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Original research article

Effect of cholecalciferol on the anticonvulsant action of some second generation antiepileptic drugs in the mouse model of maximal electroshock

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ABSTRACT

Background: From a theoretical point of view, cholecalciferol (vitamin D_3) as a precursor of calcitriol, a representative of secosteroids, may have neuroprotective properties and affect seizure phenomena.
Methods: In the present study, interactions between cholecalciferol and three second generation antiepileptic drugs (oxcarbazepine, lamotrigine, and topiramate) were studied in the maximal electroshock test in mice. Effects of drugs on motor coordination, long-term memory and explorative behavior of animals were evaluated in the chimney test, passive-avoidance task and plus-maze test, respectively.
Results: Cholecalciferol applied *ip* at doses of 37.5–75 μg/kg significantly raised the electroconvulsive threshold. Cholecalciferol, administered at the subthreshold dose of 18.75 μg, potentiated the anticonvulsant activity of oxcarbazepine and lamotrigine, but did not change their brain concentrations, therefore the revealed interactions seem to be pharmacodynamic. Furthermore, the action of cholecalciferol was not dependent on its conversion to calcitriol. The anticonvulsant effect of topiramate was enhanced by cholecalciferol applied at the higher dose of 37.5 μg/kg, at which it also increased the brain level of topiramate. As regards adverse effects, cholecalciferol, antiepileptic drugs, and their combinations did not significantly impair motor coordination or long-term memory in mice. Moreover, cholecalciferol did not show either anxiolytic or anxiogenic properties.
Conclusion: Our findings show that cholecalciferol has not only its own anticonvulsant action but also enhances efficacy of certain antiepileptic drugs, at least in experimental conditions.

Introduction

As it is widely known, cholecalciferol (vitamin D_3) after conversion to 25-hydroxycalciferol (calcidiol) and then to 1,25-dihydrocholecalciferol (calcitriol) becomes a representative of secosteroid hormones responsible for intestinal absorption of calcium, iron, magnesium, phosphate, and zinc [1]. However, calcitriol regulates also other biological functions, *e.g.*, processes related to cell cycle, reproduction or immune system [1–3]. Neuroprotective effects of 1,25-dihydrocholecalciferol may be associated with reduction of cytoplasmic concentration of calcium ions in brain cells and increased synthesis of the most potent antioxidant glutathione [1]. Moreover, vitamin D deficiency is considered to be implicated in pathogenesis of cerebrovascular, psychiatric and neurodegenerative diseases, as well as epilepsy [4–8]. Janjoppi et al. [9] reported hippocampal vitamin D receptors (VDRs) involvement in epileptogenesis of pilocarpine-induced seizures in rats. Similarly, increased severity of pentylenetetrazole (PTZ)-induced convulsions was observed in mice with partially deleted VDR gene [10]. On the other hand, anticonvulsant effects of calcitriol were manifested in experimental studies by increasing the hippocampal electroconvulsive threshold in rats [11] and reducing severity of PTZ-induced convulsions in mice [12]. Finally, correction of vitamin D deficiency improved control of pharmacoresistant epilepsy in 134 patients [13].

In our previous study, we reported that also cholecalciferol, the precursor of calcitriol, presented its own anticonvulsant properties and potentiated the anticonvulsant action of valproate and phenytoin in the maximal electroshock test in mice [14]. This observation prompted us to investigate the influence

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of cholecalciferol on the anticonvulsant action of certain second generation antiepileptic drugs (oxcarbazepine, lamotrigine, and topiramate) in the same mouse seizure model. All three medications are sodium channel blockers, similarly to valproate and phenytoin, but they have also some additional properties. Lamotrigine and oxcarbazepine block calcium channels, oxcarbazepine increases potassium conductance, while topiramate is a $GABA_A$ receptor agonist and AMPA/kainate receptor antagonist [15]. Maximal electroshock in mice is usually used as the first screening test for potential anticonvulsant compounds in the drug development research. Diverse mechanisms of action of selected antiepileptic drugs create a basis for different interactions between components of the combined treatment. In the study, plasma concentrations of calcitriol and calcium were also measured to exclude participation of calcitriol in the anticonvulsant action of cholecalciferol after its conversion to the hormonal form.

Materials and methods

Animals

In experiments we used adult male Swiss mice weighing 20–25 g. Animals were housed in colony cages (20 mice/cage), access to food and water was given *ad libitum*. Acclimatization to standardized laboratory conditions (constant temperature 21 ± 1 °C, a natural light–dark cycle) lasted 7 days. Experimental groups consisted of 8 randomly assigned mice. All procedures were conducted between 9.00 AM and 2.00 PM. Research protocols were approved by the I Local Ethics Committee for Animal Experiments at Medical University of Lublin and confirmed with the *Guide for the Care and Use of Laboratory Animals*.

Drugs

Experiments were carried out with the use of following medications: cholecalciferol (Devicap, Medana Pharma, Poland), oxcarbazepine (Novartis, Switzerland); lamotrigine (GlaxoSmithKline, Great Britain); topiramate (Janssen-Cilag, Switzerland). Antiepileptic drugs were suspended with addition of 1% solution of Tween 80 (Sigma, St. Louis, MO, USA), and cholecalciferol was dissolved in distilled water to a volume of 10 ml. All tool substances and distilled water (in control groups) were given *ip* in a volume of 0.01 ml/g body weight. Pretreatment times for drugs were as follows: cholecalciferol – 5 min, oxcarbazepine – 30 min, lamotrigine and topiramate – 60 min. Values of pretreatment times were experimentally determined and corresponded with their maximal anticonvulsant effect in the maximal electroshock test.

Electroconvulsive threshold and maximal electroshock seizure test

Electrical impulses were generated by a Hugo Sachs Rodent Shocker (type 221, Freiburg, Germany). Alternating current (50 Hz, 0.2 s, constant intensity of 25 mA, and maximum stimulation voltage of 500 V) was conducted to animals *via* ear-clip electrodes. Tonic hindlimb extension (the hind limbs of animals outstretched in the body axis) was considered as the reference point. The antielectroshock properties of antiepileptics and their combinations were shown as respective median effective doses (ED_{50}s) according to Litchfield and Wilcoxon [16]. Detailed experimental procedures were previously described by Borowicz et al. [14].

Chimney test

Potential motor deficits after administration of cholecalciferol, antiepileptic drugs, and combinations of cholecalciferol with antiepileptics were assesses in the chimney test, according to Boissier et al. [17]. The task of mice in this test was to climb backwards up a plexiglas cylinder (25-cm length, 3-cm inner diameter, threaded inner surface) within 60 s. Mice that failed the test were considered as motor-impaired. Results were expressed as the percentage of such animals.

The chimney test was carried out only in animals treated with effective drug combinations, *i.e.* in which cholecalciferol applied at the highest subprotective dose (18.75 μg/kg) enhanced the action of an antiepileptic drug.

Passive-avoidance task

The step-through passive-avoidance task is based on natural aversion of rodents to brightly illuminated areas and serves as a measure of long-term memory [18]. The apparatus consists of two chambers. One of them is illuminated, while the other is dark. Animals were administered with respective treatment and placed in the illuminated box. Mouse entry to the dark box was followed by an electric foot-shock that facilitated memory acquisition. The same animals were put into the illuminated chamber 24 h later and observed up to 180 s. The results were expressed as median time to enter the dark area with 25th and 75th percentiles. Detailed procedures may be found in the paper of Borowicz et al. [14].

Similarly to the chimney test, long-term memory was evaluated for such combinations of cholecalciferol (18.75 μg/kg) with antiepileptic drugs that were effective against the maximal electroshock.

Elevated plus-maze test

According to Komada et al. [19], the elevated plus maze test is widely used for measuring anxiety-like behavior. The test uses the natural aversion of mice for open and elevated areas and, on the other hand, their natural spontaneous exploratory behavior in the new environment. The apparatus used for this test is in the shape of a plus and consists of two open arms (30 cm × 5 cm × 0.5 cm) opposite to each other and perpendicular to two closed arms (30 cm × 5 cm × 15 cm) with a central platform (5 cm × 5 cm × 0.5 cm). The open arms have very small (0.5 cm) walls to prevent falls, whereas the closed arms have high (15 cm) walls to enclose them. The apparatus is located 50 cm above the floor. The open arms and central platform are painted white and the closed arms were painted black.

In this test, mice were applied with cholecalciferol, antiepileptic drugs or a combination of cholecalciferol with one of antiepileptics. Each mouse was placed in the central platform facing one of the open arms and was allowed to explore the maze for 5 min. The time spent in the open arms, closed arms and the time spent in the open arms relative to the total time spent in the plus maze were used as indices of mouse anxiety.

Measurement of brain concentrations of antiepileptic drugs

Before decapitation mice were administered with antiepileptic drugs alone or in combination with cholecalciferol. Brains were isolated, weighed, and homogenized with Abbott buffer (2:1 vol/weight) in an Ultra-Turrax T8 homogenizer (IKA-WERKE, Stauffen, Germany). The homogenates were centrifuged (10,000 × *g* for 10 min). Brain concentrations were measured in samples of supernatant (75 μl). Levels of topiramate were analyzed by fluorescence polarization immunoassay (FPIA) using a TDx analyzer and respective reagents (Abbott Laboratories, North Chicago, IL, USA). Oxcarbazepine and lamotrigine concentrations were assessed by high pressure liquid chromatography (HPLC) and Glison 715 software. Oxcarbazepine was separated on HyPurity