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Age-associated changes in the content of collagen, elastin and glycosaminoglycans in rats' skin and aorta

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Age-related changes in concentrations of the major connective tissue macromolecules – collagen, elastin, separate fractions of glycosaminoglycans – hyaluronic acid, chondroitin sulphates, heparan sulphate, and dermatan sulphate, as well as their proportion in both the skin and aorta were studied. An age-related increase of collagen content in both the skin and aorta and at the same time a decrease of elastin content were shown in these tissues. The content of hyaluronic acid, chondroitin sulphates, heparan sulphate and dermatan sulphate increases in the skin with age. Contrarily, in the aorta, a decrease in concentrations of hyaluronic acid and dermatan sulphate takes place with age, while those of chondroitin sulphates and heparan sulphate increase.

Key words: *age, connective tissue, collagen, elastin, glycosaminoglycans.*

Introduction

It has been shown that, at most part of ontogenesis, distinct age-related changes occur in concentrations of collagen, elastin, and glycosaminoglycans. These phenomena must lead to changes in the structural and functional properties of tissues.

Elasticity of the skin and aorta decreases with age, while their hardness or rigidity increases. It is especially important when considering mechanisms of development of age-related pathologies of the cardiovascular system, as for example, a change in the qualitative and quantitative composition of the wall of vessels, which occupies an important place in the mechanism of age-related development of sclerosis. However, it is not only changes in the content of collagen and elastin that determine the structure and respectively functional properties of the connective tissue. These properties are as well determined by polysaccharides – glycosaminoglycans, one part of which are structural components of proteoglycans, and the other – that of the amorphous "ground substance". Side chains of proteoglycans, made up of glycosaminoglycans, are interwoven with collagen fibres and create a dense three-dimentional network, which provides a selective passage of different macromolecules through it, as well as strong spatial structure (Al Jamal et al., 2001; Yanagishita, 1993). The connection between age, atherosclerosis and changes in concentration ratios of hyaluronic acid/chondroitin sulphates is generally accepted. However, there are few data that complexly reflect age-associated changes in the content of collagen, elastin and main glycosaminoglycans of the skin and aorta.

Materials and methods

Investigations were carried out using 3- and 24-months old male Wistar rats, which were forced to sleep by under-abdominal skin sodium thiopenthal administration (Stephen, 2002), after which they were decapitated. Aorta and dorsal skin samples were removed out. Those of the skin were cleaned up from hair and dermal fatty layer, defatted by, first, acetone (24 hours), and later diethyl ether (24 hours). The defatted and dried samples were weighed, cut into powder form in liquid nitrogen.

To extract skin and aorta glycosaminoglycans, a portion of the powdered sample was hydrolysed and then incubated in a sodium phosphate buffer solution (pH 7,4) that contained 1,0 mM of $CaCl_2$, 0,33 mM of $MgCl_2$, and collagenase (from *Clostridium histolyticum*, type I, Sigma-Aldrich, 200 units/ml). Incubation continued for 24 hours, at 37°C, under continuous mixing (Nagai et al., 2004).

To extract collagen from the powder, 2,5% of pepsin solution in 0,5 M CH_3COOH was added to it. Type I collagen was extracted from the obtained skin powder by 1 M solution of NaCl (Гарбузенко и др., 1997; Verziji et al., 2000), under continuous mixing, for 72 hours, at 4°C. The content of collagen and elastin in the extract were determined by way of hydroxyproline concentration in it, using the method (Утевская, Перский, 1982). At the end of extraction, collagen present in solution was precipitated by cooled up to 4°C solution of acetone, separated by centrifuging (4,500 g) and incubated enzymatically as mentioned above. After enzymatic processing, low-molecular weight peptides were precipitated by cooled up to 4°C 6% trichloroacetic acid. Residue was separated by centrifuging (4,500 g) and discarded, while the supernatant was used in subsequent extraction of glycosaminoglycans.

Glycosaminoglycans were precipitated by adding (1:10) 2% solution of cetylpyridinium chloride (Merck) to the supernatant. Residue of glycosaminoglycans was separated by centrifuging (4,500 g), washed

three times with cooled up to 4°C 95% solution of ethanol that had been saturated with NaCl (Silver et al., 2001). Washed residue was precipitated in 3 ml of 10% solution of sodium acetate, carefully mixed and fractionated (Меркурьева, Гусева, 1974).

Fractionation of isolated glycosaminoglycans was carried out by ion-exchange chromatography in a 0,9×50 cm thermostated column, filled with ion-exchange resin Dowex 1×2 (Cl^-form, 200–400 mesh, Sigma), and equilibrated with 0,2 M solution of NaCl, at 28°C. Speed of flow was 1ml/min. Eluation was step-by-step carried out by NaCl of different concentration gradients (Меркурьева, Гусева, 1974; Gillard, Mervyn, 1977). Each glycosaminoglycan fraction was eluated with 20 ml of corresponding concentration of NaCl solution. 2 ml of eluates were collected in each test tube. It has been shown, in preliminary experiments where standard samples of glycosaminoglycans (Sigma-Aldrich) were used, that exit of glycosaminoglycans from the column, in such conditions of eluation, was 87–100 %.

Contents of hyaluronic acid, heparan sulphate, and chondroitin sulphates were assessed by way of D-glucoronic acid concentration, determined in the aliquotes by carbazole reaction; that of dermatan sulphate by way of L-iduronic acid concentration, determined in the aliquotes by orceine method (Слуцкий, 1969).

Results and discussion

Results of the investigation carried out are presented in tables 1 and 2. The investigations showed, that an increase of the content of collagen in the skin (up to 32%) and in the aorta (up to 24%) takes place with ageing while at the same time the concentration of elastin in these tissues decreases by 53% and 20%, respectively (table 1). Such a change in the collagen and elastin content in these tissues leads to a change in the collagen/elastin ratio towards a reduction in elastin fraction, on the background of rise of collagen concentration (table 2).

As already known, elasticity of aorta walls, together with its high level of distensibility, is necessary in levelling up the speed of blood flow in the vessels; it is determined, firstly, by the elastinous fibres of the intercellular matrix. Collagenous fibres are less distensible and mainly cause hardness and stiffness of the vessel (Silver et al., 2001).

Table 1.

Concentrations of collagen, elastin and glycosaminoglycans in the skin and aorta of 3- and 24-months old rats

	Skin		Aorta	
	3-months old	24-months old	3-months old	24-months old
Collagen (mg%)	61,0±2,5	81,0±4,1*	25,0±1,1	31,0±1,2*
Elastin (mg%)	5,1±0,4	2,4±0,35*	16,0±0,83	13,0±0,71*
Hyaluronic acid (μg%)	282,4±18,2	372,3±57,0*	837,5±32,4	660,0±17,4*
Chondroitin sulphates (μg%)	254,2±21,7	946,8±30,9*	575,0±7,8	785,2±9,3*
Dermatan sulphate (μg%)	155,4±29,1	936,1±54,1*	1275,0±50,0	846,1±41,5*
Heparan sulphate (μg%)	20,1±3,5	241,1±61,7*	637,5±29,3	1038,4±20,6*

*Note: * indicates that changes in comparison with 3-months old rats are reliable (p<0,05).*

Table 2.

Ratios of HA/CS, HA/DS and collagen/elastin in the skin and aorta of 3- and 24-months old rats

	Skin		Aorta	
	3-months old	24-months old	3-months old	24-months old
Collagen: Elastin	1:0,083	1:0,03	1:0,64	1:0,41
Hyaluronic acid (HA): Chondroitin sulphates (CS)	1:0,90	1:2,54	1:0,68	1:1,20
Hyaluronic acid: Dermatan sulphate (DS)	1:0,55	1:2,51	1:1,52	1:1,28

It has here been shown by us, that the content of hyaluronic acid, chondroitin sulphates, heparan sulphate and dermatan sulphate in the skin increases with age. In the aorta, with age, a decrease in concentrations of hyaluronic acid and dermatan sulphate takes place, while those of chondroitin sulphates and heparan sulphate, contrarily, increase (table 1). Moreover, the concentration of each of the studied types of glycosaminoglycans changes differently by far. It can therefore be seen (table 2), that an increase in the

concentration of chondroitin sulphates in the aorta, with relation to that of hyaluronic acid, is observed with age. Contrarily, the concentration of dermatan sulphate decreases in the same organ.

It is known that an exclusive role in supporting toughness in the “family” of connective tissue polysaccharide complexes is assigned to hyaluronic acid, which together with elastin causes the resiliency of a tissue in general. When discussing the obtained results, attention must carefully be paid to the functions of higher polymeric glycans in the connective tissue, as agents that are capable of directly modifying the physical properties of collagenous fibres, thus affecting the mechanical properties of a tissue (hardness to tearing, elasticity module, volume occupied by a tissue).

The increase in chondroitin sulphates and collagen shown, on the background of a decrease in the concentration of hyaluronic acid and elastin, must lead to a decrease in the elasticity of the components of the connective tissue of the skin and aorta walls, and this also means an inadequate mechanical adaptation to factors of weight overloading. An age-related decrease of the content of hyaluronic acid and rising of chondroitin sulphates concentration may lead to a decrease in vessel wall metabolism, as a result of a decrease in the transport of nutrients. Glycosaminoglycan chains can change the conformation of a molecule, for example, enzymes, growth factors and other cytokines, and therefore, reduce their biological activity. Due to their sharply expressed anion character, chondroitin sulphates can, for example, react with cations, and this plays an important role in mineral metabolism (Hardingham, 1998; Kelly, 1998; Yanagishita, 1993). The rise, with age, of the amount of chondroitin sulphates leads to the stimulation of fibrin and calcium ion deposition. These processes, as already known, magnify during the development of atherosclerosis (Yanagishita, 1993).

Looking at the obtained results on changes in the content of hyaluronic acid in tissues, it is impossible not to take into account the role of this glycosaminoglycan in the regulation of tissue permeability. High molecular weight hyaluronic acid is a multi-acceptor of water, and this causes a high level of hydration of the connective tissue “ground substance”. Forming a three-dimensional, strongly hydrated jelly filter in the vessel wall, hyaluronic acid restricts the diffusion of high-molecular weight substances (proteins, with lipoproteins inclusive), from blood plasma into the tissue of aorta. Compared to hyaluronic acid, chondroitin sulphates have less molecular weight and hydrodynamic volume. Apparently, an age-related decrease in concentration ratio of hyaluronic acid/ chondroitin sulphates may lead to an increase in the porousness of the molecular “filter” of the vessel wall, and this makes it possible for large molecules from blood plasma, for example, fibrinogen, lipoproteins, to intensively enter the wall of the aorta. Oxidative low density lipoproteins, on the other hand, can stimulate the destruction of the glycocalyx, and this promotes the adhesion of leucocytes to the endothelium of vessels, thus advancing the conditions of atherosclerotic development (Vijayagopal, 1993).

As mentioned above, the carried out study showed, that heparan sulphate is deposited in the skin and aorta with age. It has been shown in a number of works, that an increase in heparan sulphate content in the walls of vessels leads to their thickening, narrowing (contraction) of lumen of capillaries and violation of their functions, in particular, due to a decrease in the adhesion of the endothelial cells. These violations of the extracellular matrix change the structure and function of vessels (a decrease in elasticity of the vessel wall, a change in response to vasodilation due to the action of nitrogen oxide, etc.), promote a more accelerated development of atherosclerotic process. Apart from these, an increase in the content of heparan sulphate is the reason behind the heightening of atrombogenity of the vessel wall, when deformed.

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Возрастные изменения содержания коллагена, эластина и гликозаминогликанов в коже и аорте крыс

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Исследовали возрастные изменения содержания основных макромолекул соединительной ткани – коллагена, эластина, а также отдельных фракций гликозаминогликанов – гиалуроновой кислоты, хондроитинсульфатов, гепарансульфата и дерматансульфата, и их соотношений в коже и аорте. Показано увеличение с возрастом содержания коллагена и уменьшение эластина в коже и аорте. Содержание гиалуроновой кислоты, гепарансульфата, хондроитинсульфата и дерматансульфата в коже увеличивается с возрастом. В аорте с возрастом наблюдается уменьшение концентрации гиалуроновой кислоты и дерматансульфата, в то время как концентрации хондроитинсульфатов и гепарансульфата увеличиваются.

Ключевые слова: *возраст, соединительная ткань, коллаген, эластин, гликозаминогликаны.*

Вікові зміни вмісту колагену, еластину і глікозаміногліканів в шкірі і аорті щурів

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Досліджували вікові зміни вмісту основних макромолекул сполучної тканини – колагену, еластину, а також окремих фракцій глікозаміногліканів – гіалуронової кислоти, хондроітинсульфатів, гепарансульфату і дерматансульфату та їх співвідношення в шкірі і аорті. Показано збільшення з віком вмісту колагену і зменшення еластину в шкірі і в аорті. Вміст гіалуронової кислоти, гепарансульфату і дерматансульфату та хондроітинсульфатів у шкірі збільшуються з віком. В аорті з віком спостерігається зменшення концентрації гіалуронової кислоти і дерматансульфату, у той час як концентрації хондроітинсульфатів і гепарансульфату збільшуються.

Ключові слова: *вік, сполучна тканина, колаген, еластин, глікозаміногліканы.*

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