THE CONTENT OF REDUCED GLUTATHIONE IN KIDNEY, LIVER AND SPLEEN OF MICE AFTER STREPTOZOTOCIN INJECTION

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ABSTRACT
Streptozotocin is an antibiotic with a cytostatic effect, which is used to induce experimental diabetes in experimental animals. Intraperitoneal administration of streptozotocin causes damage to liver, kidney pancreatic beta cells and inhibits the secretion of insulin. Toxic effect of streptozotocin is also associated with the generation of reactive oxygen species (ROS). Reduced glutathione is one of the most important antioxidants. The aim of work was to estimate the concentration of reduced glutathione in kidney, liver and spleen of mice after streptozotocin injection. The research was conducted on Swiss mice 12 weeks old, weight 26 g. Animals were fed with standard diet and grown in 12/12 light/dark photoperiod. The animals were segregated into four experimental and four control group. Each group consisted of 5 animals. Total number of animals was n=40 of experimental groups were injected intraperitoneally with streptozotocin in dose of 65 mg/kg. Mice of control group were injected with physiological saline. The measurements were performed 48, 72 hours and 8, 16 days after streptozotocin injection. Statistical analysis was performed using Student’s “t” test. It was observed that the level of reduced glutathione was decreased in all experimental groups compared to the control groups in all examined organs and intervals. The largest decrease was recorded in the liver. Our results indicate negative effect of streptozotocin on the level of reduced glutathione which may lead to imbalance in oxidant/antioxidant reactions.

Keywords: Streptozotocin, reduced glutathione, kidney, liver, spleen, diabetes, intraperitoneal

RESULTS AND DISCUSSION
Liver
Analysis of the results indicates that single intraperitoneal administration of streptozotocin had significant influence on the concentration of reduced glutathione in the liver. Statistically significant depletion of GSH concentration were observed in each experimental group in comparison to control at p<0.05. The largest decrease in GSH concentration was observed at 72 h after injection of streptozotocin. Results are shown in the figure 1 and table 1.
Intraperitoneal injections of streptozotocin resulted in decrease of reduced glutathione concentration in kidney of mice. Significant differences in GSH level were observed in animals of first (p=0), third (p=0) fourth experimental group (p=0) in comparison to control. Results are shown in the figure 3 and table 3.

Table 3 Average content and standard deviation ranges of reduced glutathione (μM/g) in kidney of mice

<table>
<thead>
<tr>
<th>Time</th>
<th>Control Average</th>
<th>Control SD</th>
<th>Streptozocin Average</th>
<th>Streptozocin SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>48h</td>
<td>1.41</td>
<td>0.19</td>
<td>0.91</td>
<td>0.22</td>
</tr>
<tr>
<td>72</td>
<td>1.41</td>
<td>0.31</td>
<td>1.07</td>
<td>0.16</td>
</tr>
<tr>
<td>8days</td>
<td>1.41</td>
<td>0.11</td>
<td>0.86</td>
<td>0.28</td>
</tr>
<tr>
<td>16days</td>
<td>1.41</td>
<td>0.06</td>
<td>0.78</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Our results indicate downward trend of reduced glutathione content in the kidney, liver and spleen after intraperitoneal administration of streptozotocin. Almost all groups showed significant differences in comparison to control. Only in kidney and spleen of animals examined 72 h after streptozotocin administration differences were not detected. Streptozotocin is an antibiotic easily penetrating the blood-brain barrier. Its negative effects on the body include metabolic disorders, deterioration of pancreatic beta cells and the inhibition of insulin secretion. The cytotoxic effect of the antibiotic is associated with the generation of reactive oxygen species (ROS). As a result of studies carried out by Greń et al. (2011; 2012a) it was found that streptozotocin increases the oxidative stress causing perturbation of the redox homeostasis of body (Greń et al., 2011; Greń et al., 2012a). Animal model of streptozotocin-induced diabetes is obtainable because streptozotocin affects pancreatic beta cells. In the process of self-destruction of cells macrophages, cytotoxic T lymphocytes, dendritic cells, T helper cells, as well as mediators: ROS, cytokines are involved (Knip et al., 2005). Part of scientific research conducted on animal models of streptozotocin-induced diabetes showed that the excessive production of ROS may contribute to the death of pancreatic beta cells. There are studies showing no clear effects of free radicals on the destruction of pancreatic cells (Green et al., 2004). The study showed a decrease in the concentration of reduced glutathione in the examined organs. Reduction of the level of GSH may lead to oxidative damage caused by free radicals. The excess of oxidants is destructive for proteins and cell components, DNA, lipids (Gil-del Valle et al., 2005). Greń (2012) also reported that the GSH content was decreased in diabetic mice. The decrease in GSH levels in the liver when compared to diabetic group mice could be probably due to the increased utilization of GSH by the cells to act as scavengers of free radicals caused by toxic chemical agents, or enhanced utilization of GSH by GPx (Greń, 2012b; Greń & Formicki, 2012c).

The increase in blood glucose concentration, decreased enzymatic and non-enzymatic protective mechanisms may cause oxidative stress associated with excessive production of ROS in physiological diabetes (Valko et al., 2005). The biological role of glutathione is to counteract the harmful effects of oxidative stress. This is achieved by thiol groups (-SH). The ratio of the reduced glutathione form (GSH) to its oxidized form (GSSG) is a measure of the balance between prooxidants and antioxidants (Hwang et al., 1992).

Lowering the level of reduced glutathione in the examined organs indicates a very important role in maintaining homeostasis of this tripeptide. Significant decrease of GSH in all experimental groups was observed in the liver. This result may be related to liver detoxification functions as well as the fact that this organ has the highest concentration of GSH. The unique role of the liver involves the conversion of methionine to cysteine by transsulfuration pathway, as well as the fact that the amount of GSH synthesis in hepatocytes is balanced by excreting it.
to the blood and bile (Lu, 1999). Studies carried out by de Cavanagh et al. (2001) confirmed a depletion in GSH concentration in the liver post injection streptozotocin. Injection of streptozotocin at a dose of 1mg/kg body weight causes the defensive reactions in spleen. The decrease of the GSH content in this organ indicated by our research may be explained by the protective role of GSH after intoxication. Similarly the GSH depletion in kidney suggests its role in detoxification after administration of streptozotocine. On the other hand low level of glutathione in kidney may affect some of its physiological functions such as transportation across the membranes during so called - γ-glutamyl cycle. Bartus (2007) confirmed the cytotoxic effects of streptozotocin. In his research a week after injection of streptozotocine significant increase in the concentration of glucose and production of free radicals occurred. In other studies Hosokawa et al. (2001) confirmed hyperglycemia after administration of streptozotocin to animals in a dose of 55 mg / kg body weight. This result might indicate the destruction of the beta cells of the pancreas.

CONCLUSION

The results of our studies suggest that the observed changes in the level of GSH in the liver, spleen, and kidney may result from the occurrence of free radicals after administration of streptozotocin. This leads to the suggestion that generation of free radicals together with GSH depletion are significant mechanisms of streptozotocin toxicity.

REFERENCES


