Research report

Effect of pentylenetetrazole and sound stimulation induced single and repeated convulsive seizures on the MDA, GSH and NO levels, and SOD activities in rat liver and kidney tissues

Meltem Ozlen Dillioglugil\textsuperscript{a,4}, Hale Maral Kir\textsuperscript{a}, Cennet Demir\textsuperscript{a}, Gul Ilbay\textsuperscript{b}, Deniz Sahin\textsuperscript{b}, Ozdal Dillioglugil\textsuperscript{c}, Gonul Bambal\textsuperscript{b}, Haluk Mekik\textsuperscript{d}, Nurbay Ates\textsuperscript{b}

\textsuperscript{a}Department of Clinical Biochemistry, Faculty of Medicine, Kocaeli University, Kocaeli, Turkey
\textsuperscript{b}Department of Physiology, Faculty of Medicine, Kocaeli University, Kocaeli, Turkey
\textsuperscript{c}Department of Urology, Faculty of Medicine, Kocaeli University, Kocaeli, Turkey
\textsuperscript{d}Laboratory of Büyük İstanbul, Çorlu-Tekirdag, Turkey

\textsuperscript{4}Corresponding author at: Kocaeli University Faculty of Medicine, Department of Biochemistry, Umuttepe Kampüsü-41380, Kocaeli, Turkey. Tel.: +90 262 3591233; fax: +90 262 3038003.
E-mail address: mozden@superonline.com (M.O. Dillioglugil).

1. Introduction

Epilepsies constitute a large group of neurological diseases that affect more than 50 million people worldwide\textsuperscript{25}. Pentylenetetrazole (PTZ) induces dose dependent convulsive seizures in animals. In this model, single and repeated injection of the convulsant PTZ...
causes generalized tonic–clonic seizures [30,1,27,23]. Generalized epilepsy is a chronic disorder characterized by recurrent seizures which has been shown to increase reactive oxygen species (ROS) generation in the brain [33]. Prolonged seizure activity in animals results in increased production of ROS, and thus increased oxidative stress. Oxidative stress and mitochondrial dysfunction occur as a consequence of prolonged epileptic seizure and may contribute to seizure-induced brain damage [25].

Oxidative stress in the central nervous system has been shown to be increased in several models of experimental epilepsy described so far, such as the amygdala kindling model [9], the kainic acid model [10], the PTZ model [11,28], sound stimulation (key ringing) model [38], and the electroshock model [29,2].

In the body, liver and the kidney are among the tissues which act as the organs of detoxification, protecting body from dietary, environmental and metabolic chemicals and toxins. Therefore liver and kidney tissues were chosen in this study because they are the sensitive organs to oxidative damage [32]. The objective of this study was to evaluate the activity of superoxide dismutase (SOD) and the levels of glutathione (GSH), malondialdehyde (MDA), and nitric oxide (NO) in, liver and kidney tissues in a rat model of single or repeated seizures produced by PTZ and sound stimulation with key ringing.

2. Materials and methods

2.1. Subjects

Male Wistar adult rats (n = 48), weighing 200–250 g, were used in the experiment. Ethical approval was given by the Ethics Committee of Kocaeli University Faculty of Medicine. The animals were divided into six groups: (1) Single Seizure Control Group (SS-Control; n = 8); rats were treated with single dose of i.p. saline, (2) Repeated Seizures Control Group (RS-Control; n = 8); rats were treated with repeated doses of i.p. saline every other day for six times, (3) PTZ induced Single Seizure Group (SS-PTZ Group; n = 8); rats were treated with single dose of i.p. PTZ (60 mg/kg), (4) PTZ induced Repeated Seizures Group (RS-PTZ Group; n = 8); rats were treated with repeated doses of i.p. PTZ (60 mg/kg), every other day for six times (each dose of 60 mg/kg PTZ induced generalized tonic–clonic seizures in all animals), (5) Key-Ringing Induced Single Seizure Group (SS-KEY Group; n = 8); the rats were subjected to the sounds produced by key ringing until the induction of a seizure, (6) Key-Ringing Induced Repeated Seizures Group (RS-KEY Group; n = 8); the rats were subjected to the sounds produced by key ringing until the induction of a seizure every other day for six times.

2.2. Seizure models

In key ringing groups seizures were induced by sound stimulations. The sound stimulations were provided by a short manual shake of a bunch of keys (5–10 metal door keys on a metal key-ring) held at 50 cm above the floor of an observation box (35 cm × 35 cm × 35 cm). The frequency and intensity of the sound were measured by Biopac MP36 Data Acquisition System (St Barbara, CA, USA), and by sound level meters Lutron SL-4012 (Taipei, Taiwan). The peak frequency of sound stimulation was around 6.7 kHz, with a wide range of 2–14 kHz. The intensity of the sound ranged from 80 to 90 dB. To evaluate the progression of audiogenic seizure, a Wistar audiogenic rat was placed into a box (60 cm × 60 cm × 60 cm) and sound stimulation was applied for 60 s. As a result of this sound stimulation several phases of seizure occurs: wild running, clonus, and tonus [38].

Key-Ringing model was also used, because we wanted to obtain convulsive seizures not influenced by chemical effect of PTZ [30]. Sound stimulation provokes convulsive seizures in a subpopulation of Wistar rats. This ranges from 15% to 30% [38,20,16]. We used 98 rats (48 for SS-KEY Group and 50 for RS-KEY Group) and obtained convulsive seizures in 16 (8 in SS-KEY and 8 in RS-KEY group) of them.

Following PTZ injections and sound stimulations rats were observed for convulsive activity for 30 min. Activity occurred as generalized seizures, which started with the clonus of the facial and the forelimb muscles, and continued with the neck and tail extensions, loss of straightening reflex with tonic flexion–extension and usually with extended clonic activities. Behavioral characteristics of seizures and duration of convulsions were recorded [30,15]. Animals were sacrificed by guillotine 24 h after PTZ and key ringing-induced seizure (single or last seizure) or saline administration.

2.3. Tissue assessment and methods

Liver and kidney tissues were removed and washed three times in cold isotonic saline (0.9%). Tissues were homogenized with cold Tris–HCl buffer (pH 7.4) to obtain 10% homogenate (w/v). Lipid peroxide levels, expressed in terms of MDA, were determined according to the method of Buege and Aust [3]. GSH was measured according to the method of Ellman [8]. Cu-ZnSOD activity was measured kinetically by the method of Sun et al. [34]. Nitrite and nitrate were estimated as an index of NO production [4]. The method for nitrite and nitrate levels was based on the Griess reagent which consists of sulphanilamide and N-(1-naphthyl) ethylenediamine. The protein concentrations of tissue homogenates were determined by the method of Lowry et al. [18].

2.4. Statistics

All data were expressed as mean ± SD. The SPSS statistics package (SPSS Inc., Chicago, IL) was used for all statistical analyses. Statistical comparisons were performed using analysis of variance (ANOVA) followed by post-ANOVA (Tukey’s HSD) test to compare changes among individual groups. Differences between groups were considered to be statistically significant at p < 0.05.

3. Results

Table 1 includes all results (MDA, NO and GSH levels and SOD activities in rat liver and kidney) in all (six) groups. There was no significant difference between SS-Control and RS-Control groups (p > 0.05) in none of the examined parameters in liver and kidney tissues. There was no significant difference between SS-PTZ and SS-KEY groups (p > 0.05) in none of the examined parameters in liver and kidney tissues. There was also no significant difference between RS-PTZ and RS-KEY groups (p > 0.05) in none of the examined parameters in liver and kidney tissues.

The liver and kidney levels of MDA and NO in SS-PTZ group were found to be significantly higher than the SS-Control group (p < 0.05). In SS-KEY group, the liver and kidney levels of MDA and NO were found to be significantly higher and GSH levels were significantly lower than the SS-Control group (p < 0.05).

While liver and kidney levels of MDA in RS-PTZ and RS-KEY groups were found to be significantly higher than the RS-Control group (p < 0.05), liver and kidney GSH levels were significantly lower (p < 0.05). The liver levels of NO in RS-PTZ and RS-KEY groups were found to be significantly higher than the RS-Control group (p < 0.05). Kidney SOD activities in RS-PTZ and RS-KEY groups were found to be significantly lower than the RS-Control group (p < 0.05). When RS-PTZ group is compared with the SS-PTZ group, the liver SOD activity and kidney NO level were found to be significantly lower in the RS-PTZ group (p < 0.05).

While the liver NO level and GSH level in RS-KEY group were significantly higher than the SS-KEY group, SOD activity was significantly lower in the RS-KEY group (p < 0.05). When RS-KEY group was compared with SS-KEY group, the kidney NO level and SOD activity were found to be significantly lower in the RS-KEY group (p < 0.05).

4. Discussion

Free radicals, such as NO generation results in lipid peroxidation (MDA) which may also induce epileptic seizure activity by direct inactivation of glutamine synthase, thereby permitting an abnormal build up of major excitatory neurotransmitter glutamate [33,24]. L-glutamate mediates its actions via activation of receptors. These receptors play role in a variety of central nervous system disorders such as epilepsy, pain, ischemia and neurodegenerative diseases [19]. Oxidative stress is thought to be an important consequence of glutamate receptor activation and excitotoxicity [6,26]. N-methyl-D-aspartate-receptor subtype of glutamate, which is coupled with NO synthesis by activation of NO synthase (NOS), play a crucial role in the production of kindling seizures and to induce epileptic neurodegeneration [37,31].

It is accepted that GSH and SOD are one of the most important physiological antioxidants against free radicals and that they prevent subsequent lipid peroxidation [32,35]. Lipid peroxidation,
Table 1
Liver and kidney MDA, NO, GSH levels and SOD activities in control (SS-Control and RS-Control), pentylenetetrazole induced single seizure (SS-PTZ), pentylenetetrazole induced repeated seizures (RS-PTZ), key-ringing induced single seizure (SS-KEY), and key-ringing induced repeated seizures (RS-KEY) groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (nmol/100 mg Prt)</th>
<th>NO (μmol/100 mg Prt)</th>
<th>GSH (nmol/mg Prt)</th>
<th>SOD (U/100 mg Prt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS-Control (n=8)</td>
<td>13.41 ± 4.35</td>
<td>8.86 ± 3.77</td>
<td>53.94 ± 25.32</td>
<td>72.06 ± 27.10</td>
</tr>
<tr>
<td>RS-PTZ (n=8)</td>
<td>25.46 ± 6.53</td>
<td>13.30 ± 5.22</td>
<td>39.42 ± 17.97</td>
<td>7.49 ± 3.07</td>
</tr>
<tr>
<td>SS-KEY (n=8)</td>
<td>31.06 ± 2.88</td>
<td>29.69 ± 5.24</td>
<td>41.86 ± 16.70</td>
<td>25.46 ± 6.53</td>
</tr>
<tr>
<td>RS-KEY (n=8)</td>
<td>18.29 ± 4.35</td>
<td>9.16 ± 2.34</td>
<td>114.25 ± 55.86</td>
<td>25.46 ± 6.53</td>
</tr>
</tbody>
</table>

Key:
- SS-Control: Single seizure control group.
- RS-PTZ: Pentylenetetrazole induced repeated seizures (RS-PTZ).
- SS-KEY: Key-ringing induced single seizure (SS-KEY).
- RS-KEY: Key-ringing induced repeated seizures (RS-KEY).

Significantly different from RS-Control, p < 0.05.

Liver results

In the present study, we found that mean liver and kidney levels of MDA in all single and repeated seizure groups (i.e., SS-KEY, SS-PTZ, RS-KEY, and RS-PTZ) were significantly higher than the corresponding control groups (i.e., SS-Control and RS-Control). This is concordant with the findings in other studies. Significant decrease in liver SOD activities after PTZ exposure [36]. They found that PTZ induced seizure did not produce significant difference in kidney GSH levels compared with controls. However, the kidney level of GSH in our study was significantly lower in RS-PTZ and RS-KEY groups when compared with RS-Control group. We failed to find any study regarding kidney tissue levels of SOD following induced seizure. Kidney SOD activities were found to be significantly lower in the RS groups (RS-PTZ and RS-KEY groups) when compared with their control (RS-Control group). On the other hand, we also found that the liver SOD activity was significantly lower in the RS-KEY group when compared with its control (RS-Control) group.

The literature suggests that epileptic seizures produce stress on liver. In a previous study with epilepsy, it was observed that fulminant hepatic failure is a rare complication of status epilepticus [5]. In the present study, we found that mean liver and kidney levels of MDA in all single and repeated seizure groups (i.e., SS-KEY, SS-PTZ, RS-KEY, and RS-PTZ) were significantly higher than the corresponding control groups (i.e., SS-Control and RS-Control). This is concordant with the findings in other studies. Significant decrease in liver SOD activities after PTZ exposure [36]. They found that PTZ induced seizure did not produce significant difference in kidney GSH levels compared with controls. However, the kidney level of GSH in our study was significantly lower in RS-PTZ and RS-KEY groups when compared with RS-Control group. We failed to find any study regarding kidney tissue levels of SOD following induced seizure. Kidney SOD activities were found to be significantly lower in the RS groups (RS-PTZ and RS-KEY groups) when compared with their control (RS-Control group).
significantly lower in RS-PTZ and RS-KEY groups when compared with their control (RS-Control) in our study.

When repeated seizure groups are compared with single seizure groups, we found differences (more pronounced in the key-ringing groups) between oxidant and antioxidant parameters. Furthermore, seizures impaired the balance between the oxidant and antioxidant systems in liver and kidney tissues. In a recent study, maximal electroshock-induced seizures in mice were examined with respect to changes in total antioxidant capacity (TAC), lipid peroxidation intensity (LPI), and glutathione peroxidase (GSH-Px) activity in brain tissue, plasma and erythrocytes. Pronounced changes in oxidative/antioxidative status were found after both convulsive and subconvulsive stimuli when compared with controls. The authors suggested that oxidative stress may participate in seizures and the pathophysiology of epilepsy, and this issue needs to be studied by further studies [22].

Our study was designed to test the ominous effects of single and repeated seizures on kidney and liver tissues by measuring oxidant and antioxidant parameters. Damage in these tissues may also cause accompanying systemic disorders, such as fulminant hepatitis, and furthermore this damage may be responsible for the emergence of side effects of the drugs used for epilepsy.

In conclusion, key-ringing or PTZ induced single and repeated seizures result in increased oxidative damage and lipid peroxidation, and decreased antioxidant defense mechanisms.

References


