancing [5] activities. Experimental in vitro studies have revealed that indapamide inhibits calcium entry into vascular smooth muscle cell [6], blocks the slow component of the delayed rectifier potassium current and inhibits sodium and L-type calcium currents [7].

Generally, diuretics are prescribed for patients with mild or moderate hypertension, which may appear also in patients with epilepsy [8-10]. In such cases, both epilepsy control and reduction of blood pressure is required using specific drugs (antiepileptic and diuretic drugs). The therapeutic regimen in these patients should consist of simultaneous application of both drugs. However, concomitant application of two or more

drugs is usually associated with appearance of pharmacokinetic and/or pharmacodynamic interactions, which may theoretically lead to worse seizure control, no changes or increasing seizure control in patients by enhancing the anticonvulsant action of the administered antiepileptic drugs. On the other hand, the excessive application of diuretics may evoke loss of water with hyponatremia, which in turns may produce seizures, as it has been reported in patients taking indapamide in combination with amiloride [11].

Recently, it has been documented that furosemide (a loop diuretic) and amiloride (a potassium-sparing diuretic) potentiated the anticonvulsant action of some selected antiepileptic drugs in the mouse maximal electroshock-induced seizure model [12,13]. However, there was a difference between amiloride and furosemide in terms of the enhancement of the anticonvulsant action of conventional and second-generation antiepileptic drugs. It has been reported that furosemide potentiated only the anticonvulsant action of valproate, and remained inactive when combined with carbamazepine, lamotrigine, oxcarbazepine, phenobarbital, and topiramate [12]. In contrast, amiloride enhanced the anticonvulsant activity of all studied antiepileptic drugs (i.e., carbamazepine, oxcarbazepine, phenobarbital, topiramate, and valproate) except for lamotrigine, in the mouse maximal electroshock seizure model [13].

Since there has been documented a difference between furosemide and amiloride in terms of their potentiating effect on the anticonvulsant activity of antiepileptic drugs in the mouse maximal electroshock seizure model, it was of pivotal importance to evaluate the effects evoked by indapamide on the threshold for maximal electroconvulsions and on the antielectroshock action of some selected antiepileptic drugs in mice. Both, the threshold for maximal electroconvulsions and the maximal electroshock seizure test are thought to be experimental models of tonic-clonic seizures and, to a certain extent, of partial seizures with or without secondary generalization [14]. Noteworthy, in these experimental tests one can readily assess the anticonvulsant potential of agents and compounds possessing the antiepileptic properties, as well as, to determine their effects on conventional and second-generation antiepileptic drugs, fully effective in suppressing tonic-clonic seizures in humans [14]. Therefore, it was appropriate to use both tests in order to evaluate the effects of indapamide on the protective action of the various antiepileptic drugs in the mouse maximal electroshock seizure model. Additionally, we investigated the combinations of indapamide with conventional and second-generation antiepileptic drugs in relation to impairment of motor coordination, long-term memory and muscular strength by the use of the chimney test, step-through passive avoidance task and grip-strength test, respectively. Finally, total brain antiepileptic drug concentrations were measured with immunofluorescence in order to ascertain whether any observed effects were consequent to a pharmacodynamic and/or a pharmacokinetic interaction.

The purpose of this study was to determine the effect of indapamide on the threshold for maximal electroconvulsions and to assess its influence on the protective activity of numerous conventional and second-generation antiepileptic drugs (carbamazepine, lamotrigine, oxcarbazepine, phenobarbital, topiramate, and valproate) in the mouse maximal electroshock seizure test.

MATERIALS AND METHODS

Animals and experimental conditions

Adult male Swiss mice (weighing 22 – 26 g) that were kept in colony cages with free access to food and tap water, under standardized housing conditions (natural light-dark cycle, temperature of 23 ± 1 ºC, relative humidity of 55 ± 5 %), were used. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups each comprised of 8 mice. Each mouse was used only once and all tests were performed between 08.00 and 15.00 hours. Procedures involving animals and their care were conducted in accordance with current European Community and Polish legislation on animal experimentation. 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 described in this manuscript were approved by the Local Ethics Committee at the Medical University of Lublin (License no.: 27/2006) and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Drugs

The following drugs were used: indapamide (Sigma, St. Louis, MO, USA), carbamazepine (a gift from Polpharma, Starogard, Poland), lamotrigine (Lamictal®, Glaxo Wellcome, Middlesex, UK), oxcarbazepine (Trileptal®, Novartis Pharma AG, Basel, Switzerland), phenobarbital (Polfa, Krakow, Poland), topiramate (Topamax®, Cilag AG, Schaffhausen, Switzerland), and valproate (magnesium salt - kindly donated by ICN-Polfa S.A., Rzeszow, Poland). All drugs, except for valproate, were suspended in a 1 % solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water, while valproate was dissolved in distilled water. All drugs were administered intraperitoneally (i.p.) as a single injection, in a volume of 5 ml/kg body weight. Fresh drug solutions were prepared on each day of experimentation and administered as follows: indapamide - 120 min, lamotrigine, phenobarbital, and topiramate - 60 min, carbamazepine, oxcarbazepine and valproate - 30 min before the initiation of electroconvulsions, motor coordination, grip-strength and long-term memory tests, as well as, before brain sampling for the measurement of antiepileptic drug concentrations. The pretreatment times before testing of the antiepileptic drugs were based upon information about their biological activity from the literature and our previous experiments [12,13,15,16]. The times to the peak of maximum anticonvulsant effects for all antiepileptic drugs were used as the reference times in all behavioral tests and pharmacokinetic

estimation of total brain antiepileptic drug concentrations. The route of i.p. administration of indapamide and the pretreatment time before testing of its antielectroshock effect were based upon information from previous experiments [17].

Maximal electroconvulsions

Electroconvulsions were produced by a current (sine-wave, 500 V, 0.2 s stimulus duration) delivered *via* ear-clip electrodes by a Rodent Shocker generator (Type 221, Hugo Sachs Elektronik, Freiburg, Germany). The criterion for the occurrence of seizure activity was the tonic hind limb extension (i.e., the hind limbs of animals outstretched 180° to the plane of the body axis). In this experiment, two experimental models of maximal electroconvulsions were used: (1) maximal electroshock seizure threshold test and (2) maximal electroshock seizure test.

Maximal electroshock seizure threshold test

To evaluate the threshold for maximal electroconvulsions, at least 4 groups of control mice, consisting of 8 animals per group, were challenged with electroshocks of various current intensities ranging between 5 and 8 mA to yield 10 – 30%, 30 – 50%, 50 – 70%, and 70 – 90% of animals with seizures. Then, a current intensity-response relationship curve was constructed, according to a log-probit method by Litchfield and Wilcoxon [18], from which a median current strength (CS_{50} in mA) for the control animals was calculated. Each CS_{50} value represents the current intensity required to induce tonic hindlimb extension in 50 % of the mice challenged. Next, after administration of a single dose of indapamide to 4 groups of animals, the mice were subjected to electroconvulsions (each group with a constant current intensity ranging between 5 and 9 mA). The threshold for maximal electroconvulsions was recorded for 3 different doses of indapamide: 1.5, 3 and 6 mg/kg. The experimental procedure has been described in more detail in our earlier studies [12,13,15,16,19].

Maximal electroshock seizure test

The protective activity of carbamazepine, lamotrigine, oxcarbazepine, phenobarbital, topiramate, and valproate was determined as their median effective doses (ED_{50} values in mg/kg) against maximal electroshock-induced seizures (fixed current intensity of 25 mA). The animals were administered with different drug doses so as to obtain a variable percentage of protection against maximal electroshock-induced seizures, allowing for the construction of a dose-response relationship curve for each antiepileptic drug administered alone, according to Litchfield and Wilcoxon [18]. Each ED_{50} value represents the dose of a drug required to protect 50% of the animals tested against maximal electroshock-induced seizures. Similarly, the anticonvulsant activity of a mixture of an antiepileptic drug with indapamide was evaluated and expressed as the ED_{50} value, corresponding to a dose of an antiepileptic drug necessary to protect 50% of mice against tonic hindlimb extension in the maximal electroshock seizure test. In the present study, carbamazepine and oxcarbazepine were administered at doses ranging between 6 – 12 mg/kg, lamotrigine at doses ranging between 3 – 7 mg/kg, phenobarbital at doses ranging between 8 – 24 mg/kg, topiramate at doses ranging between 25 – 50 mg/kg, and valproate at doses ranging between 150 – 250 mg/kg. This experimental procedure has been described in detail in our earlier studies [12,13,15,16,19].

Measurement of total brain antiepileptic drug concentrations

Pharmacokinetic evaluation of total brain antiepileptic drug concentrations was performed only for those combinations of indapamide with antiepileptic drugs, whose anticonvulsant effect in the maximal electroshock seizure test was significantly greater than that for control (an antiepileptic drug + vehicle-treated) animals. Thus, the measurement of total brain concentrations of carbamazepine, phenobarbital and valproate, but not those of lamotrigine, oxcarbazepine or topiramate was undertaken at the doses, which corresponded to their ED_{50} values from the maximal electroshock seizure test. Mice were killed by decapitation at times reflecting the peak of maximum anticonvulsant effects for the drugs in the maximal electroshock seizure test. The whole brains of mice were removed from skulls, weighed, harvested and homogenized using Abbott buffer (1:2 weight/volume) in an Ultra-Turrax T8 homogenizer (IKA Werke, Staufen, Germany). The homogenates were centrifuged at 10,000 *g* for 10 min. The supernatant samples (75 µl) were analyzed by fluorescence polarization immunoassay for carbamazepine, phenobarbital, and valproate content using a TDx analyzer and reagents exactly as described by the manufacturer (Abbott Laboratories, North Chicago, IL, USA). Total brain antiepileptic drug concentrations were expressed in µg/ml of brain supernatants as means ± S.D. of 8 separate brain preparations.

Chimney test

The chimney test of Boissier *et al*. [20] was used to quantify the adverse effect potential of conventional and second-generation antiepileptic drugs administered in combination with indapamide on motor performance in mice. In this test, the animals had to climb backwards up a plastic tube (3 cm inner diameter, 30 cm long), and impairment of motor performance was indicated by the inability of the mice to climb backward up the transparent tube within 60 s. The acute adverse effect potentials for the combinations of conventional and second-generation antiepileptic drugs with indapamide were determined for the antiepileptic drugs administered at doses corresponding to their ED_{50} values from the maximal electroshock seizure test when combined with indapamide. This experimental procedure has been described in detail in our earlier studies [12,13,15,16,19].

Grip-strength test

The effects of combinations of indapamide with conventional and second-generation antiepileptic drugs at their ED_{50} values from the maximal electroshock seizure test, on skeletal

Table 1. **Effect of indapamide on the threshold for electroconvulsions in mice.**

Treatment (mg/kg)	CS_{50} (mA)	n	S.E.M.
Vehicle	5.54 (4.88 – 6.29)	16	0.357
Indapamide (1.5)	5.66 (4.98 – 6.43)	16	0.369
Indapamide (3.0)	6.21 (5.07 – 7.62)	8	0.645
Indapamide (6.0)	7.61 (6.72 – 8.62) **	24	0.482
F (3; 60) = 5.211; P = 0.0029			

Data are presented as median current strengths (CS_{50} values with 95% confidence limits in parentheses), required to produce tonic hindlimb extension in 50% of animals tested in the maximal electroshock seizure threshold test. Indapamide was administered i.p. 120 min before the test. Statistical evaluation of the data was performed with log-probit method [18] associated with one-way ANOVA [19] followed by the post-hoc Tukey-Kramer test for multiple comparisons. *n* – number of animals tested at those current strength intensities, whose seizure effects ranged between 16% and 84%; S.E.M. – standard error of the mean of CS_{50} values; *F* – F-statistics from one-way ANOVA; *P* – probability value from one-way ANOVA.

**P<0.01 vs. the respective control group (vehicle-treated animals).

muscular strength in mice were quantified by the grip-strength test of Meyer *et al*. [21]. The time before the commencement of the grip-strength test (after drug administration) was identical to that for the maximal electroshock seizure test. The grip-strength apparatus (BioSeb, Chaville, France) comprised a wire grid (8 × 8 cm) connected to an isometric force transducer (dynamometer). The mice were lifted by the tails so that their forepaws could grasp the grid. The mice were then gently pulled backward by the tail until the grid was released. The maximal force exerted by the mouse before losing grip was recorded. The mean of 3 measurements for each animal was calculated and subsequently, the mean maximal force of 8 animals per group was determined. The muscular strength in mice was expressed in N (newtons) as means ± S.E.M. of 8 determinations. This experimental procedure has been described in detail in our earlier studies [12,13,15,16,22].

Step-through passive avoidance task

Each animal was administered an antiepileptic drug either singly or in combination with indapamide on the first day before training. The time before the commencement of the training session (after drug administration) was identical to that for the maximal electroshock seizure test. Subsequently, animals were placed in an illuminated box (10 × 13 × 15 cm) connected to a larger dark box (25 × 20 × 15 cm) equipped with an electric grid floor. Entrance of animals to the dark box was punished by an adequate electric footshock (0.6 mA for 2 s). The animals that did not enter the dark compartment were excluded from subsequent experimentation. On the following day (24 h later), the pre-trained animals were placed again into the illuminated box and observed up to 180 s. Mice that avoided the dark compartment for 180 s were considered to remember the task. The time that the mice took to enter the dark box, was noted and the median latencies (retention times) with 25th and 75th percentiles were calculated. The step-through passive avoidance task gives information about ability to acquire the task (learning) and to recall the task (retrieval). Therefore, it may be regarded as a measure of long-term memory [23]. This experimental procedure has been described in detail in our earlier studies [24,25].

Statistics

Both, CS_{50} and ED_{50} values with their 95 % confidence limits were calculated by computer log-probit analysis according to Litchfield and Wilcoxon [18]. Subsequently, the respective 95% confidence limits were transformed to S.E.M. as described previously [19]. Statistical analysis of data from the maximal electroshock seizure tests was performed with one-way analysis of variance (ANOVA) followed by the post-hoc Tukey-Kramer test for multiple comparisons. Total brain antiepileptic drug concentrations were statistically compared using the unpaired Student's *t*-test. Qualitative variables from the chimney test were compared by use of the Fisher's exact probability test, whereas, the results obtained in the step-through passive avoidance task were statistically evaluated using Kruskal-Wallis nonparametric ANOVA. The results from the grip-strength test were verified with one-way ANOVA. Differences among values were considered statistically significant if P<0.05. All statistical tests were performed using commercially available GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Influence of indapamide on the threshold for maximal electroconvulsions

Indapamide administered systemically (i.p., 120 min prior to the test), at doses of 1.5 and 3 mg/kg did not affect the threshold for maximal electroconvulsions in mice (*Tab. 1*). In this case, the experimentally derived CS_{50} values for animals receiving indapamide did not differ significantly from the CS_{50} value as determined for control animals in the maximal electroshock seizure threshold test in mice (*Tab. 1*). In contrast, indapamide administered at a dose of 6 mg/kg significantly elevated (by 37 %) the threshold for maximal electroconvulsions in mice (P<0.01; *Tab. 1*).

Table 2. **Effect of indapamide on the protective action of conventional and second-generation antiepileptic drugs in the maximal electroshock-induced seizure test in mice.**

Treatment (mg/kg)	ED_{50} (mg/kg)	n	S.E.M.
Carbamazepine + vehicle	11.3 (10.2 – 12.6)	24	0.602
Carbamazepine + indapamide (1.5)	10.0 (8.6 – 11.6)	16	0.769
Carbamazepine + indapamide (3.0)	8.5 (6.8 – 10.6) *	16	0.955
F (2; 53) = 3.574; P = 0.035			
Lamotrigine + vehicle	4.8 (3.5 – 6.6)	16	0.771
Lamotrigine + indapamide (1.5)	4.3 (3.1 – 6.0)	24	0.739
Lamotrigine + indapamide (3.0)	3.8 (2.9 – 4.9)	16	0.511
F (2; 53) = 0.418; P = 0.660			
Oxcarbazepine + vehicle	9.9 (8.3 – 11.7)	8	0.871
Oxcarbazepine + indapamide (1.5)	8.5 (6.8 – 10.6)	16	0.955
Oxcarbazepine + indapamide (3.0)	6.6 (5.2 – 8.4)	16	0.801
F (2; 37) = 2.861; P = 0.070			
Phenobarbital + vehicle	19.9 (17.3 – 22.9)	24	1.422
Phenobarbital + indapamide (1.5)	17.4 (14.9 – 20.2)	16	1.352
Phenobarbital + indapamide (3.0)	11.4 (8.8 – 14.6) **	24	1.459
F (2; 61) = 10.10; P = 0.0002			
Topiramate + vehicle	37.4 (30.7 – 45.6)	16	3.760
Topiramate + indapamide (1.5)	39.1 (31.6 – 48.6)	24	4.298
Topiramate + indapamide (3.0)	36.3 (29.9 – 44.1)	16	3.599
F (2; 53) = 0.126; P = 0.882			
Valproate + vehicle	230.4 (209.2 – 253.7)	16	11.309
Valproate + indapamide (1.5)	210.6 (188.8 – 235.0)	16	11.752
Valproate + indapamide (3.0)	188.0 (163.3 – 216.5) *	24	11.502
F (2; 53) = 3.412; P = 0.040			

Results are presented as median effective doses (ED_{50} in mg/kg, with 95% confidence limits in parentheses) of antiepileptic drugs, protecting 50% of animals tested against maximal electroshock-induced hindlimb extension. The drugs were administered i.p.: indapamide – 120 min, phenobarbital, lamotrigine and topiramate – 60 min, carbamazepine, oxcarbazepine and valproate – 30 min prior to the maximal electroshock seizure test. Statistical analysis of data was performed with one-way ANOVA followed by the post-hoc Tukey-Kramer test for multiple comparisons. *n* – total number of animals used at those doses whose anticonvulsant effects ranged between 4 and 6 probits; S.E.M. – standard error of the mean of ED_{50} values; F – F-statistics from one-way ANOVA; P – probability value from one-way ANOVA.
*P<0.05 and **P<0.001 vs. the respective control group (antiepileptic drug + vehicle-treated animals).

Effects of indapamide on the protective action of conventional and second-generation antiepileptic drugs in the mouse maximal electroshock seizure model

All studied antiepileptic drugs (carbamazepine, lamotrigine, oxcarbazepine, phenobarbital, topiramate and valproate) administered singly exhibited a clear-cut anticonvulsant activity in the maximal electroshock seizure test in mice and their ED_{50} values are presented in *Tab. 2*. When indapamide at a dose of 3 mg/kg was co-administered with carbamazepine, it significantly enhanced the anticonvulsant action of the latter drug in the maximal electroshock seizure test by reducing its ED_{50} value from 11.3 mg/kg to 8.5 mg/kg (by 25 %; P<0.05; *Tab. 2*). Similarly, indapamide at a dose of 3 mg/kg significantly potentiated the protective action of phenobarbital against maximal electroshock-induced seizures by reducing the ED_{50} value of the latter drug from 19.9 mg/kg to 11.4 mg/kg (by 43 %; P<0.001; *Tab. 2*). Indapamide (3 mg/kg) also significantly enhanced the anticonvulsant action of valproate in the maximal electroshock seizure test in mice by diminishing its ED_{50} value from 230.4 mg/kg to 188.0 mg/kg (by 18 %; P<0.05; *Tab. 2*). In contrast, indapamide at a lower dose of 1.5 mg/kg had no impact on the anticonvulsant potency of carbamazepine, phenobarbital and valproate in the maximal electroshock seizure test. Moreover, one-way ANOVA followed by the post-hoc Tukey-Kramer test for multiple comparisons revealed that indapamide at doses of 1.5 and 3 mg/kg did not significantly affect the anticonvulsant action of lamotrigine, oxcarbazepine and topiramate in the maximal electroshock seizure test in mice (*Tab. 2*).

Table 3. **Total brain concentrations of the studied antiepileptic drugs when administered singly or in combination with indapamide.**

Treatment (mg/kg)	Brain concentration (μg/ml)
Carbamazepine (8.5) + vehicle	1.446 ± 0.192
Carbamazepine (8.5) + indapamide (3.0)	1.435 ± 0.305
Phenobarbital (11.4) + vehicle	2.191 ± 0.350
Phenobarbital (11.4) + indapamide (3.0)	2.644 ± 0.427*
Valproate (188.0) + vehicle	40.08 ± 3.464
Valproate (188.0) + indapamide (3.0)	41.20 ± 3.132

Data are presented as means ± S.D. (n = 8). Total brain antiepileptic drug concentrations were determined with fluorescence polarization immunoassay. Statistical evaluation of data was performed using the unpaired Student's t-test. The drugs were administered i.p. at times scheduled from the maximal electroshock seizure test and at doses corresponding to their ED_{50} values against maximal electroshock-induced seizures (for more detail see the legend to Tab. 2).
*$P<0.05$ vs. the respective control group (antiepileptic drug + vehicle-treated animals).

Influence of indapamide on total brain antiepileptic drug concentrations

Fluorescence polarization immunoassay revealed that indapamide at a dose of 3 mg/kg significantly increased (by 21 %) total brain concentration of phenobarbital co-administered at a dose of 11.4 mg/kg ($P<0.05$), as compared to when phenobarbital was administered alone (*Tab. 3*). In contrast, indapamide at a dose of 3 mg/kg did not significantly alter total brain concentrations of carbamazepine or valproate in mice (*Tab. 3*). The total brain concentrations of lamotrigine, oxcarbazepine and topiramate combined with indapamide were not measured and pharmacokinetically verified in the present study because indapamide did not significantly potentiate the anticonvulsant potency of the second-generation antiepileptic drugs in the maximal electroshock seizure test in mice.

Effects of indapamide in combination with various antiepileptic drugs on motor performance, long-term memory, and muscular strength of animals in the chimney, step-through passive avoidance and grip-strength tests

When indapamide was administered in combination with carbamazepine, lamotrigine, oxcarbazepine, phenobarbital, topiramate, and valproate at doses corresponding to their ED_{50} values from the maximal electroshock seizure test, motor performance as assessed by the chimney test was unaffected (*Tab. 4*). Furthermore, none of the combinations studied impaired long-term memory as determined in the passive avoidance test (*Tab. 4*). Similarly, indapamide concomitantly administered with the studied antiepileptic drugs had no significant impact on skeletal muscular strength of the animals as assessed by the grip-strength test (*Tab. 4*).

Table 4. **Effects of indapamide, antiepileptic drugs and their combinations on long-term memory, skeletal muscular strength and motor performance in mice.**

Treatment (mg/kg)	Retention time (s)	Grip-strength (N)	Motor coordination impairment (%)
Control	180 (180; 180)	92.08 ± 5.68	0
Indapamide (3.0) + vehicle	180 (170; 180)	88.50 ± 5.27	12.5
Carbamazepine (8.5) + vehicle	180 (180; 180)	89.93 ± 5.74	0
Indapamide (3.0) + carbamazepine (8.5)	180 (180; 180)	87.63 ± 5.67	12.5
Lamotrigine (3.8) + vehicle	180 (180; 180)	89.92 ± 5.47	0
Indapamide (3.0) + lamotrigine (3.8)	180 (175; 180)	89.50 ± 5.36	12.5
Oxcarbazepine (6.6) + vehicle	180 (180; 180)	89.44 ± 5.64	0
Indapamide (3.0) + oxcarbazepine (6.6)	180 (162.5; 180)	88.91 ± 5.44	0
Phenobarbital (11.4) + vehicle	180 (180; 180)	91.04 ± 5.69	0
Indapamide (3.0) + phenobarbital (11.4)	180 (165.5; 180)	89.77 ± 5.55	25
Topiramate (36.3) + vehicle	180 (180; 180)	90.63 ± 5.52	0
Indapamide (3.0) + topiramate (36.3)	180 (155; 180)	88.97 ± 5.91	12.5
Valproate (188.0) + vehicle	180 (155.5; 180)	90.15 ± 5.78	0
Indapamide (3.0) + valproate (188.0)	170.5 (135.5; 180)	87.99 ± 5.62	25

Results are presented as: 1) median retention times (in seconds with 25^{th} and 75^{th} percentiles in parentheses) from the passive avoidance task, assessing long-term memory in mice; 2) mean grip-strengths (in newtons ± S.E.M.; n = 8) from the grip-strength test, assessing muscular strength in mice; and 3) percentage of animals showing motor coordination impairment in the chimney test. Each experimental group consisted of 8 mice. Statistical analysis of data from the passive avoidance task was performed with nonparametric Kruskal-Wallis ANOVA test, whereas those from the grip-strength test were analyzed with one-way ANOVA. The Fisher's exact probability test was used to analyze the results from the chimney test. All drugs were administered i.p. at times scheduled from the maximal electroshock seizure test and at doses corresponding to their ED_{50} values against maximal electroshock-induced seizures (for more detail see the legend to Tab. 2).

DISCUSSION

Here it was found that indapamide administered systemically (i.p.) at 120 min before the maximal electroshock seizure threshold test, in a dose-dependent manner increased the threshold for maximal electroconvulsions in mice. Moreover, indapamide at a dose of 3 mg/kg, which *per se* did not affect the electroconvulsive threshold in experimental animals, significantly enhanced the anticonvulsant action of conventional antiepileptic drugs (carbamazepine, phenobarbital and valproate) in the maximal electroshock seizure test in mice. However, it was documented that indapamide at the dose of 3 mg/kg did not affect the antielectroshock action of second-generation antiepileptic drugs (lamotrigine, oxcarbazepine, and topiramate) in the maximal electroshock seizure test in mice, although a slight reduction in the ED_{50} values for the studied antiepileptic drugs was observed. The lack of significant effects of indapamide on the anticonvulsant profile of second-generation antiepileptic drugs is surprising because of the fact that these antiepileptic drugs are sometimes more effective in terms of seizure suppression than conventional antiepileptic drugs [26]. Therefore, the potency of enhancement of the anticonvulsant action of second-generation antiepileptic drugs should theoretically be higher than that for conventional antiepileptic drugs in the maximal electroshock seizure test in mice.

Moreover, the pharmacokinetic evaluation of total brain concentrations of conventional antiepileptic drugs revealed that indapamide significantly increased total brain phenobarbital concentrations and thus, the observed interaction between indapamide and phenobarbital in the maximal electroshock seizure test was pharmacokinetic in nature. However, in the maximal electroshock seizure test, it was documented that the anticonvulsant action of phenobarbital after co-administration of indapamide at 3 mg/kg was enhanced by 43%, as compared to control (phenobarbital + vehicle-treated) animals. Since the pharmacokinetic study revealed that indapamide increased total brain phenobarbital concentrations by 21%, it was evident that this increase in total brain phenobarbital concentration could not be completely responsible for a 43% enhancement of the anticonvulsant action of phenobarbital after administration of indapamide. Therefore, one should ascertain that the combination of indapamide with phenobarbital is both, pharmacokinetic and pharmacodynamic in nature. In the case of the combinations of indapamide with carbamazepine and valproate, it was found that indapamide had no significant impact on the total brain concentrations of the former antiepileptic drugs and thus, the observed interactions in the maximal electroshock seizure test between drugs were pharmacodynamic in nature.

As mentioned in the introduction, it has recently been documented that furosemide administered i.p. at a dose of 100 mg/kg enhanced the anticonvulsant action of valproate, but not that of carbamazepine, lamotrigine, phenobarbital, oxcarbazepine, or topiramate in the mouse maximal electroshock seizure model [12]. The observed interaction between furosemide and valproate was pharmacodynamic in nature since neither free plasma, nor total brain concentrations of valproate were altered after the co-administration of furosemide [12]. On the other hand, amiloride administered i.p. at doses of 75 and 100 mg/kg, potentiated the anticonvulsant action of carbamazepine, phenobarbital, valproate, topiramate, and oxcarbazepine, but not that of lamotrigine in the maximal electroshock seizure test in mice [13]. The pharmacokinetic estimation of total brain antiepileptic drug concentrations revealed that the observed interactions between amiloride and carbamazepine, oxcarbazepine and phenobarbital were pharmacokinetic, while the interactions of amiloride with lamotrigine, topiramate and valproate were pharmacodynamic in nature [13]. Considering the above-mentioned effects evoked by furosemide and amiloride in combination with conventional and second-generation antiepileptic drugs in the maximal electroshock seizure test in mice, as well as, the results presented in this study, one can ascertain that indapamide takes a middle place between amiloride and furosemide as regards the effects evoked by the diuretics on the anticonvulsant action of conventional and second-generation antiepileptic drugs.

Furthermore, it is worth mentioning that the combinations of indapamide with conventional and second-generation antiepileptic drugs (administered at doses corresponding to their ED_{50} values from the maximal electroshock seizure test) neither altered motor coordination in animals as documented in the chimney test, nor disturbed long-term memory in mice subjected to the passive avoidance task. Additionally, it was reported that indapamide in combination with the studied antiepileptic drugs had no impact on skeletal muscular strength in mice challenged with the grip-strength test.

CONCLUSIONS

The enhancement of the anticonvulsant action of carbamazepine and valproate by indapamide accompanied with no pharmacokinetic interactions between drugs, and no acute adverse effects exerted by the antiepileptic drugs combined with indapamide make these combinations of pivotal importance for patients receiving carbamazepine or valproate and indapamide. The utmost caution is advised when combining indapamide with phenobarbital due to the appearance of pharmacokinetic interactions between drugs in further clinical practice. In the case of the other antiepileptic drugs studied (i.e., lamotrigine, oxcarbazepine and topiramate) in combination with indapamide, the observed interactions between drugs seem to be neutral when considering both, the anticonvulsant and acute adverse-effect profiles of the studied antiepileptic drugs after co-administration with indapamide.

ACKNOWLEDGEMENTS

Project supported by grants from Medical University of Lublin (DS 475/2006-2008 and PW 557/2008). The authors are grateful for the generous gifts of valproate from ICN-Polfa S.A. (Rzeszow, Poland) and carbamazepine from Polpharma S.A. (Starogard, Poland).

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