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Acute adverse-effect profile of ethosuximide and stiripentol in the grip-strength test in mice

Jarogniew J. Łuszczki[1,2]

[1] *Department of Pathophysiology, Medical University, Lublin, Poland*
[2] *Department of Physiopathology, Institute of Agricultural Medicine, Lublin, Poland*

Abstract: The aim of the study was assessment of acute adverse (neurotoxic) effects of ethosuximide and stiripentol (two antiepileptic drugs) 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 determination of the doses of antiepileptic drugs that reduced the grip-strength in mice by 50%, compared with control animals. Results indicate that the experimentally-derived dose reducing muscular strength in mice by 50% (D_{50}) for ethosuximide was 755.4 mg/kg and that for stiripentol was 971.2 mg/kg. The studied antiepileptic drugs reduced skeletal muscular strength in mice in a dose-dependent manner.

Key words: antiepileptic drugs, ethosuximide, stiripentol, grip-strength test, acute adverse-effect profile

INTRODUCTION

In spite of progress in our understanding of the pathophysiological processes underlying seizure initiation, amplification and propagation in the brain, and a wide range of classical (first-generation), newer (second-generation) and novel (third-generation) antiepileptic drugs, there are still approximately 30% of epileptic patients who fail to respond satisfactorily to current antiepileptic drug therapies [1, 2]. The selection and choice of antiepileptic drugs for the treatment of patients with epilepsy is based primarily on the anticonvulsant properties of the antiepileptic drugs with respect to suppressing specific seizure types, and additionally on their adverse-effect (neurotoxic) profiles observed in patients [3, 4]. In clinical settings, the adverse effects produced by antiepileptic drugs appear either after acute administration, or after accumulation of the drugs and/or chronic intoxication of the human organism [3, 5-7].

Generally, acute adverse effects produced by antiepileptic drugs are related to their influence on normal brain functioning; therefore, the first signs of acute intoxication with antiepileptic drugs in epileptic patients are manifested in the form of some neurotoxic symptoms such as: ataxia, somnolence, headache, sedation, depression, agitation, changes in skeletal muscular strength, or other neurological dysfunctions [3].

In preclinical studies on animals, the antiepileptic drugs also produce acute adverse effects such as: sedation, ataxia, tremor, motor coordination impairment, disturbances in locomotor activity and/or alterations in muscular strength [8].

It has also been reported that some classical and second-generation antiepileptic drugs (i.e., carbamazepine, valproate, clonazepam, phenytoin, phenobarbital, lamotrigine, oxcarbazepine and topiramate) produce impairment of skeletal muscular strength in mice in a dose-dependent manner [9, 10]. The aim of this study therefore was to determine the acute adverse (neurotoxic) effects produced by ethosuximide and stiripentol (two other antiepileptic drugs) with respect to their propensity to impair skeletal muscular strength in the grip-strength test in mice. The experimental design utilised in this study allowed the assessment of dose-response relationship between drug doses and their resultant changes in muscular strength. Thus, the doses of the studied antiepileptic drugs that reduced muscular strength by 50% (D_{50}), 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 [11].

MATERIAL AND METHODS

Animals and experimental conditions. Adult male Swiss mice were used (8-weeks-old, weighing 22–26 g), 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 comprising of 8 mice each. Each mouse was used only once and all tests were performed between 08:00-03:00. 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 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).

Corresponding author: Prof. Jarogniew Łuszczki, Department of Pathophysiology,
Medical University, Jaczewskiego 8, 20-090 Lublin, Poland.
E-mail: jarogniew.luszczki@am.lublin.pl / jluszczki@yahoo.com

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Drugs. The following antiepileptic drugs were used in this study: ethosuximide (Sigma, St. Louis, MO, USA) and stiripentol (Laboratoires Biocodex, Gentilly, France). The antiepileptic drugs were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water and administered intraperitoneally (*i.p.*) as a single injection in a volume of 5 ml/kg body weight. Fresh drug solutions were administered as follows: ethosuximide – 45 min. and stiripentol – 60 min. before grip-strength testing. The pretreatment times before testing of the antiepileptic drugs reflect the times to peak of maximum anticonvulsant effects produced by these antiepileptic drugs [12-14].

Grip-strength test. The effects of ethosuximide and stiripentol (administered alone in increasing doses) on skeletal muscular strength in mice were quantified by the grip-strength test of Meyer et al. [15]. 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 ± S.E. of at least 8 determinations. This experimental procedure has been described in detail elsewhere [9, 16].

Doses of the antiepileptic drugs were plotted on the X-axis of the Cartesian coordinate system, whereas their corresponding grip-strength values were plotted on the Y-axis. Subsequently, dose-response relationship lines for the studied antiepileptic drugs were constructed using linear regression analysis according to Motulsky and Christopoulos [11]. The doses of ethosuximide and stiripentol were selected by considering their degree of strength impairment by the drugs in experimental animals, compared to control (vehicle-treated) animals. Only the doses of the studied antiepileptic drugs that reduced grip-strength were placed on the Cartesian coordinate system and analyzed with linear regression to determine the dose-response relationship lines and strength reducing doses by 50% (D_{50}). All required calculations and dose-response relationship analysis were performed using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA).

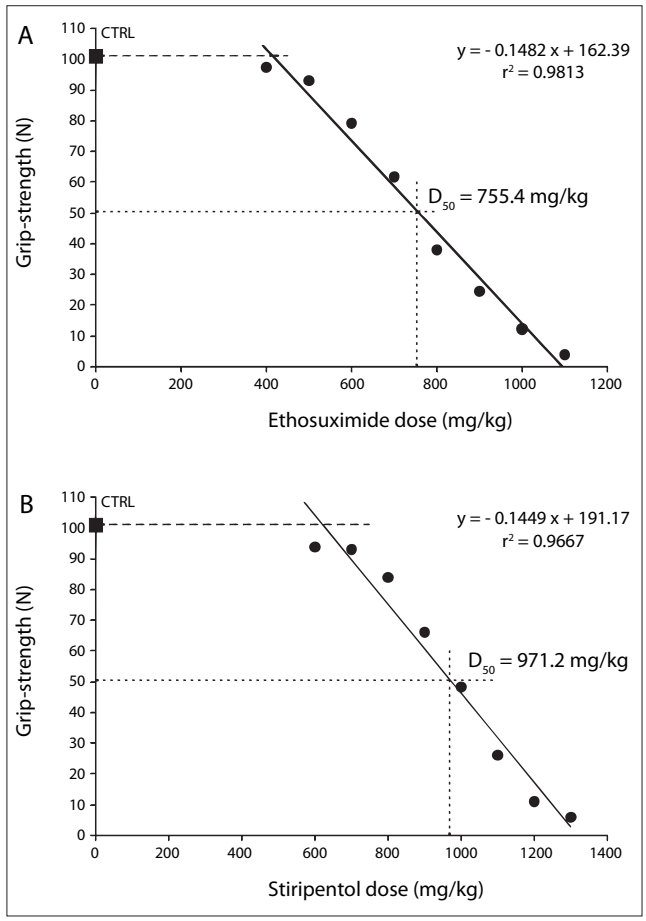

Figure 1A-B Dose-response relationship between ethosuximide and stiripentol doses and their corresponding skeletal muscular strength in the grip-strength test in mice. Doses of ethosuximide and stiripentol (in mg/kg) are plotted graphically on the X-axis, whereas their resultant skeletal muscular strength (in Newtons) is plotted on the Y-axis. The solid line between black circular points on each graph reflects the dose-response relationship line for the antiepileptic drug doses and their corresponding grip-strengths. The dashed line on each graph represents the mean muscular strength in control (CTRL) animals receiving the adequate amount of vehicle – 101.2 N. The dotted lines indicate the strength reducing dose by 50% (D_{50}), compared to the muscular strength in CTRL animals. The equations of dose-response relationships for the antiepileptic drug doses and their corresponding muscular strength in mice are presented on each graph, where y – muscular strength value, x – drug dose, and r^2 – coefficient of determination. D_{50} – dose of an antiepileptic drug for which the strength in experimental animals was reduced by 50%. Linear regression analysis was performed according to Motulsky and Christopoulos [17].

RESULTS

Influence of ethosuximide and stiripentol on skeletal muscular strength in the grip-strength test in mice. The experimentally-derived skeletal muscular strength in control (vehicle-treated) animals was 101.2 N. In the grip-strength test, ethosuximide was administered at increasing doses from 400 – 1,100 mg/kg, and the resulting skeletal muscular strength in mice was plotted in the Cartesian coordinate system (Fig. 1A). Subsequently, linear regression analysis of dose-response relationship between drug doses and their resultant effects allowed determination of the the equation for ethosuximide as follows: $y = -0.1482 x + 162.39$ [$r^2 = 0.9813$]; where y – muscular strength value, x – the drug dose, and r^2 – the coefficient of determination (Fig. 1A).

From the equation of dose-response relationship, the dose of ethosuximide that reduced muscular grip-strength in mice by 50% (D_{50}), compared to the control animals, was calculated. In this case, the D_{50} for ethosuximide was 755.4 mg/kg (Fig. 1A). In the case of stiripentol, the drug was administered at increasing doses ranging between 600 – 1,300 mg/kg, and the linear regression equation for stiripentol ($y = -0.1449 x + 191.17$ [$r^2 = 0.9667$]) allowed calculation of the D_{50} of stiripentol that reduced muscular strength in mice by 50% – 971.2 mg/kg (Fig. 1B).

DISCUSSION

The objective of this study was to determine the effect of ethosuximide and stiripentol on skeletal muscular strength in mice. The experimental design used in this study allowed calculation of the doses of the antiepileptic drugs that reduced

Table Comparison of doses of the studied antiepileptic drugs in various experimental tests assessing acute adverse (neurotoxic) effects and anticonvulsant activity in mice.

Drug	Grip-strength [a]	Chimney [b]	Pentylene-tetrazole [b]	Protective index [c]
Ethosuximide	755.4	722.1	147.8	5.11
Stiripentol	971.2	640.2	221.3	4.39

Values are presented either as doses of antiepileptic drugs (in mg/kg) reducing the strength by 50% (D_{50}) in animals subjected to the grip-strength test or as median toxic doses (TD_{50}), impairing motor coordination in 50% of animals challenged with the chimney test. Values in the pentylenetetrazole column reflect median effective doses (ED_{50} in mg/kg), protecting 50% of animals against clonic phase of pentylenetetrazole-induced seizures.
[a] column displays the results from this study;
[b] column represents the results from [11];
[c] Protective index was calculated as D_{50} to ED_{50} ratio based on the grip-strength and pentylenetetrazole-induced seizure tests. The protective index is considered an index of the margin of safety and tolerability between anticonvulsant doses and doses of the antiepileptic drugs exerting acute adverse effects (e.g. sedation, ataxia, loss of muscular strength, impairment of motor coordination or other neurotoxic manifestations) in preclinical studies [10].

muscular grip strength by 50% (D_{50}), compared to control animals. It was found that the studied antiepileptic drugs, in a dose dependent manner, decreased skeletal muscular grip-strength in mice. To assess unequivocally the neurotoxic potential of antiepileptic drugs, the effects produced by the antiepileptic drugs in the grip-strength test were compared to the effects exerted by the same drugs in the chimney test in mice. It is important to note that the chimney test evaluates the effects of drugs on muscular strength and movement synchronization in rodents [8]. The comparison of median toxic doses (TD_{50} values) for the studied antiepileptic drugs, as determined in the chimney test with strength reducing doses by 50% (D_{50} values), as denoted in the grip-strength test, revealed that the D_{50} values for ethosuximide and stiripentol were higher than those required to impair motor coordination in the chimney test in mice (Tab. 1). On the other hand, direct comparison of the D_{50} values, as denoted in the grip-strength test, with median effective doses (ED_{50}) of the respective antiepileptic drugs, as determined in the mouse pentylenetetrazole-induced seizure model, revealed that the doses of the studied antiepileptic drugs (ethosuximide and stiripentol) required to impair skeletal muscular strength in animals were ~4.4-5.1-fold higher than the anticonvulsant doses, necessary to protect the animals against pentylenetetrazole-induced seizures (Tab. 1). Calculation of the protective index (a ratio of D_{50} and ED_{50} values from the grip-strength and pentylenetetrazole-induced seizure tests) for ethosuximide and stiripentol, provided additional information about the margin of safety and tolerability between the anticonvulsant doses (offering the suppression of pentylenetetrazole-induced clonic seizures) and doses of the antiepileptic drugs, exerting acute adverse effects (e.g. loss of muscular strength) in preclinical studies [8].

As mentioned in the Introduction, it has been documented that carbamazepine, valproate, clonazepam, phenytoin, phenobarbital, lamotrigine, oxcarbazepine and topiramate exert clear-cut, acute adverse effects in the grip-strength test, and the experimentally-derived D_{50} values for these antiepileptic drugs were also higher than their corresponding TD_{50} values, as assessed in the chimney test [12-14, 9, 10].

To explain and correctly interpret the results observed in experimental animals administered with ethosuximide and stiripentol, one should consider the molecular mechanisms of action of the studied antiepileptic drugs. Ethosuximide preferentially binds to the inactivated state of low-threshold T-type Ca^{2+} channels and selectively inhibits pathological firing without any effect on normal neuronal activity [17, 18]. Furthermore, ethosuximide decreases the Ca^{2+}-activated K^+ current in thalamocortical neurons [17], and partially reduces the non-inactivating Na^+ current [19]. All these changes in Na^+, K^+, and Ca^{2+} currents following ethosuximide administration are responsible for disrupting thalamocortical synchronized activity of neurons during spike and wave discharges *in vivo* [20]. Moreover, in an *in vitro* study, ethosuximide inhibits G protein-activated inwardly rectifying K^+ (GIRK) channels [21]. As regards stiripentol, the drug inhibits the metabolism of GABA through the blockade of GABA-transaminase activity [22] and reduces synaptosomal uptake of GABA [23], leading to an increase in GABA brain content. Stiripentol, however, has no affinity for $GABA_A$ and $GABA_B$ receptors [22]. The antiepileptic drug markedly increases the mean open duration of $GABA_A$ receptor dependent chloride channels by a barbiturate-like mechanism [24]. Stiripentol is a racemic mixture of 2 enantiomers: R(+)-stiripentol and S(–)-stiripentol, of which R(+)-stiripentol has a 2.4-fold greater anticonvulsant potency, but is also associated with ~3-fold faster elimination rate [25].

Considering the above-mentioned molecular mechanisms of action of ethosuximide and stiripentol, it is difficult to unequivocally indicate the mechanism(s) which is (are) responsible for the observed impairment of muscular strength in mice. Generally, neurochemical and electrophysiological studies are preformed to reveal the mechanisms associated with anticonvulsant activity of the antiepileptic drugs, but not those producing neurotoxic effects or impairing muscular strength in experimental animals [26]. It is highly likely that ethosuximide and stiripentol impair muscular strength in animals through the similar mechanisms of action as those responsible for their anticonvulsant effects. On the other hand, it is not excluded that ethosuximide and stiripentol, when administered at high doses that impair muscular strength, might exert their neurotoxic effects *via* different mechanisms than those reported for the antiepileptic activity *in vivo*. Therefore, more advanced studies are required to elucidate the exact mechanisms responsible for the impairment of muscular strength in experimental animals.

Finally, based on this preclinical study, one can conclude that the grip-strength test allows the evaluation of acute adverse-effect potential of antiepileptic drugs at high (neurotoxic) doses with respect to the reduction of muscular strength. Ethosuximide and stiripentol impaired, in a dose dependent manner, skeletal muscular strength in mice; the experimentally-derived doses for ethosuximide and stiripentol that reduced muscular strength in mice by 50% (D_{50} values) were 755.4 and 971.2 mg/kg, respectively. The D_{50} values perfectly characterize propensity of the drugs to diminish skeletal muscular strength.

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