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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 itraperitoneally 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

INTRODUCTION

Streptozotocin is an antibiotic and cytostatic exhibiting toxic effects on tumor cells. It is used in the treatment of malignant lymphoma, lymphoma, myeloma and cancer of the pancreas. Streptozotocin selectively destroys pancreatic beta cells and it is therefore used to induce experimental diabetes in laboratory animals. The antibiotic is achieved from *Streptomyces griseu*. After intravenous administration it is quickly concentrated in organs such as kidney or liver where causes damage (**Orlowski, 1988**). The cytotoxic effects of streptozotocin is associated with the production of ROS.
Glutathione is an important factor of the first line of defence against different toxins. It enters the oxidation and reduction reactions with electrophilic reagents such as xenobiotics, toxins, free radicals, organic hyperoxides (**Bouaicha *et al.*, 2004**). Glutathione is composed of 3 aminoacids i.e. cysteine, glycine and glutamate. The tripeptide glutathione is one of the most important antioxidants which protects cells against reactive oxygen species (ROS). The ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) is 10:1 in normal cytosol. Total glutathione concentration in cells depends on the type of cells and reaches from 5 to 10 mmol/l. High glutathione concentration usually occurs in cytoplasm, nucleus and mitochondria. Endoplasmic reticulum contains lower concentration of glutathione - 2 mmol/l (**Meister, 1984**). GSH is considered as the most important thiol buffer and GSH/GSSG ration indicates prooxidant/antioxidant balance in cells.

MATERIAL AND METHODS

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: eksperimental group I – decapitation after 48h after streptozotocin injection; eksperimental group II with decapitation after 72h of streptozotocin injection; eksperimental group III – decapitation after 8 days of streptozotocin injection; eksperimental group IV – decapitation after 16 days. Each group consisted of 5 animals. Total number of animals was n=40. Animals 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, 8 and 16 days after streptozotocin injection. Animals were narcotized with CO_2 and decapitated. The dissected organs were homogenized in 6 ml of cool (4°C) phosphate buffer. The homogenates were centrifuged in a centrifuge MPW-365 at 4°C for 15 minutes and 15 000 revolutions/minute. Then the resulting homogenates were deproteinized by mixing 500 µl of supernatant, 500 µl of 10% TCA and 500 µl EDTA. Samples were placed in a refrigerator for 10 minutes at 4°C. After 10 minutes the samples were centrifuged in a centrifuge MPW-365 for 5 min, 15000 rpm/min. The concentrations of GSH were analysed using Ellman's method (1970). Protein concentration was measured using Bradford's method (1976) with microplate reader Sunrise TECAN Austria.Statistical analysis was performed using Student's "t" test. The significance level was established at $p<0.05$.

RESULTS AND DISCUSSION

Liver

Analysis of the results indicates that single intreperitoneal administration of strepotozotocin 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. 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.

Table 1 Average content and standard deviation ranges of reduced glutathione in liver (µM/g)

	Control		Streptozotocin	
Time	**Average**	**SD**	**Average**	**SD**
48h	1.54	0.18	0.96	0.13
72	1.5	0.09	0.55	0.09
8days	1.53	0.17	0.68	0.13
16days	1.56	0.09	0.64	0.16

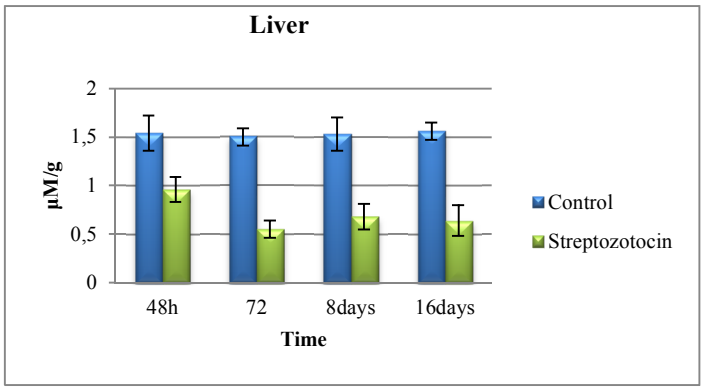

Figure 1 The content of reduced glutathione (µM/g) in liver of mice after streptozotocin injection

Spleen

Application of streptozotocin resulted in decrease of reduced glutathione content in spleen of the experimental animals compared to control groups. The decreases were observed in all experimental groups, although in some groups the noted changes were statistically insignificant. Statistically significant depletions of GSH concentration were observed in first experimental group (p=0), second experimental group (p=0) and fourth experimental group in relation to the control (p=0). Results are shown in the figure 2 and table 2.

Table 2 Average content and standard deviation ranges of reduced glutathione (µM/g) in spleen

	Control		Streptozotocin	
Time	**Average**	**SD**	**Average**	**SD**
48h	1.41	0.19	0.92	0.09
72	1.41	0.31	1.07	0.07
8days	1.41	0.06	0.86	0.09
16days	1.41	0.11	0.78	0.19

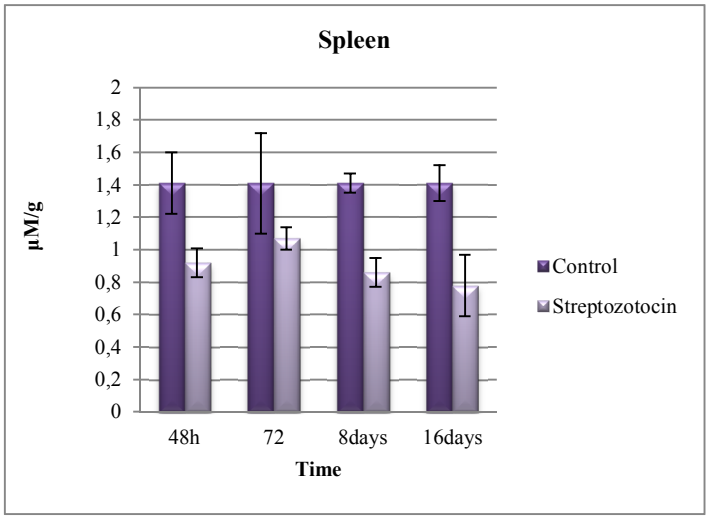

Figure 2 The content of reduced glutathione (µM/g) in spleen of mice after streptozotocin injection

Kidney

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

	Control		Streptozotocin	
Time	**Average**	**SD**	**Average**	**SD**
48h	1.41	0.19	0.91	0.22
72	1.41	031	1.07	0.16
8days	1.41	0.06	0.86	0.26
16days	1.41	0.11	0.78	0.28

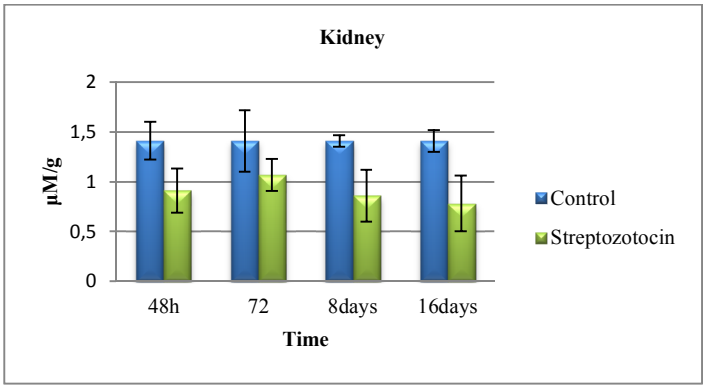

Figure 3 The content of reduced glutathione (µM/g) in kidney of mice after streptozotocin injection

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 either 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 streptozotocin 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

BARTUŚ, M., 2007. Badania nad mechanizmem działania 1-metylonikotynamidu (MNA+) w doświadczalnych modelach dysfunkcji śródbłonka. Collegium Medicum UJ Wydział Lekarski, Kraków.

BOUAICHA, N., MAATOUK, I. 2004. Microcystin - LR and nodularin induce intracellular glutathione alteration, reactive oxygen species production and lipid peroxidation in primary cultured rat hepatocytes. *Toxicology Letters*, 48, 53-63.

BRADFORD, M.M. 1976. A rapid and sensitive method for the quantitaties of microgram quantities of protein, utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 72, 248-54.

DE CAVANAGH, EM, INSERRA, F., TOBLLI, J., STELLA, I., FRAGA, C.G., FERDER, L. 2001. Enalapril attenuates oxidative stress in diabetic rats. *Hypertension*. 38, 1130-1136.

ELLMAN, G.L. 1970. SH groups determination in biological fluids. *Analytical Biochemistry*. 46, 237.

GIL-DEL VALLE, L., MILIAN, L.C., TOLEDO, A., et al. 2005. Altered redox status in patients with diabetes mellitus type 1. *Pharmacological Research*. 51, 375-380.

GREEN, K., BRAND, M.D., MURPHY, M.P. 2004. Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes. *Diabetes*. 53, 110-118.

GREŃ, A., FORMICKI, G., GOC, Z., MUCHACKA R., KŇAŽICKÁ Z., LUKÁČ N., MASSÁNYI P. 2012a. Effects of *Trigonella foenum graecum* on the leukocytes in diabetes mice. *Journal of Microbiology, Biotechnology and Food Sciences*, 2(2), 467-476.

GREŃ, A. 2012b. Antioxidant activities of white mulberry, phaseolus vulgaris and ginkgo biloba preparations in streptozotocin diabetic mice. *Postępy Fitoterapii*. 4, 220-225.

GREŃ, A., FORMICKI, G. 2012c. Antioxidants in therapy and prevention of diabetes mellitus. in: *Xenobiotics : soil, food and human health interactions* (Pasternakiewicz A., Stawarz R., Witek B. eds.), Rzeszów : Wydawnictwo Uniwersytetu Rzeszowskiego, 276-291.

GREŃ, A., FORMICKI, G., STAWARZ, R., GUZIK, M., SZAROMA, W. 2011. The effect of plant preparations on selected parameters of oxidative stress in animal models of diabetes mellitus. *Journal of Physiology and Pharmacology*. 62, 47.

HOSOKAWA, M., DOLCI, W., THORENS, B. 2001. Differential sensitivity of GLUT1- and GLUT2 – expressing beta cells to streptozotocin. *Biochemical and Biophysical Research Communication*. 289, 1114-1117.

HWANG, C., SINSKEY, A.J., LODISH, H.F. 1992. Oxidized redox state of glutathione in the endoplasmatic reticulum. *Science*. 257, 1496-1502.

KNIP, M., VEIJOLA, R., VIRTANEM, S.M., HYOTY, H., VAARALA, O., AKERBLOM, H.K. 2005. Environmental triggers and determinants of type 1 diabetes. *Diabetes*. 54 (2), 125-136.

LU, S.C. 1999. Regulation of hepatic glutathione synthesis: current concepts and controversies. *FASEB Journal*. 13, 1169-1183.

MEISTER, A., 1984. Selective modifications of glutathione metabolism. *Science*, 220, 472-477.

ORŁOWSKI, W. 1988. Nauka o chorobach wewnętrznych. Wydawnictwo PZWL, Warszawa.

VALKO, M., MORRIS, H., CRONIN, M.T.D. 2005. Metals, toxity and oxidative stress. *Current Medicinal Chemistry*. 12, 1161-1208.