ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXII, N 2, 20, 2009 SECTIO DDD DOI: 10.2478/v10080-008-0191-x

Human Anatomy Department, Medical University of Lublin

ZBIGNIEW WÓJTOWICZ, WOJCIECH DWORZAŃSKI, AGATA KĘDZIERAWSKA-KURYLCIO, JAKUB DWORZAŃSKI, JOANNA G. KIŚ, TERESA HERMANOWICZ-DRYKA, JACEK Z. KIŚ, JOLANTA LISIECKA, ELŻBIETA A. JĘDRZEJEWSKA, FRANCISZEK BURDAN

Activity of testicular lysosomal enzymes in the course of experimental diabetes in rabbits

Aktywność enzymów lizosomalnych w jądrze królika w cukrzycy doświadczalnej

Lysosomal enzymes are sensitive markers used in various biochemical and toxicological investigations [2–5, 8, 17, 20–26]. They are synthesized in the rybosomes and secreted into cytozol, where they receive a mannose-6-phosphate tag that targets them for the lysosome. Aberrant lysosomal targeting is the key event in the inclusion-cell disease, whereby enzymes do not properly reach the lysosome, resulting in accumulation of waste within these organelles. Lysosomal enzymes such as lipase, carbohydrases, proteases and nucleases play a crucial role in the macromolecules digestion during autophagy, phago- and endocytosis [1].

In the past few years lysosomal enzymes were extensively studied in various pathological conditions, including diabetes mellitus, acute pancreatitis, neuronal degeneration, etc [2, 4, 5, 8, 17]. The aim of the present study was to establish the activity of selected lysosomal enzymes in rabbit testis in the course of the experimentally induced diabetes mellitus. The current paper is a part of a large scientific project to evaluate enzymatic and morphological changes in the experimental diabetes in rabbits.

MATERIAL AND METHODS

The study was based on an animal experimental model, designed according to the general principles for the animal experimentation [9] and the guidelines of the Bioethical Committee of the Medical University of Lublin. Mature, male New Zealand rabbits were obtained from the commercial breeder (Chorzelów, Poland) and housed in proper stainless steel cages under standard laboratory conditions. An laboratory chow LSM® (Agropol SA, Motycz, Poland) and filtrated tap water were provided *ad libitum*.

The used methodology is the same as in previous studies [12–14, 20–26]. Briefly, diabetes mellitus was developed by the alloxan (Sigma Chemical Co., St. Louis, USA) intravenous injection to the marginal auricular vein. A week later, the glucose level was measured in the sera by the automatic enzymatic method (Dextrostix; BAYER, Austria). In case the level was over 11.1 mmol/l (200 mg%)

diabetes mellitus was diagnosed and animals were randomly selected into four experimental groups (Table 1 and 2). The experimental day 7 was regarded as the first day of diabetes mellitus. Animals from each experimental group were sacrificed by the spinal cord dislocation, on days 21, 42 and 180, respectively. Except the initial dose of alloxan, no other xenobiotics were administered thoughout the study. According to principles of the Bioethical Committee, only one control, untreated group was designated. The control animals were sacrificed on experimental day 180.

During the autopsy testis were removed, weighed and immediately frozen in liquid nitrogen and stored at -20^{o}C until biochemical studies. Each organ, after being defrosted at the temperature of melting ice, was dissected and a half was taken for further biochemical investigations. The activity of the bound and free fraction of acid phosphatase, beta-galactosidase, beta-N-acetyl-glucosaminidase (NAGL); cathepsin B, D and L, lipase, and sulphatase were determined spectrophotometrically (Specol 21 Carl Zeiss; Jena, Germany) in homogenates by the methods described in detail elsewhere [5]. All the reagents were obtained from Sigma Chemical Co (St. Luise, USA).

The data was statistically evaluated using STATISTICA 5.0 software. The homogeneity of variance was examined using Kolmogoroff-Smirnoff test. Because of normal distribution, the data was analyzed by ANOVA followed by Ducan test. An α=0.05 ($p<0.05$) was considered significant.

RESULTS

The activity of the bound fraction of NAGL, cathepsins and lipase was increased on day 21 of diabetes (Table 1). On that day, changes of the free fraction of all the examined lysosomal enzymes were insignificant (Table 2). On day 42, the activity of the bound fraction of NAGL, cathepsin B and sulphatase were significantly decreased, while the activity of bound cathepsin D fraction was increased. Simultaneously, a significant elevation of the free faction of acid phosphatase and cathepsin D was revealed on day 42. On day 90 a significant increase of both fractions of cathepsin L was noted. No significant changes of the evaluated lysosomal enzymes were found on the last day of the experiment.

Table 1. The bound fraction activity (Mean±SD) of acid phosphatase, beta-galactosidase, beta-N-acetyl-glucosaminidase, lipase, (pmol/mg of protein/hour), cathepsin B, D and L, and sulphatase (nmol/mg of protein/hour) in rabbit testes

	Control	Diabetic day			
		21	42	90	180
Number of animals	17	15	13	17	17
Acid phosphatase	181.23±43.20	186.72±51.62	146.31±69.82	170.32±56.07	73.88±59.74
Beta-galactosidase	2.42±0.55	2.71±0.33	1.76±0.56	1.89±0.94	2.24±1.19
Beta-N-acetyl-glucosaminidase	90.28±16.09	96.67±14.55*	71.67±23.11*	114.22±46.02	121.29±68.14
Cathepsin B	14.88±2.69	16.07±4.09*	11.96±3.98*	18.14±8.35	13.14±3.45
Cathepsin D	89.40±25.81	84.18±18.28	125.62±78.44*	47.13±20.88	125.29±21.82
Cathepsin L	36.10±11.58	40.17±5.84	56.04±38.58	48.21±33.28*	30.87±5.42
Lipase	133.21±50.03	201.24±38.63*	128.03±91.12	168.97±60.44	188.7±80.55
Sulphatase	0.19±0.06	0.15±0.08	0.10±0.05*	0.13±0.07	0.10±0.04

*$p<0.05$ vs control

Table 2. The free fraction activity (Mean±SD) of acid phosphatase, beta-galactosidase, beta-N-acetyl-glucosaminidase, lipase, (pmol/mg of protein/hour), cathepsin B, D and L, and sulphatase (nmol/mg of protein/hour) in rabbit testes

	Control	Diabetic day			
		21	42	90	180
Number of animals	17	15	13	17	17
Acid phosphatase	280.31±52.63	316.94±47.80	300.27±69.82*	261.52±141.16	415.52±126.06
Beta-galactosidase	4.36±1.47	6.19±4.10	5.62±4.73	5.62±4.73	4.89±1.01
Beta-N-acetyl-glucosaminidase	245.90±47.35	269.68±98.79	307.50±116.98	392.01±132.93	317.72±154.44
Cathepsin B	8.36±1.40	15.97±3.28	16.58±8.54	17.24±17.07	13.14±3.45
Cathepsin D	98.40±25.81	84.18±18.28	125.62±78.44*	47.13±20.88	125.29±21.82
Cathepsin L	36.10±11.58	40.17±5.84	56.04±38.58	48.21±38.28*	78.94±15.80
Lipase	196.76±32.44	243.69±1.16	231.26±91.12	334.11±269.44	340.86±110.90
Sulphatase	0.56±0.14	0.63±0.07	0.86±0.44	0.86±0.65	0.89±0.17

*$p<0.05$ vs control

DISCUSSION

The presented data indicate that diabetes mellitus alerts the activity of acid phosphatase, beta-N-acetyl-glucosaminidase, lipase, sulphatase, cathepsin B, D and L, especially in the early phase of the pathological process. The bound fraction stored mainly in lysosomes [1] was the most affected one. The free fraction, also known as extralysosomal one [1], was significantly changed only for acid phosphatase, cathepsin L and D. The enzyme profiles were normalized during the experiment, and on the last day of the study (day 180) lack of any differences were found.

The obtained results are similar to the previous observations. Time-dependent changes of the free and bound fractions of the same lysosomal enzymes were also noted in arterial wall [21, 22], cerebral cortex [20], gingiva [23], liver [24], pancreas [13], parotid and submandibular glands [12], skeletal muscle [26], thyroid gland [25], as well as thymus [15] in the same group of animals. Additional biochemical changes typical of diabetes, such as abnormal glucose, electrolyte, cholesterol, and lipids levels were also found [14]. Similar biochemical findings were reported in humans as well [2, 8, 17].

The revealed enzymatic changes may explain low sperm quality and decrease fertilization capacity that led to male infertility – a typical late reproductive complication of diabetes mellitus [3, 10, 16]. A subsequent impairment of the embryo development was also described [10]. Bener et al. [3] found that infertility in diabetic men was also dependent on overweight, and highly associated with hypertension as well as erectile dysfunction. The increase of animal body weight and changes of electrolytes' profile was also found in currently reported animals [14, 24]. Moreover, epidemiological studies suggest a direct correlation between plasma level of testosterone and insulin sensitivity. It is also known that a low testosterone levels is associated with an increased risk of type 2 diabetes mellitus [11, 15]. Opposite data indicated, however, that abnormal testosterone level could be a consequence of diabetes [7]. However, the male infertility in the studied group of animals is only a theoretical divagation, without further histological evaluation of testes and sperm analysis.

CONCLUSIONS

The obtained data suggest altered activity of selected (acid phosphatase, beta-N-acetyl-glucosaminidase, lipase, sulphatase, cathepsin B, D and L) testicular lysosomal enzymes in experimental diabetes mellitus. Such an effect may impair male fertility. However, other experimental and epidemiological studies are required to prove such a hypothesis.

REFERENCES

1. Barrett A. J.: Lysosomal enzymes. In: Dingle J. T. (ed) Lysosomes. North-Holland Publishing Co, Amsterdam 1972.
2. Beattie G. M. et al.: Acid beta-galactosidase: a developmentally regulated marker of endocrine cell precursors in the human fetal pancreas. J. Clin. Endocrinol. Metab., 78, 1232, 1994.
3. Bener A. et al.: Is male fertility associated with type 2 diabetes mellitus?. Int. Urol. Nephrol. 2009 – in press.
4. Burdan F. et al.: The activity and immunoexpression of cathepsin D in rat male reproductive organs. Folia Morphol., 65, 111, 2006.
5. Burdan F. et al.: Hepatic lysosomal enzymes activity and liver morphology after short-time omeprazole administration. Exp. Toxicol. Pathol., 53, 453, 2002.
6. Burlina A. B. et al.: Behaviour of some lysosomal enzymes in the plasma of insulin dependent diabetic patients during artificial pancreas treatment. Clin. Biochem., 20, 423, 1987.
7. Corona G. et al.: Why can patients with erectile dysfunction be considered lucky? The association with testosterone deficiency and metabolic syndrome. Aging Male, 11, 193, 2008.
8. Garcia-Pascual J. J. et al.: Enzymatic glycosidase activities in experimental obesity. Horm. Metab. Res., 24, 412, 1992.
9. Górska P.: Principles in laboratory animal research for experimental purposes. Med. Sci. Monit., 6, 171, 2000.
10. Kim S. T., Moley K. H.: Paternal effect on embryo quality in diabetic mice is related to poor sperm quality and associated with decreased glucose transporter expression. Reproduction, 136, 313, 2008.
11. La Vignera S. et al.: Andrological characterization of the patient with diabetes mellitus. Minerva Endocrinol., 34, 1, 2009.
12. Maciejewski R. et. al.: Changes of the ATPase, acid phosphatase and alkaline phosphatase reaction intensity in the parotid and submandibular glands of rabbits in experimental diabetes. Folia Histochem. Cytobiol., 37, 99, 1999.
13. Maciejewski R. et al.: Changes in the activities of pancreatic cathepsins D and L and acid phosphatase in the course of alloxan-induced diabetes mellitus in rabbit. Med. Sci. Res., 28, 113, 2000.
14. Maciejewski R. et. al.: Changes in pancreatic lysosomal enzymes activity as the potential factors leading to diabetic enteropathy. J. Physiol. Pharmacol., 52, 823, 2001.
15. Saad F., Gooren L.: The role of testosterone in the metabolic syndrome: a review. J. Steroid Biochem. Mol. Biol., 114, 40, 2009.
16. Shirneshan K. et al.: Directed overexpression of insulin in Leydig cells causes a progressive loss of germ cells. Mol. Cell Endocrinol., 295, 79, 2008.
17. Strawser L. D., Touster O.: The cellular processing of lysosomal enzymes and related proteins. Rev. Physiol. Biochem. Pharmacol., 87, 169, 1980.

18. T a c z a ł a S. et. al.: The activity of some lysosomal enzymes of the thymus in rabbits. Annales UMCS, Sect. D, 49, 87, 1994.
19. T o n g P. C. et al.: Association of testosterone, insulin-like growth factor-I, and C-reactive protein with metabolic syndrome in Chinese middle-aged men with a family history of type 2 diabetes Med. Sci. Monit., 6, 171, 2000.
20. W ó j t o w i c z Z. et. al.: The activity of lysosomal enzymes in visual cortex of rabbits during experimental diabetes. Folia Neuropathol., 33, 159, 1995.
21. W ó j t o w i c z Z. et. al.: The activity of lysosomal enzymes of the basilar artery wall on rabbits of different breed. Annales UMCS, Sect. D, 49, 141, 1994.
22. W ó j t o w i c z Z. et. al.: Lysosomal enzyme activity of internal and middle layers of renal arterial wall in rabbits. Annales UMCS, Sect. D, 53, 153, 1998.
23. W ó j t o w i c z Z. et. al.: The activity of lysosomal enzymes of rabbits gingiva mucosa during induced experimental diabetes. Annales UMCS, Sect. D, 60, 369, 2005.
24. W ó j t o w i c z Z. et al.: Hepatic lysosomal enzymes activity turning the course of the experimental diabetes in rabbit. Annales UMCS, Sect. D, 60, 908, 2005.
25. W ó j t o w i c z Z. et al.: The activity of lysosomal enzymes of rabbit thyroid gland during induced experimental diabetes. Annales UMCS, Sect. D, 61, 748, 2006.
26. W ó j t o w i c z Z. et al.: The activity of lysosomal enzymes of rabbit skeletal muscles during induced experimental diabetes. Annales UMCS, Sect. D, 60, 369, 2005.

SUMMARY

Diabetes mellitus is a syndrome of disordered metabolism, usually due to a combination of hereditary and environmental causes. One of its complications is male infertility and endocrine dysfunction, including abnormality of the testosterone level. The aim of the study was to evaluate the activity of selected lysosomal enzymes in rabbit testis. Mature male New Zealand rabbits were randomly selected into a control and the experimental groups in which diabetes was developed by the intravenous injection of alloxan. Animals were sacrificed and the testicles were removed during autopsy done on days 21, 42, 90 and 180. The activity of the bound and free fraction of acid phosphatase, beta-galactosidase, beta-N-acetyl-glucosaminidase (NAGL), cathepsin B, D and L, lipase, and sulphatase were determined spectrophotometrically in testicular homogenates. The activity of the bound fraction of NAGL, cathepsin and lipase was increased on day 21 of diabetes. On day 42 the activity of the bound fraction of NAGL, cathepsin B and sulphatase were significantly decreased, while the activity of bound cathepsin D fraction was increased. Simultaneously, a significant elevation of the free faction of acid phosphatase and cathepsin D was revealed. On day 90 a significant increase of both fractions of cathepsin L was noted. No significant changes of the evaluated lysosomal enzymes were found on the last day of the experiment. The obtained data suggest that diabetes mellitus alerts the activity of most studied lysosomal enzymes in rabbit testis. Such an effect may impair male fertility.

STRESZCZENIE

Cukrzyca jest schorzeniem metabolicznym, powstającym na podłożu genetycznym w wyniku działania niekorzystnych czynników środowiskowych. Jednym z jej powikłań jest niepłodność męska i zaburzenia hormonalne, w tym nieprawidłowy poziom testosteronu. Celem pracy była ocena aktywności wybranych enzymów lizosomalnych w jądrach królika w przebiegu cukrzycy

doświadczalnej. Dorosłe samce królika rasy nowozelandzkiej białej były losowo przydzielane do grupy kontrolnej i grup doświadczalnych, w których cukrzycę wywoływano poprzez dożylne podanie alloksanu. Zwierzęta usypiano w 21, 42, 90 i 180 dniu, a podczas sekcji pobierano jądra. W homogenatach jąder oznaczono spektrofotometrycznie aktywność frakcji wolnej i związanej fosfatazy kwaśnej, beta-galaktozydazy, beta-N-acetylo-glukozaminidazy (NAGL), katepsyny B, D i L, lipazy oraz sulfatazy. Aktywność frakcji związanej NAGL, katepsyn i lipazy była istotnie zwiększona w 21 dniu od rozpoznania cukrzycy. Znamienne obniżenie aktywności frakcji związanej NAGL, katepsyny B i sulfatazy oraz zwiększenie aktywności katepsyny D wykazano w 42 dniu. Jednocześnie zanotowano istotny wzrost aktywności frakcji wolnej fosfatazy kwaśnej i katepsyny D. W 90 dniu obserwowano ponadto wzrost aktywności obu frakcji katepsyny L. Aktywność badanych enzymów nie wykazywała istotnych różnic w ostatnim dniu doświadczenia. Otrzymane wyniki wskazują na związek zaburzeń aktywności większości badanych enzymów lizosomalnych w jądrach z niepłodnością męską.