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ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2020;9(4):978-81

Beneficial effects of ambroxol hydrochloride on pentylenetetrazol-induced convulsion model in rats

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Received 14 July 2020; Accepted 09 September 2020
Available online 20.11.2020 with doi: 10.5455/medscience.2020.07.136

Abstract

In present experimental study, we purposed to research if ambroxol would have beneficial acute effects on PTZ-induced convulsions by EEG records. 48 rats were randomly divided into two groups; group A for EEG recordings and B for behavioral evaluations. Groups A and B determined as; Group A1 and B1 control, Group A2 and B2 saline, Group A3 and B3 10 mg / kg ambroxol and Group A4 and B4 20 mg / kg ambroxol group. Drugs were given intraperitoneally 30 minutes before pentylenetetrazol (PTZ) administration. While 35 mg / kg PTZ was used for EEG recordings and 70 mg / kg PTZ was used for behavioral evaluations. Racine Convulsion Scale (RCS) and "first myoclonic jerk" (FMJ) times were used for the seizure evaluations. Racine's convulsion scale was significantly lower in control group compared PTZ (70 mg/kg) and saline group ($p < 0.001$). It was lower in ambroxol hydrochloride group compared with PTZ (70 mg/kg) and saline group (70 mg/kg). FMJ onset time was significantly shorter in ambroxol hydrochloride group compared with PTZ (70 mg/kg) and saline group. Spike percentage EEG recordings were significantly lower in ambroxol hydrochloride group compared with PTZ (70 mg/kg) and saline group (70 mg/kg). The statistical significance increased based on the administration doses of ambroxol hydrochloride. Ambroxol hydrochloride has a positive effect on PTZ-induced convulsions, but by more detailed further experimental and clinical studies, involving advanced biochemical measurements, are needed to understand the exact mechanism of action.

Keywords: Epilepsy, ambroxol hydrochloride, pentylenetetrazol, racine convulsion scale, first myoclonic jerk time

Introduction

Epilepsy is a relatively common neurological condition that affects approximately 0.5% to 1% of the general population. In addition to being a cause of considerable expenditure for health systems, it has a mortality risk of around 2% to 3% in patients who are admitted to emergency services [1]. Clinically, it is characterized by generalized seizures and leads to cognitive, psychological and social dysfunctions due to seizures and associated neurobiological factors [2]. It is well established that epileptic seizures are the consequences of abnormal, excessive and synchronous excitation (bursts) of neuronal populations [3].

Antiepileptic drugs are designed to suppress the occurrence neuronal bursts and decrease hyper-synchronization; thus inhibiting the development and spread of abnormal neuronal firing [4]. However, treatments are often unsatisfactory.

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Seizure freedom rates have remain unchanged during the last few decades in which around 20 medications have been introduced [5], even though drug tolerability has improved [6]. Therefore, it is clear that there is still a need for the development of more sophisticated drugs in the treatment of epilepsy [6].

Ambroxol is currently marketed as an expectorant and mucolytic for lung diseases and has local anesthetic properties. It also has several effects on some body systems. Ambroxol is a potent inhibitor of sodium and calcium channels in central nervous system (CNS), which is assumed to cause anti-glutamatergic effects. Furthermore, ambroxol was also demonstrated to have direct antioxidative properties that seemed to be beneficial in ALS [7]. Ambroxol also binds and stimulates the lysosomal glucocerebrosidase (GBA1) enzyme in the cytosol, thereby enhancing GBA1 activity. The same study also showed a significant chaperone effect resulting in the modulation of GBA1 expression [8]. There have been several studies which showed beneficial effects of ambroxol on Gaucher disease, Parkinson disease and ALS [9].

Pentylenetetrazol (PTZ) is a selective blocker of the GABA-A

receptor. It is used as a chemical agent to induce convulsion and generalized tonic-clonic seizures in a dose-dependent manner [10,11]. Although there have been several studies which showed beneficial effects of ambroxol on some neurological diseases, there are no studies in which the effects of ambroxol on epileptic seizures have been investigated. In the present experimental study, we sought to determine whether ambroxol had beneficial acute effects on PTZ-induced convulsions by performing electroencephalography (EEG) and behavioral investigations.

Material and Methods

Animal and Laboratory

The experimental procedures employed in present study were approved by the Animal Ethics Committee (approval number: 2019/049a). All experiments were carried out according to the Guide for the Care and Use of Laboratory Animals put forth by the National Institutes of Health (U.S.A)

Forty-eight male (24 for EEG and 24 for behavioral studies) Sprague–Dawley rats, weighing 200–250 g each were utilized for this study. The rats were kept on a 12-hour light–dark cycle (light from 07.00 to 19.00) in quiet rooms with an ambient temperature of 22–24 °C ambient temperature. They were fed with standard rodent chow and tap water, ad libitum.

Experimental Procedures

The 48 rats were randomly divided in two groups: Group A for EEG recordings and Group B for behavioral assessment. In Group A; Rats were deeply anesthetized and stereotaxic surgery was undertaken to drill a small hole. The electrodes (Polyamide-coated stainless steel wires, 0.1 mm in diameter with an electrical resistance of <1Ω/10 mm) were implanted on the dura over the left frontal cortex (2.0 mm lateral to the midline, 1.5 mm anterior to the bregma) and the reference electrode was implanted over the cerebellum (1.5 mm posterior to the lambda, on the midline) (9, 10) for EEG recording. The electrodes were fixed by using dental acrylic (dental acrylic is a mixture of numerous alloys used for dental restoration). Rats were deeply anesthetized with ketamine (80 mg/kg) and xylazine (4 mg/kg) intraperitoneally (i.p.). It must be noted that 35 mg/kg is ideal for observing changes in EEG spikes but does not consistently produce observable behavioral changes, while 70 mg/kg consistently produces behavioral changes but EEG readings usually have small signal-to-noise ratio that complicate the assessment of different drug concentrations. Twelve days after the implantation of electrodes, 24 rats were divided randomly into 4 groups (n=6): Group A1, A2, A3, A4

Group A1 was defined as the control group and given no medication. Group A2 was administered saline i.p, Group A3 was administered 20 mg/kg ambroxol hydrochloride i.p. (Sekrol 3 mg/ml, BILIM) and Group A4 was administered 10 mg/kg ambroxol hydrochloride i.p. The drugs were administered 30 minutes prior to pentylenetetrazol (PTZ) (35 mg/kg, i.p.) injection. All groups, except Group A1, received 35 mg/kg PTZ and EEG recording was initiated 5 minutes after PTZ administration while rats were conscious and in a special container.

All EEG recordings and behavioral assessment protocols were performed as previously described [12]. In summary, EEG measurements were recorded for 60 minutes, the signals were amplified 10 thousand times and filtered within a range of 1-60 Hz. The BIOPAC MP150 Data Acquisition System (Biopac System Inc., Santa Barbara, CA, USA) was used to evaluate spike percentage. Two clinical neurophysiologists scored EEG data with regard to spike percentage. This approach has been shown to be a reproducible method of quantifying epileptiform activity via the assessment of the percentage of 1-second bins with at least one spike-wave, called "spike-wave percentage" [12]. The onset and cessation of these complexes were identified by the presence of a higher amplitude (at least two-fold) compared to the baseline characteristics of the EEG. The cumulative duration of spike-waves was detected within 2-minute intervals.

Then, the groups were rearranged with the remaining 24 rats (Group B) for behavioral analyses. These rats were similarly separated into 4 groups (n = 6): Group B1, B2, B3 and B4. The first group (Group B1) was defined as control and given no medication. Group B2 was administered saline i.p, Group B3 received 10 mg/kg ambroxol hydrochloride i.p., Group B4 received 20 mg/kg ambroxol hydrochloride i.p. The drugs were administered 30 minutes prior to PTZ (70 mg/kg, i.p.) injection.

Racine's Convulsion Scale (RCS) (14) and onset time of the first myoclonic jerk (FMJ) was used to evaluate the seizures (with the use of 70 mg/kg PTZ only) as follows: 0 = no convulsion; 1 = twitching of vibrissae and pinnae; 2 = motor arrest with more pronounced twitching; 3 = motor arrest with generalized myoclonic jerks (time until the development of this finding was recorded as the onset time of FMJ); 4 = tonic clonic seizure with the animal remaining on its feet (righting reflex present); 5 = tonic–clonic seizure with loss of righting reflex; 6 = lethal seizure. Rats were observed for the onset time of FMJ as described in a previous study [13]. The onset time was recorded in seconds. Almost all animals showing tonic generalized extension died due to the seizure. The observation period for PTZ-induced seizures was limited to a period of 30 minutes [13]. Any animals that were alive after 30 minutes were euthanized.

Statistical Analysis

Results were expressed as a mean ± standard error of mean (SEM). Data analyses were performed by utilizing SPSS version 20.0 for Windows. The Shapiro-Wilk test is used to determine if variable had normal distribution. The Racine convulsion scores were evaluated and compared by the Kruskal Wallis test. The onset times of the FMJ were compared by the two-tailed one-way analysis of variance (ANOVA) test. Post-hoc Bonferroni correction and the Mann Whitney U test was utilized to identify differences between the experimental groups. The p-value of <0.05 was accepted as the threshold of statistical significance.

Results

Assessment of groups in terms of Racine convulsion stage and FMJ onset time

Racine's convulsion scale demonstrated significantly lower results in the control group compared to the PTZ (70 mg/kg) and saline groups ($p < 0.001$). The RCS results were also significantly lower in both the 10 mg/kg and 20 mg/kg ambroxol hydrochloride groups when compared to the PTZ (70 mg/kg) and saline groups (70 mg/

kg) ($p<0.05$ for comparison with 10 mg/kg ambroxol, $p<0.01$ for comparison with the 20 mg/kg ambroxol) (Table 1).

The FMJ onset time was significantly shorter in the control group compared to the PTZ (70 mg/kg) and saline groups ($p < 0.001$). It was shorter in the 10 mg/kg ambroxol hydrochloride group compared with PTZ (70 mg/kg) and saline group (70 mg/kg) ($p<0.05$). In addition, results were shorter in the 20 mg/kg ambroxol hydrochloride group compared to the PTZ (70 mg/kg) and saline groups (70 mg/kg) ($p<0.01$) (Table 1).

Assessment of groups in terms of spike percentage EEG recordings

Spike percentage results were significantly lower in the control group compared to the PTZ (70 mg/kg) and saline groups ($p < 0.001$). The results in both the 10 mg/kg and 20 mg/kg ambroxol hydrochloride groups were significantly lower when compared to the PTZ (70 mg/kg) and saline groups (70 mg/kg) ($p<0.05$ and $p<0.001$, respectively) (Table 2) (Figure 1).

Table 1. Comparisons of groups in terms of Racine convulsion stage and FMJ onset time

Drugs Group	Convulsion Stage (Racine)	FMJ onset time (sec)
Control	0	0
PTZ (70 mg/kg) and saline	5.8 ± 0.48 *	57.4 ± 8.6 *
PTZ (70 mg/kg) and 10 mg/kg ambroxol hydrochloride	4.4 ± 0.22 #	146.3 ± 23.9 #
PTZ (70 mg/kg) and 20 mg/kg ambroxol hydrochloride	3.7 ± 0.18##	209.7 ± 21.5.##

* p<0.001, Control Group compared PTZ (70 mg/kg) and saline group
# p<0.05 PTZ (70 mg/kg) and saline Group compared PTZ (70 mg/kg) and 10 mg/kg ambroxol hydrochloride Group
p<0.001 PTZ (70 mg/kg) and saline Group compared PTZ (70 mg/kg) and 20 mg/kg ambroxol hydrochloride Group

Table 2. Assessment of groups in terms of spike percentage EEG recordings of the rats

Drugs Group	Spike Percentage
Control	% 0
PTZ (35 mg/kg) and saline	% 78.9 ± 10.3 *
PTZ (35 mg/kg) and 10 mg/kg ambroxol hydrochloride	% 62.1 ± 7.4 #
PTZ (35 mg/kg) and 20 mg/kg ambroxol hydrochloride	% 47.4 ± 4.5 ##

* p<0.001, Control Group compared PTZ (70 mg/kg) and saline group
# p<0.05 PTZ (70 mg/kg) and saline Group compared PTZ (70 mg/kg) and 10 mg/kg ambroxol hydrochloride Group
p<0.001 PTZ (70 mg/kg) and saline Group compared PTZ (70 mg/kg) and 20 mg/kg ambroxol hydrochloride Group

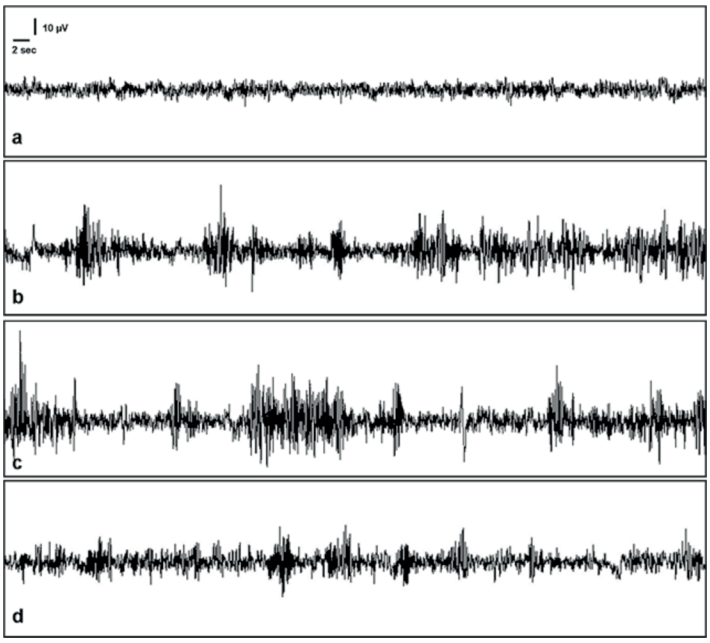

Figure1. EEG recording (a):Control group, (b): PTZ and saline group, (c): PTZ and 10 mg/kg ambroxol hydrochloride citrate group, (d): PTZ and 20 mg/kg ambroxol hydrochloride group

Discussion

We were able to show that ambroxol hydrochloride treatment had significant positive effects on the seizures induced with PTZ in rats. The anticonvulsant effects of ambroxol hydrochloride were evident in both EEG and clinical (behavioral) evaluations. The rats that received ambroxol hydrochloride treatment had fewer spikes in EEG recordings, had significantly lower RCS results and longer FMJ onset time when compared to rats that received saline. The beneficial effects of ambroxol hydrochloride were also found to be greater when rats were given a higher concentration of ambroxol hydrochloride (10 mg/kg versus 20 mg/kg).

As mentioned in the introduction section, ambroxol is currently marketed as an expectorant and mucolytic for lung diseases. However, recent data shows that ambroxol may have considerable effects in the CNS via the inhibition of sodium and calcium channels and its anti-glutamatergic properties. Additionally, Weiser et al. showed that ambroxol blocked glutamate induced currents, but with relatively low potency [7].

When the literature on this topic is reviewed, contrary to our results, Lapenda et al. [14] reported that an epileptic patient suffered from convulsions that were triggered by an expectorant syrup that contained ambroxol. Although the evidence was anectodal, it was noted that her ictal EEG recordings demonstrated epileptic discharges after taking the drug. There may be some explanations for this issue, including the possible role of other ingredients and a specific vulnerability of the patient to ambroxol. Other than this case report, there is no evidence indicating possible pro-convulsant effects of ambroxol.

Ambroxol has been used to treat patients with type I Gaucher disease in a pilot trial. In this pilot study, it was suggested that physiological relevance of ambroxol on glucosylceramide

activity contributed to the beneficial effects [12]. Data shows that ambroxol may alter the reduction rate of dihydroethidium oxidation in Gaucher disease and its possible relationships with GBA mutations in Parkinson's disease. Beside its chaperone-like activity, it has been established that ambroxol is an anti-oxidant [15]. Another interesting feature of ambroxol has been put forth by its decreasing effect on alpha synuclein levels in a cell line overexpressing its levels [16]. These characteristics would not be very relevant if ambroxol hydrochloride had not been shown to easily cross the blood–brain barrier [17]. Thus, it is possible that ambroxol can act as an anti-oxidant while enhancing the clearance of alpha-synuclein. A relatively recent study demonstrated that high dose ambroxol was effective in decreasing the severity of neurological symptoms in patients with the neuropathic form of Gaucher's disease [18]. Furthermore, in a recent study, it has been shown and concluded that ambroxol hydrochloride promotes and protects motor units and improves axonal plasticity, thus indicating a promising role for ambroxol in ALS [9].

To our knowledge, this is the first study that investigated the effects of ambroxol hydrochloride in epilepsy. The beneficial effects of ambroxol hydrochloride on seizures can be explained by some mechanisms. Firstly, ambroxol hydrochloride can pass through the blood-brain barrier and inhibits sodium and calcium channels in the CNS [7] –which is the basic mechanism of the majority of antiepileptic drugs. Glutamate is the leading excitatory neurotransmitter in CNS. It acts through ionotropic (NMDA, AMPA, and kainite) and metabotropic receptors and plays a significant role in the initiation, spread, and maintenance of epileptic activity [19]. Accumulating data has shown that epilepsy is associated with the dysfunction in the glutamate system at different levels, including genetic factors, neurotransmitter release and receptor expression [20,21]. Researchers have demonstrated increased plasma levels of glutamate in epilepsy [22,23]. Furthermore, several antiepileptics alter glutamate receptors selectively and nonselectively. Thus, a possible mechanism of action of ambroxol hydrochloride can be associated with its anti-glutamatergic property.

Limitations: we have shown positive effects of ambroxole on PTZ-induced convulsions; however, we were not able to evaluate any biochemical pathways due to technical and financial insufficiencies, so the exact mechanism of action of ambroxol remains unclear.

Conclusion

Our study is the first for demonstrating beneficial effects of ambroxol hydrochloride on PTZ-induced seizures in rats. Ambroxol hydrochloride has a positive effect on PTZ-induced convulsions; but detailed animal studies involving advanced biochemical evaluations are needed to confirm our findings and understand its mechanism of action.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
Ethics Committee Approval number: 2019/049a

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