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temat pracy: *„Parametry stresu oksydacyjnego w tkance gruczołów ślinowych w przebiegu eksperymentalnej cukrzycy typu 1”*

Summary

In a living organism in physiological conditions there is a balance between production and neutralisation of ROS. For that reason neither intensified oxidative modification of cellular components, nor enhanced anti-oxidative response is observed. In diabetes the redox balance becomes shifted towards the reactions of oxidation. A significant increase in levels of numerous products of oxidative modification of cellular components is observed in many organs and in serum, both in patients and in experimental in vitro systems. However, not much is known about diabetes-associated oxidative stress and oxidative injury in salivary glands. Changes in activity of the anti-oxidative barrier in the form of a reduced level of endogenous, non-enzymatic antioxidants, or enhanced/reduced activity of antioxidative enzymes were used for the assessment of presence or intensification of oxidative stress in the salivary glands of rats with streptozotocin-induced diabetes. Those are erroneous practices, as increased levels of oxidatively modified bio-molecules are the most reliable marker of oxidative stress. There are many markers indicating the intensity of oxidative stress and stress-induced oxidative injury. In the case of lipid peroxidation, some commonly used methods include determination of the level of 8-isoprostanes (8-isoP), adducts of oxidized low density lipoproteins/malondialdehyde (oxyLDL/MDA), adducts of 4-hydroxynonenal with proteins, as well as determination of carbonyl groups (PC) in the case of proteins, and 8-hydroxy-D-guanosine (8-OHdG) in the case of DNA. The oxidative stress index (OSI) is a recommended parameter for demonstration of intensity of oxidative stress and assessment of the redox imbalance level.

Study objective:

1. The assessment of the occurrence and intensity of oxidative injury of salivary glands in rats, measured with levels of carbonyl groups, 8-isoprostanes, adducts of 4HNE with protein, oxyLDL/MDA, 8-OHdG and OSI during two stages of streptozotocin-induced diabetes.

2. A comparison of oxidative injury and redox imbalance measured with levels of carbonyl groups, 8-isoprostanes, adducts of 4HNE with proteins, oxyLDL/MDA, 8-OHdG and OSI between the parotic gland and the submandibular gland during two stages of experimental diabetes.

3. The assessment of the secretory function of salivary glands in rats during two stages of streptozotocin-induced diabetes.

The study was also aimed at obtaining answers to the following questions:

4. Is there an association between the tested parameters of oxidative stress measured in blood serum and measured in salivary glands of rats during two stages of streptozotocin-induced diabetes?

5. Is there an association between the tested parameters of oxidative stress in rats' salivary glands and the secretory function of those glands during two stages of experimental diabetes?

6. Is there an association between hyperglycaemia and oxidative injury of salivary glands during two stages of experimental diabetes?

Experiments were carried out on male Wistar rats (approximately 6 weeks old) with a baseline body weight of 180-210 g. For the whole time of the experiment the animals were kept in standardised conditions. The period of adaptation for new environmental conditions was 7 days. Animals were randomly divided into two main groups: control (16 animals) and study (16 animals), with two sub-groups (of 8 animals) in each of them: depending on the duration of diabetes (7 and 14 days). Diabetes was induced by a single, intraperitoneal injection of streptozotocin in citrate buffer, pH 4.5, at the dose of 50 mg/kg body weight. The control group received a single intraperitoneal injection of citrate buffer, pH 4.5, at a volume of 0.5 mL. The glucose level in venous blood was determined in all rats in blood collected from the tail vein 48 hours after the injection. Glucose levels of >250 mg/mL were found in the study group, indicating diabetes. Rats in both groups were sacrificed on day 7 or 14 post induction of diabetes in the study group. The following were collected for tests: stimulated and non-stimulated saliva, blood from the abdominal aorta, both submandibular and parotid salivary glands. Insulin, glucose, free fatty acid levels and pH were determined in blood serum from the rats with induced diabetes and from the control rats. Salivary glands were homogenised, sonicated and centrifuged. Supernatant was used for further tests.

The following were determined in supernatants and blood serum samples: adducts of 4-HNE with proteins, 8-isoP, oxy-LDL/MDA, 8-OHdG - using the ELISA method; and total protein and PC levels, TAS, TOS - using colorimetric methods, and OSI was calculated.

The statistical analysis was completed using Statistica 10.0 software (Stat Soft). The Kruskal-Wallis ANOVA test was used for comparison of dependent variables. The correlation coefficient was estimated using the Spearman test. The non-parametric Mann-Whitney U test was used for the assessment of groups for comparison of quantitative variables demonstrating a non-normal distribution. The value of $p<0.05$ was considered statistically significant.

Results

Median non-stimulated saliva secretion was significantly reduced in the group of rats with STZ-induced diabetes compared to the control group in week 2 of the experiment (p=0.034). The median stimulated saliva secretion was significantly reduced in the group of rats with STZ-induced diabetes compared to the control group in week 1 and 2 of the experiment (p=0.03 and p=0.011, respectively).

For the whole time of the experiment (both week 1 and 2) a significant increase of PC level (p=0.046 and p=0.049, respectively), of adducts of 4-HNE with proteins (p=0.00001 and p=0.00001, respectively), of oxyLDL/MDA (p=0.009 and p=0.0006, respectively), of 8-isoP (p=0.01 and p=0.007, respectively) was observed, as well as significantly higher values of TOS (p=0.0001 and p=0.0002, respectively) and OSI (p=0.003 and p=0.001, respectively) in blood serum from rats with STZ-induced diabetes compared to controls. In the second week of the experiment, a significant reduction of the TAS value (p=0.003) and a significant increase of the 8-OHdG level (p=0.009) was found in blood serum from rats with STZ-induced diabetes compared to the control group.

Both in week 1 and week 2 of the experiment the median TAS value (p=0.002 and p=0.0001, respectively) was significantly lower, and median values of TOS (p=0.006 and p=0.0001, respectively) and OSI (p=0.0001 and p=0.00001, respectively) and levels of adducts of 4-HNE with proteins (p=0.001 and p=0.0001, respectively) and of PC (p=0.03 and p=0.01, respectively) were significantly higher in homogenates of parotid salivary glands from rats with STZ-induced diabetes compared to the control group. In experimental week 2, the median concentrations of oxyLDL/MDA (p=0.03), 8-isoP (p=0.004), as well as 8-OHdG (p=0.001) were significantly higher in homogenates of parotid glands from rats with STZ-induced diabetes compared to the control group.

The median TAS value was significantly higher (p=0.04) in week 1 and significantly lower (p=0.04) in week 2 in homogenates of submandibular salivary glands from rats with STZ-induced diabetes compared to controls. Both in week 1 and week 2 of the experiment, the median values of TOS (p=0.03 and p=0.02, respectively) and OSI (p=0.03 and p=0.002, respectively) were significantly higher in homogenates of submandibular salivary glands from rats with STZ-induced diabetes compared to the control group. In experimental week 2, the median concentrations of oxyLDL/MDA (p=0.02), and adducts of 4-HNE with proteins (p=0.002) were significantly higher in homogenates of submandibular glands from rats with STZ-induced diabetes compared to the control group.

In experimental week 1 the median TAS value (p=0.039), and in experimental week 2 the median 8-OHdG level (p=0.001), and in both experimental week 1 and 2 the median TOS (p=0.0002 and p=0.002, respectively), OSI (p=0.0001 and p=0.0001, respectively) values and median PC

(p=0.02 and p=0.04, respectively), adducts of 4-HNE with proteins (p=0.032 and p=0.001, respectively), oxyLDL/MDA (p=0.03 and p=0.03, respectively) and 8-isoP (p=0.04 and p=0.039, respectively) concentrations in homogenates of parotid salivary glands from rats with STZ-induced diabetes **were significantly higher** compared to corresponding values obtained for homogenates of submandibular salivary glands from that group of rats.

Both in experimental week 1 and 2 the median TAS (p=0.001 and p=0.001, respectively) and TOS (p=0.0001 and p=0.001, respectively) values in homogenates of parotid salivary glands from control rats were significantly higher compared to values for homogenates of submandibular salivary glands from that group of rats.

The following conclusions were drawn based on the obtained results:

1. In both salivary glands the phenomenon of oxidative stress becomes intensified with the duration of experimental diabetes.
2. Some more pronounced redox imbalance and a higher variability of oxidative modifications are observed in the parotid gland compared to the submandibular gland, regardless of the duration of the disease.
3. The reduction of stimulated saliva secretion is intensified with the duration of experimental diabetes. Non-stimulated saliva secretion is reduced in the advanced stage of streptozotocin-induced diabetes. Regardless the stage of streptozotocin-induced diabetes, the function of both salivary glands is disturbed. The fact is manifested by a significant reduction of the total protein content.
4. Absence of correlation between oxidative stress markers in serum and salivary glands suggests that intensification of oxidative modifications of their cellular components is a result of pathological processes taking place in salivary glands, independent of systemic oxidative stress.
5. The observed negative correlation between concentration of adducts of 4-HNE with proteins in the parotid gland and the stimulated secretion may be a cause of reduced secretion of that type of saliva in the course of STZ-induced diabetes.
6. Hyperglycaemia seems to be associated with oxidative injury of both types of salivary glands in the advanced stage of the disease, with no effect on the redox balance during the early stage.