The effects of chloramphenicol, thiamphenicol and florfenicol on glucose 6-phosphate dehydrogenase (G6PD), reduced glutathione (GSH) enzyme activities and some haematological parameters in mice

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Summary: The aim of present study was to investigate the effects of chloramphenicol thiamphenicol and florfenicol on activities of reduced glutathione (GSH) and glucose-6 phosphate dehydrogenase (G6PD) enzymes that play an important role in the free radical metabolism of erythrocytes and haematological parameters in the mice. In the study, 70 male Swiss albino mice were used. Animals were divided into seven groups, each having 10 mice. Each antibiotic were given ad libitum in drinking water at levels of 0 (control), 100 mg/kg and 200 mg/kg for 7 days. Biochemical and haematological analysis were studied on days 1, 7 and following 14. Both dosages of chloramphenicol and thiamphenicol, but not florfenicol were induced a significant reduction (p < 0.05) in GSH and G6PD enzyme activities on days 7 and 14 compared to control group. However, only high dosage of thiamphenicol and both dosage of chloramphenicol were induced a significant (p < 0.05) decrease in red blood cell (RBC) count, hemoglobin (Hb) value, packed cell volume (PCV), white blood cell (WBC) and neutrophil percentage on day 14 compared to control group. The present findings indicate that chloramphenicol and thiamphenicol have significant inhibition effects on the activities of erythrocytes GSH, G6PD enzymes and some haematological parameters in mice.

Key Words: Antibiotic, Glucose-6 phosphate dehydrogenase, Reduced glutathione, Haematological parameter, mouse

Özet: Bu çalışmanın amacı farelerde, eritrositlerin serbest radikal metabolizmasında rol oynayan gluкоz-6 fosfat dehidrogenaz (G6PD) ve glutatyon (GSH) enzim aktiviteleri ile kan değerleri üzerine kloramfenikol, tiamfenikol ve florfenikolün etkilerini araştırmaktır. Çalışmada 70 adet Swiss albino erkek fare kullanıldı. Hayvanlar herbirinde 10 adet fare olacak şekilde 7 gruba ayrıldı. Her bir antibiotik içme suralar ile ad libitum olarak 0 (kontrol), 100 mg/kg ve 200 mg/kg dozlarında 7 gün uygulandı. Biyokimyasal ve hematolojik analizler uygulanan 1. ve 7. günlerde takip edildi. Kloramfenikol ve tiamfenikol her iki dozu GSH ve G6PD enzim aktivitelerini kontrol grubuna oranla 7. ve 14. günlerde istatistiksel olarak önemli oranda düşürtürken (p < 0.05) florfenikolün etkisi Yapmadığı tespit edildi. Bununla birlikte tiamfenikolun bazı hematolojik parametreler üzerine engelleyici bir etkiye sahip olduğunu gösterdi.

Anahtar Sözcükler: Antibiyotik, Gluкоz-6 fosfat dehidrogenaz, Redükte glutatyon, Hematolojik parametre, Fare

INTRODUCTION

Reduction in glutathione (GSH) and glucose 6-phosphate dehydrogenase (G6PD) enzymes activities in erythrocytes in organism is clinically important, GSH and G6PD activity changes in the presence of various illnesses and some other circumstances such as drug usage. A decrease in GSH activity causes an increase in hydrogen peroxide and leads cell damage (Schaeffer and Stainer, 1978). G6PD is a very important enzyme which catalyzes first step of the pentose phosphate metabolic pathway (Srivastava and Beutler, 1989). This metabolic pathway is a unique source of NADPH in erythrocyte and synthesis of NADPH decreases in the G6PD deficiency (Yuregir et al., 1994). The essential function of NADPH in erythrocytes is regeneration of reduced glutathione which prevents hemoglobin denaturation, protects the integrity of the red blood cell membrane sulfhydryl groups, and detoxifies hydrogen peroxide and oxygen radicals in and on the red blood cells (Lehninger et al., 2000). Decrease of G6PD results in NADPH and GSH deficiency in erythrocyte. Erythrocyte GSH insufficiency causes an early hemolysis in spleen (Andrews and Mooney, 1994). Many antibiotics are being used in physician and veterinarian therapies. There are a few
published papers in relation to changes in these enzyme activities (Yuregir et al., 1994; Ciftci et al., 2000). For many years, chloramphenicol was considered as an ideal antibiotic. Apart from being inexpensive and relatively nontoxic to the animals, it has a broad spectrum of antibacterial activity and penetrates well in tissues and cerebrospinal fluid (Yunis, 1988). However, the drug is haemotoxic in man inducing a reversible anaemia or irreversible fatal aplastic anaemia and leukaemia (Manyan et al., 1972; Holt et al., 1998).

Thiamphenicol differs structurally from chloramphenicol. Thus, it is thought that the aromatic nitro group chloramphenicol-induced aplastic anaemia which has been replaced by a methylsulfonyl group. Thiamphenicol exhibits antibacterial activity similar to chloramphenicol but it is effect weaker than that of chloramphenicol (De Renzo et al., 1981; Skolimowski et al., 1983).

Flofenicol, like thiamphenicol, has lack of the nitro group located on the chloramphenicol aromatic ring that has been associated with chloramphenicol induced nondose related irreversible aplastic anaemia in human (Sams, 1994; Sams, 1995). However, chloramphenicol and thiamphenicol also cause dose dependent, reversible bone marrow suppression in some animals and human (Krako et al., 1955) due to mitochondrial injury (Nijhof and Kroon, 1974). It is theoretically possible that flofenicol could cause some dose-dependent, reversible bone marrow suppression, but it has not been clinically reported.

The aim of the present study was to compare the effects of chloramphenicol, thiamphenicol and flofenicol on G6PD activity, GSH activity and some haematological parameters in mice.

**MATERIALS and METHODS**

**Animals, Diet and Housing Conditions**

In present study, seventy adult inbred male Swiss albino mice (n = 10 x 7) weighing about 30 g were used. The animals were fed by standard mice pellets and drinking water ad libitum. The mice were housed in individual cages (360 x 200 x 190 mm). Housing was started 15 days before the experiments. All animals were housed in stainless cages under standard laboratory conditions (light period 07.00 a.m. to 8.00 p.m., 21 ± 2 °C, and relative humidity 55 %). All experiments in this study were performed in accordance with the European guidelines, European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purpose, 1996.

**Experimental Design**

The average consumption of drink water (5 ml/day) was determined for each mouse. Thus, thiamphenicol, chloramphenicol and flofenicol were dissolved in water in concentration of 600 mg/L and 1200 mg/L. The solutions were given ad libitum orally for 7 days into six experimental groups to provide a dose of 100 mg/kg and 200 mg/kg of mouse body weight. Control animals received only drinking water without antibiotic in the same manner. Free access to basal diet was maintained during the study.

**Haematological and Biochemical Analysis**

Blood samples were collected by cardiac puncture from experimental groups on days 1, 7 and following treatments 14. Red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb), packed cell volume (PCV), neutrophil and lymphocyte countings were determined. RBC and WBC counting methods were based on the dilution of obtained blood with fluids (Hayem and Turk) in RBC and WBC counting pipettes (Mitruka and Rawnsley, 1977). Individual cells were then counted in the counting chamber (haemocytometer). Giemsa’s staining method was used in determination of leucocyte and neutrophil percentage. PCV was measured by the microhaematocrit centrifuge (Mitruka and Rawnsley, 1977). Hb concentration was determined by the cyanomethemoglobin technique (Mitruka and Rawnsley, 1977).

G6PD activity was measured as described Beutler’s (1971). The concentration of GSH in hemolysates was determined as previously described by Barhoumi et al. (1995) and Chaudierej et al. (1999).

**Statistical Analysis**

All data were expressed as mean ± S.E. Statistical analysis of data was performed using a one-way analysis of variance (ANOVA) and Tukey’s posttest. A value of p < 0.05 was considered statistically significant.

**RESULTS**

Results of the present study are presented in Table 1 and Table 2 for all groups. Both dosages of chloramphenicol and thiamphenicol were induced a significant reduction (p < 0.05) in GSH and G6PD activities on both days 7 and 14.

It was considered that in the present study, a marked anemia immediately post dosing could be achieved by administering both dosage of chloramphenicol and only higher dosage of thiamphenicol were induced a significant decrease (p <0.05) in RBC count, PCV, Hb value on days 7 and 14. The WBC count, neutrophil and lymphocyte percentages were decreased (p < 0.05) in both dosages of chloramphenicol and thiamphenicol administrated animals on day 14, but and on day 7 these parameters were not affected by antibiotic administration. However, flofenicol was found ineffective on GSH activity, G6PD activity and haematological parameters.
DISCUSSION

It is known that many drugs have adverse effects on the organism when they are used for therapeutic or other purposes. These effects may be dramatic and systematic (Christensen et al., 1982). G6PD catalyzes the initial step in the hexose monophosphate shunt, oxidizing glucose-6-phosphate to 6-phosphogluconolactone and reducing nicotinamide adenine dinucleotide phosphate (NADP) to NADPH. The main function of the hexose monophosphate shunt is to protect red blood cells against oxidative injury via the production of NADPH (Yuregir et al., 1994).

Red blood cells contain relatively high concentrations of GSH which is protective against oxidative injury. The oxidants such as super oxide anion (O₂⁻) and hydrogen peroxide (H₂O₂) are formed within red blood cells due to reactions of hemoglobin with oxygen and due to drugs or infections. They accumulate within red blood cells and cause oxidation of hemoglobin and other proteins leading to loss of function and cell death (Ciftci et al., 2000). Under normal circumstances, these oxidants are rapidly inactivated by GSH in conjunction with glutathione peroxidase. The depleted GSH levels are restored by glutathione reductase which catalyzes the reduction of oxidized glutathione (GSSG) to GSH. This reaction requires the NADPH generated by G6PD. For this reason, G6PD was considered as an antioxidant enzyme (Srivastava and Beutler, 1989). In the present study, the in vivo effects of some antibiotics used by veterinarian and physician on GSH, G6PD and some hematological parameters have been investigated.

The antibacterial efficiency of chloramphenicol, thiamphenicol and florfenicol against bacterial pathogens has been investigated by many authors (Neu and Fu, 1980; Prescott and Baggot, 1993; Ueda and Suenag, 1995). However, the inhibitory effects of these antibiotics on GSH and G6PD in mice have not been studied. The effects of chloramphenicol, thiamphenicol and florfenicol on GSH and G6PD enzyme activities are summarized in Table 1. These data show that chloramphenicol had the highest inhibitor effect.

Table 1. The effects of chloramphenicol, thiamphenicol and florfenicol on the activities of reduced glutathione (GSH) and glucose-6-phosphate dehydrogenase (G6PD) enzymes in mice (n = 10 for each group).

<table>
<thead>
<tr>
<th>Parameters / Days</th>
<th>Control</th>
<th>Thiamphenicol (100 mg/kg)</th>
<th>Thiamphenicol (200 mg/kg)</th>
<th>Chloramphenicol (100 mg/kg)</th>
<th>Chloramphenicol (200 mg/kg)</th>
<th>Florfenicol (100 mg/kg)</th>
<th>Florfenicol (200 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (μmol/g)</td>
<td>10.43 ± 0.51</td>
<td>10.73 ± 0.24</td>
<td>10.30 ± 0.05</td>
<td>11.11 ± 0.42</td>
<td>10.73 ± 0.25</td>
<td>10.52 ± 0.46</td>
<td>10.17 ± 0.55</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>9.56 ± 0.11</td>
<td>9.44 ± 0.09</td>
<td>9.30 ± 0.25</td>
<td>9.03 ± 0.18</td>
<td>9.21 ± 0.21</td>
<td>9.19 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>10.51 ± 0.64</td>
<td>9.52 ± 0.21</td>
<td>8.56 ± 0.78</td>
<td>8.75 ± 0.26</td>
<td>8.14 ± 0.09</td>
<td>9.97 ± 0.56</td>
</tr>
<tr>
<td>G6PD (μg Hb)</td>
<td>1</td>
<td>12.27 ± 0.61</td>
<td>12.64 ± 0.70</td>
<td>12.61 ± 0.68</td>
<td>13.23 ± 0.06</td>
<td>12.81 ± 0.25</td>
<td>13.60 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>12.90 ± 0.12</td>
<td>12.65 ± 0.59</td>
<td>11.28 ± 0.20</td>
<td>11.55 ± 0.14</td>
<td>11.04 ± 0.17</td>
<td>12.85 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>12.99 ± 0.70</td>
<td>11.73 ± 0.61</td>
<td>10.21 ± 0.83</td>
<td>10.17 ± 0.38</td>
<td>9.72 ± 0.26</td>
<td>12.19 ± 0.33</td>
</tr>
<tr>
<td>RBC (10⁶/mm³)</td>
<td>1</td>
<td>45.20 ± 1.25</td>
<td>45.30 ± 1.50</td>
<td>42.30 ± 2.64</td>
<td>44.40 ± 1.21</td>
<td>43.50 ± 1.50</td>
<td>43.10 ± 1.47</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>46.50 ± 1.48</td>
<td>42.70 ± 1.75</td>
<td>40.40 ± 1.38</td>
<td>41.50 ± 1.50</td>
<td>41.50 ± 1.25</td>
<td>42.80 ± 1.31</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>44.00 ± 1.57</td>
<td>42.00 ± 1.11</td>
<td>39.50 ± 1.37</td>
<td>40.50 ± 1.52</td>
<td>39.50 ± 1.07</td>
<td>41.50 ± 1.73</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>1</td>
<td>11.85 ± 0.25</td>
<td>12.05 ± 0.95</td>
<td>11.20 ± 0.54</td>
<td>11.98 ± 0.65</td>
<td>12.57 ± 0.58</td>
<td>11.54 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>10.28 ± 0.60</td>
<td>11.58 ± 0.18</td>
<td>10.38 ± 0.33</td>
<td>11.12 ± 0.95</td>
<td>9.98 ± 0.52</td>
<td>10.67 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>10.83 ± 0.25</td>
<td>11.04 ± 0.20</td>
<td>9.83 ± 0.45</td>
<td>9.60 ± 0.80</td>
<td>8.20 ± 0.20</td>
<td>10.35 ± 0.35</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>1</td>
<td>23.00 ± 1.10</td>
<td>25.50 ± 5.50</td>
<td>21.00 ± 3.70</td>
<td>22.00 ± 2.50</td>
<td>25.00 ± 2.45</td>
<td>24.50 ± 1.50</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>21.50 ± 2.50</td>
<td>23.00 ± 5.20</td>
<td>19.50 ± 5.50</td>
<td>19.00 ± 2.60</td>
<td>19.00 ± 1.20</td>
<td>22.50 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>22.00 ± 1.10</td>
<td>19.30 ± 2.00</td>
<td>16.50 ± 2.40</td>
<td>15.20 ± 0.90</td>
<td>16.00 ± 2.60</td>
<td>21.50 ± 0.70</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>1</td>
<td>66.00 ± 2.80</td>
<td>64.50 ± 2.50</td>
<td>70.00 ± 3.80</td>
<td>62.50 ± 3.50</td>
<td>67.50 ± 2.30</td>
<td>66.50 ± 2.50</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>65.00 ± 1.20</td>
<td>61.50 ± 4.50</td>
<td>63.60 ± 2.40</td>
<td>63.00 ± 1.23</td>
<td>69.00 ± 3.10</td>
<td>62.50 ± 2.50</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>68.50 ± 1.40</td>
<td>60.50 ± 5.50</td>
<td>62.10 ± 2.90</td>
<td>60.00 ± 2.90</td>
<td>62.00 ± 2.40</td>
<td>65.50 ± 3.50</td>
</tr>
</tbody>
</table>

*Significantly different from the control value for each parameter in the same line (p < 0.05).
followed by thiamphenicol (p < 0.05). GSH and G6PD were not affected by florfenicol administration. There are lack of reference on chloramphenicol, thiamphenicol and florfenicol inhibition on G6PD and GSH enzyme activities in mice and other animal species. Only Erdogan et al. (2004) stated that thiamphenicol decreases erythrocyte G6PD enzyme level. However inhibitory effects of some antibiotics on enzymatic activities in other animal species and human beings have been reported in many investigations. For example, pamaquine caused severe anemia and death because of severe G6PD deficiency (Telefoncu and Telefoncu, 1989). Ampicillin, netilmisim and metamizol inhibit human red blood cells G6PD enzyme. In addition, it has been reported that sodium cefuroxime, sodium ceftriaxone, ampicillin and metamizol inhibit rat carbonic anhydrase and G6PD enzyme activities in vivo (Beydemir et al., 2000; Ciftci et al., 2000; Ciftci et al., 2002). Penicillin, sulbactam, ceftazolin and amikacine inhibit the G6PD enzyme activity in vitro (Ciftci et al., 2001).

In our study, it is observed that the results for G6PD and GSH enzyme activities have reflected on erythrocyte and leucocyte values (Table 2). Since, it is observed that haematological values of chloramphenicol and thiamphenicol applied groups decreased gradually after day 7 of application and this decrease was evident on day 14. On the other hand, there wasn’t any change in haematological values in florfenicol administrated groups. It is thought that chloramphenicol causes DNA damage in cells because of nitro group existing in the structure of chloramphenicol. Due to this fact it causes depression in the bone marrow and anemia. In previous studies, it has been stated that thiamphenicol causes anemia by damaging mitochondrial protein synthesis in red blood cells (Manyan and Yunis, 1970). We believe that anemia occurring in chloramphenicol and thiamphenicol applied groups may exist due to the decrease in G6PD and GSH enzyme activity levels in red blood cell except above reasons, because G6PD and GSH enzyme activities show protective effect against oxidative damage occurring in the metabolism of red blood cell. The results of the previous studies support our hypothesis. For example, Manyan and Yunis (1970) and Manyan et al. (1972) demonstrated that chloramphenicol inhibited mitochondrial ferrochelatase activity in dog marrow cells by blocking haem synthesis and a depressing of erythropoiesis. Ferrari and Pajola (1981) stated that inhibition of haematozois with reduced Hb and reticulocytopenia; occasionally WBC and platelet counts decreased. Turton et al. (1999), in an earlier study with chloramphenicol in the female CD-1 mouse, showed a decrease in RBC, PCV, Hb and reticulocyte count. In experimental animals, thiamphenicol exerts toxicity to haematopoietic organs and induces bone marrow suppression in rabbits (Takamizawa, 1984) and rats (Ando et al., 1997), similar to the human case (Keiser, 1974; Tomoeda and Yamamoto, 1981). However, there is no documentation of dose-dependent, reversible bone marrow suppression caused by florfenicol application to then animals.

As a result of this study, it was concluded that chloramphenicol and thiamphenicol had a decreasing effect on erythrocyte GSH and G6PD enzyme activities in mice. It was observed that a decrease in enzyme activities reflected on blood parameters and blood values were decreased. However, it was determined that florfenicol did not have any damage on these parameters. While using these drugs, it is necessary to decide on appropriate dosage and watchful for the adverse effects such as anemia.

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