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ORIGINAL ARTICLE

Characterization of acute adverse-effect profile of carbamazepine and valproate in the grip-strength test in mice

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Abstract: The aim of this study was to assess the acute adverse (neurotoxic) effects of two conventional antiepileptic drugs (carbamazepine and valproate) in the grip-strength test by measuring skeletal muscular strength in mice. Linear regression analysis of dose-response relationship for drug doses and their corresponding skeletal muscular strength allowed the determination of the doses of carbamazepine and valproate that reduced the grip-strength in mice by 50%, as compared to control mice. The experimentally-derived strength reducing dose by 50% for carbamazepine was 80.8 mg/kg, and that for valproate – 501.2 mg/kg. Based on this preclinical study, one can conclude that carbamazepine and valproate reduced in a dose-dependent manner skeletal muscular strength in mice. The grip-strength test can be applied as a paradigm for the evaluation of acute adverse-effect (neurotoxic) profile of drugs with respect to their influence on muscular strength in experimental mice.

Key words: carbamazepine, valproate, grip-strength, dose-response relationship analysis, acute adverse-effect profile

INTRODUCTION

The selection and choice of an antiepileptic drug (AED) for the treatment of epilepsy patients is based primarily on the anticonvulsant properties of an AED in terms of suppressing specific seizure types and, additionally, on its adverse-effect (neurotoxic) profile, observed in epilepsy patients [1]. The application of an AED to epileptic patients is very often terminated due to some acute (harmful or life-threatening) adverse effects exerted by an AED, despite the complete protection of the drug against seizures [2]. In clinical settings, the side effects produced by an AED appear either after acute administration of the drug in high (intolerable) doses or after accumulation of drug doses and chronic intoxication of the human organism [2]. On the other hand, some epileptic patients, during the chronic treatment with AEDs, are able to tolerate high doses of AEDs without any signs of adverse effects [2]. Generally, the acute adverse effects produced by AEDs are related to their influence on normal brain functioning; therefore, the first signs of acute intoxication with AEDs in epileptic patients are manifested in the form of some neurotoxic symptoms, such as: ataxia, nystagmus, dysarthria, somnolence, diplopia, headache, dizziness, nausea, tremor, loss of weight, drowsiness, sedation, depression, agitation, changes in skeletal muscular strength, or other neurological dysfunctions [1].

In preclinical studies on animals, the AEDs also produce acute adverse effects such as: sedation, ataxia, tremor, motor coordination impairment, disturbances in locomotor activity and/or alterations in muscular strength [3]. Therefore, we endeavoured to determine the acute adverse (neurotoxic) effects produced by two commonly used conventional AEDs (carbamazepine [CBZ] and valproate [VPA]) with respect to their propensity to impair skeletal muscular strength in the grip-strength test in mice. Experimental design in this study allowed the assessment of the dose-response relationship between doses of both drugs, and their resultant decrease in muscular strength. Thus, the doses of CBZ and VPA that reduced muscular strength by 50% (D_{50}), as compared to control animals in the grip-strength test in mice, were calculated using linear regression analysis of dose-response relationship according to Motulsky and Christopoulos [4].

MATERIAL AND METHODS

Animals and experimental conditions. Adult male Swiss mice (7-week-old; weighing 22 – 26 g) 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°C, relative humidity of 55 ± 5%). After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups, each group comprising 8 mice. Each mouse was used only once and all tests were performed between 08.00 – 15.00. Procedures involving mice 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 mice necessary to produce reliable scientific data. The experimental protocols and procedures described in this article were approved by the First Local Ethics Committee in Lublin (License No. 543/2005/585/2005) and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

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Drugs. The following AEDs were used in the study: CBZ (a gift from Polfa, Starogard, Poland), and VPA - magnesium salt (kindly donated by ICN-Polfa SA, Rzeszow, Poland). CBZ was suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water, while VPA was directly dissolved in distilled water. All drugs were administered intraperitoneally (i.p.) as a single injection, in a volume of 5 ml/kg body wt. Fresh drug solutions were prepared on each day of experimentation and administered 30 min. before grip-strength testing. The pretreatment time before the testing of CBZ and VPA reflects the time to peak of maximum anticonvulsant effects produced by these AEDs [5, 6].

Grip-strength test. The effects of CBZ and VPA (administered alone at increasing doses) on skeletal muscular strength in mice were quantified by the grip-strength test. The time before the commencement of the grip-strength test (after drug administration) was 30 min. 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 skeletal muscular strength in mice was expressed in N (Newtons) as means ± SEM of 8 determinations. This experimental procedure has been described in detail elsewhere [7].

Doses of CBZ and VPA were plotted on the X-axis of the Cartesian plot system, whereas their corresponding grip-strength values were plotted on the Y-axis. Subsequently, dose-response relationship lines for CBZ and VPA were constructed using linear regression analysis according to Motulsky and Christopoulos [4]. It should be noted that the doses of CBZ and VPA were selected by considering their degree of strength impairment by the drugs in the experimental mice, compared to control (vehicle-treated) mice. Only doses of CBZ and VPA that reduced grip-strength were placed on the Cartesian plot system and analyzed with linear regression to determine the dose-response relationship lines and strength reducing doses by 50% (D_{50}). This is the reason why CBZ was administered at doses ranging between 40-110 mg/kg, and VPA within the range 200-700 mg/kg. All required calculations and dose-response relationship analyses were performed using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Influence of CBZ on skeletal muscular strength in the grip-strength test in mice. In the grip-strength test, CBZ was administered separately at increasing doses from 40 – 110 mg/kg and the resultant skeletal muscular strength in mice was plotted in the Cartesian plot system (Figure 1). Subsequently, linear regression analysis of dose-response relationship between drug doses and their resultant effects allowed determination of the equation for CBZ as follows: $y = -1.589\ x + 180.4$ [$r^2 = 0.950$]; where y – is the muscular strength value, x – the drug dose, and r^2 – the coefficient of determination (Figure 1). From the equation of dose-response relationship, the dose of CBZ that reduced muscular grip-strength in mice by 50% (D_{50}), as compared to the control mice, was calculated. In this case, the D_{50} for CBZ was 80.8 mg/kg (Figure 1). The experimentally-derived grip-strength in control (vehicle-treated) mice was 102.6 N (result not shown).

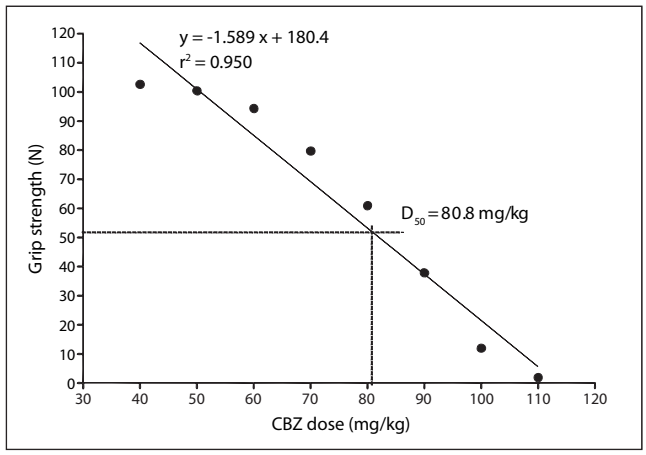

Figure 1 Dose-response relationship between carbamazepine (CBZ) doses and their corresponding skeletal muscular strength in the grip-strength test in mice.

Doses of carbamazepine (CBZ) are plotted graphically on the X-axis; resultant skeletal muscular strength is plotted on the Y-axis. The solid line between black circular points on the graph reflects the dose-response relationship line for CBZ doses and their corresponding grip-strengths. The dotted lines indicate the strength reducing dose by 50% (D_{50}), compared with the strength of control animals, which was 102.6 N. CBZ was administered i.p. at 30 min. before the strength testing. The equation of dose-response relationship for CBZ doses and their corresponding muscular strength in mice is as follows: $y = -1.589\ x + 180.4$ [$r^2 = 0.950$]; where y – is the muscular strength value, x – is the drug dose, and r^2 – the coefficient of determination which assesses the goodness-to-fit for the denoted dose-response line. D_{50} – dose of CBZ for which the strength in animals was reduced by 50%.

Effect of VPA on skeletal muscular strength in the grip-strength test in mice. VPA was administered separately at increasing doses from 200 – 700 mg/kg and their resultant muscular strength in mice was plotted in the Cartesian plot system (Figure 2). Subsequently, linear regression analysis of dose-response relationship between drug doses and their resultant effects allowed the determination of the equation for VPA as follows: $y = -0.2036\ x + 152.8$ [$r^2 = 0.9386$] (Figure 2).

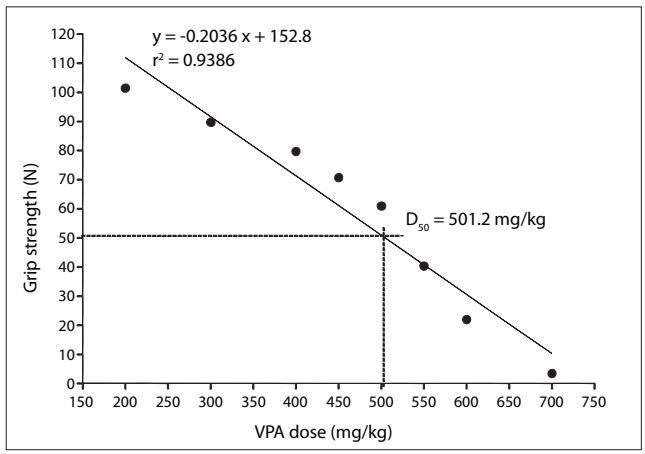

Figure 2 Dose-response relationship between valproate (VPA) doses and their corresponding skeletal muscular strength in the grip-strength test in mice.

Doses of valproate (VPA) are plotted graphically on the X-axis; resultant skeletal muscular strength is plotted on the Y-axis. The solid line between black circular points reflects the dose-response relationship line for VPA doses and their corresponding grip-strength values. VPA was administered i.p. at 30 min. before the strength testing. For more details see legend to Figure 1.

Based on this linear equation, the dose of VPA reducing muscular strength in mice by 50% (D_{50}) was 501.2 mg/kg (Figure 2).

DISCUSSION

The purpose of this study was to assess the effect of two conventional AEDs (CBZ and VPA) on skeletal muscular strength in mice. The experimental design in this study allowed the determination of the doses of both drugs that reduced muscular grip strength by 50% (D_{50}), as compared to control mice. For the first time, it was found that CBZ and VPA dose-dependently reduced skeletal muscular grip-strength in mice. To assess unequivocally the neurotoxic potential of AEDs, the effects produced by the AEDs in the grip-strength test were compared with the effects exerted by the same drugs in other experimental models, evaluating acute adverse effects, i.e., in the rotarod and chimney tests in mice. Generally, the rotarod test evaluates the influence of drugs on motor coordination and balance during movements, whereas the chimney test assesses the effects of drugs on muscular strength and movement synchronization in rodents [3]. Comparison of median toxic doses (TD_{50} values) for CBZ and VPA, as determined in the chimney and rotarod tests, with strength reducing doses by 50% (D_{50} values), as denoted in the grip-strength test (Table 1), revealed that the D_{50} values in mice were substantially higher than those required to impair motor coordination in the rotarod and chimney tests in mice. Therefore, the grip-strength test can be used as a supplementary test in order to differentiate between the adverse effects produced by the drugs, especially to verify whether the observed impairment of motor coordination in animals is evoked by impairment of balance and de-synchronization of movements, or is evoked by loss of skeletal muscular strength in mice. It is noteworthy that the D_{50} values in this study were calculated using linear regression analysis according to Motulsky and Christopoulos [4], whereas the TD_{50} values in the rotarod and chimney tests were calculated using log-probit analysis according to Litchfield and Wilcoxon [8]. There is no doubt that the chimney and rotarod tests are alternative tests, because they evaluate similar adverse effects (motor coordination) in animals, and the conventional AEDs have quite similar TD_{50} values (Table 1).

Finally, based on this preclinical study, one can conclude that the grip-strength test is able to evaluate the acute adverse-effect potential of AEDs at high (neurotoxic) doses with respect to the reduction of muscular strength. The experimental procedure described here for determining D_{50} values in the grip-strength test for AEDs can be readily applied in preclinical studies to determine the D_{50} values for other drugs affecting CNS. In our opinion, the D_{50} values perfectly characterize the drug propensity to diminish muscular grip-strength. Despite the fact that D_{50} values for CBZ and VPA in mice were higher than their corresponding TD_{50} values in the chimney and rotarod tests, the grip-strength test could be applied as an alternative test to assess acute adverse effects with respect to the reduction of muscular strength after AED administration at high doses.

Table 1 Comparison of acute adverse effects of carbamazepine (CBZ) and valproate (VPA) in various experimental tests in mice.

Experimental test	CBZ (mg/kg)	VPA (mg/kg)	References
Grip-strength	80.8	501	present study
Rotarod	53.6	363	[5]
Chimney	53.3	393	[6]

Values are presented as doses of AEDs reducing the strength by 50% (D_{50}) in animals subjected to the grip-strength test and as median toxic doses (TD_{50}), impairing motor coordination in 50% of animals challenged with the rotarod and chimney tests.

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