Comparison of the anticonvulsant potency of various diuretic drugs in the maximal electroshock-induced seizure threshold test in mice

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Conflict of interest

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Abstract

Background. The coexistence of seizures and arterial hypertension requires an adequate and efficacious treatment involving both protection from seizures and reduction of high arterial blood pressure. Accumulating evidence indicates that some diuretic drugs (with a well-established position in the treatment of arterial hypertension) also possess anticonvulsant properties in various experimental models of epilepsy.

Objectives. The aim of this study was to assess the anticonvulsant potency of 6 commonly used diuretic drugs (i.e., amiloride, ethacrynic acid, furosemide, hydrochlorothiazide, indapamide, and spironolactone) in the maximal electroshock-induced seizure threshold (MEST) test in mice.

Material and methods. Doses of the studied diuretics and their corresponding threshold increases were linearly related, allowing for the determination of doses which increase the threshold for electroconvulsions in drug-treated animals by 20% (TID₂₀ values) over the threshold in control animals.

Results. Amiloride, hydrochlorothiazide and indapamide administered systemically (intraperitoneally – i.p.) increased the threshold for maximal electroconvulsions in mice, and the experimentally-derived TID₂₀ values in the maximal electroshock seizure threshold test were 30.2 mg/kg for amiloride, 68.2 mg/kg for hydrochlorothiazide and 3.9 mg/kg for indapamide. In contrast, ethacrynic acid (up to 100 mg/kg), furosemide (up to 100 mg/kg) and spironolactone (up to 50 mg/kg) administered i.p. had no significant impact on the threshold for electroconvulsions in mice.

Conclusions. The studied diuretics can be arranged with respect to their anticonvulsant potency in the MEST test as follows: indapamide > amiloride > hydrochlorothiazide. No anticonvulsant effects were observed for ethacrynic acid, furosemide or spironolactone in the MEST test in mice.

Key words: diuretic drugs, threshold for electroconvulsions, TID₂₀ values

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Introduction

Arterial hypertension is the most common cardiovascular disease affecting approx. 26% of the worldwide population, and thus, it is a major public health problem in both developed and developing countries.¹ In contrast, epilepsy is a serious neurological disorder affecting approx. 1% of the population in every country throughout the world.² The coexistence of both diseases (seizures and hypertension) requires adequate and efficacious treatment which simultaneously protects against seizures and reduces arterial blood pressure. In such cases, a combined treatment of antihypertensive and antiepileptic drugs is prescribed for these patients. However, any combined pharmacotherapy is associated with the appearance of interactions, whose nature may be pharmacodynamic, pharmacokinetic, or mixed.³ The most notable group of drugs with a well-established position in the treatment of arterial hypertension are diuretics.⁴

Accumulating experimental evidence indicates that some diuretic drugs also possess anticonvulsant properties in various experimental models of epilepsy.^{5–8} Previously, it was discovered that amiloride, hydrochlorothiazide and indapamide (3 classical diuretics) elevated the threshold for maximal electroconvulsions in experimental animals.^{9–11} Unfortunately, the anticonvulsant properties of the diuretics were not directly compared to each other. However, to unequivocally assess the anticonvulsant potential of agents or drugs that increase the threshold for electroconvulsions, Swinyard et al. recommended determining doses of the compounds that increase the electroconvulsive threshold in drug-treated animals 20% over the threshold in control animals (i.e., TID₂₀ values).¹² Thus, the TID₂₀ values allow researchers to precisely assess the anticonvulsant potency of drugs or agents in preclinical studies.^{12,13}

Considering the abovementioned facts, it was of pivotal importance to determine the TID₂₀ values for 6 commonly prescribed diuretics (i.e., furosemide, spironolactone, amiloride, ethacrynic acid, hydrochlorothiazide, and indapamide) in the maximal electroshock-induced seizure threshold (MEST) test in mice in order to assess their anticonvulsant potency in this seizure model. There is no doubt that diuretic drugs with anticonvulsant properties can be preferentially recommended for patients with both high arterial blood pressure and seizures.

Material and methods

Animals and experimental conditions

Experiments were performed on adult male albino Swiss mice weighing 22–26 g. The experimental protocol described in this study conformed to the “Guide for the Care and Use of Laboratory Animals” and was approved by the Local Ethics Committee.¹⁴

Drugs

Ethacrynic acid, indapamide and spironolactone (all 3 drugs from Sigma-Aldrich, Poznań, Poland) as well as hydrochlorothiazide (Polpharma S.A., Starogard Gdański, Poland) were suspended in a 1% solution of Tween 80 (Sigma-Aldrich) in distilled water. Amiloride hydrochloride hydrate (Sigma-Aldrich) and furosemide (for injection 10 mg/mL; Polpharma S.A.) were dissolved in distilled water. All diuretic drugs were administered intraperitoneally (i.p.) in a volume of 0.005 mL/g body weight as follows: ethacrynic acid and furosemide at 30 min before the MEST-induced seizures, amiloride at 60 min before, and hydrochlorothiazide, indapamide and spironolactone at 120 min before.

Maximal electroshock seizure threshold test and the calculation of threshold increasing doses by 20% (TID₂₀) values

Seizure activity (electroconvulsions) was evoked by a current (sine-wave, 0.2 s stimulus duration, 50 Hz, 500 V) delivered via auricular electrodes by a Rodent Shocker generator (Hugo Sachs Elektronik, Freiburg, Germany).¹⁵ To assess the threshold for maximal electroconvulsions, 4 groups of mice (8 mice per group) were subjected to electroshocks of varying intensities (ranging from 4 to 10 mA) to yield seizures in 10–30%, 30–50%, 50–70%, and 70–90% of animals. The log-probit method was used to calculate the median current strength (CS₅₀ [mA]), representing the current intensity necessary to induce tonic hind limb extension in 50% of the mice challenged.¹⁶ The threshold (CS₅₀ values) for maximal electroconvulsions in mice receiving amiloride, ethacrynic acid, furosemide, hydrochlorothiazide, indapamide, and spironolactone (all diuretics in increasing doses), were experimentally determined as described in detail previously.^{17,18} Subsequently, the percentage of increase in CS₅₀ values for animals injected with increasing doses of diuretics over the control CS₅₀ value was calculated. The doses of diuretics and their resultant percentage of threshold increase over the control were graphically plotted in rectangular coordinates of the Cartesian plot system and examined with the least-squares linear regression analysis. From linear regression equations, the TID₂₀ values were calculated as recommended earlier.^{12,13}

Statistics

The CS₅₀ values with their standard error of mean (SEM) were statistically analyzed with one-way analysis of variance (ANOVA) followed by the Tukey-Kramer post-hoc test. Differences among values were considered statistically significant if $p < 0.05$.

Results

Effects of various diuretic drugs on the threshold for maximal electroshock-induced seizures

Amiloride administered systemically (i.p.) at doses of 5, 10, 50, 75, and 100 mg/kg elevated the threshold for MEST-induced seizures over the controls by 11.2%, 12.7%, 24.2%, 37.2%, and 51.2%, respectively (Table 1). With one-way ANOVA and the Tukey-Kramer post-hoc test, only amiloride (100 mg/kg) significantly raised ($p < 0.05$) the threshold for electroconvulsions in mice ($f(5; 90) = 2.889$; $p = 0.0182$) (Table 1). Similarly, hydrochlorothiazide administered i.p. at doses of 25, 50 and 100 mg/kg increased the threshold for maximal electroconvulsions by 11.5%, 15.4% and 26.9%, respectively (Table 1). Statistical analysis of data with one-way ANOVA revealed that hydrochlorothiazide (100 mg/kg) significantly elevated ($p < 0.05$) the electroconvulsive

threshold in mice ($f(3; 76) = 2.535$; $p = 0.0631$) (Table 1). Moreover, indapamide administered i.p. at doses of 1.5, 3 and 6 mg/kg raised the threshold for MEST-induced seizures by 2.2%, 12.1% and 37.4%, respectively (Table 1). In this case, indapamide (6 mg/kg) significantly increased ($p < 0.05$) the threshold for electroconvulsions in mice ($f(3; 60) = 3.718$; $p = 0.0161$) (Table 1). In the case of ethacrynic acid, the drug at a maximally tested dose of 100 mg/kg elevated the threshold only by 5.9%, which was not statistically significant (Table 1). In contrast, furosemide and spironolactone (both at a maximally tested dose of 100 mg/kg) decreased the threshold for electroconvulsions in experimental animals in the MEST test. The observed changes, however, were not statistically significant (Table 1).

Subsequently, the doses of amiloride, hydrochlorothiazide and indapamide were linearly related to the threshold increases, and the respective equations describing these relations are illustrated in Fig. 1. The experimentally-derived TID_{20} values in the MEST test in mice were 30.2 mg/kg

Table 1. Influence of 6 diuretic drugs in the maximal electroshock seizure threshold test in mice

Drug [mg/kg]	CS ₅₀ [mA]	n	TI [%]	One-way ANOVA
Control	5.92 ± 0.77	16	–	$f(5; 90) = 2.889$; $p = 0.0182$
Amiloride (5)	6.58 ± 0.59	16	11.2	
Amiloride (10)	6.67 ± 0.61	24	12.7	
Amiloride (50)	7.35 ± 0.45	8	24.2	
Amiloride (75)	8.12 ± 0.72	16	37.2	
Amiloride (100)	8.95 ± 0.53*	16	51.2	$f(2; 53) = 0.0976$; $p = 0.9072$
Control	6.8 ± 0.61	24	–	
Ethacrynic acid (50)	7.0 ± 0.72	16	2.9	
Ethacrynic acid (100)	7.2 ± 0.62	16	5.9	$f(2; 45) = 0.0168$; $p = 0.9833$
Control	4.89 ± 0.40	16	–	
Furosemide (50)	4.76 ± 0.45	8	2.7	
Furosemide (100)	4.84 ± 0.36	24	1.0	$f(3; 76) = 2.535$; $p = 0.0631$
Control	5.2 ± 0.37	16	–	
Hydrochlorothiazide (25)	5.8 ± 0.29	24	11.5	
Hydrochlorothiazide (50)	6.0 ± 0.35	16	15.4	$f(3; 60) = 3.718$; $p = 0.0161$
Hydrochlorothiazide (100)	6.6 ± 0.40*	24	26.9	
Control	5.54 ± 0.42	16	–	
Indapamide (1.5)	5.66 ± 0.51	16	2.2	$f(5; 74) = 0.2891$; $p = 0.9176$
Indapamide (3.0)	6.21 ± 0.48	16	12.1	
Indapamide (6.0)	7.61 ± 0.55*	16	37.4	
Control	5.5 ± 0.47	16	–	$f(5; 74) = 0.2891$; $p = 0.9176$
Spironolactone (12.5)	5.4 ± 0.42	8	–1.8	
Spironolactone (25)	5.2 ± 0.35	24	–5.5	
Spironolactone (37.5)	5.1 ± 0.45	8	–7.3	
Spironolactone (50)	5.0 ± 0.40	16	–9.1	
Spironolactone (100)	4.8 ± 0.46	8	–12.7	

Results are median current strengths (CS₅₀ [mA] ± SEM) necessary to produce tonic convulsions in 50% of the animals tested; control groups of animals received a vehicle (1% of solution of Tween 80 in distilled water); n – number of animals tested at those current strength intensities whose seizure effects ranged between 16% and 84%; TI – threshold increasing values are presented as percentage of increase in CS₅₀ values for animals injected with increasing doses of diuretics over the control (vehicle-treated) animals; statistical analysis of data was performed with one-way ANOVA followed by the Tukey-Kramer post-hoc test; * $p < 0.05$ vs the control (vehicle-treated) animals.

Fig. 1. Linear regression analysis of doses of various diuretic drugs and their corresponding threshold increases in the MEST test in mice

y – threshold increase [%]; x – dose of the diuretic; R² – coefficient of determination. The dashed line reflects the TID₂₀ values (threshold increasing doses by 20%) in the MEST test.

for amiloride, 68.2 mg/kg for hydrochlorothiazide and 3.9 mg/kg for indapamide, respectively.

Discussion

Results indicate that there was a close correlation between the doses of 3 diuretic drugs (i.e., amiloride, hydrochlorothiazide and indapamide) and their anticonvulsant properties in mice subjected to the MEST test. In this study, linear regression analysis allowed for the determination of the TID₂₀ values for amiloride, hydrochlorothiazide and indapamide. In the case of ethacrynic acid, the drug had no impact on the threshold for electroconvulsions. In contrast, furosemide and spironolactone diminished the threshold for electroconvulsions in mice, although the data did not reach the level of statistical significance.

Of note, the TID₂₀ values presented in this study for diuretic drugs can be readily compared to those reported earlier for some second-generation antiepileptic drugs (AEDs). The TID₂₀ values for some AEDs were as follows: 70 mg/kg for gabapentin,¹⁸ 44 mg/kg for levetiracetam,¹⁹ 103.2 mg/kg for stiripentol,²⁰ 4.4 mg/kg for tiagabine,¹⁷ and 226.2 mg/kg for vigabatrin.^{17,18} A direct comparison of TID₂₀ values of the second-generation AEDs with those calculated for the 3 diuretics revealed that indapamide has the lowest TID₂₀ value (3.9 mg/kg). In contrast, gabapentin, levetiracetam, stiripentol, and vigabatrin have higher TID₂₀ values than those calculated for amiloride (30.2 mg/kg). In the case of hydrochlorothiazide, its TID₂₀ value (68.2 mg/kg) is lower than that of gabapentin, stiripentol and vigabatrin in the MEST test. Thus, considering the anticonvulsant potency of the studied diuretics, the drugs can be arranged from the lowest to the highest TID₂₀ values as follows: indapamide > amiloride > hydrochlorothiazide. This comparative study allowed for the assessment of the most favorable diuretic drug with the lowest TID₂₀ value (indapamide), which is effective in suppressing MEST-induced tonic seizures in experimental animals.

There is another fact worth keeping in mind while translating the results from this study to a clinical setting. Since the TID₂₀ value for indapamide in mice was 3.9 mg/kg, it can be readily converted to a dose of indapamide used in humans, according to the method presented by Reagan-Shaw et al.²¹ The converted dose of indapamide for a 60 kg human should be 18.72 mg (i.e., 0.312 mg/kg of body weight). The recommended maximum daily dose of indapamide in humans is 5 mg.²² Thus, it seems that the anticonvulsant effects of indapamide would appear if the daily dose of indapamide exceeded ~4 times the recommended maximum. Similarly, the TID₂₀ values for amiloride and hydrochlorothiazide were 30.2 mg/kg and 68.2 mg/kg, respectively. Thus, the corresponding doses of the diuretics in a 60 kg human should amount to 145 mg for amiloride and 327.4 mg for hydrochlorothiazide. The recommended maximum daily dose of amiloride is 20 mg,²³ and for hydrochlorothiazide it is 100 mg,²⁴ so the anticonvulsant effects of amiloride would be expected if the daily dose exceeded ~7 times the recommended maximum, and that of hydrochlorothiazide ~3 times. We are fully aware of such limitations in translational studies. However, it has to be noted that diuretic drugs cannot be used alone as anticonvulsant agents in patients with epilepsy, but always in combination with classical and novel AEDs in order to provide epilepsy patients with the most effective treatment against both arterial hypertension and seizures. Obviously, combinations of diuretics and AEDs always produce interactions of a pharmacodynamic, pharmacokinetic, or mixed nature.³ However, preclinical studies on animals allowed us to characterize the interactions between diuretics and AEDs. For instance, it has been reported that indapamide significantly potentiated the anticonvulsant action of carbamazepine, phenobarbital and valproate, although the effects observed for phenobarbital in the mouse maximal electroshock seizure (MES) model were of a pharmacokinetic nature.¹¹ Only the interactions between indapamide and carbamazepine and valproate were pharmacodynamic in the mouse MES model, suggesting their clinical applicability in epilepsy patients.¹¹ In the case of hydrochlorothiazide, the diuretic drug exclusively enhanced the anticonvulsant activity of carbamazepine in the mouse MES model.¹⁰ As regards amiloride, the drug potentiated the anticonvulsant activity of carbamazepine, oxcarbazepine, phenobarbital, topiramate, and valproate in the mouse MES model.⁹ Unfortunately, amiloride pharmacokinetically potentiated the effects of carbamazepine, oxcarbazepine and phenobarbital in mice challenged with the MEST test. Only the pharmacodynamic interactions between amiloride and topiramate, and valproate deserve recommendation for their clinical use in epilepsy patients.

In conclusion, indapamide – with the lowest TID₂₀ value in the mouse MEST test – deserves recommendation for clinical use as an add-on therapy in epilepsy patients who additionally require the application of diuretic drugs.

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