BIOGERONTOLOGY

Study of Biological Activity of Lys-Glu-Asp-Trp-NH_2 Endogenous Tetrapeptide

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Biological effect of natural tetrapeptide Lys-Glu-Asp-Trp-NH_2 was evaluated during the ontogeny and in experimental streptozotocin-induced diabetes mellitus (a model of rapid experimental aging) by the sum of metabolic parameters characterizing apoptosis.

Key Words: *gerontology; geroprotectors; streptozotocin-induced diabetes mellitus; ontogeny*

Experimental studies confirmed the possibility of life prolongation by means of geroprotectors modulating the metabolic processes and thus producing an integral effect on aging organism, its metabolic processes and function, which prevents the development of age-associated diseases.

The search for geroprotectors among natural regulators of human functions is the most promising trend of research in gerontology [3,4]. Natural bioactive substances taken with food (this is the most physiological approach to regulation) [2] can play an important role.

Tetrapeptide Lys-Glu-Asp-Trp-NH_2 (KEDWa), a synthetic structural analog of the peptide isolated from cattle pancreas, is a prospective peptide bioregulator for pharmacological correction of metabolic disorders during aging. Hypoglycemic effect of this tetrapeptide was demonstrated [5,6].

We evaluated biological effect of KEDWa in the ontogeny and on the model of local pathology (streptozotocin-induced diabetes mellitus) associated with partial necrosis of pancreatic β-cells and formation of insulin insufficiency and (by the metabolic parameters characterizing apoptosis) regarded as a model of rapid experimental aging.

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MATERIALS AND METHODS

The study was carried out on male Wistar rats fed standard vivarium ration.

Three age groups were selected: 1, 3, and 9 months, corresponding to infant, young, and mature ages of animals. Each group consisted of 20 animals (10 controls and 10 experimental rats). Experimental groups received KEDWa water solution (400 μg/kg) orally for 10 days until sacrifice. The blood was collected into tubes with heparin and the liver was perfused with cold saline for 30-40 sec. All biomaterials were frozen at -80°C.

Diabetes was induced by single intraperitoneal injection of streptozotocin (Sigma-Aldrich; 50 mg/kg) [10]. Blood glucose level served as the control. For creating local disease model, 3-month-old male rats were divided into 3 groups, 10 per group: control, streptozotocin, and streptozotocin+KEDWa.

Standard metabolic values were measured in the serum on a ConeLab biochemical analyzer.

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TABLE 1. Ontogenetic Model (*M*±*m*)

Parameter	Animal age					
	1 month		3 months		9 months	
	control	KEDWa	control	KEDWa	control	KEDWa
Blood cis-hydroxyproline, μg/ml	16.1±0.9	16.6±2.1	23.1±1.3	25.1±1.1	20.0±0.3	16.9±1.8
Liver caspase-3, arb. units/h×g	2.61±0.12	2.06±0.17*	1.62±0.28	1.80±0.15	2.43±0.17	2.87±0.26
Liver cathepsin B, μmol/min×g tissue	0.63±0.02	0.57±0.01*	0.89±0.02	0.94±0.02	1.24±0.02	1.17±0.03
TNF-α, μg/ml	4.80±0.19	—	7.32±0.31	3.46±0.19*	3.42±0.34	3.39±0.17
IGF-I, μg/liter	320±29	301±57	588±101	476±65	414±81	604±49*

Note. Here and in Table 2: *$p<0.05$ compared to the control.

Liver caspase-3 was evaluated by a modified method using Apo Target Caspase-3/CPP32 Colorimetric Protease Assay kits (BioSource International). Liver samples were homogenized on ice in 50 mM Tris-HCl buffer (pH 7.4) in 1:2 weight/volume ratio. Dilution buffer (250 μl) was put into the reaction well, after which 50 μl homogenate and 5 μl 1 M dithiotreitol were added. The mixture was then incubated (37°C, 10 min) with shaking and the substrate (5 μl 4 mM DEVD-pNA) was added; the reaction was performed in the darkness. The measurements were carried out directly after the substrate addition and after 1 h at 405 nm *vs.* control (no substrate).

Activity of cathepsin B was measured as described previously [1].

The levels of TNF-α and insulin-like growth factor (IGF-I) were measured by enzyme immunoassay with Bender MedSystems kits with antibodies for laboratory rats.

The content of cis-hydroxyproline was measured by HPLC on an Agilent 1100 device with fluorescent detector [8] at the following separation parameters: Eclipse XDD-C18 5 μ column, 2.1×150 mm (Agilent); mobile phase: A: 0.02 M KH_2PO_4 (pH 7.8); B: methanol:acetonitrile:water (225:225:5 ml); derivatization agent FMOC 98%.

The results were statistically processed using Student's *t* test.

RESULTS

Plasma content of cis-hydroxyproline (aging biomarker) in 1-month-old rats was significantly (20-30%; $p<0.05$) lower than in 3- and 9-month old rats, which suggests that the ontogenetic model was adequate (Table 1).

In 1-month rats, injection of KEDWa led to a significant decrease in caspase-3 and cathepsin B activities, while at later stages of the ontogeny the injection of KEDWa led to a 53% drop of TNF-α level in 3-month-old rats and to a 46% elevation of IGF-I in 9-month-old rats, activities of cathepsin B and caspase-3 virtually not changing.

TABLE 2. Model of Streptozotocin-Induced Diabetes Mellitus (*M*±*m*)

Parameter	Group of animals		
	control (*n*=10)	streptozotocin (*n*=10)	streptozotocin+KEDWa (*n*=10)
Liver caspase-3, arb. units/h×g	1.28±0.08	1.83±0.11*	1.14±0.10
Liver cathepsin B, μmol/min×g tissue	0.92±0.03	1.58±0.02*	0.98±0.03
TNF-α, μg/ml	4.45±0.85	3.15±0.72	4.81±0.88
IGF-I, μg/liter	706±644	543±65*	521±29*
Glucose, mmol/liter	5.15±0.21	9.12±0.34*	5.03±0.24

The results of studies on the model of streptozotocin-induced diabetes mellitus (glucose concentration by 77% surpassed the control; Table 2) are presented. As was previously shown, injection of KEDWa led to normalization of blood glucose level in experimental animals [5,6]. It is noteworthy that significant activation of liver caspase-3 and cathepsin B (by 43 and 72%, respectively; $p<0.05$) was virtually completely abolished by injection of KEDWa, after which enzyme activities returned to normal.

No appreciable changes in the content of TNF-α under the effects of streptozotocin and KEDWa were detected, while the content of IGF-I decreased significantly (by 23%; $p<0.05$) after streptozotocin and did not change after KEDWa. These results suggest that the biological effect of KEDWa under conditions of natural biological aging is realized at the level of the IGF-I "survival factor" antiapoptotic effect induction. Activated receptors of this factor are involved in the transduction of the antiapoptotic signal [11]. The results obtained on the rapid aging model indicate an indirect biological effect of KEDWa consisting in suppression of the apoptotic enzymatic component at the level of cathepsin B endopeptidase performing its peculiar trigger functions [7,9] and the initiatory caspase-3 [11]. Analysis of the results gives good grounds to suggest that, apart from the known KEDWa interactions with the promotor ggcagg site of the preproinsulin gene DNA [6], the target of the tetrapeptide effect can be located at the level of proteolytic processing.

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