Effect of phenobarbital on antioxidant enzyme activities and blood gas parameters in Balb/C mice

O. DEMIR, E. YAZAR*, V. ALTUNOK, M. ELMAS and V. OZDEMIR

SUMMARY

In this study, effect of phenobarbital was investigated on antioxidant enzyme activities and blood gas parameters in Balb/C mice. Forty male Balb/C mice were used. Ten mice were served as a control group, and thirty mice were administered phenobarbital (80 mg/kg body weight, orally, single administration). Blood, brain and liver samples were taken at 6, 12 and 24 hours after administration. Brain and liver tissues superoxide dismutase and glutathione peroxidase activities were measured by spectrophotometry, and blood gas parameters were measured with blood gas analyzer. As results, phenobarbital caused temporary respiratory acidosis and a decrease in brain tissue superoxide dismutase activity.

KEY-WORDS: phenobarbital - superoxide dismutase - glutathione peroxidase - blood gas parameters - mice.

1. Introduction

Phenobarbital (PB) is a major drug in the treatment of canine, feline and human epilepsy and can significantly reduce the rate of severity of seizures. PB raises the threshold for seizure discharge and inhibits the initiation, diffusion, and spread of discharge from the neural focus. Common side effects of PB are ataxia, sedation, polyuria, polydipsia, polyphagias, and may depress both the respiratory drive and the mechanisms responsible for the rhythmic character of respiration [3, 5, 9, 13]. Drug is rapidly and completely absorbed after oral doses, and maximum concentrations in plasma are reached 4 to 8 hours after administration. Blood level of PB is clinically effective 12 to 24 hours after oral administration. PB is metabolized by liver and extracted by the kidney [3].

Phenobarbital is a known inducer of microsomal enzymes (cytochrome P-450 (CYP), NADPH- cytochrome P-450 reductase, NADPH oxidase, glutathione-S-transferase), which are responsible for the metabolic breakdown of a large number of endogenous and exogenous chemical compounds [1, 3, 8, 32].

Superoxide dismutase (SOD) and glutathione peroxidase (GPX) called antioxidant enzymes protect the cells against reactive oxygen radicals (singlet oxygen : \( ^1 \text{O}_2 \), superoxide radical : \( \text{O}_2^- \), hydroxyl radical : \( \cdot \text{OH} \) and hydrogen peroxide : \( \text{H}_2\text{O}_2 \)). Reactive oxygen radicals (ROR) may become toxic to tissues by initiating lipid peroxidation [20, 21], and they may be implicated in epilepsy [26]. SOD catalyzes the dismutation of two superoxide radicals to \( \text{O}_2 \) and \( \text{H}_2\text{O}_2 \) [27]. GPX detoxifies \( \text{H}_2\text{O}_2 \) to \( \text{H}_2\text{O} \) and \( \text{O}_2 \), and converts lipid hydroperoxides to nontoxic alcohols [11, 34]. Serum SOD and GPX activities do not reflect the changes of these enzyme activities derived from tissues. It was reported that these enzymes must be measured directly from affected organ [7].
The aim of this study was to investigate the effect of phenobarbital on brain and liver tissues SOD and GPX activities. The effect of PB on blood gas parameters were also investigate, because PB may cause a respiratory depression and this depression may change the blood gas parameters.

2. Materials and methods

Forty male Balb/C (approximately 5 months, 38-44 gram) mice were used as materials (Selcuk University, Experimental Medicine, Research and Application Center, Konya, Turkey). Mice were fed on standard pellet diet and tap water ad libitum all experimental period. Ten mice were used as a control group. Thirty mice were orally administered phenobarbital (Luminalletten®, Bayer, Istanbul) via intragastric gavage at dose 80 mg/kg body wt [6, 28, 37]. After the administration of phenobarbital, blood samples were taken from (400-500 µl) the heart by cardiac puncture under light ether anesthesia at hours 6, 12 and 24. Mice were immediately killed after just bleeding. Same procedure was performed in control group.

pH, partial carbondioxyde pressure (pCO₂), partial oxygen pressure (pO₂), actual bicarbonate (HCO₃a), standard bicarbonate (HCO₃s), total carbondioxyde (tCO₂), base excess in vivo (BE(vv)), base excess in vitro (BE(vt)), oxygen saturation (O₂sat), oxygen concentration (O₂ct), haemoglobin (Hb), packed cell volume (PCV), sodium (Na), potassium (K) and ionized calcium (ICa) were measured by blood gas analyzer (288 Ciba-Corning®). Brain (cerebrum) and liver (300 mg, lobus hepatis sinister) samples were immediately removed after killing and washed with cold saline solution. Samples were homogenized with 500 µl of cold homogenate solution [0.25 M sucrose (Sigma) + 10 mM Tris (Sigma) + 1 mM EDTA (Pharmacia Biotech), pH 7.4] into ice [31, 35]. The homogenates were centrifuged (10,000 rpm, 15 minutes, +4 °C), and the supernatants were carefully removed and stored for analysis (-80 °C). Brain tissues and hepatic SOD (Randox-Ransod, SD125) and GPX (Randox-Ransel, RS 505) activities and total protein (Sigma, 541-2) level were assayed in supernatants by using commercially available kits for spectrophotometer (Schimadzu UV 2100). Enzyme activities were expressed as U mg⁻¹ tissue protein.

All values were expressed as mean ± standard error (SE). The results were analyzed by Duncan multiple range test (SPSS for windows, release 6.0). In all cases, probability of error of less than 0.05 was selected as the criterion for statistical significance from the control values.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>6th hour</th>
<th>12th hour</th>
<th>24th hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.40±0.02</td>
<td>7.28±0.02*</td>
<td>7.37±0.01</td>
<td>7.38±0.02</td>
</tr>
<tr>
<td>pCO₂ mmHg</td>
<td>33.5±1.90</td>
<td>46.4±2.30*</td>
<td>40.7±1.80</td>
<td>37.5±5.80</td>
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<tr>
<td>pO₂ mmHg</td>
<td>90.3±1.71</td>
<td>62.3±4.71*</td>
<td>66.0±6.82</td>
<td>66.7±10.28</td>
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<td>HCO₃a mmol/L</td>
<td>20.9±1.56</td>
<td>21.6±0.47</td>
<td>23.5±0.69</td>
<td>21.2±2.76</td>
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<td>HCO₃s mmol/L</td>
<td>22.5±1.35</td>
<td>20.2±0.66</td>
<td>23.4±0.39</td>
<td>21.9±1.39</td>
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<td>tCO₂ mmol/L</td>
<td>21.9±1.62</td>
<td>23.0±0.48</td>
<td>24.8±0.75</td>
<td>22.4±2.94</td>
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<tr>
<td>BE(vt) mmol/L</td>
<td>-2.4±1.65</td>
<td>-4.2±0.63</td>
<td>-0.9±0.51</td>
<td>-2.9±1.87</td>
</tr>
<tr>
<td>BE(vv) mmol/L</td>
<td>-3.0±1.68</td>
<td>-4.3±0.61</td>
<td>-1.2±0.54</td>
<td>-3.4±2.26</td>
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<td>O₂sat %</td>
<td>96.9±0.27</td>
<td>85.9±3.28</td>
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<td>O₂ct mL/dL</td>
<td>19.0±0.53</td>
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<td>16.3±1.13</td>
<td>16.1±1.61</td>
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<td>Hb g/dL</td>
<td>13.9±0.40</td>
<td>13.1±0.36</td>
<td>13.4±0.33</td>
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<td>PCV %</td>
<td>41.1±1.16</td>
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<td>39.5±0.96</td>
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<td>Na mmol/L</td>
<td>144.4±2.21</td>
<td>137.3±1.89</td>
<td>134.9±1.33*</td>
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<td>K mmol/L</td>
<td>5.7±0.24</td>
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<td>ICa mmol/L</td>
<td>0.91±0.06</td>
<td>1.08±0.02*</td>
<td>0.95±0.05</td>
<td>1.01±0.03</td>
</tr>
</tbody>
</table>

* p < 0.05 statistically different from control group.

Table I. — Effect of a single dose of phenobarbital (80 mg/kg orally) on blood gas parameters in Balb/C mice (mean ± SE, n = 10).
3. Results

Blood gas parameters, and brain and liver tissues antioxidant enzyme (SOD and GPX) activities are given in Table I and II, respectively.

In the blood gas analysis, while pH and pO2 levels decreased (p < 0.05), pCO2 level increased (p < 0.05) at 6 hours, and brain tissue SOD activity decreased (p < 0.05) at 24 hours when compared to control group. Sodium, potassium and ionized calcium levels were found statistically different from control group, but these results stayed within reference ranges [16].

4. Discussions and conclusions

In the present study, PB caused a temporary respiratory acidosis. At 6 hours, it was observed that PB decreased (p < 0.05) pH and pO2 levels and increased (p < 0.05) pCO2 level. It has been reported that PB may cause a respiratory depression [5, 13], and similar blood gas result was reported by SCAILLES et al [23].

PB was evaluated in rodent models of epilepsy in many studies [2, 17]. It was stated that free radicals might be implicated in epilepsy [26]. PB caused increases in lipid peroxidation, CYP and ROS (Reactive oxygen species) production [14, 22, 30, 32]. In addition to this, induced CYP may produce hydrogen peroxide [1, 4, 15, 24], and hydrogen peroxide may directly inactivate SOD activity [10, 25]. It was suggested that decreases in antioxidant enzyme activities may be attributed to an increase in CYP-mediated reactions [33]. In this study, PB caused a decrease in brain tissue SOD activity. Produced ROS by PB might cause a decrease in brain tissue SOD activity. Similar results have been reported in red blood cell [19, 36]. Interestingly, the same result was not found in hepatic SOD activity. In the present study, hepatic SOD (9.42 U/mg protein) and GPX (1.36 U/mg protein) activities were found higher than brain tissue SOD (8.15 U/mg protein) and GPX (0.09 U/mg protein) activities. It may be due to the fact that liver possesses high antioxidant capacity [12, 29] and composes the produced ROS. This result indicated that capacity of SOD induction to ROS is different from tissue to tissue.

As results, PB caused temporary acidosis and decreases in brain tissue SOD activity. Temporary acidosis may be due to the respiratory depression, and decreased brain tissue SOD activity may be due to produced ROS by PB. For fully determination of cause of decreased brain tissue SOD activity, further investigations are required, particularly malondialdehyde, which is indicator of lipid peroxidation [18].

References


<table>
<thead>
<tr>
<th>SOD brain U/mg</th>
<th>GPX brain U/mg</th>
<th>SOD hepatic U/mg</th>
<th>GPX hepatic U/mg</th>
</tr>
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<tr>
<td>8.5±0.83</td>
<td>0.09±0.01</td>
<td>9.42±1.67</td>
<td>1.36±0.11</td>
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<tr>
<td>8.0±0.42</td>
<td>0.07±0.01</td>
<td>9.37±0.59</td>
<td>1.67±0.11</td>
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<tr>
<td>6.5±0.64</td>
<td>0.08±0.01</td>
<td>8.78±0.91</td>
<td>1.17±0.05</td>
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<td>4.8±0.54*</td>
<td>0.09±0.01</td>
<td>10.88±0.59</td>
<td>1.23±0.09</td>
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</tbody>
</table>

* p < 0.05 statistically different from control group.

Table II. — Effect of a single dose of phenobarbital (80 mg/kg orally) on brain and liver tissues superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities in Balb/C mice (mean ± SE, n = 10).


