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Short communication

Vague effects of chronic topiramate administration on maximal electroshock-induced seizures in mice

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ABSTRACT

Background: Almost all experimental studies evaluating interactions between antiepileptic and non-antiepileptic drugs are based on their single administration, whereas epileptic patients require chronic pharmacotherapy. Herein, we attempted to figure out whether single and repeated administration of topiramate leads to the same anticonvulsant and undesired effects.
Methods: Experiments were conducted in the model of maximal electroshock in mice. Motor coordination was evaluated in the chimney test. Brain concentrations of topiramate were determined by high-performance liquid chromatography and triple quadrupole mass spectrometry.
Results: The anticonvulsant activity of topiramate administered once or twice a day for 7 days did not significantly differ from the respective effect of topiramate given acutely in a single injection. However, calculating of 50% effective doses for topiramate applied in 14-days protocol (once or two times a day) was impossible. The antiepileptic administered at the dose range of 80–150 mg/kg did not offer protection in more than 50% of mice. This phenomenon cannot be attributed to pharmacokinetic events, because there were no significant differences between plasma and brain concentrations of topiramate after its acute and chronic administration. Topiramate (150 mg/kg) did not affect motor performance in mice.
Conclusions: Maximal electroshock in mice does not seem to be an appropriate seizure model to test anticonvulsant effects of chronic topiramate.

Introduction

In many respects, topiramate seems to be a very interesting medication. Heterogeneous mechanisms of action makes it unique among antiepileptic drugs. Topiramate activates $GABA_A$ receptors, blocks glutamatergic AMPA/kainate, without affecting NMDA receptors. It also inhibits type L calcium channels, decreasing release of calcium-dependent secondary transmitters, and activates potassium channels. The list of actions is completed by weak inhibition of carbonic anhydrase activity [1]. At present, topiramate is approved for the treatment of epilepsy (partial and generalized tonic-clonic seizures, Lennox–Gastaut syndrome) and prevention of migraine. However, promising effects of topiramate were also observed in treatment of other disorders, like alcohol dependence, eating disorder, neuropathic pain, depression, schizophrenia, the posttraumatic stress disorders, essential tremor or Tourette syndrome [2]. In experimental conditions, topiramate is effective in the maximal electroshock in mice, amygdala-kindled rats, and genetic absence epilepsy rats, weakly effective against pentylenetetrazole-induced convulsions in mice, and ineffective in blocking seizures induced by picrotoxin and bicuculline [3]. Numerous studies were conducted in the maximal electroshock, the leading animal model of generalized tonic–clonic convulsions. In one of them, isobolographic analysis showed synergistic interaction between topiramate and lamotrigine in terms of the anticonvulsant action, and antagonism in terms of undesired effects [4]. Such an interaction is the most favorable one can imagine. Nevertheless, from unknown reasons these results do not translate into clinical conditions. In our opinion, it may be, at least partially, due to the fact that antiepileptic drugs are applied in a single injection in experimental studies, whereas epileptic patients undergo chronic treatment. Therefore, we decided to evaluate the anticonvulsant action and undesired effects of topiramate after its chronic administration. It would be a basis for further studies

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involving interactions between topiramate and other antiepileptic drugs in chronic protocol of administration.

Materials and methods

Animals

Experiments were carried out on male Swiss mice weighing 20–25 g. The animals were housed in colony cages with free access to food (chow pellets) and tap water. The experiments started after 7-day acclimatization to standardized laboratory conditions (temperature 21 ± 1 °C, a natural light–dark cycle). The tested groups, consisting of 8 animals, were randomly assigned. All experiments were performed in spring months (from March to June) between 9:00 a.m. and 2:00 p.m. Each mouse was used only once. The Local Ethical Committee of Lublin Medical University approved all experimental procedures of this study.

Drugs

In the study we used topiramate (Sequoia Research, Pangbourne, UK), which was suspended in a 1% solution of Tween 80 (Sigma–Aldrich, St. Louis, MO, USA). All drugs were prepared each day as fresh solutions or suspensions and administered intraperitoneally (*ip*) in a volume of 0.01 ml/g body weight. Topiramate was applied in four chronic protocols: once or twice a day for 7 days and once or twice a day for 14 days. Single administration served as control.

Chemicals and reagents used in chromatographic analysis: acetonitrile, methanol, (Chromasolv® LC–MS, Fluka, Germany), formic acid (Fluka, Germany), diazepam (Cerilliant, USA). Standard solutions of topiramate and diazepam were prepared in methanol. The working solutions of different concentrations were prepared by dilution of the standard solution with methanol. The stock solution and standard solutions were stored at −20 °C.

Maximal electroshock seizure test in mice

Electrically induced seizures in rodents are a well-known animal model of tonic–clonic convulsions [5].

Electroconvulsions were produced by a Hugo Sachs generator (Rodent Shocker, type 221, Freiburg, Germany). An alternating current (50 Hz, fixed current intensity of 25 mA, maximum stimulation voltage of 500 V, 0.2 s stimulus duration) was delivered *via* ear-clip electrodes. The generator is equipped with an internal stabilization system providing self adjustable constant current stimulation, i.e., changes in impedance did not alter current intensity. Tonic hindlimb extension (the hindlimbs of animals outstretched 180° to the plane of the body axis) was considered as the endpoint.

The protective efficacy of topiramate was determined as ability to protect 50% of animals against the maximal electroshock-induced tonic hindlimb extension and expressed as respective values of the median effective dose (ED_{50}). To evaluate each ED_{50} value (in mg/kg), at least four groups of mice received progressive doses of an antiepileptic drug and were challenged with the maximal electroshock test. A dose–response curve was constructed based on the percentage of mice protected [6].

Chimney test

The effect of chronic topiramate, applied in four chronic protocols, on motor coordination was quantified in the chimney test [7], in which animals had to climb backward up the plastic tube (25 cm length, 3 cm inner diameter). Motor impairment was indicated by the inability of mice to perform this test within 60 s.

Measurement of brain concentrations of topiramate

Instrumentation

Chromatographic analysis was performed using high-performance liquid chromatograph (HPLC 1260, Agilent Technologies, Germany). Separation was done employing a Poroshell 120 EC-C18 column 3.0 mm × 50 mm; 2.7 μm (Agilent Technologies, USA) with a thermostat at 40 °C. A mixture of 0.1% formic acid in water (A) and acetonitrile (B) was used as a mobile phase. The injection volume was 5 μl.

Detection of the investigated compounds was achieved by using triple quadrupole mass spectrometer (QqQ 6460, Agilent Technologies, USA). The spectrometer was equipped with an electrospray ion source (ESI). Quantitative analysis was carried out in the multiple reaction monitoring (MRM) mode. Retention time for topiramate and diazepam are 2.64 min and 2.92 min, respectively. The lowest calibration level (LCL) of topiramate in rat serum and brain homogenate was determined to be 25 ng/ml. The correlation coefficient (r^2) of calibration curve were >0.997 in both cases (Fig. 1).

Sample preparation

Mice were administered topiramate according to the respective chronic protocol. Animals were killed by decapitation at times respective to those scheduled for the maximal electroshock test. 200 μl ten-fold diluted of rat plasma or brain homogenate was transferred to 2 ml Eppendorf tubes adding 10 μl internal standard (diazepam at concentration 1 μg/ml). During vortex mixing for 0.5 min. 800 μl acetonitrile was being added dropwise and vortexed for another 30 s, 3 min later samples was centrifuged for 10 min at 15,000 rpm at 10 °C, the supernatant was transferred to the new tubes and evaporated to dryness under a stream of nitrogen (at 50 °C). The extract was dissolved in 50 μl of methanol and analyzed by HPLC-QqQ-MS/MS. All concentrations of topiramate were expressed in micrograms per milliliter of plasma or brain supernatants as means ± standard deviation (SD) of at least 8 determinations.

Statistics

ED_{50} values with their respective 95% confidence limits were estimated using computer log-probit analysis according to Litchfield and Wilcoxon [6]. Subsequently, standard error (SEM) of the mean values were calculated on the basis of confidence limits and ED_{50} values were compared with the Student's *t* test [8].

Qualitative variables from the chimney test were compared by the Fisher's exact probability test.

Total brain concentrations of topiramate were evaluated by the use of the unpaired Student's *t* test. The significance level was set at $p \leq 0.05$.

Results

Maximal electroshock test

The control ED_{50} of topiramate applied acutely was 55.6 ± 4.86 mg/kg. The corresponding value for topiramate administered once a day for 7 days was 51.0 ± 6.54 mg/kg, while that for topiramate given twice a day for 7 days was equal 77.3 ± 15.18 mg/kg. Differences between the two values and control did not reach the level of significance (Table 1).

Interestingly, calculating of ED_{50}s for topiramate applied in two-week protocol (once or two times a day) was impossible. The antiepileptic administered at the dose range of 80–150 mg/kg resulted in plateau effect – degree of protection against tonic convulsions fluctuated around 50% (Table 2).