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Age-dependent change in aldo-keto reductases composition in the blood of rats

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Abstract: Resistance of the organism to the stress injury changes during ontogenesis. In this regard, the incidence of cardiovascular, central nervous, endocrine systems, etc. increases at certain stages of individual development. Taking into account the fact that the development of stress-caused lesions is associated with accumulation of carbonyl products of free radical oxidation in cells, we have suggested that the one of the reasons for this phenomenon is age-related changes in the efficiency of the scavenging of endogenous aldehydes in the organism. In view of this fact, spectrum of aldo-keto reductases in blood serum of rats at different stages of ontogenesis was investigated by means of electrophoresis. Identical changes in the composition of aldo-keto reductases spectrum of blood in early immature age and in aging have been shown. Change in aldo-keto reductases spectrum modulates the role of reductive pathway of endogenous aldehydes scavenging in the organism. Thus, in child age and aging efficiency of scavenging of carbonyl products of free radical oxidation in cells is limited and especially when they are intensively produced. This results in increase of the susceptibility of the organism to oxidative stress.

Keywords: Aldo-Keto Reductases, Blood, Ontogenesis, Oxidative Stress, Stress

1. Introduction

It is known that aging and pubertal age are accompanied by increase in the incidence of cardiovascular disease, central nervous, endocrine systems, gastrointestinal tract, etc. [1 – 3]. Stress plays an important role in the occurrence of these diseases [4, 5]. The stimulation of free radical processes serves as an important part of the pathogenesis of stress damage of the visceral organs [3, 6, 7]. Such stimulation is followed by accumulation of a large number of carbonyl products, especially aldehydes in the cells [8, 9]. Being highly reactive, they exhibit pronounced cytotoxicity and genotoxicity [9 – 12], and cause cell damage. According to K. Uchida (2000), aldehydes act as a specific messenger of cell damage under conditions of free radical processes stimulation [8].

A special enzymatic system of protection against endogenous aldehydes has been formed in the evolution process. It includes aldehyde dehydrogenases, aldo-keto reductases, and glutathione transferases [11, 13, 14]. Aldo-keto reductases which catalyze reduction of aldehydes into less toxic alcohols are of particular importance [15, 16]. Free aldehydes, as well as their glutathione conjugates can undergo enzymatic reduction in the cells [17]. It should be noted that conjugation with glutathione is the main route of aldehydes catabolism [11, 13, 14].

Aldo-keto reductases are a large family of enzymes, which includes aldose reductases, aldehyde reductases, and carbonyl reductases. They differ from each other in structure, substrate specificity, and regulatory properties [18]. It can be assumed that alteration of the stress sensitivity of the organism at certain stages of individual development may be caused by limitation of the reductive catabolism of endogenous aldehydes due to age-dependent

change in aldo-keto reductases spectrum.

Taking this into account, the purpose of the present study was the investigation of aldo-keto reductases spectrum of blood of rats at different stages of ontogenesis.

2. Materials and Methods

30 Male Wistar rats of six different age groups were employed in the study: 1 – 0.5-months-old (immature); 2 – 1.5-months-old (early pubertal); 3 – 2-months-old (late pubertal); 4 – 3-months-old (early mature); 5 – 12-months-old (adult); 6 – 24-months-old (old) rats. The animals were kept on vivarium standard diet.

Aldo-keto reductases spectrum from blood serum was investigated using the electrophoresis method on agarose plates. A kit of plates and reagents for proteins separation Cormay Gel Protein 100 (Cormay) was used for this purpose.

Serum portion of 5 μL was placed on the plate. Fractionation of aldo-keto reductases was performed for 30 minutes at 100 V using tris-barbital buffer as an electrode buffer from set of plates.

Dyeing of plates after electrophoresis was carried out in a special staining solution for 30 minutes at 37 ^{0}C. Staining solution was prepared by dissolving 30 mg of NAD, 17.5 mg of nitro blue tetrazolium, and 1 mg of phenazine methosulfate in 45 ml of 0.1 M glycine-NaOH buffer (pH 10,0). After filtration, 0.486 ml of benzyl alcohol dissolved in 0.75 ml of methanol was added to the solution [19].

Stained plates were thoroughly washed, air dried and scanned on densitometer. Electrophoresis apparatus “Solar” and densitometer DM 2120 (Belarus) were used in the investigation.

Statistical calculations were done by Wilcoxon-Mann-Witney test.

3. Results and Discussion

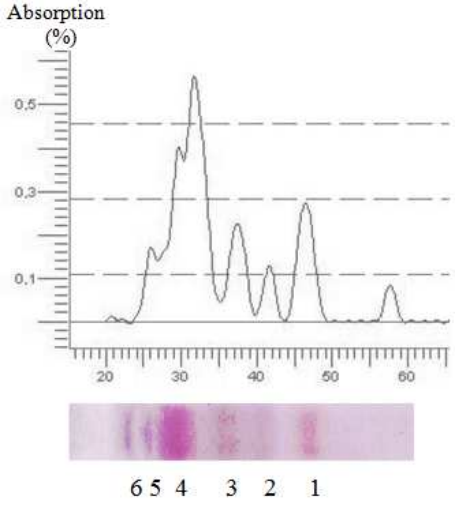

Figure 1. *Densitogram and photograph of the plate with separated fractions of aldo-keto reductases of the blood of rats. Numerals indicate the fractions numbers on electrophoregram.*

Studies have shown occurrence of four invariable aldo-keto reductases fractions in the blood of rats of different ages (Fig. 1). Fraction 4 is a predominant one in the spectrum. At the same time, portion of fractions 3 and 4 is less than that of fractions 1 and 4. Fractions 5 and 6 are the most electrophoretically mobile. But they can not be found in all investigated age groups of animals.

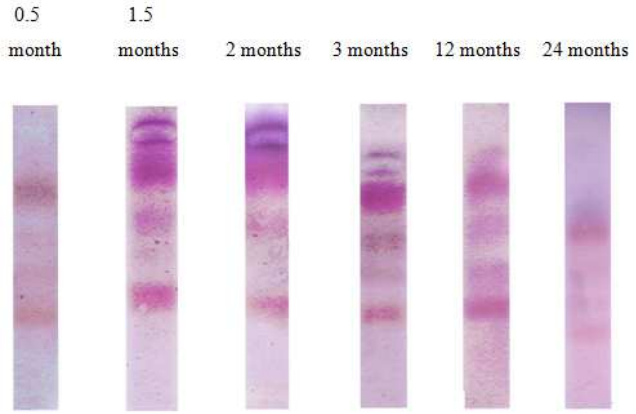

Figure 2. *Photograph of the plates with separated fractions of aldo-keto reductases of the blood of various age groups rats.*

Fig. 2 represents photos of plates with separated fractions of aldo-keto reductases of animals of different ages. It shows that aldo-keto reductases spectrum of blood changes during ontogenesis. This includes alteration of number of isozymes spectrum fractions and of ratio between the fractions.

Table 1. *Aldo-keto reductases spectrum of blood serum of rats of different ages (% of sum total)*

Age (months)	Electrophoretic fractions						
	start	1	2	3	4	5	6
0,5	9,2	28,7	15,0	5,0	42,0	-	-
1,5	2,6	18,6	7,5	11,9	37,1	22,0	-
2,0	5,3	18,7	7,9	14,4	26,4	27,0	-
3,0	3,2	16,0	5,3	13,3	39,8	15,2	7,1
12,0	5,5	19,2	11,9	14,4	29,1	19,9	-
24,0	8,4	13,9	17,3	21,8	38,4	-	-

Note: average values of 4 – 5 tests

Table 1 shows that immature and old rats (0.5-, and 24-months) have similar isozyme composition of blood aldo-keto reductases. Only four electrophoretic fractions of enzymes are revealed in animals of these age groups in the course of electrophoresis. However, isozyme spectra of aldo-keto reductases in immature and old rats are different. They have various portions of fractions 1 and 3 in the isozyme spectrum of blood. The old rats have half the proportion of fraction 1 (least mobile isozyme) and, conversely, three times higher the proportion of fraction 3 in the aldo-keto reductases spectrum than that in 0.5-months-old rats.

Pubertal and 3-4-month-old rats show increase of the portion of fraction 3 in aldo-keto reductases spectrum as compared with that of 0.5-months-old animals. Pubertal rats also show the presence of the additional 5th fraction. However, it is variable and is not detected in all animals of this age group. There are 6 fractions of aldo-keto reductases in the blood of 3-4-month-old rats and two of them possess highest electrophoretic mobility. Fractions 3 and 4 have equal portions in the aldo-keto reductases spectrum in 12-month-old animals and the 5th fraction becomes variable.

The results indicate the change of aldo-keto reductases spectrum of blood during ontogenesis. It is known that the isozymes have different catalytic and regulatory properties, and also different affinity to various substrates [20, 21]. In this regard, the alteration of the aldo-keto reductases spectrum will create prerequisites for changing in the efficiency of reductive utilization of endogenous aldehydes, and modulating of metabolic response of tissues to increasing rate of their synthesis during oxidative stress conditions.

Taking into account the significance of this pathway in the removal of cytotoxic carbonyl free radical oxidation products, we can assume that in child age and aging efficiency of scavenging of carbonyl products of free radical oxidation in cells is limited and especially when they are intensively produced. This, in turn, increases the susceptibility of the organism to adverse external factors implementing negative effect through the formation of oxidative stress. Probably it acts as one of the reasons for decreasing of resistance of the organism to the stress damage at these stages of ontogenesis [22, 23].

Changing in aldo-keto reductases spectrum during ontogenesis may be due to age-dependent peculiarities in the regulation of expression of genes of enzymes which catalyze the reduction pathway of endogenous aldehydes scavenging at certain stages of individual development in the organism. It is significant that early immature rats (0.5 months) and aged rats (24 months) have similar composition of the blood aldo-keto reductases spectrum. Probably it is due to the age specificity of functioning of the endocrine system, because hormones act as natural regulators of gene expression. Moreover, previously we have reported about potential role of testosterone in the change of production of aldo-keto reductases isozymes [24]. It should be noted that there are some common features in regulation of production and secretion of this hormone in early postnatal development and in aging. Child (immature) age is characterized by endocrine system functional immaturity and the absence of synthesis and secretion of testosterone, while late ontogenesis is characterized by manifestations of involution of gonads and, accordingly, by sharp decrease in the level of secretion of this hormone. Still, it should be noted, that the regulation of synthesis of aldo-keto reductases isozymes is provided not only by testosterone, but also by other hormones.

However, the nature of the relationship between the state of endocrine regulation and aldo-keto reductases spectrum of the organism remains unclear. Its revealing will open new perspectives in developing new approaches to prevention of age-related pathologies associated with the direct impact on the reducing pathway of endogenous aldehydes catabolism. This in turn will allow to develop a new approach to the prevention and treatment of stress-induced diseases. This approach will aim to increase efficiency of catabolism of free radical oxidation carbonyl products in the organism.

4. Conclusions

1. Four invariable electrophoretic fractions of aldo-keto reductases are revealed in the blood of rats of different ages.
2. Two additional aldo-keto reductases fractions possessing higher electrophoretic mobility appear at certain stages of ontogenesis. Ratio between different fractions also changes.
3. Compositions of aldo-keto reductases of blood are very similar in 0.5-month and 24-months-old rats.
4. Age-dependent change of aldo-keto reductases spectrum influences the role of reductive catabolic pathway of free radical oxidation carbonyl products in the organism.

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