

Department of Human Anatomy, Medical University of Lublin

ZBIGNIEW WÓJTOWICZ, PIOTR SZKODZIAK, GRAŻYNA KIŚ,
MAGDALENA BŁASZCZAK, MAŁGORZATA PLISZCZYŃSKA-STEUDEN,
ANNA HADAŁA-KIŚ

*Hepatic lysosomal enzymes activity during the course
of the experimental diabetes in rabbit*

According to the literature, activity of lysosomal enzymes changes during the course of diabetes mellitus in various viscera organs (2). Therefore, we decided to evaluate the enzymatic changes in liver during experimental diabetes in rabbit.

MATERIAL AND METHODS

This experiment was designed in accordance with the guidelines of the Local Bioethical Committee. The study was carried out on 56 male rabbits of New Zealand breed, obtained from Experimental Animal Laboratory, Chorzełów n/Warsaw, Poland. All the animals were housed and maintained in an animal care facility, as described before (1). Diabetes mellitus was induced by a single injection of alloxan (Sigma Chemical Co., St. Louis, MO, USA) at the dose of 10 mg/kg into the auricular vein. For further study only animals with glucose serum level of at least 11.1 mmol were qualified. The experimental protocol was the same as described before (1). The enzyme activity was measured spectrophotometrically in the liver homogenates using methods described in detail elsewhere (1, 2).

The obtained data were compared between the experimental groups using the Kolmogorov-Smirnov test. The differences were analysed by ANOVA and followed by Duncan test. In case of inhomogeneous distribution Mann-Whitney U test was employed. The 0.05 level ($p < 0.05$) of probability was used as the criterion of significance.

RESULTS

Free and bound lysosomal liver enzymes in rabbit during the course of the experimental diabetes are presented in Table 1 and 2, respectively. It is obvious that the activity of free acid phosphatase was insignificantly increased during the first 6 weeks of disease. Three months later the enzyme activity was decreased by 25.9%. After 6 months the activity was even lower. The difference was 17.8% when compared to the control group. The activity of the bound fraction of acid phosphatase was lower compared to the free fraction during the first 3 months of diabetes, but in the 6th month it increased by 8.4%.

The activity of the free fraction of β -galactosidase was increasing until the 6th week of diabetes, then it was dropping down, and in the 6th month it was 29.5% lower than that of control. The activity of the bound fraction was lower than that of the free fraction.

Statistically significant increase of the activity of the N-acetylo- β -D-glucosamidase (NAGL) free fraction was noted at the end of the first weeks of diabetes. Then the level was decreasing, and in the 6th month it was 18.8% lower ($p > 0.05$) when compared to the corresponding control value. The activity

of the free fraction was lower comparing to control. At the beginning it was increasing and in the 6th month of disease it was nearly identical as in the control group.

Table 1. Activity of free fraction of lysosomal enzymes ($\mu\text{mol}/\text{mg}$ of protein /1 hour of incubation) in rabbit during the course of experimental diabetes

	Control	Duration of diabetes			
		3 rd week	6 th week	3 rd month	6 th month
Acid phosphatase	0.1323 ± 0.0584	0.1875 ± 0.0795	0.1889 ± 0.0811	0.1396 ± 0.0609	0.1087 ± 0.0283
β -galactosidase	0.0359 ± 0.0253	0.0471 ± 0.0270	0.0579 ± 0.0546	0.0319 ± 0.0120	0.0253 ± 0.0082
NAGL	0.1170 ± 0.0541	0.1974 ± 0.0952	0.1927 ± 0.1197	0.1178 ± 0.0441	0.0950 ± 0.0398
Cathepsin B	29.2412 $\pm 15,4915$	47.7999 $\pm 26,2583$	42.2209 ± 12.0387	45.5912 ± 12.0768	34.7986 ± 12.3904
Cathepsin D	58.3345 ± 18.5954	76.8744 ± 31.6234	79.9807 ± 27.9595	74.4340 ± 17.1839	66.3483 ± 18.1707
Cathepsin L	21.9127 ± 13.1796	56.2524 ± 20.6790	48.6473 ± 18.8429	50.7410 ± 14.9900	35.5660 ± 16.1331
Lipase	0.1955 ± 0.0295	0.1753 ± 0.0674	0.1755 ± 0.0584	0.1971 ± 0.0365	0.1586 ± 0.0190
Sulphatase	0.000955 ± 0.000527	0.002598 ± 0.001604	0.001973 ± 0.001184	0.001273 ± 0.000603	0.000706 ± 0.000214

Table 2. Activity of bound fraction of lysosomal enzymes ($\mu\text{mol}/\text{mg}$ of protein /1 hour of incubation) in rabbit during the course of experimental diabetes

	Control	Duration of diabetes			
		3 rd week	6 th week	3 rd month	6 th month
Acid phosphatase	0.0677 ± 0.0281	0.1122 ± 0.0763	0.0928 ± 0.0322	0.1059 ± 0.0579	0.1178 ± 0.0929
β -galactosidase	0.0250 ± 0.0111	0.0172 ± 0.0113	0.0155 ± 0.0079	0.0194 ± 0.0137	0.0185 ± 0.0065
NAGL	0.0424 ± 0.0130	0.0547 ± 0.0343	0.0431 ± 0.0219	0.0518 ± 0.0234	0.0423 ± 0.0099
Cathepsin B	16,4205 ± 8.3820	19.9053 ± 10.0553	13.4790 ± 6.3141	23.6494 ± 9.9131	19.7854 ± 10.5780
Cathepsin D	64.2751 ± 12.4664	63.5916 ± 28.1137	53.4359 ± 17.6937	102.9664 ± 45.4063	74.2761 ± 31.2411
Cathepsin L	5.3398 ± 5.7002	27.1687 ± 23.9787	18.1709 ± 11.0745	33.5108 ± 26.0875	19.4238 ± 13.7126
Lipase	0.3493 ± 0.0864	0.2272 ± 0.0652	0.2049 ± 0.0409	0.3545 ± 0.1817	0.3062 ± 0.1020
Sulphatase	0.000420 ± 0.000266	0.000355 ± 0.000157	0.000283 ± 0.000134	0.000451 ± 0.000177	0.000381 ± 0.000102

The activity of the cathepsin B free fraction was higher than in the control group. It was significant at the end of the 3rd week and 3rd month of experiment. The activity of the cathepsin B bound fraction was lower comparing to the bound fraction in all the studied groups. During the course of experiment the activity varied, and in the 3rd month it was highly significant in the control group.

The activity of cathepsin D was higher than that of cathepsin B. During the study it was higher than in control, but not significantly.

The activity of the cathepsin L free fraction in the course of diabetes was increasing to the highest level in 3rd week exceeding by 156.7% the level of the control group. At the end of the 3rd and 6th week, and 3rd month of the experiment the effect was highly significant when compared with the control value. The activity of the bound fraction was higher than that of the free fraction. In the diabetes animals it was increasing, exceeding by 627.6% in 3-month-old animals comparing to control. Significant differences were noted at the end of the 3rd and 6th week, as well as the 3rd and 6th month of the study.

The activity of the free lipase fraction was insignificantly and slowly decreasing, but not in the 3rd month of experiment. The activity of the lipase bound fraction in the 3rd and 6th week was significantly decreasing comparing to the control group.

The activity of the free sulphatase fraction was the lowest of all the assessed enzymes. In the rabbits with 3 and 6 weeks' diabetes it was significantly higher than in the control group. In the further course of diabetes it was decreasing and the activity of the bound fraction was lower than that of the free fraction. At the end of the 3rd and 6th week the activity insignificantly decreased by 15.4% and 32.6% respectively in comparison to control. In the further course of the experiment it was increasing, and in the 3rd month it overcame the control by 7.4%.

REFERENCES

1. Wójtowicz Z. et al.: The activity of lysosomal enzymes of rabbit's gingiva mucosa during induced experimental diabetes. *Annales UMCS, Sectio D*, 60 N1, 2005.
2. Wójtowicz Z. et al.: Serum total cholesterol, triglycerides and high density lipoproteins (HDL) levels in rabbit during the course of experimental diabetes. *Annales UMCS, Sectio D*, 59 N2, 144, 2004.

SUMMARY

The experiment was carried out on 56 New Zealand White rabbits. The diabetes was initiated by the intravenous administration of alloxan. For further study only animals with glucose serum level of at least 11.1 mmol were qualified. The enzyme activity was measured spectrophotometrically. The highest activity was noted for cathepsins, lower for lipase, acid phosphatase and NAGL, and the lowest for β -galactosidase and sulphatase. In the course of experimental diabetes the activity of lysosomal enzymes was labile.

Aktywność enzymów lizosomalnych wątroby u królika w przebiegu cukrzycy doświadczalnej

Badanie wykonano na 56 królikach rasy nowozelandzkiej. Cukrzyca była wywoływana poprzez dożylną iniekcję aloksanu. Do doświadczenia użyto zwierząt z poziomem glukozy we krwi co najmniej równym 11,1 mmol. Aktywność enzymów lizosomalnych mierzono spektrofotometrycznie. Najwyższą aktywność obserwowano dla katepsyn, mniejszą dla lipazy, fosfatazy kwasnej i NAGL. Najniższą aktywność wykazano dla β -galaktozydazy i sulfatazy. Aktywność badanych enzymów była zmienna w trakcie trwania cukrzycy doświadczalnej.