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Short communication

The effects of cimetidine chronic treatment on conventional antiepileptic drugs in mice

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ABSTRACT

Purpose: The aim of this study was to evaluate the effects of 1-day, 7-day and 14-day administrations of cimetidine on the anticonvulsant activity of conventional antiepileptic drugs (AEDs; valproate, carbamazepine, phenytoin and phenobarbital) against maximal electroshock (MES)-induced convulsions in mice.
Methods: Electroconvulsions were evoked in Albino Swiss mice by a current delivered *via* ear-clip electrodes. In addition, the effects of cimetidine, AEDs alone and their combinations were studied on performance and long-term memory tests. Pharmacokinetic changes in plasma and brain concentrations of AEDs after cimetidine administration were evaluated with immunofluorescence.
Results: Cimetidine (up to 100 mg/kg) after 1-day administration did not affect the electroconvulsive threshold in animals. Moreover, in the 14-day treatment, cimetidine administered at a dose of 40 mg/kg did not significantly change the electroconvulsive threshold in the MES-test, cimetidine administered 14-day (at 20 mg/kg) significantly increased the anticonvulsant activity of carbamazepine, staying without effects after a 1-day and 7-day studies. In contrast, both the 7-day and 14-day administrations of cimetidine resulted in significant reductions of protective efficacy of the phenobarbital. Only valproate and phenytoin were not affected by cimetidine (20 mg/kg) in all experimental period. Cimetidine administered 1-day, did not alter total brain concentrations and free plasma levels of all AEDs tested, whilst the 14-day study elevated carbamazepine plasma and brain concentration and reduced phenobarbital brain concentration. Cimetidine co-applied with AEDs did not impair performance of mice evaluated in the chimney test however, it worsened long-term memory in animals.
Conclusions: Based on this preclinical study, a special caution is advised when treating epileptic patients with combinations of phenobarbital or carbamazepine with cimetidine.

Introduction

Histamine is present in humans at different concentrations in the majority of human body organs (*i.e.*, brain, lungs, stomach, intestine, uterus, and ureters). Since the mid-seventies of the XX century, when Garbarg et al. [1] had traced an ascending histamine neuronal pathway, numbers of research studies on the role of histaminergic neurons in the brain have been launched. The widespread distribution and magnitude of the human histamine system suggests that it must be functionally important [2]. For many years, three main histamine receptor subtypes have been distinguished as follows: H_1, H_2 and H_3. Noticeably, all these histamine receptor subtypes are present in the brain [3]. Relatively recently, Tasaka et al. [4] have confirmed the existence of a novel histamine receptor subtype (H_4).

Previously, it has been found that H_1 receptor antagonists provoked seizures in rodents, or even epilepsy attacks in humans (especially in very young children). Pro-seizure effects of H_1 receptor antagonists may probably be caused by an insufficient concentration of histamine in the brain. First, enunciation of possible harmful adverse effects of histamine receptor antagonists have been described just after their approval for clinical practice [5,6]. Also, preclinical experiments on animals have indicated that the reduction in brain histamine concentration may lead to a

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decrease in the convulsive threshold in various models of epilepsy [7–9]. In contrast, an increase in the histamine level in the CNS inhibits both maximal electroshock (MES)- [10] and pentylenetetrazole-induced seizures in rodents [11].

Cimetidine and other H_2 receptor antagonists are widely used in clinical practice, as an antiulcer drug, although, clinical reports indicate various incidences of adverse effects from the CNS, including headache, drowsiness, disturbance of consciousness, mental confusion, hallucination or even convulsions [12,13].

Numerous experimental and clinical studies have reported that the administration of cimetidine or other H_2 histamine receptor antagonists may be associated with a higher risk of the appearance of convulsive attacks [14–17]. Moreover, it has been found that seizures evoked by cimetidine or other H_2 histamine receptor antagonists were blocked by muscimol – a $GABA_A$ receptor agonist [18]. On the other hand, brain endogenous histamine plays a protective role during the development of seizures in pentylenetetrazole-kindled rats [19]. However, the brain concentration of endogenous histamine seemed to be independent on the administration of H_2 receptor antagonists [20]. Relatively recently, Cannon et al. [20] have suggested that some cimetidine-like drugs (*i.e.*, famotidine, tiotidine, ranitidine and improgan) do not produce seizures *via* H_2 and $GABA_A$ receptors, but through other, as of yet unknown mechanisms that might be involved in CNS adverse effects.

Accumulating clinical data indicates that H_2 receptor antagonists may also produce convulsions, which are difficult to be treated with standard antiepileptic drugs (AEDs), but they can be successfully blocked with physostigmine [21] or thiopental [15]. Experimental studies have shown that cimetidine and ranitidine (another H_2 receptor antagonist) were able to induce seizures in mice after their intracerebral injection [16]. Moreover, it has been documented that muscimol, aminooxyacetic acid (AOAA) and diamino-*n*-butyric acid reversed tonic convulsions induced by intraperitoneal injection of cimetidine [14].

The present study was aimed at examining the effects of the H_2 receptor antagonist cimetidine upon the anticonvulsant potential of conventional AEDs against MES-induced seizures in mice. Cimetidine was administered either acutely (1-day) or in repeated doses, once daily for 7 and 14 days. In addition, the adverse-effect profiles of combinations of cimetidine with conventional AEDs were studied in the chimney test (motor coordination) and passive avoidance task (long-term memory) following 1-day and 14-day administration of this H_2 receptor antagonist. The existence of possible pharmacokinetic interactions between this H_2 antagonist and conventional AEDs was also verified in the plasma or brain of experimental animals by using fluorescent polarization immunoassay.

Materials and methods

Animals and experimental conditions

The 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 experimental temperature was 21 ± 1 °C and the mice were kept in a natural light-dark cycle. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups (consisting of 8–12 animals). Each mouse was used only once and all tests were performed between 08:00 and 15:00 h. The 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 any animal suffering and to use only the number of animals necessary to produce reliable scientific data. The experimental protocols and procedures described in this study were approved by the Local Ethics Committee at the Medial University of Lublin and complied with the European Communities Council Directive of November 24th, 1986 (86/609/EEC).

Drugs

The following drugs were used in this study: cimetidine (Polfa, Warszawa, Poland), valproate (magnesium salt, Dipromal, ICN Polfa, Rzeszów, Poland), carbamazepine (Amizepin), phenytoin (Phenytoinum), and phenobarbital (sodium salt, Luminalum Natrium, all three drugs from Polfa Warszawa, Poland). Valproate, cimetidine and phenobarbital were dissolved in distilled water, whereas carbamazepine and phenytoin were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water. All AEDs were administered intraperitoneally (*ip*) in a volume of 5 ml/kg body weight. Pretreatment times for valproate and carbamazepine were 30 min, phenobarbital and cimetidine – 60 min, and phenytoin – 120 min prior to the electroconvulsive and behavioral tests.

Electroconvulsions

Electroconvulsions were induced by applying alternating current (50 Hz; 500 V) *via* ear-clip electrodes from a rodent shocker generator (type 221; Hugo Sachs Elektronik, Freiburg, Germany). Stimulus duration was 0.2 s. Tonic hind limb extension was used as the endpoint. This apparatus was used to induce seizures in 2 methodologically different experimental approaches: MES seizure threshold test and MES seizure test [22].

MES seizure threshold test

The MES seizure threshold test was used to assess the anticonvulsant effects of cimetidine administered alone. The convulsive threshold was evaluated as median current strength (CS_{50} in mA), which is necessary to produce tonic hind limb extension in 50% of the animals tested. To estimate the convulsive threshold, at least four groups of mice (8 animals per group) were challenged with electroshocks of various intensities. Statistical analysis of data was performed with one-way ANOVA followed by the *post hoc* Tukey–Kramer test for multiple comparisons. Cimetidine at a dose of 40 mg/kg, which did not significantly affect the seizure threshold in the MES seizure threshold test, was selected for testing in combination with the four AEDs in the MES seizure test. This approach allowed us to rule out any contribution of the intrinsic anticonvulsant efficacy of cimetidine in the effects observed in combination with the AEDs in the MES seizure test.

MES seizure test

In the MES seizure test, mice were challenged with a current of fixed intensity that was 4–5-fold higher than the CS_{50} value in controlled-treated mice [22]. To evaluate median effective doses (ED_{50}, values, corresponding to the doses of AEDs, protecting 50% of the animals against tonic hind limb extension) for valproate, carbamazepine, phenytoin and phenobarbital, the animals pretreated with different doses of AEDs, alone or with cimetidine, were challenged with a MES (25 mA). At least four groups of mice, with 8 mice per group, were used to estimate each ED_{50} value (in mg/kg). A dose-effect curve was constructed, based on the percentage of mice protected against maximal electroconvulsions, and calculated according to the method of Litchfield and Wilcoxon [23] and one-way ANOVA followed by the *post hoc* Tukey–Kramer test for multiple comparisons.