Comparative Study of Age-Related Activity of Monoamine Oxidase-B, Antioxidant Defense Enzymes, and Tolerance to Oxidative Stress in Various Segments of Human Spinal Cord

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Activities of monoamine oxidase B, Cu-Zn-dependent superoxide dismutase (SOD), and catalase, the concentration of enzyme-active ceruloplasmin, and resistance of the nerve tissue to oxidative stress were examined in spinal cord preparations from humans (n=43) died at the age of 21-92 years. Age-related activation of monoamine oxidase B was found only in thoracic segments, while age-related decrease in SOD activity was demonstrated in thoracic segments and cervical intumescence of the spinal cord. Age-related accumulation of enzyme-active ceruloplasmin was observed in all segments of the spinal cord. Activation of catalase and increased sensitivity to oxidative stress were observed in the cervical and lumbosacral intumescences. Heterotopic changes in the examined indices suggest that activity of monoamine oxidase B cannot serve as a universal factor of age-related changes in antioxidant defense in the spinal cord and its sensitivity to oxidative stress.

Key Words: *spinal cord; monoamine oxidase B; antioxidant defense; sensitivity to oxidative stress*

Monoamine oxidase (MAO; EC 1.4.3.4) catalyzes intracellular catabolism of monoamine neurotransmitters and plays a key role in neurochemical regulation of behavior [5]. H_2O_2, a substrate-independent products of MAO, inhibits Cu-Zn-dependent superoxide dismutase (SOD, EC 1.15.1.1) [4] and triggers free-radical damage to biological membranes [8]. MAO-B is a predominant form of this enzyme in human brain [11]. Activity of this enzyme increases with age in parallel with age-related decrease in SOD activity and LPO intensification [1,10]. These features suggest the existence of a MAO-B-dependent mechanism of suppression of the antioxidant defense (AOD) in aging brain [1]. The opposite age-related changes in MAO-B and SOD activities were clearly demonstrated for brainstem (especially, for bulbar structures) [1]. However, there are no data on the role of MAO-B in the regulation of AOD enzymes in the spinal cord.

Here we compared age-related changes in activity of MAO-B and enzymes eliminating reactive oxygen species, and the resistance of nerve tissue to oxidative stress in various spinal cord segments in humans.

MATERIALS AND METHODS

Spinal cord specimens were obtained from 43 humans (28 males and 15 females, 22-92 years) dies from injuries or diseases not directly involving CNS. The specimens were collected within 12 h postmortem. Four age groups were selected: adults I (21-35 years for women and 22-35 years for men), adults II (36-55 years for women and 36-60 years for men), elderly (56-74 years for women and 61-74 years for men), and senile (over 75 years).

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Activities of MAO-B and AOD enzymes including SOD, catalase (CAT, EC 1.11.16), and enzyme-active ceruloplasmin (EACP, EC 1.16.3.1), and the resistance to oxidative stress were estimated in tissue homogenates isolated from cervical and lumbosacral intumescences and thoracic segments of the spinal cord. MAO-B activity was measured by spectrophotometry using benzylamine hydrochloride as the substrate [2,3], activity of Cu-Zn-dependent SOD was determined colorimetrically [9], and CAT activity was evaluated by the rate of H_2O_2 utilization in the incuba-tion medium [7]. The content of EACP was estimated by a modified method [6] with incubation time prolonged to 180 min. To examine tolerance of lipids to free-radical oxidation, we assessed accumulation of the substances, which reacted with 2-thiobarbituric acid (TBA) in 2.5% spinal cord tissue homogenates incubated for 1 h *in vitro* in open air at 37°C [2]. The integral index of tolerance to oxidative stress *in vitro* (oxidizability) was expressed in percent of increment of TBA-reactive species compared to initial level.

The data were processed statistically using Student's *t* test and non-parametric Mann—Whitney, Wald—Wolfowitz, and Kolmogorov—Smirnov tests.

RESULTS

Our study confirmed qualitative identity of age-related changes in activity of MAO-B and AOD enzymes in human spinal cord (Table 1) and brain [1]. MAO activity increased with age in thoracic segments of the spinal cord, and attained a maximum in elderly and senile individuals (184-187% of the corresponding value in adult I group). It is noteworthy that the increase in MAO-B activity in the spinal cord was less pronounced than in most cerebral structures [1]. Only a tendency to age-related increase in MAO-B activity was observed in the cervical and lumbosacral intumescences. The rate of age-related decrease in SOD activity in the spinal cord (Table 1) was also lower than in the brain: in some cerebral structures SOD activity dropped 2-fold in adults II [1]. A significant decrease in activity of this enzyme was observed only in the cervical intumescence and thoracic segments of senile individuals (by 1.58-2.2 times in comparison with adult I group). The lowest activity of spinal CAT was observed in adult I group (Table 1). More than 2-fold increase in CAT activity was revealed in the cervical and lumbosacral intumescences of adults II, and persisted in elderly and senile individuals. In

TABLE 1. Age-Related Changes in Activities of MAO-B (per 1 mg tissue), SOD and CAT (per 1 g tissue), Content of EACP (per 10 g tissue), and LPO Intensity in Various Structures of Human Spinal Cord (*M*±*m*)

Spinal cord subdivision	Age			
	adult I	adult II	elderly	senile
Cervical intumescence				
MAO, nM/min	0.0811±0.0215	0.1367±0.0214	0.1530±0.0305	0.1430±0.0265
SOD, units/min	0.0347±0.0044	0.0266±0.0031	0.0231±0.0038	0.0219±0.0036*
CAT, nM/sec	0.5494±0.1328	1.1919±0.1072*	1.7265±0.1665*+	1.8632±0.4883*
EACP, mg	1.1473±0.1618	1.4702±0.3824	2.0594±0.2759	3.0845±0.2970*+ϵ
oxidizability, %	33.00±7.73	53.50±11.55	73.5±19.1	100.40±15.01*+
Thoracic segments				
MAO, nM/min	0.0669±0.0142	0.0996±0.0135	0.1249±0.0299+	0.1229±0.0169*
SOD, units/min	0.0219±0.0041	0.0217±0.0072	0.0215±0.0078	0.0100±0.0018*
CAT, nM/sec	1.0027±0.2238	1.6963±0.2463	1.7175±0.5265	2.4202±0.5112*
EACP, mg	1.4779±0.1704	2.2519±0.4636	2.5494±0.4216	3.1154±0.1168*
oxidizability, %	39.70±3.18	37.80±11.06	57.20±11.48	119.8±68.47
Lumbosacral intumescence				
MAO, nM/min	0.0855±0.0179	0.0955±0.0091	0.1049±0.0248	0.1466±0.0289
SOD, units/min	0.0269±0.0073	0.0233±0.0053	0.0228±0.0029	0.0170±0.0057
CAT, nM/sec	0.9412±0.1944	2.1243±0.2460*	1.8241±0.4359*	2.1693±0.1377*
EACP, mg	0.4084±0.2209	0.9217±0.3414	1.9136±0.3786*	2.1469±0.2039*+
oxidizability, %	30.30±7.73	36.10±5.23	60.40±9.57*	51.50±17.58

Note. $p<0.05$: *compared to adults I, +compared to adults II, °compared to elderly individuals.

elderly individuals, CAT activity in the cervical intumescence significantly surpassed the corresponding values not only in adult I, but also in adult II group. The senile age group was characterized by increment of EACP content in all three subdivisions of the spinal cord. In this group, the lowest increment of EACP content (by 2.69 times) was observed in the cervical intumescence, while the greatest increase (by 5.25 times) was noted in the lumbosacral intumescence, where a significant increment in EACP content was established not only in senile, but also in the elderly age group. Similar but less pronounced changes in CAT activity and in the content of EACP in the brain were found previously [1].

Thus, the spinal cord is characterized by a poorly expressed age-related increment in MAO-B activity and a moderate inhibition of SOD against the background of pronouncedly expressed increase in the content of EACP and CAT activity. Probably, the increase in the content of EACP and CAT activity is directed toward compensation of the age-related inhibition of SOD in the spinal cord of aging human. This compensation is especially efficient in the thoracic subdivision of the spinal cord, where, despite a significant increase in MAO activity and decrease in SOD activity, no significant rise in oxidizability was observed in elderly and senile groups (Table 1). The age-related redistribution in AOD enzymes was less effective in the lumbosacral intumescence. Despite increased CAT activity, absence of significant ontogenetic decrease of SOD activity, and increased MAO-B activity, LPO intensity in this spinal subdivision increased 2-fold in elderly group in comparison with adult I group. The most pronounced deficiency was observed in the cervical intumescence of senile individuals: oxidizability increased 3-fold compared to adult I group, and by 27% compared to elderly individuals. The decrease in tolerance to oxidative stress in the cervical intumescence was developed in senile age group in parallel with increases in EACP level and CAT activity against background statistical stability of MAO-B activity.

Evident heterotopic pattern of ontogenetic shifts in LPO intensity, MAO and SOD activity in the spinal cord does no allow us to consider MAO-B-dependent [1] H_2O_2-mediated SOD [4] inhibition as a universal mechanism of suppression of AOD system and age-related increase in the sensitivity of the nerve tissue in the spinal cord to oxidative stress. It cannot be excluded that most pronounced age-related changes in AOD activity and lipid oxidizability in cervical and lumbosacral intumescences are caused by their greater functional load in comparison with thoracic segments of the spinal cord.

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