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Changes in glucose, cholesterol and serum lipid fraction levels in experimental diabetes

Diabetes has become one of the most important health problems in the world. This disease is associated with mostly absolute insulin deficiency (type I) or mostly relative insulin deficiency (type II), which causes disorders in the metabolism of carbohydrates, proteins, and fats, as well as morphological changes in many organs (13). This disease affects two to four percent of the global population. During the recent 20 years, a twofold increase has been observed in the incidence of insulin-dependent diabetes among people over the age of 65 (3). Many researchers often investigate changes in the pancreas concomitantly with those in the parotid gland; however, the abnormalities in the parotid gland in the course of diabetes are considerably less researched. Changes in ultrastructure of the parotid gland appeared by the 21st day of the diabetes and their intensification was observed during the course of the disease.

OBJECTIVE

The objective of this research was to trace the changes in serum glucose, cholesterol and lipid fractions during progression of diabetes in rabbits.

MATERIAL AND METHODS

89 male rabbits, New Zealand breed (Experimental Animals Laboratory, Chorzelow n/Warsaw, Poland), weighing 2.750-3.300 kg were used in experiment. Animals were housed one per cage under 12h/12h light/dark cycle at 21 ± 2°C temperature and 50% relative humidity with standard granulated food (Motycz, Poland) and water available. Body weight was measured before induction of diabetes and before decapitation. Bioethical Committee of the Medical University of Lublin approved the experimental protocol. Diabetes mellitus was induced by a single injection of alloxan (Sigma Chemical Company, St. Louis, MO, USA) at a dose of 10 mg/kg into the auricular vein (9). On day 7 the glucose level in the whole blood was measured by a glucometer (Boehringer, Germany) to confirm the presence of diabetes. From this day the time of disease was counted. The rabbits were divided into the following

groups: Group 1 – controls (n=18), Group 2 – 21 days diabetes mellitus (n=18), Group 3 – 42 days diabetes mellitus (n=17), group 4 – 90 days diabetes mellitus (n=19), group 5 – 180 days diabetes mellitus (n=17). After above-mentioned periods blood samples were taken and the rabbits were killed by decapitation.

The final level of glucose in the sera was determined spectrophotometrically by the enzymatic method using ready kit GS-120L (Cormay, Lublin, Poland) at wavelength 500 nm (16). The method of cholesterol measurement was based on oxidation of free cholesterol to cholestenon releasing hydrogen peroxide, which in the presence of 4-aminophenason and phenol is converted quantitatively to chromogen. Boehringer Mannheim (Austria, Wien) set was used in that measurement (15).

Measurement of lipid fractions was based on indirect methods consisting in precipitation of specific lipoprotein fractions and measurement, in enzymatic manner, of cholesterol included in supernatant of a given fraction (10). HDL cholesterol was determined in supernatant after LDL and VLDL had been precipitated using magnesium ions and phosphotungstic acid (12). LDL cholesterol concentration was determined after its selective precipitation using polyvinyl sulphate. LDL cholesterol was a difference between the total cholesterol concentration and concentration in supernatant left after sediment removing by centrifugation (2).

Extinction was measured on spectrophotometer SPM 90A at the wavelength 546 nm. Concentration was calculated by multiplying results by coefficient of extinction. Results are presented in mmol/l.

The statistical analysis was done using the SAS system v. 6.11 (SAS Institute Inc., SAS Campus Drive, Carry, NC 27513, USA). Results are expressed as mean ± SD. Differences between the groups were analysed by analysis of variance (ANOVA). If $P <= 0.05$, differences between the mean values were considered statistically significant and highly significant – if $P < 0.01$. The obtained mean values were shown in the figures.

RESULTS

Blood glucose levels and animals body weights were examined. Initial mean blood glucose level in rabbits was 6.36 mmol/l. It increased up to 21.79 mmol/l after 21 days and was the highest on 42nd day (mean 32.02 mmol/l), Figure 1. Decrease in blood glucose level was observed after that period, especially in the 5th experimental group, reaching value of 23.15mmol/l.

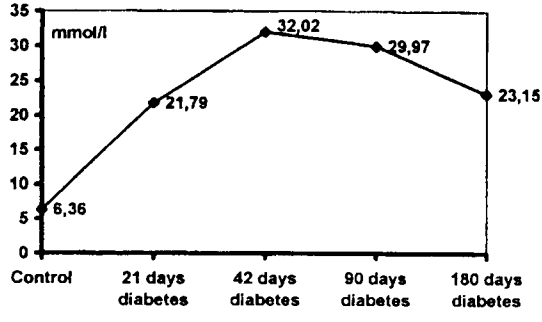

Fig. 1. Serum glucose level in rabbits in the course of experimental diabetes

Changes in animals body weight were also observed during the experiment. The highest decrease in body weight occurred in 180-days diabetes group, slightly lower in 21 days' diabetes group and the lowest in 42-days diabetes group. There were no significant changes in body weight in 90 days' diabetes group.

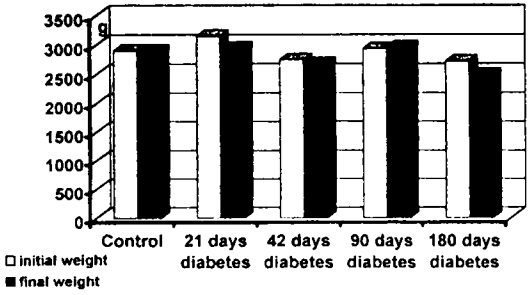

Fig. 2. Rabbits body weight changes in the course of experimental diabetes

Initial total cholesterol ranged from 0.82 to 1.72 mmol/l (mean 1.35 mmol/l). Increase of total cholesterol was observed in the course of diabetes, it was differentiated depending on diabetes duration. Animals with 180 days' diabetes revealed the highest – 61% increase according to initial value, lower one – 42% in 21 days' diabetes, evidently lower – 25% in 90 days' disease, and the lowest one in 42 days' group – 20%. Control rabbits revealed highly significant ($p<0.01$) or significant ($p<0.05$) correlation between initial and final cholesterol and glucose levels. It was not observed in diabetic rabbits (Fig. 3).

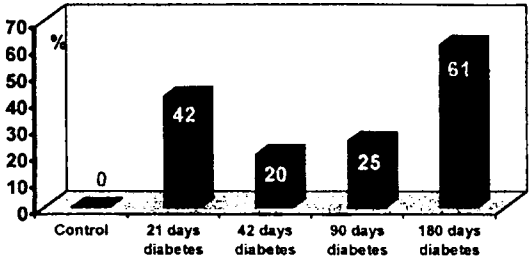

Fig. 3. Cholesterol level changes in various experimental groups of rabbits

HDL and LDL levels in individual examination groups are shown in Figure 4. HDL level was the highest on 21st day of diabetes (0.61 mmol/l) in comparison with control. Then it remained on a slightly lower level, and decreased to a very low value in the 180 days' diabetes group. LDL levels more than doubled in the first 42 days of disease in comparison with control. Afterwards it decreased significantly, reaching the half of initial value after 90 days, and later increased again. Highly significant correlation ($p<0.01$) was found between LDL and total cholesterol concentration on 21st and 42nd day of diabetes. Similar correlation was observed between HDL and total cholesterol concentration on 90th day of the course of disease.

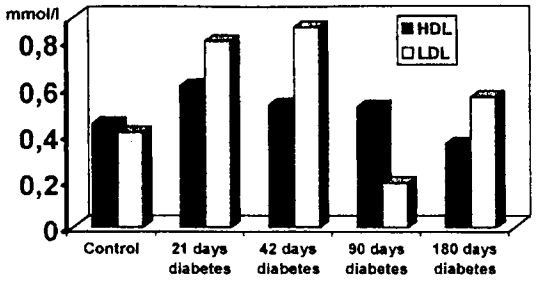

Fig. 4 HDL and LDL serum lipid fraction concentration in the course of diabetes

DISCUSSION

Alloxan is known for its specific cytotoxic influence on beta cells in the pancreas, and is used to produce experimental diabetes in various laboratory animals. Most of reported cases on lipid disorders in diabetes were based on observation of rat models. The model of alloxan-induced diabetes in rabbits is interesting in particular, due to a fact that considering lipid accumulation, a rabbit model is more like human, than rat's (4, 5, 6). D a v i d s o n and co-workers report that in the course of diabetes in humans, lipids accumulate in stroma rather than inside parenchyma cells (7). A contrary observation was made in diabetic rats, in which lipids accumulate inside the intracellular space.

D i x i t et al. reported in 1962 that insulin contents in beta cells decreased to 5% of normal value in 48 hours after alloxan administration (8). In our experiment, in the same period of time, there was observed a significant increase of blood glucose level reaching its maximum value on 42nd day. It remained on a high level until 90th day, and slightly decreased on 180th day. Even at this time it was three times higher than the control. Disorders in blood glucose levels were accompanied by other biochemical changes, which is consistent with reports by many authors (10, 11). Body weight loss reported by numerous authors is often regarded as a second, next to hyperglycaemia characteristic feature of diabetes.

Disorders in serum lipid fractions were also observed. HDL level, after initial increase on 21st day of diabetes, was decreasing to values lower than in control. Such a course of this "protective" lipid fraction is observed in diabetic humans and animals. LDL fraction revealed a significant drop on 90th day of disease to a value lower than the control one, and afterwards an increase of its concentrations on 180thday. Such a low LDL level on 90th day of disease may be correlated to a low level of cholesterol as well as lipoproteins in this experimental group. We concluded that significant disorders of lipid metabolism occur in the course of alloxan-induced diabetes in rabbits, manifested by total cholesterol level increase and changes in proportions and levels of serum lipid fractions.

REFERENCES

1. A r b e e n y C. M. et al.: Hyperlipoproteinemia in spontaneously diabetic guinea pigs. Metabolism, 38, 895-900, 1989.
2. A s s m a n n G.: Lipiddiagnostik heute. In: Lipoproteine und Herzinfarkt. Greten H. (ed.) Witzstrock-Verlag, Baden-Baden, 1979.
3. C h e r r y - P e p p e r s G. et al.: Salivary gland function and glucose metabolic status. J.

Gerontol., 47, 130-134, 1992.
4. Chisolm G. M. et al.: Lipoprotein oxidation and lipoprotein-induced cell injury in diabetes. Diabetes, Suppl. 2, 61-66, 1992.
5. Conaway H. H. et al.: Spontaneous diabetes mellitus in the New Zealand white rabbit: history, classification, and genetic analysis. J. Hered., 71, 179-186, 1980.
6. Conaway H. H. et al.: Spontaneous diabetes mellitus in the New Zealand white rabbit: physiologic characteristics. Metabolism, 30, 50-56, 1981.
7. Davidson D. et al.: Asymptomatic parotid gland enlargement in diabetes mellitus. Ann. Intern. Med., 70, 31-38, 1969.
8. Dixit P. K. et al.: Effect of Alloxan on the insulin content of micro-dissected mammalian pancreatic islets. Nature, 195, 388-389, 1962.
9. Kuschner B. et al.: Resistance of rabbits and guinea pigs to the diabetogenic effect of streptozotocin. Diabetes, 18, 542-544, 1969.
10. Kohlmeier M.: Direct enzymic measurement of glycerides in cells and in lipoprotein fractions. Clin. Chem, 32, 63-66, 1986.
11. Kortlandt W. et al.: Glycated low density lipoprotein catabolism is increased in rabbits with Alloxan-induced diabetes mellitus. Diabetologia, 35, 202-207, 1992.
12. Lopes-Virella M. F. et al.: Cholesterol determination in High Density Lipoproteins separated by three different methods. Clin. Chem., 23, 882-884, 1977.
13. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care, 20, 1183-1197, 1997.
14. Reuterving C. O. et al.: Salivary glands in long term alloxan diabetic rats. A quantitative light and microscopic study. Acta Pathol. Microbiol. Immunol. Scand, 95, 131-136, 1987.
15. Siedel J. et al.: Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. Clin. Chem., 29, 1075-1080, 1983.
16. Trinder P.: Determination of blood glucose using 4-aminophenazone as oxygen acceptor. J. Clin. Pathol., 22, 246-250, 1969.

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SUMMARY

The objective of this research was to trace the changes in serum glucose, cholesterol and lipid fractions during progression of diabetes in rabbits. 89 male rabbits, New Zealand breed were used in the experiment. Diabetes mellitus was induced by a single injection of alloxan. On day 7 the glucose level in the whole blood was measured by a glucometer to confirm the presence of diabetes. From this day the time of disease was counted. The rabbits were divided into the following groups: Group 1 – controls (n=18), Group 2 – 21 days diabetes mellitus (n=18), Group 3 – 42 days diabetes mellitus (n=17), group 4 – 90 days diabetes mellitus (n=19), group 5 – 180 days diabetes mellitus (n=17). After above-mentioned periods blood samples were taken and the rabbits were killed by decapitation. The final level of glucose in the sera was determined spectrophotometrically by enzymatic method. The method of cholesterol measurement was based on oxidation of free cholesterol to cholestenon releasing hydrogen peroxide. Measurement of lipid fractions was based on indirect methods consisting in precipitation of specific lipoprotein fractions. Control rabbits revealed highly significant ($p<0.01$) or significant ($p<0.05$) correlation between initial and final cholesterol and glucose levels. It was not observed in diabetic rabbits. Highly significant correlation ($p<0.01$) was found between LDL and

total cholesterol concentration in 21 and 42 day of diabetes. Similar correlation was observed between HDL and total cholesterol concentration on 90th day of the course of disease. We concluded that significant disorders of lipid metabolism occur in the course of alloxan-induced diabetes in rabbits, manifested by total cholesterol level increase and changes in proportions and levels of serum lipid fractions.

Zmiany poziomu glukozy, cholesterolu i frakcji lipidowych osocza krwi w cukrzycy doświadczalnej

Celem pracy była ocena zmian biochemicznych zachodzących w organizmie królików pod wpływem cukrzycy doświadczalnej. Badano poziom glukozy, cholesterolu całkowitego, jego frakcji HDL i LDL, oraz oceniano ciężar ciała. Pracę wykonano na 89 królikach rasy nowozelandzkiej białej, o masie ciała od 2,7 do 3,3 kg, którym do żyły usznej podano pojedynczą dawkę alloksanu (10 mg/kg). Poziom badanych wskaźników biochemicznych oceniano ponownie po upływie 21, 42, 90 i 180 dni, a następnie ważono zwierzęta. Maksymalny wzrost stężenia glukozy stwierdzono po 42 dniach doświadczenia (32,02 mmol/l), co w porównaniu z grupą kontrolną (6,36 mmol/l) było wzrostem prawie pięciokrotnym. Po 180 dniach choroby poziom glukozy spadł do wielkości 23,15 mmol/l. Wzrost poziomu cholesterolu całkowitego wyrażony w procentach w stosunku do grupy kontrolnej był największy po 180 dniach i wynosił 60%. Poziom LDL wzrósł prawie dwukrotnie po 42 dniach choroby (0,93 mmol/l), ale następnie w 90 dniu spadł poniżej wartości kontrolnej (0,48mmol/l). Najmniejszym zmianom podlegał poziom HDL, który we wszystkich grupach zwierząt oscylował wokół wartości średniej i odchylenia standardowego z grupy kontrolnej (0,5mmol/l). Stwierdzono istnienie korelacji między wielkością zmian biochemicznych i stopniem nasilenia zmian morfologicznych w narządach zwierząt, co było przedmiotem osobnych publikacji.