Neuroendocrinology Letters ISSN 0172–780X

ORIGINAL ARTICLE

The insulin, glucose and cholesterol level and activity of lysosomal enzymes in the course of the model alloxan diabetes

Bozena Witek,[1] Teodora Król[1] Adam Kołataj,[2] Ewa Ochwanowska,[1] Iwona Stanisławska[1] & Alina Slewa[1]

1. Department of Genetics, Institute of Biology, Swietokrzyska Academy, Poland.
2. Institute of Genetics and Animal Breeding, Polish Academy of Sciences in Jastrzebiec,Wólka Kosowska near Warsaw, Poland.

Correspondence to: Dr. Bozena Witek,
Department of Genetics, Institute of Biology, Swietokrzyska Academy
Swietokrzyska 15, 25-406 Kielce, Poland.
E-MAIL b.witek@pu.kielce.pl

Submitted: *July 23, 2001*
Accepted: *July 30, 2001*

Key words: **Alloxan diabetes; insulin level; glycaemia level; cholesterol level; lysosomal enzymes; rabbits**

*Neuroendocrinology Letters 2001; **22**:238–242 pii: NEL220401A02 Copyright © Neuroendocrinology Letters 2001*

Abstract

OBJECTIVES: The study was carried out on fifty male rabbits of the New Zealand White breed. Diabetes was caused by a single, intravenous alloxan injection. Rabbits which had glycaemia 7th day after the alloxan administration higher than 11 millimol/litre were selected for the studies. They were divided into 5 groups: I – control (without diabetes); II – 3-week diabetes; III – 6-week diabetes; IV – 3-month diabetes; V – 6-month diabetes.

METHODS: In control and experimental rabbits the activity of β-glucuronidase, N-acetyl-β-glucosaminidase, lysosomal acid phosphatase, leucine aminopeptidase, cathepsin D, and lysosomal arylesterase was determined in lysosomal fractions of the liver and kidney.

RESULTS: Alloxan caused lowering of the activity of all the investigated enzymes in the kidney and liver except lysosomal arylesterase.

CONCLUSION: Alloxan injection caused a significant increase in the activity of all the investigated enzymes. The advisable lysosomal enzymes may be useful for the monitoring of the course and effectiveness of diabetes therapy.

Introduction

It is known that diabetes causes profound metabolic disturbances of carbohydrate, protein and lipid metabolism which leads to pathological changes in the organs and subsequent complications of the large blood vessels of the heart, brain, kidney and legs known as diabetic macroangiopathy and the small vessels of the heart, kidney and eye retina, known as diabetic microangiopathy [1–6].

A prompt diagnosis of these disturbances and a proper treatment are not easy because they are highly unspecific. Therefore, laboratory diagnostics of diabetes is extremely important because it significantly influences the quality and length of a patient's life.

Contemporary diagnostics more often analyses the rate and scope of biochemical transformations in cells on the basis of the activity of the lysosomal substructure. Lysosomal enzymes have become the object of investigations aimed at finding, among other things, the indices of stress reactivity, apoptose and degradation of energetic substrates in various spaces of cellular protoplasm. Lysosomal enzymes are beginning to be recognised as indicators of various diseases [7–16].

Material and methods

The experiment was carried out on fifty 12-month-old male rabbits of the New Zealand White breed, coming from the Research Institute of Experimental and Laboratory Animals in Chorzelów (Poland). The animals were kept in standard cages in a ventilated room at 18°C with 12 h daylight and 12 h darkness. They received standard, homogenous industrial food mixture, 19% of crude protein (Altromin Standard Diets 1320 Totally Pathogen Free TPF; International, Germany) in a quantity of 100–200 gram/individual/day and they had a constant access to water. Before the alloxan administration and on the day of slaughter, the rabbits were weighed and the glucose concentration in blood plasma and total cholesterol was determined on the basis of enzymatic methods using diagnostic tests of the „Bio- Lachema-Test" Co., Brno, Slovak Republic. It was expressed in millimol/litre of blood plasma.

Diabetes was caused by single injection of 2 ml of 10% alloxan solution to *vena marginalis auris* (Sigma St. Louis, USA) dissolved in a solution 0.9% natrium chloride, in the concentration 100 mg/kg of body weight. Rabbits in which glucose concentration in blood plasma on the 7th day after alloxan injection was higher than 11 millimol/litre were selected for the experiment. At the same time, the control rabbits received 2 ml of 0.9% natrium chloride solution. In the control group the mean level of glucose in blood plasma was 5.73 millimol/litre.

During the experiment the glycemia level was measured at 7-day intervals by using glucometer and Destrostix belts (Bayer GmbH Vienna, Austria).

Rabbits were divided into five groups (n = 10 in each group): I control (with 0.9% natrium chloride - without diabetes); II – 3-week diabetes (21 days); III – 6-week diabetes (42 days); IV - 3-month diabetes (90 days); and V - 6-month diabetes (180 days).

After 21, 42, 90 and 180 days after the diagnosis of diabetes, the rabbits from the experimental and control groups were killed by intravenous *thiopental* injection and slices of the liver and kidney were immediately taken.

Weighed slices of the liver were perfused with 0.9% solution natrium chloride cooled to +5°C and similarly with the slices of kidney were suspended in 0.1 M phosphate buffer cooled to +5°C at pH 7.0 (500 mg of tissue/5 ml buffer). The whole was homogenized at +5°C in a Potter homogenizer with a teflon piston at 200 rot./min. The liver and kidney homogenates were subjected to differentiated centrifuging according to [17].

In the lysosomal fractions of the liver and kidney the activity (nanomol/mg of protein/hour) of beta-glucuronidase (BGRD, EC 3.2.1.31) was determined according to [18]; acid phosphatase (AP, EC 3.1.3.2) according to [19]; leucine aminopeptidase (LAP, EC 3.4.11.1) according [20]; lysosomal arylesterase (EL, EC 3.1.1.2) according [21]; cathepsin D (Cath.D, EC 3.4.23.5) according to [22] using as substrate 2% azocasein in 6M urea. All substrates were from Serva Feinbiochemica GmbH & Co., Heidelberg, Germany.

Protein was also determined in the lysosomal fractions [23], insulin concentration was determined by the Radio-Test (Slovak Republic, and glucose concentration using the "Bio-Lachema-Test" tests (Slovak Republic). The experiments have been confirmed by the University Ethics Commission for Animals Research of the Swietokrzyska Academy in Kielce. The results obtained were analysed statistically according to the Student's ***t*** test.

Results

As can be seen in Table 1, the greatest decrease of body weight gain was observed in the group of rabbits with 6-month diabetes (226 g). A similar decrease was revealed in rabbits with 3-week diabetes (208 g) and a much lower decrease in rabbits with 6 weeks diabetes (69 g). In rabbits with 3-month diabetes, a slight increase in body weight gain (39 g) was found.

Table 1. Values of the body weight, insulin, glucose and cholesterol level ($\bar{x}$ ± SD) in blood plasma in the course of the model alloxan diabetes;

Traits	Control	Model alloxan diabetes			
		three weeks	six weeks	three months	six months
Body weight (g)	2880 ± 120	2900* (-208)	2850 (-69)	2775 (+ 39)	2625* (-226)
Insulin level (µU/ml)	2.51 ± 0.91	*** 17.53 ± 3.83	*** 28.48 ± 6.15	*** 27.91 ± 9.77	*** 18.64 ± 3.76
Glucose level (mmol/l)	5.73 ± 1.53	*** 21.79 ± 3.78	*** 32.02 ± 4.32	*** 31.33 ± 4.22	*** 23.15 ± 2.89
Cholesterol level (mmol/l)	1.37 ± 0.921	*** 1.94 ± 0.877	* 1.65 ± 0.7 63	* 1.71 ± 0.566	** 2.20 ± 0.764

* P < 0.05; ** P < 0.01; *** P < 0.001 - the differences statistically confirmed

Table 2. The activity of lysosomal enzymes ($\bar{x}$ ± SD) in the liver of rabbits (in nanomol/mg of protein/hour) in the course of the model alloxan diabetes; control = 100%;

Enzyme	Control	Model alloxan diabetes							
		three weeks	%	six weeks	%	three months	%	six months	%
NAG	0.535 ± 0.05	*** 1.22 ± 0.09	**228**	*** 1.10 ± 0.05	**206**	* 0.669 ± 0.07	**125**	*** 1.20 ± 0.05	**224**
BGRD	0.431 ± 0.05	*** 0.845 ±0.06	**196**	*** 0.746 ±0.07	**173**	*** 0.655 ± 0.06	**152**	*** 0.866 ± 0.04	**201**
AP	0.474 ± 0.07	0.520 ± 0.05	**110**	* 0.569 ± 0.07	**120**	*** 0.706 ± 0.03	**149**	*** 0.720 ± 0.07	**152**
Cath.D and L	0.530 ± 0.01	* 0.662 ± 0.07	**125**	*** 0.853 ± 0.09	**161**	** 0.689 ± 0.05	**130**	** 0.742 ± 0.06	**140**
LAP	0.496 ± 0.04	0.580 ± 0.03	**117**	* 0.595 ± 0.06	**120**	* 0.620 ± 0.06	**125**	** 0.640 ± 0.06	**129**
EL	0.271 ± 0.07	0.255 ± 0.09	**94**	0.221 ± 0.02	**81**	* 0.214 ± .006	**79**	*** 0.103 ± 0.05	**38**

* P < 0.05; ** P < 0.01; *** P < 0.001 - the differences statistically confirmed

Table 3. The activity of lysosomal enzymes ($\bar{x}$ ± SD) in the kidney of rabbits (in nanomol/mg of protein/hour) in the course of the model alloxan diabetes; control = 100%;

Enzyme	Control	Model alloxan diabetes							
		three weeks	%	six weeks	%	three months	%	six months	%
NAG	0.410 ± 0.05	** 0.635 ± 0.02	**155**	0.425 ± 0.01	**104**	*** 0.704 ± 0.08	**172**	*** 0.830 ± 0.01	**202**
BGRD	0.320 ± 0.03	*** 0.734 ± 0.03	**229**	*** 0.536 ± 0.05	**167**	*** 0.895 ± 0.02	**280**	*** 0.976 ± 0.03	**305**
AP	0.268 ± 0.07	*** 0.600 ± 0.06	**224**	*** 0.790 ± 0.05	**295**	*** 0.730 ± 0.01	**273**	* 0.334 ± 0.08	**125**
Cath.D and L	0.370 ± 0.02	0.377 ± 0.09	**102**	* 0.480 ± 0.07	**130**	*** 0.822 ± 0.07	**222**	*** 0.531 ± 0.07	**143**
LAP	0.130 ± 0.05	0.136 ± 0.01	**105**	* 0.158 ± 0.04	**121**	*** 0.260 ± 0.02	**200**	*** 0.235 ± 0.03	**181**
EL	0.285 ± 0.01	*** 0.480 ± 0.07	**168**	** 0.433 ± 0.04	**152**	*** 0.403 ± .004	**141**	*** 0.500 ± 0.06	**175**

* P < 0.05; ** P < 0.01; *** P < 0.001 - the differences statistically confirmed

The insulin level increased already in animals with 3-week diabetes to 17.53 μU/ml and in rabbits with 6-week diabetes – to 28.48 μU/ml. In rabbits with 3-month and 6-month diabetes the insulin concentration decreased slightly to 27.91 and 18.64 μU/ml suitable. The mean glucose level in the control group was 5.73 millimol/litre; in animals with 3-week diabetes it increased to 21.79 millimol/litre, and after 6 weeks – to 32.02 millimol/litre. In rabbits with 3-month diabetes the glycemia level decreased slightly to 31.33 millimol/litre, and it decreased to 23.15 millimol/litre in rabbits with 6-month diabetes.

The total cholesterol level in the control group was on average 1.37 millimol/litre and it increased in the course of 3-week diabetes to 1.94 millimol/litre. After 6 weeks and 3 months from diabetes induction it decreased slightly to 1.65 millimol/litre and 1.71 millimol/litre, but after 6 months it increased significantly to 2.20 millimol/litre.

As can be seen in Table 2, in the course of alloxan diabetes a significant increase in the activity of all the investigated lysosomal hydrolases was found in the liver of the rabbits except EL. The highest activity of NAG was recorded in the third week of alloxan diabetes; BGRD and AP in the sixth month; Cath.D and L in the sixth week, but the highest decrease of EL activity was found in the sixth month of the disease.

As is shown in Table 3, alloxan injection caused a statistically confirmed increase in the activity of all the investigated lysosomal hydrolases in the kidney. The highest NAG, BGRD and EL activities were found in the sixth month of diabetes; Cath.D and L, and LAP in the third month and AP in the sixth week of experimental alloxan diabetes.

Discussion

In spite of the fact that contemporary medicine, physiology and endocrinology made significant progress in the interpretation of diabetes mechanisms, the essence of this disease is still unknown [24-25].

In the applied model of alloxan diabetes, apart from the increase of the insulin level, glycemia and total cholesterol level in blood plasma, we also found highly significant changes in the activity of the investigated lysosomal enzymes. Increase of the activity of the studied NAG and BGRD glycosidases was observed. Glycosidases take part in the degradation of glycoproteins, glycolipids and glycosaminoglycans and hyperglycaemia, which accompanies diabetes, causes an increase of glycosilation of many proteins. The highest increase in NAG activity was recorded in the liver in the third week, and in the sixth month in the kidney after diabetes induction. The highest BGRD activity in the liver and kidney was recorded in the sixth month of the disease. The glycosidases in the course of diabetes were the subject of some publications [26-29].

An increase of activity was recorded for Cath.D and L, and LAP too. An increase of activity of the investigated lysosomal enzymes in the course of model alloxan diabetes probably suggests the vast adaptation processes during hyperglycaemia, beginning with labilisation of the membranes of the lysosomal system through an increase in their permeability for particular substrates and intensive degradation processes.

We would like to suggest that the advisable lysosomal enzymes may be useful for the monitoring of the course and effectiveness of diabetes therapy [30–34].

REFERENCES

1 Botion LM, Green A. Long-term regulation of lipolysis and hormone –sensitive lipase by insulin and glucose. Diabetes 1999; **48**:1691-1698.
2 Corder CN, Kalkhoff RK. Hepatic lipid metabolism in alloxan diabetic rats. J Lab Clin Med 1969; **73**:551-563.
3 Lewis GF, Zinman B, Groenewoud Y, Vranic M, Giacca A. Hepatic glucose production is regulated both by direct hepatic and extrahepatic effects of insulin in humans. Diabetes 1996; **45**:454-460.
4 Marks JB, Raskin P. Neuropathy and hypertension in diabetes. Med Clin North Amer 1998; **82**:877-909
5 Nonogaki K, Uguchi A. Stress, acute hyperglycemia and hyperlipidemia. Role of the autonomic nervous system and cytokines. Trends Endocrinol Metabolism 1997; **8**:192-199.
6 Ritz E, Orth SR. Primary cars: Nephropathy in patients with 2 diabetes mellitus. New Engl J Med 1999; **341**:1127-1134.
7 Cuervo AM, Knecht E, Terlecky SR, Dice JF. Activation of a selective pathway of lysosomal proteolysis in rat liver prolonged starvation. Amer J Physiol 1995; **269**:C1200-C1208.
8 Elqun S, Karaayvaz M, Durak I. Alanine aminopeptidase activities in cancerous and noncancerous human breast tissues. Eur J Clin Chem Clin 1997; Biochem **35**:249-254.
9 Goi G, Fabi A, Lorenzi R, Lombardo A, Tettamanti G, Burlina AB, Pinelli L, Gaburro D. Serum enzymes of lysosomal origin as indicators of the metabolic control diabetes: comparison with glycated hemoglobin and albumin. Acta Diabetol Lat 1986; **23**:117-125.
10 Hill JO. Energy metabolism and obesity In: Draznin B and Rizza R. editors, Clinical Research in Diabetes and Obesity Human Press Inc 1997; 392-350.
11 Kashihara H, Shi ZQ, Yu JZ, McNeill JH, Tibbits GF. Effects of diabetes and hypertension on myocardial Na^+ - Ca^{2+} exchange. Can J Physiol Pharmacol 2000; **78**:12–20.
12 Kołataj A, Sommer A, Witek B, Nitray J, Flak P. The effect of exogenous glucose on the activity of lysosomal enzymes, the glucose and insulin concentration in the bulls. Arch Anim Breed 1998; **41**:11-17.
13 Morano S, Tiberti C, Cristina G, Sensi M, Cipriani R, Guidobaldi L, Torresi P, Medici F, Anastasi E, Di Mario V. Autoimmune markers and neurological complications in non-dependent diabetes mellitus. Hum Immunol 1999; **60**:848-855.
14 Sommer A, Witek B, Kołataj A.The effect of exogenous glycerol on the reactivity of lysosomal enzymes in the blood plasma of

young bulls. Archiv Anim Breed 1999; **42**:451-458.
15 Witek B, Kołataj A. Effect of Ethanol Administration on Activities of Some Lysosomal hydrolases in the Mouse. Gen Pharmacol 1999; **32**:163-168.
16 Witek B, Kołataj A, Król T. Adaptative changes in the glucose level and activity of some lysosome enzymes in plasma of starved rabbits. Archiv Anim Breed 1995; **38**:341-345.
17 Beaufay H. Methods for the isolation of lysosomes. In: Dingle JT, editor, Lysosomes: A Laboratory Handbook North-Holland Amsterdam 1972; 1-135.Barrett AJ. Lysosomal enzymes. In: Dingle JT. editor, Lysosomes: A Laboratory Handbook North-Holland Amsterdam 1972; 46-135.
19 Hollander VP. Acid phosphate. In: Boyer PD editor, The enzymes, Academic Press London 1970; **4**:449-498.
20 Pfleiderer G, Celliers PG. Isolierung einer Aminopeptidase aus Nierenpartikeln. Biochem Zeitschr 1963; 339:186-189.
21 Main AR. The purification of the enzyme hydrolyzing diethyl p-nitrophenyl phosphate (paraoxon) in sheep serum. J Biol Chem 1960; **74**:11-20.
22 Langner J, Wakil A, Zimmermann M, Ansorge S, Bohley P, Kirschke H, Wiederanders B. Aktivitätsbestimmung proteolytischer Enzyme mit Azokasein als Substrat. Acta Biol Med Germ 1973; **31**:1-18.
23 Kirschke H, Wiederanders B. Methoden zur Aktivitätsbestimmung von Proteinasen 1984; Martin Luther Universität Halle-Wittenberg Wissenschaftl Beitr Halle/Salle 11-30.
24 Ceriello A. Hyperglycemia: the bridge between non-enzymatic glycation and oxidative stress in the pathogenesis of diabetic complications. Diabetes Nutr Metab 1999; **12**:42-449.
25 Jacob S, Hauer B, Becker R, Artzner S, Grauer P, Loblein K, Nielsen M, Renn W, Rett K, Wahl HG. Lipolysis in skeletal muscle in rapidly regulated by low physiological doses of insulin. Diabetologia 1999; **42**:1171-1175.
26 Belfiore F, Lo Vecchio L, Napoli E, Borzi U. Increased β-N- acetyl-glucosaminidase activity in diabetes mellitus. Clin Chem 1974; **20**:1229-1230.
27 Kelly L, Wopdward SH. Alterations in the activities of lysosomal glycosidases in human diabetes. Med Sci Res 1988; **16**:491-496.
28 Muzzarelli RA. Analytical biochemistry and clinical significance of N-acetyl-beta-D-glucosaminidase and related enzymes. 1999; EXS **87**:235-247.
29 Perez-Blanco FJ, Garbin-Fuentes J, Perez-Chica G, Moreno-Terribs G, Rodriqez-Cuartero A. Urinary activity of N-acetyl-β-glucosaminidase and progression of retinopathy in non-insulin-dependent diabetes mellitus. Clin Nephrol 1997; **48**:388-389.
30 Anderson GH. Sugars and health. Nutrition. Research 1997; **17**:1485-1490.
31 Louis-Sylvestre J. Glucose utilization dynamics and food intake. Brit J Nutr 1999; **82**:427-431.
32 Montminy C, Galigois I. Role of protein and fibersource nature on glucose metabolism in rats. Nutr 1994; 10:144-150.
33 Strubbe JH, Steffens AB. Neural control of insulin secretion. Horm Metab Res 1993; **25**:507-512.
34 Sugiyama Y, Shimura Y, Ikeda H. Pathogenesis of hyperglycemia in genetically obese-hyperglycemic rats Wistar fatty: presence of hepatic insulin resistance. Endocrinol Jpn 1989; **36**:65-73.