The Effect of Nitrofurantoin on Tonic Seizures Induced by MES in Male Mice

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Abstract- Antibiotics are medicines that fight against bacterial infections and are usually considered safe drugs. However, they can simultaneously cause several adverse reactions. Nitrofurantoin, which is mainly administered for treatment and prevention of urinary tract infections, causes seizure reportedly. Therefore, further research is required to be conducted to simulate the case report situations and examine whether nitrofurantoin is the main factor leading to seizures. To do this, NMRI male mice (20-30 gr) were chosen and classified into different groups in both acute and chronic phases. Each phase contained mice treated with nitrofurantoin, phenytoin and the combination of both drugs as well as untreated control group. An Electroshock device was used to induce seizure in mice and then the effect of nitrofurantoin and phenytoin was examined in acute and chronic phases. Seizure induction in mice was examined 30 minutes and one week after injection in acute and chronic phases, respectively. Results indicated that THE (Tonic Hind-limb Extension) duration was different among the studied groups. Nitrofurantoin-injected mice were revealed to have a higher THE duration in comparison with control group, while phenytoin-injected group showed a lower THE duration. Furthermore, administration of nitrofurantoin and phenytoin combination reduced THE duration in both acute and chronic phases. Our conclusion is that nitrofurantoin can possess convulsive effects and cause seizure as a side effect.

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Keywords: Antibiotics; Electroshock; Nitrofurantoin; Phenytoin; Seizure

Introduction

Antibiotics, which are being widely administered for treatment of bacterial infections, are categorized into two different groups including bactericidal and bacteriostatic antibiotics. More exactly, bactericidal antibiotics exert their inhibitory effect by inducing bacterial death, while bacteriostatic antibiotics negatively affect bacterial growth (1-2). The most prevalent bacterial infections are those that occur in the urinary tracts, both upper and lower ones. Women are more likely to develop Urinary Tract Infections (UTIs) due to their smaller urinary systems (3). Nitrofurantoin, a synthetic nitrofuran derivative with a Hydantoin structure, is an effective antibiotic that possesses both bactericide and bacteriostatic activities and thus is widely used to treat or prevent UTIs (4). It is of foremost importance to note that like other antibiotics, nitrofurantoin might pose a number of neurological complications including peripheral neuropathy, cerebral dysfunction, cluster headaches, dizziness, vertigo and intracranial hypertension (5). These side effects have been particularly observed to occur in women and elderlies, the etiology of which has been attributed to axon loss (6-7). Of note, post-antibiotic neurological complications have been reported in a myriad of studies (8-10). It has been proposed that these adverse side effects might stem from nitric oxide (NO) production by nitrofurantoin (11). NO is a neurotransmitter, acting as a potent vasodilator that contributes to regulation of vascular tone and generation of cluster headaches (12-14). Hitherto, it has not been completely understood that whether nitrofurantoin is the main reason for seizures or not. Therefore, the purpose of this investigation was to examine the impact of nitrofurantoin on seizure induction in acute and chronic phases.

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Materials and Methods

Chemicals and route of administration

Nitrofurantoin was purchased from Soha Pharmaceutical Company, Tehran, Iran, and phenytoin was obtained from Caspian Tamin Pharmaceutical Company, Rasht, Iran. Normal saline 0.9% was provided from Shahid Ghazi Pharmaceutical Company, Tabriz, Iran. Preparation of nitrofurantoin was performed by dissolving the drug in 0.9% normal saline. The drugs and vehicles were given to each mouse via intraperitoneal injection (i.p.) at a constant volume of 10 ml/kg body weight.

Animals

Male Naval Medical Research Institute (NMRI) mice (20-30 g) were acquired from Pasteur Institute, Tehran, Iran. Animals were classified into different groups including control group (n≥6), which was injected with Normal Saline 0.9% and treatment groups (n≥6), which were treated i.p. with 25, 50, 75 and 100 mg/kg of nitrofurantoin, 5, 10 and 20 mg/kg of phenytoin, and the combination of 75 mg/kg nitrofurantoin and 5 mg/kg phenytoin. The animals were kept in a room with a controlled temperature (23±2° C), with a 12-hour cycle of light and darkness, and were given unrestricted access to food and water unless injected or tested. Mice were housed in regular poly carbonate cages and allowed to adjust for a minimum of two days before any experiments were conducted. All experiments were carried out between 10:00 a.m. and 2:00 p.m. The animal and experimental committee at Islamic Azad University Pharmaceutical Sciences (IAUPS), Tehran, Iran, approved all study protocols.

Drug treatment and maximal electroshock-induced seizures

The injection of Nitrofurantoin and Phenytoin was done in acute and chronic phases.

To induce seizure in the acute phase, all groups were shocked with an Electroshock device, right 30 min after injections. In the chronic phase, seizure induction was carried out by shocking the mice one week after injection which means for each injection, we should inject our substance at first, and then inducing seizure after one-week interval. In brief, after drug treatment, tonic-clonic seizures in mice were induced using an electro-convulsiometer by administering electroshock (Ugo Basile electroshock machine, Model 7800, Camerio, Italy), at 50 Hz and 75 mA of intermittent current for 0.2 s through electrodes affixed to the mouse ears. To enhance electrode contact, the electrodes were moistened with normal saline before being attached to the ear of the mouse. The occurrence of THLE (180° extension of mouse hindlimbs in relation to the body axis) was used as a measure of seizure activity. Then the duration of THE was measured using Stopwatch. If our substance increases the duration of THE, we can consider it as convulsive and if it causes a reduction in this time, it can be an anticonvulsant one.

Statistical analysis

The data that was collected was analyzed using a one-way analysis of variance (ANOVA) with GraphPad Prism software version 8.0 (San Diego, USA), and were expressed as mean±SD of three replicates. Data that had a *P* of less than 0.05 were deemed statistically significant.

Results

Evaluation of the convulsive effect of nitrofurantoin in acute phase

The effect of acute i.p. injection of nitrofurantoin (25, 50, 75 and 100 mg/kg) on CST (clonic seizure threshold) are shown in Figure 1. What stands out from this figure is that the duration of THE registered an upward trend in a nitrofurantoin dose dependent manner. More importantly, it was found that the most significantly high duration of THE was related to the administration of 100 mg nitrofurantoin (*P<0.0001*).

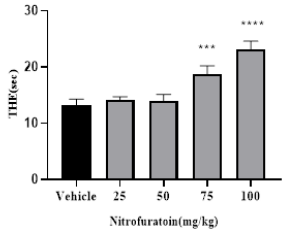

Figure 1. The effect of acute administration of different doses of nitrofurantoin (25, 50, 75 and 100 mg/kg) on Tonic Hind-limb Extension duration time. Results are expressed as mean±SEM. Each group contained at least six mice, and the data was examined through using one-way ANOVA. ***$P<0.001$, ****$P<0.0001$ Significant difference in comparison to the control group (Vehicle)

Evaluation of the convulsive effect of nitrofurantoin in chronic phase

In the chronic administration, 50 and 75 mg/kg concentrations of nitrofurantoin were injected into mice and its impact was determined after a week. Measured duration of THE after-nitrofurantoin injection (i.p. injection) has been represented in figure 2. As it can be

seen from this figure, 75 mg of nitrofurantoin could potentially increase the THE duration as compared to 50 mg of the antibiotic and vehicle control (*P<0.05*).

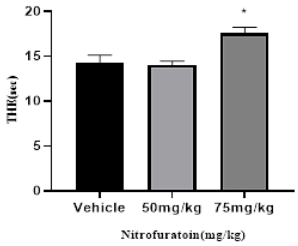

Figure 2. The effects of chronic administration of nitrofurantoin (50 and 75mg/kg) on Tonic Hind-limb Extension duration time. Results are expressed as mean±SEM. Each group contained at least six mice, and the data was examined through using one-way ANOVA. **P*<0.05 Significant difference in comparison to the control group (Vehicle)

Evaluation of the anticonvulsive effect of phenytoin in acute and chronic phases

In order to examine the effect of an anticonvulsant drug on mice, we used 5, 10 and 20 mg/kg of phenytoin in both acute and chronic phases. Figure 3 illustrates THE duration after phenytoin administration. It is apparent from this figure that only 20 mg/kg of phenytoin administration in both acute and chronic phases resulted in a notable reduction in the duration of THE (*P<0.01*).

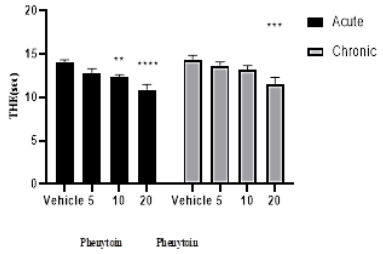

Figure 3. The effects of acute and chronic administration of phenytoin (5, 10 and 20 mg/kg) on Tonic Hind-limb Extension duration time. Results are expressed as mean ± SEM. Each group contained at least six mice, and the data was examined through using one-way ANOVA. ***P*<0.01, ****P*<0.001, *****P*<0.0001 Significant difference in comparison to the control group (Vehicle)

Simultaneous comparison of the convulsive effect of nitrofurantoin and anticonvulsive effect of phenytoin in both acute and chronic phase

We investigated the effect of combined nitrofurantoin (75 mg/kg) and phenytoin (5 mg/kg) on the THE duration in both acute and chronic phases. As figure 4 shows, the combination of nitrofurantoin and phenytoin had no statistically significant effect on the THE duration in the acute phase. However, we noticed that the administration of combined nitrofurantoin and phenytoin in the chronic phase led to a remarkable decrease in the THE duration (*P<0.05*).

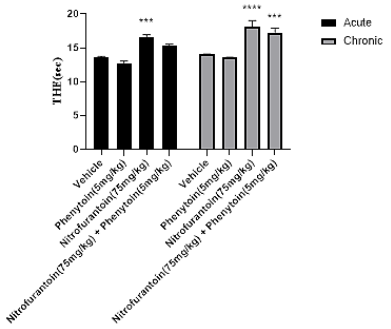

Figure 4. The effect of acute and chronic administration of the combination of nitrofurantoin (75 mg/kg) and phenytoin (5 mg/kg) on Tonic Hind-limb Extension duration time. Results are expressed as mean ± SEM. Each group contained at least six mice, and the data was examined through using one-way ANOVA. ****P*<0.001, *****P*<0.0001 Significant difference in comparison to the control group (Vehicle)

Discussion

Generally, a seizure is defined as electrical malfunction of brain cells (15). Our brain consists of several local excitatory and inhibitory circuits, the disturbance of which may cause seizures (16). GABAergic system inhibition, excitatory amino acid activation, and voltage-dependent sodium channel derangement are considered major contributors to the seizures (17). A wide array of drugs and toxins can cause seizure as a result of indirect effects on brain function (18). There exists a bunch of studies on surged risk of developing seizure as adverse side effect of antibiotic therapy (7-8).Taking these pharmacological observations into account and what we mentioned to about nitrofurantoin adverse effect in introduction according to some reports, this study, aimed to delineate the role of nitrofurantoin in the induction of seizure as a major neurological side effect and check whether nitrofurantoin can induce seizure or not. Herein, we used electroshock therapy to stimulate seizure in NMRI mice. Interestingly, a high number of earlier similar studies have applied Maximal Electroshock to induce seizures in rodents (19-22). In the present study, after injecting nitrofurantoin to mice and checking the duration of THE in both acute and chronic phase, we found that administration of nitrofurantoin in these two phases could potently increase

the THE time which is our sign for causing seizure and lead to induction of seizure in mice. Further, to examine the assumption that nitrofurantoin can exert convulsive effect, we used phenytoin as an anticonvulsant drug. Notably, we chose phenytoin according to a hypothesis, which shows the similarity between nitrofurantoin and neurotoxins like ciguatoxin that cause neurotoxicity via opening the sodium channels. Interestingly, phenytoin plays its anticonvulsive role through blocking sodium channels and thus, selecting phenytoin was the best option to show its opposite effect to the nitrofurantoin (22). Considering this information, it can be postulated that nitrofurantoin might possess convulsive effect (23). However, the mechanism upon which nitrofurantoin causes neurological impairment differs from toxins like Ciguatoxin, which might be associated with their different ligand binding sites in the sodium channels (24). Therefore, this hypothesis is refused. We also examined the possibility of neuropathy induction by nitrofurantoin to assess the plausibility of the second hypothesis. This antibiotic can interfere with oxidation of pyruvate and thereby disturbing its conversion to acetyl coenzyme-A, which is the predominant factor in the process of ATP production (25). Given that ATP is the main energy used by the brain (26), its deficit causes reduced GABA-ergic potentials and the subsequent seizure induction (27). In addition, by disturbing pyruvate oxidation, it has to enter another pathway which is lactic acid synthesis pathway. The acidic feature of lactic acid may cause neurological impairment like seizures (28). Interestingly enough, it has been demonstrated that antibiotics with modes of action of targeting bacterial translation can also affect mitochondrial translation and lead to impaired mitochondrial function, which in turn can affect neurodevelopment and brain function (26). These mechanisms indicate that the nitrofurantoin can possibly exert convulsive effects. Further investigation is required to comprehend the precise mechanisms, through which nitrofurantoin triggers seizure induction.

At the end, we examined combination therapy of nitrofurantoin and phenytoin to study and compare the treatment of them in the same situation with each other. Opposite behavior of these two drugs can be seen in Fig 4. In other words, nitrofurantoin increased the THE time in comparison with that in vehicle and phenytoin has the opposite action, means decreasing effect on the THE time. Moreover, we observed that the combination of convulsive nitrofurantoin and anticonvulsive phenytoin has no significant effect on THE time in the acute phase, while this combination therapy led to a remarkably increased THE time in the chronic phase. A possible explanation for this might be drug interactions that could be more probable in chronic phase than the acute phase. Nevertheless, the outcomes of the present investigation do not corroborate the prior research, which demonstrated that co-administration of antibiotics and anticonvulsant drugs may lead to enhanced seizure risk due to drug interactions (7).

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