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Effect of Pancragen on Blood Glucose Level, Capillary Permeability and Adhesion in Rats with Experimental Diabetes Mellitus

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The effects of tetrapeptide pancragen (Lys-Glu-Asp-Trp-NH₂) on blood glucose level and permeability and adhesion of mesenteric capillaries were studied in Wistar rats with experimental streptozotocin-induced diabetes mellitus. Oral pancragen produced a pronounced hypoglycemic effect during treatment. Intramuscular pancragen normalized the adhesion of mesenteric capillary endothelium, but did not modify capillary permeability. The results indicate homeostatic and endothelioprotective effects of pancragen during the early period of diabetes mellitus.

Key Words: *pancragen; streptozotocin; diabetes mellitus; hyperglycemia; endothelium*

Diabetes mellitus (DM) ranks third in the world by prevalence after cardiovascular diseases and cancer [3]. The most frequent causes of disability and mortality of diabetics are diabetic angiopathies (generalized involvement of blood capillaries linked with hemostasis disorders) [1,4]. Diabetes is associated with systemic organ dysfunctions caused by disorders in microcirculation and transcapillary exchange, the endothelium playing the key role in both. The metabolic dysfunctions, primarily hyperglycemia, trigger the development of endothelial dysfunction in DM [6]. Diabetic hyperglycemia promotes an increase in the synthesis of mucopolysaccharides, which leads to an increase in their serum level and deposition in capillaries of the kidney, retinal, and other organs. Glucose is transformed mainly by the polyolic pathway in insulin-

dependent tissues of diabetics, with activation of aldoreductase, as a result of which sorbitol and fructose accumulate in the vascular wall, lens of the eye, nerves, and kidneys in high osmotically active concentrations. These hydrophilic polyolic products promote the development of hydropic edema of the cells and impair active transmembrane transport of substances [1]. In addition, high glucose concentration leads to nonenzymatic glycosylation of proteins, lipids, and other components of the vascular wall, which modifies their antigenic and functional characteristics, causes disorders in capillary wall permeability, stenosis of the lumen, and shrinkage of their inner surface area with the development of ischemia and tissue dystrophy. One of the most severe complications of DM is atherosclerosis [1,5].

Tetrapeptide pancragen (Lys-Glu-Asp-Trp-NH₂; PG), synthesized at St. Petersburg Institute of Bioregulation and Gerontology is a prospective bioregulators for drug correction of metabolic disorders and endothelial dysfunction in DM.

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We studied the hypoglycemic effect of PG and its effect on endothelial function in chronic hyperglycemia on the model of DM in rats.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (aged 10-12 weeks, 170-210 g). The animals were kept with free access to fodder (Inform-Korm K-120 standard ration for laboratory rats) and water.

Diabetes mellitus was induced by single intraperitoneal injection of streptozotocin (ST; 50 mg/kg in 1 ml 0.9% NaCl). Streptozotocin produces a selective cytotoxic effect on rat pancreatic β -cells, and experimental animals develop DM (permanent hyperglycemia, polyuria, glucosuria) 2-3 weeks after ST injection.

After ST injection, group 1 animals were intramuscularly injected with PG (10 μ g/ml saline) daily for 10 days. Group 2 animals received PG orally through a tube (pulverized tablet containing 100 μ g PG in 2 ml suspension) daily for 10 days after ST injection. Group 3 rats received injections of saline according to analogous protocol. Healthy intact animals served as controls. Each group consisted of 20 animals.

Blood glucose was measured by an Accu Chek glucometer after overnight fasting (the fodder was removed from cages 14 h before blood collection) on days 10, 15, and 20 after ST injection. On day 20 after ST injection, diuresis and urinary glucose were evaluated by the universal method on a COBAS biochemical analyzer and endothelial permeability and adhesion were studied.

The function of capillary endothelium was studied in narcotized animals (50 mg/kg sodium thiopental intraperitoneally) by complex life-time microscopy [2]. A fragment of the small intestine was mobilized for evaluation of the mesenteric microcirculation. The small intestinal mesentery, adjacent to the mesoappendix, was placed on the light guide of warmed table. The temperature of the table and objective was automatically maintained at 37.5-38.0°C. The studied mesenteric portion was constantly (at a rate of 0.5 ml/min) irrigated with warm (37.5°C) saline. All studies of the endothelial function were carried out on the same type of capillaries (venules 20-35 μ in diameter) using a video-microscopic complex consisting of an MT-9 microscope (LOMO), CCD videocamera (ISTA Ltd.), SLV-X55ME videotape recorder (Sony), and a KV-2185MT TV set (Sony). Capillary endothelium permeability for Na fluorescein was evaluated by complex life-time biomicroscopy. Appropriate photofilters were used in the optical system, the transmitted luminous flux

was transformed into reflected, the fluorescent agent was intravenously injected, and the time course of fluorescence of the selected area was videorecorded. Videomaterials were processed using a programmed complex including the videotape recorder, AV Master analog digital transformer (Fast Multimedia AG) attached to an IBM-compatible PC, and Fast Cap 2.5.0 (FAS Multimedia Inc.) and VideoTest 5.0 (ISTA Ltd.) software. Due to this complex it was possible to carry out a dynamic geometrical, velocity, and quantitative analysis of the digital videoimage.

The main parameter characterizing vascular permeability is permeability coefficient (P) determined as the amount of substance penetrating through a certain area of the vascular wall. Na-Fluorescein fluorochrome (mol. weight 376 Da) in a dose of 2.5 mg/kg was selected as the indicator for studies of capillary permeability. The indicator was dissolved in saline directly before the experiment and injected intravenously (0.2 ml solution).

The permeability coefficient was calculated using the equation:

$$P=0.25 \times dIi(t) \times D \times [Ii(0)/Iv(0)]/[Iv(t)-Ii(t) \times dt],$$

where P is permeability coefficient (cm/sec); $dIi(t)$ increment in fluorochrome fluorescence intensity in the interstitium (luminance/sec units); D vessel diameter; $Ii(0)$ initial mean luminance outside the capillary in transmitted luminous flux (luminance units); $Iv(0)$ initial mean luminance inside the vessel in transmitted luminous flux (luminance units); $Iv(t)$ fluorochrome fluorescence intensity inside the vessel (luminance units); $Ii(t)$ fluorochrome fluorescence intensity in the interstitial space (luminance units); and dt time (sec).

The initial picture of microcirculation for studies of the process of leukocyte adhesion to vascular wall was videorecorded in transmitted light without photofilters. Videomaterials were processed using a programmed complex for life-time biomicroscopy. The adhesion characteristics of the endothelium were studied on unstained leukocyte. The best contrast was attained by transmitting the illumination luminous flux through the photofilter with the transmission wavelength of 360-560 nm. Clearly contrasted leukocytes, immobilized during the entire period of measurement (5 sec), were considered adherent. The ratio of adherent leukocytes to the vascular wall area was calculated.

Experimental data were statistically processed by multidimensional statistical analysis using Student's t and Mann-Whitney U tests.

TABLE 1. Effect of PG on Blood Glucose Level, Capillary Adhesion and Permeability in Animals with ST-Induced DM ($M \pm m$)

Parameter	Intact animals	Group		
		1	2	3
Blood glucose, mmol/liter				
initial level	4.2±0.4	4.5±0.3	4.5±0.4	4.6±0.4
day 10	4.5±0.3	8.2±4.7*	8.0±4.7*	5.6±1.5**
day 20	4.4±0.5	9.1±4.8*	8.9±4.0**	9.2±5.3*
Diuresis on day 20, ml/h	0.16±0.05	0.50±0.40**	0.61±0.52**	0.95±0.82**
Urinary glucose on day 20, mmol/liter	0	31.4±26.0**	31.4±26.0**	33.3±25.1**
Permeability coefficient on day 20, $\times 10^{-5}$ cm/sec	1.03±0.21	0.72±0.32*	0.68±0.30*	0.65±0.36*
Adhesion on day 20	1.93±0.34	1.30±0.58*	1.93±0.31*	1.22±0.32**

Note. * $p < 0.05$, ** $p < 0.01$ compared to healthy animals; * $p < 0.05$, ** $p < 0.01$ compared to group 3.

RESULTS

Diabetes mellitus was diagnosed by biochemical analysis of the blood and urine on day 10 after ST injection in 30% and on day 20 in the majority of animals, which corresponded to the model used in the study (Table 1, Fig. 1). A pronounced hypo-

glycemic effect of PG was noted in group 2 rats on day 10: the number of animals with hyperglycemia was 2-fold lower than in the controls injected with ST. The effect was not retained after discontinuation of PG treatment, and by day 20 the parameters in this group virtually did not differ from the control (Fig. 1).

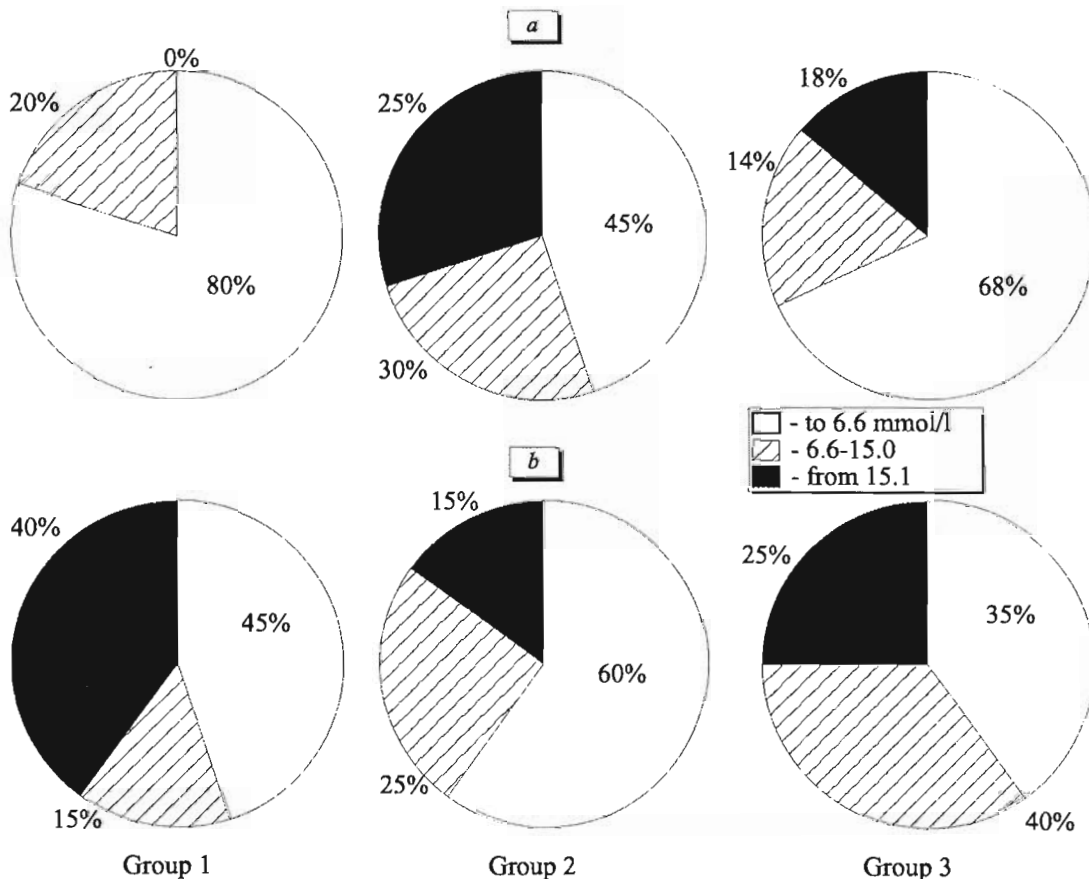


Fig. 1. Distribution of rats with ST-induced DM by the level of glycemia (mmol/liter) on days 10 (a) and 20 (b) of experiment.

The parameters reflecting the endothelial morphology and function changed significantly by day 20 after ST injection. Vascular wall permeability decreased in the majority of diabetic animals in comparison with the control. Pancragen did not modify this parameter of transcapillary exchange during intramuscular and oral treatment (Table 1). Vascular wall endothelial adhesion reduced in group 3 animals. Intramuscular injection of PG restored the endothelial adhesive characteristics, the values virtually not differing from those in normal animals, irrespective of the blood glucose level. Oral PG did not modify the endothelial adhesion (Table 1). Hence, long-term parenteral PG treatment produced a long-lasting endothelium-protective effect,

while oral treatment had a short-term hypoglycemic effect (observed only during the treatment).

REFERENCES

1. M. I. Balabolkin, *Kardiologiya*, No. 10, 74-87 (2000).
2. N. A. Gavrisheva, I. A. Mikhailova, T. D. Vlasov, *et al.*, *Microcirculation and Hemostasis Pathophysiology* [in Russian], Ed. N. N. Petrishchev, St. Petersburg (1998), pp. 246-262.
3. E. Adegate, P. Schattner, and E. Dunn, *Ann. N. Y. Acad. Sci.*, **1084**, 1-29 (2006).
4. E. Arikawa, C. Cheung, I. Sekirov, *et al.*, *Can. J. Physiol. Pharmacol.*, **84**, Nos. 8-9, 823-833 (2006).
5. N. S. Harhaj, E. A. Felinski, E. B. Wolpert, *et al.*, *Invest. Ophthalmol. Vis. Sci.*, **47**, No. 11, 5106-5115 (2006).
6. L. Rodella, B. D. Lamon, R. Rezzani, *et al.*, *Free Radic. Biol. Med.*, **40**, No. 12, 2198-2205 (2006).