PRELIMINARY COMMUNICATION

ERYTHROCYTE GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN LABORATORY RATS TREATED WITH AMOXICLAV, LIDAPRIM AND 1-CHLORO-2,4-DINITROBENZEN

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Abstract: The enzyme glucose-6-phosphate dehydrogenase (G6P–DH, EC.1.1.1.49) catalyzes the oxidation of glucose-6-phosphate in 6-phosphogluconat which is indispensable in the defence of erythrocytes from oxidative insult. The aim of this study was to examine the influence of commonly used drugs in our medical practice, amoxiclav (amoxicillin-clavulanate combination) and lidaprim (trimethoprim-sulfamethrole combination) respectively, upon the erythrocyte G6P-DH activity in experimental rats. In addition, the effect of the toxic drug 1-chloro-2,4-dinitrobenzen (CDNB) on the activity of G6P-DH was examined. The experiment was conducted in fresh blood haemolysates of white laboratory rats, Wistar type, of both genders (n = 80). The enzyme activity was determined by “Boehringer-Mannheim” diagnostic assay kits (Kornberg et al., 1955). However, the measured enzyme activity in the control group of rats was found to be a statistically insignificant difference between the genders (140.2 ± 21.2 mU/10⁹Er in male rats, 144.3 ± 20.6 mU/10⁹ in the female group). Hence, the established enzyme activity does not differ from the activity of the same enzyme in healthy human subjects. The administered dose of lidaprim did not affect the activity of G6P-DH in the treated group of rats, thus attaining levels similar to the control group. By contrast, amoxiclav administration provoked a significant reduction in enzyme activity of 13.6% in male and 19.5% in female rats (p < 0.001), while the treatment with CDNB significantly increased the activity of the latter to 49.7% in male and 30.1% in female rats (p < 0.001) in comparison with the control ones. Testing of haemolitical potential is strongly recommended prior to the use of new drugs, particularly in the
Mediterranean region, were this enzymopathy is found to be frequent bearing in mind that there is an established list of drugs which affect the G6P-DH activity in the erythrocytes. The above-mentioned method may be used in experimental animal models allowing for administration of a wider selection of drugs in this type of research.

**Key words:** erythrocyte G6P-DH, rats, amoxiclav, lidaprim, 1-chloro-2,4-dinitrobenzen.

### Introduction

Under normal physiologic conditions, 10% of glucose in the erythrocytes is metabolized via the pentose-phosphate pathway of carbohydrate metabolism.

The enzyme glucose-6-phosphate dehydrogenase (G6P-DH, EC.1.1.1.49) catalyses the oxidation of glucose-6-phosphate in 6-phosphogluconate, causing the production of reduced nicotinamide adenine dinucleotide phosphate coenzyme (NADPH) which is indispensable in the erythrocyte defence from oxidative insult [1, 2, 3, 4]. G6P-DH deficiency is the most common human enzymopathy particularly in the Mediterranean area [1]. The majority of drugs with a haemolytic potential (antimalarics, analgetics, antipyretics, sulfonamides, chloramphenicol, etc) are additional factors for clinical manifestation of this enzyme deficiency [5, 6].

G6P-DH deficiency was discovered as a result of a series of investigations performed on persons with developed haemolytic anaemia after the ingestion of primaquine as an antimalarian drug [7].

The aim of this study was to examine the influence of commonly-used drugs in our human medical practice, *amoxiclav* and *lidaprim* respectively, upon the erythrocyte G6P-DH activity in experimental rats. In addition, the effect of the toxic drug 1-chloro-2,4-dinitrobenzen (CDNB) on G6P-DH activity was examined because of its well-known influence leading to irreversible depletion of erythrocyte reduced glutathione (GSH), for the recurrence of which G6P-DH activity is necessary.

### Materials and methods

White laboratory rats, *Wistar* type of both genders (n = 80), weighing approximately 170–250 g, each at the start of the experiment, were used in this experimental research. The laboratory animals were obtained from the animal facility at the Institute of Biology of the Faculty of Natural Sciences and Mathematics, Skopje. All animals were fed with standard feed for laboratory animals and water *ad libitum*.
The study was performed with the following groups of rats: (I) a control group of rats (n = 48, A, B); (II) rats receiving 40 mg/kg/day suspension of amoxiclav (amoxicillin-clavulanate combination), made by Lek-Ljubljana (n = 20, A1, B1); (III) rats receiving 20 mg/kg/day suspension of lidaprim (trimethoprim-sulfamethrole combination), made by Alkaloid-Skopje (n = 20, A2, B2); (IV) rats receiving one dose of 50 mg/100g CDNB (n = 20, A4, B4) administered one hour before determination of the enzyme activity and (V) rats receiving 20 mg/kg/day suspension of lidaprim followed with administration of 50 mg/100g CDNB dose as the afore-mentioned, one hour before determination of the enzyme activity (n = 20, A3, B3).

The drug suspensions were administered orally during the 11 days, orally with a probe at the same time of day, while only a dose of CDNB was applied as a substance.

The enzyme activity measured in mU/10^9 Er, was determined in fresh blood haemolysates with the quantitative method of Kornberg et al., 1955 [8], using “Boehringer-Mannheim” diagnostic assay kits [8,9].

The principle of the above-method is based on the reaction catalysed by G6P-DH activity, where the rate of NADPH production is a measure of the enzyme activity. The change of absorbance at 340nm was read on a Cecil 2021 type spectrophotometer.

The following haematological parameters were determined prior to the function of the biochemical features of experimental animals: erythrocyte count (×10^12/l); leukocyte count (×10^9/l); total haemoglobin level (g/l); haematocrite (%); MCV (mean corpuscular volume – fl), MCH (mean corpuscular haemoglobin – pg), MCHC (mean corpuscular haemoglobin concentration – g/dl) and platelet count (×10^9/L).

The haematological data were performed with automated equipment – a veterinary cell counter for in vitro diagnostics (Celly-Hycel, France).

The results were submitted to statistical analysis using the Student’s t-test, p < 0.05 was considered significant. The results in Tables 1, 2 and Figure 1 are presented as a mean value ± SD.

**Results**

In the control group of rats a G6P-DH activity of 140.16 ± 21.18 mU/10^9 Er in male and 144.26 ± 20.55 mU/10^9 Er in female rats was measured. The difference of 2.8% between the genders was statistically insignificant (Figure 1).
Amoxiclav administration provoked a significant reduction (p < 0.001) in the enzyme activity for 13.6% of male (121.14 ± 8.26 mU/10⁹Er) and 19.4% of female rats (116.28 ± 17.53 mU/10⁹Er), in comparison to the control group-baseline (Fig. 1). The difference of 4.2% in enzyme activity between the genders did not differ significantly.

Despite the above-mentioned treatment, the administered dose of lida-prim did not affect the activity of G6P-DH in the treated group of rats; therefore this attained level was similar to the control group. However, the G6P-DH activity was reduced by 4.9% of male (133.25 ± 6.37 mU/10⁹Er) and 8.8% of female rats (131.61 ± 3.91 mU/10⁹Er), (Fig. 1). The difference of 1.1% in enzyme activity between the genders was also statistically insignificant.
The treatment with CDNB resulted in a significant enhancement (p < 0.001) in the enzyme activity by 49.7% in male (209.76 ± 13.07 mU/10^9Er) and 30.1% in female rats (187.67 ± 5.31 mU/10^9Er) as compared with the values achieved in enzyme activity in the control groups of rats. The difference of 11.8% in enzyme activity between genders was also statistically significant (p < 0.05, Fig. 1).

In contrast, the lidaprim treatment followed with CDNB administration did not significantly alter the enzyme activity in either genders of rats. In male rats the measured G6P-DH activity amounted to 138.69 ± 9.97 mU/10^9Er, while in female rats the enzyme activity amounted to 136.73 ± 13.17 mU/10^9Er, with an insignificant difference in the enzyme activity between both groups of animals (Fig. 1).

Summarizing the results of the measured haematological parameters, it can be assumed that they were in the function of the biochemical properties of the control and treated group of rats of both genders (Tables 1 and 2).

The haematological data obtained in the control rats corresponds to the same data presented in literature [10].

Table 1 – Таблица 1

Haematological data obtained with automated equipment (counter) in the male controls and treated rats

<table>
<thead>
<tr>
<th>Haematological parameters (mean value± SD)</th>
<th>Control group</th>
<th>Treatment with amoxiclav</th>
<th>Treatment with lidaprim</th>
<th>Treatment with CDNB</th>
<th>Treatment with lidaprim+CDNB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er (×10^{12}/l)</td>
<td>8,2±0,6</td>
<td>8,2±0,49</td>
<td>7,8±0,88</td>
<td>7,42±0,56</td>
<td>8,05±0,96</td>
</tr>
<tr>
<td>Hb (g/l)</td>
<td>150,5±13,42</td>
<td>152,4±8,43</td>
<td>141,8±11,96</td>
<td>134,80±13,54</td>
<td>148,40±17,11</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>44,9±3,32</td>
<td>44,2±2,22</td>
<td>43±4,06</td>
<td>40,94±3,56</td>
<td>44,52±5,98</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>54,6±2,04</td>
<td>53,9±2,26</td>
<td>55,1±1,99</td>
<td>56,05±0,98</td>
<td>55,18±1,93</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>18,31±0,95</td>
<td>18,5±0,70</td>
<td>18,2±0,97</td>
<td>18,50±0,62</td>
<td>18,44±0,46</td>
</tr>
<tr>
<td>MCHC (g/l)</td>
<td>335,3±1,60</td>
<td>344,4±1,28</td>
<td>330,3±0,87</td>
<td>331,2±0,58</td>
<td>334,4±0,88</td>
</tr>
<tr>
<td>Le (×10^9/l)</td>
<td>8,03±1,89</td>
<td>9,1±2,81</td>
<td>7,9±1,46</td>
<td>9,90±3,44</td>
<td>9,06±2,50</td>
</tr>
<tr>
<td>Plt (×10^9/l)</td>
<td>868,9±294,3</td>
<td>1000,1±71,7</td>
<td>874,0±150,9</td>
<td>898,2±141,21</td>
<td>881,20±102,07</td>
</tr>
</tbody>
</table>
Table 2 – Тabela 2

Haematological data obtained with automated equipment (counter) in the female control and treated rats

Хематологски параметри кај контролните и третираните тровини, од женски пол

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>Control group</th>
<th>Treatment with amoxiclav</th>
<th>Treatment with lidaprim</th>
<th>Treatment with CDNB</th>
<th>Treatment with lidaprim+CDNB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er (×10^{12}/l)</td>
<td>7.7±0.84</td>
<td>8.0±0.53</td>
<td>6.6±0.62</td>
<td>6.5±0.63</td>
<td>7.5±0.22</td>
</tr>
<tr>
<td>Hb (g/l)</td>
<td>146.5±15.70</td>
<td>150±6.35</td>
<td>130.8±10.06</td>
<td>133.20±11.29</td>
<td>126.00±6.02</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>42.5±4.16</td>
<td>44±2.10</td>
<td>38.4±2.55</td>
<td>38.32±3.16</td>
<td>39.82±1.46</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>55.6±2.40</td>
<td>55.3±2.40</td>
<td>57.3±2.44</td>
<td>59.66±3.12</td>
<td>56.94±1.89</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>19.16±0.78</td>
<td>18.2±0.75</td>
<td>19.9±1.19</td>
<td>20.16±0.79</td>
<td>17.64±0.69</td>
</tr>
<tr>
<td>MCHC (g/l)</td>
<td>344.7±0.64</td>
<td>341.3±0.47</td>
<td>339.2±1.03</td>
<td>340.4±1.43</td>
<td>304.6±1.84</td>
</tr>
<tr>
<td>Le (×10^{9}/l)</td>
<td>8±3.24</td>
<td>11.6±3.57</td>
<td>7.6±1.21</td>
<td>10.88±2.73</td>
<td>7.56±0.57</td>
</tr>
<tr>
<td>Plt (×10^{9}/l)</td>
<td>761.9±269.5</td>
<td>862.7±345.8</td>
<td>1030.8±57.5</td>
<td>825.60±100.84</td>
<td>914.40±33.06</td>
</tr>
</tbody>
</table>

The amoxiclav and lidaprim administration changed significantly few haematological parameters only in female rats, as compared with the same control values. However, the treatment with amoxiclav provoked a significant enhancement in leukocyte count by 44.4%. The lidaprim application caused a significant reduction in the erythrocyte count and total haemoglobin level by 14% and 10.7% respectively, while the platelet count was significantly increased by 35.5% (Table 2). The treatment with lidaprim followed with administration of a dose of CDNB caused a decline in the total haemoglobin level of 14% while the exposure to only a dose of CDNB, established a significant decrease in the erythrocyte count by 14.8% (p < 0.05), in female rats (Table 2).

Discussion

The results in the present study which refer to the measured enzyme activity of glucose-6-phosphate dehydrogenase in control rats, indicate that there is no difference in derived values between the genders, and correspond with the referent data achieved by the Kornberg et al. method [8,9]. The latter results are consistent with the results for enzyme activity measured in a healthy human population [11, 12]. Therefore, comparison between the G6P-DH activity in experimental animals and the human population is possible.

The objective of this study was to examine the influence of commonly-used drugs in our medical practice, amoxiclav and lidaprim, on the G6P-DH activity.
activity in the erythrocytes of experimental rats and their possible implication on other biochemical parameters.

Amoxiclav administration provoked a significant decrease of the enzyme activity in rats of both genders (p < 0.001). We assumed that this type of amoxiclav influence is probably due to the presence of amoxicillin (halvesynthetic penicillin) as its own active component. In support of the above results are the results expressed in laboratory rats (Sprague-Dawley) which suggest that the ampicillin as a halvesynthetic penicillin causes a significant decrease of erythrocyte G6P-DH activity, provoking uncompetitive inhibition [5].

The groups of animals exposed to lidaprim administration id not demonstrate a statistical change of enzyme activity in rats of either gender as compared with intact rats. Lidaprim belongs in the group of sulphonamides which are on the list of drugs with haemolitical potential and cause a clinical manifestation of G6P-DH deficiency.

The enzyme activity measured before the treatment on experimental animals does not indicate enzyme deficiency, hence lidaprim did not fulfil its haemolitical potential. In the available literature we did not find data about lidaprim's influence on erythrocyte G6P-DH activity in rats, but data about the haemolitical potential of the analogues on its compound components in G6P-DH deficient people are described and discussed. Actually, sulphamethoxazole and sulphanilamide and sulphamethoxazole induce clinically significant haemolysis by a decrease of the intracellular reduced glutathione (GSH) concentration in erythrocyte G6P-DH deficient people [6, 13, 14, 15].

The treatment with CDNB significantly increased the erythrocyte G6P-DH activity in of both rat genders, because erythrocyte glutathione (GSH) can be rapidly depleted by incubating the cells with CDNB, which forms an undergraded GSH-CDNB conjugate with GSH through the reaction catalysed by erythrocyte glutathione-S-transferase (E.C.2.5.1.18). Namely, with incubation with CDNB at 37°C, more than 80% of erythrocyte GSH is conjugated within 5 minutes, while it is completely depleted in an hour. The depletion of erythrocyte GSH by 1-chloro-2, 4-dinitrobenzen results in a rapid oxidation of haemoglobin to methaemoglobin, so GSH is important in maintaining the reduced environment within the erythrocytes, for which synthesis is a necessary NADPH-product of the reaction catalyzed by G6P-DH [16, 17].

The ability of CDNB to erythrocyte GSH depletion on the hereditary G6P-DH deficiency level is useful for experimental aims such as proving a significantly increased risk of haemoglobin glycolysation, which leads to high damage of GSH and G6P-DH deficient-erythrocytes [18]. CDNB administration is considered to provoke oxidative stress in erythrocytes, which is a crucial factor for cell disintegration, haemolysis with concomitant development of haemopoethic stress and reticulocytosis as a response to the oxidative injury.
The reticulocytes have a 10 times higher glycolytical and G6P-DH enzyme activity than the mature erythrocytes, because of their intensive metabolism [19, 20]. In addition to the opinion about the causing of erythrocyte oxidative damage by toxic substances, there is a study about rats treated with 2000-ppm lead acetate in drinking water for a period of 5 weeks. The demonstrated signs of anemia and lipid peroxidation, as evidenced by increased malondialdehyde content, as well as decreases in reduced glutathione (GSH) and increases in catalase and G6P-DH activity were noted in erythrocytes from lead-treated rats, suggesting lead-induced oxidative stress [21].

In contrast to exposure to only a dose of CDNB, the treatment with lidaprim followed with CDNB administration of a dose, did not demonstrate any significant difference in erythrocyte G6P-DH activity, probably as a result of the suppressible impact of lidaprim on the toxic substance. The latter could not be confirmed in the available literature.

Conclusions

1. The registered values for erythrocyte G6P-DH enzyme activity in control rats correspond with similar studies and with the referent values as by Kornberg et al. method [8].

2. The treatment with amoxiclav marked a significant reduction in enzyme activity in genders both of rat as compared with the control values.

3. The administered dose of lidaprim did not affect erythrocyte G6P-DH activity in the treated group of rats, thus attaining levels similar to the control group.

4. The exposure to CDNB significantly increased the enzyme activity in rats of both genders in comparison to the control group.

5. Lidaprim administration reveals a non-significant reliance in G6P-DH activity in the group of rats treated with lidaprim followed by one dose of CDNB.

6. The method described above may be successfully applied in laboratory research for testing the haemolitical potential of new drugs, particularly in areas where G6P-DH enzymopathy is a common disease.

REFERENCES


Резиме

ЕРИТРОЦИТНА ГЛУКОЗА-6-ФОСФАТ ДЕХИДРОГЕНАЗА КАЈ СТАОРЦИ ТРЕТИРАНИ СО АМОКСИКЛАВ, ЛИДАПРИМ И 1-ХЛОРО-2,4-ДИНИТРОБЕНЗЕН

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Глукозо-6-фосфат дехидрогеназата – G6P-DH (EC.1.1.1.49) ја катализира оксидацијата на глукозо-6-фосфат во 6-фосфоглукозенат, при што се продуцира редуктирани никотинамид аденин динуклеотид фосфат (NADPH), неопходен за одбрана од еритроцитите од оксидативно огледување.

Целта на овој труд е да се испита влијанието на амоксициллин (комбинација на амоксициллин и клавулонска киселина) и лидаприм (комбинација на триметоприн и сулфаметрол), врз активноста на G6P-DH во еритроцитите на стаорци. Истражувањата се направени во свеж хемолизат на еритроцити, ќе би лабораториjsки стаорци (n = 80), соj Wistar, од двата пола. За експериментални цели се испитувале влијането на токсичниот 1-хлоро-2,4-дINITРОБЕНЗЕН (CDNB), врз активноста на ензимот.


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(140,16±21,18 mU/10⁹Er) и женската контролна група (144,26±20,55 mU/10⁹Er), а вредностите се во согласност со оние добиени кај хуманата популација. Исто така лидаприм кај стаорците од двата пола не предизвика статистички значајна промена во активноста на ензимот, во однос на контролата. Третманот со амоксиклав ја намали активноста на ензимот за 13,6% кај мажките и 19,4% кај женските стаорци (p < 0,001), додека третманот со CDBN ја зголеми активноста на истиот за 49,7% кај мажките стаорци и за 30,1% кај женските (p < 0,001), во однос на контролните стаорци.

Се препорачува тестирање на хемолитичкиот потенцијал на новите лекови пред нивната употреба, особено во подрачјето на Медитеранот каде што оваа ензимопатија е честа, имајќи го предвид податокот дека постои листа на лекови кои имаат влијание врз активноста на G6P-DH во еритроцитите. Испитувањата на белите лабораториски стаорци како експериментален модел отвора широки можности за настамошнин истиражувања на ова поле.

Ключни зборови: еритроцитна G6P-DH, стаорци, амоксиклав, лидаприм, 1-хлоро-2,4-дINITробензен.