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MAO Inhibitors in Aging: Can They Serve as Protective Agents in Cardiac Tissue Against Oxidative Stress?

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Abstract: Biological damage mediated by reactive oxygen species has been increasingly recognized in aging phenomena in recent decades. The increase in catecholamine metabolism is yet another physiological change in aging, predisposing the organism to supranormal hydrogen peroxide production as a by-product. This study was undertaken to investigate the alterations in oxidant-antioxidant status in rat cardiac tissue during aging. The effects of acute administration of monoamine oxidase (MAO) inhibitors on this status were also investigated to find possible preventive strategies in conjugate aging. MAO activities, malondialdehyde MDA and diene conjugate levels as well as superoxide dismutase and catalase activities were determined in the cardiac tissues of young and aging groups, each consisting of 10 rats. MAO inhibitors (deprenyl and pargyline) were administered to another two groups of young and aging animals (10 in each group) and the same parameters were also determined in these groups. Cardiac tissue MAO activities ($p < 0.005$) as well as diene conjugates ($p < 0.0001$) and MDA levels ($p < 0.005$) were found to be significantly

higher in aging compared to young control groups. The increases in these parameters were all correlated. Acute MAO inhibitor administration caused significant decreases in MAO activities in both young ($p < 0.0005$ with deprenyl and $p < 0.0001$ with pargyline) and aging groups ($p < 0.0001$ with deprenyl and $p < 0.00001$ with pargyline). Parallel to this finding, diene conjugate and MDA levels were also found to be decreased after MAO administration. Although the aging control group had manifested a decrease in antioxidant enzymes; SOD and catalase ($p < 0.05$, both), acute administration of MAO inhibitors did not induce any significant changes in these parameters. In conclusion, our experimental data suggest that catecholamine metabolism and MAO activity may play a role in the pathological alterations in the aging cardiac tissue by serving as an important source for increased lipid peroxidation, and inhibition of this activity may play a role in limiting the clinical sequelae of these alterations.

Key Words: Monoamine oxidase inhibitors, lipid peroxidation, antioxidant enzymes, cardiac tissue, aging.

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Introduction

As the elderly population increases due to the advances in the modern world, a knowledge of the physiology and fundamental causes of aging, in particular, the role of reactive oxygen species (ROS) therein, is a prerequisite in understanding geriatric problems. The intricate causes of the aging process are still a matter of extensive speculation giving rise to many theories (1, 2).

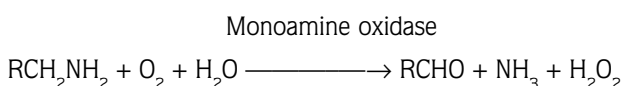
Biological damage mediated by ROS has increasingly come to be recognized in recent decades as an important factor in human disease, ranging from inflammatory conditions to cancer. ROS, generated continuously in the

cells either as accidental by-products of metabolism or deliberately during the presence of phagocytosis, ionizing radiation, drugs capable of redox cycling, or metabolism of xenobiotics are also considered to be critical factors involved in aging processes (3, 4, 5).

Cells have developed a comprehensive array of antioxidant defences to limit the formation or deleterious effects of ROS. Among these are enzymes that decompose peroxides, proteins that sequester transition metals and a range of compounds that scavenge free radicals (4, 5).

The increases in catecholamine metabolism have also

received attention among the physiological changes in aging, and monoamine oxidase (EC 1.4.3.4; amine: oxygen oxidoreductase (deaminating) (flavin containing) (MAO), a key enzyme in this process, has recently been studied extensively (6). MAO catalyzes the oxidative deamination of amines according to the overall reaction, as shown below:



Hydrogen peroxide (H_2O_2), the readily diffusible by-product of MAO activity, is an example of ROS which can cross cell membranes, causing damage at sites distant from its area of generation. This molecule, although not a free radical, falls into the category of ROS and can act as either a destructive species by itself or as an intermediate in the production of the hydroxyl radical (OH^*). Recent studies have shown that H_2O_2 is an important mediator of cardiac ischemia-reperfusion injury causing perturbations in cardiomyocyte carbohydrate metabolism and heart-muscle energy balance (7).

MAO inhibitors (mainly deprenyl) have been widely used in depression and Parkinson's disease due to their effects on catecholamine metabolism. These agents have been shown to reduce the catabolism of dopamine, exert an endogenous amphetamine-like tonic effect and, most importantly, suppress the generation of endogenous neurotoxic free radicals (8).

Based on the fact that free radicals and their effects (mainly lipid peroxidation) might have significant roles in the aging organism, this study was undertaken first to examine the role of oxidative stress and antioxidant enzymes with regard to the increased MAO activity in the aging rat heart. Since MAO activity is a possible source of H_2O_2 , the second aim of the study was to investigate the variations in the related parameters induced by the acute administration of MAO inhibitors.

Materials and Methods

Chemicals

All chemicals were analytical grade. EDTA, kynuramine, thiobarbituric acid, epinephrine, and bovine erythrocyte SOD standard and bovine serum albumin standard were purchased from Sigma. While cyclohexan was obtained from Riedel de Haen, all the rest of the chemicals (saccharose, KH_2PO_4 , Na_2HPO_4 , K_2HPO_4 , 4-Hydroxyquinoline, NaOH, TCA, chloroform, methanol,

Na_2CO_3 , NaHCO_3 , hydrogen peroxide, Na-K tartarate, CuSO_4 , and the reactivities of Folin-chiocalteu) were purchased from Merck & Darmstadt Co.

MAO inhibitors, Deprenyl (selegiline) was purchased from Kocak Drug Company (Turkey), while pargyline was purchased from Sigma.

Animals

Eight groups of male Swiss Albino rats were formed, as shown in Table 1.

Preparation of the heart tissue

The animals were decapitated and their hearts quickly excised and washed thoroughly in cold (4°C) saline. The heart tissue was minced to three fractions; while a homogenate for MAO activity was prepared with EDTA/saccharose (1/10, w/v), a homogenate with phosphate buffer (KH_2PO_4 , $\text{Na}_2\text{HPO}_4 \cdot 2 \text{H}_2\text{O}$, 50 mM, $\text{pH}=7.0$) (1/10, w/v) was prepared for diene conjugates, SOD and catalase determinations. For MDA analysis, the last fraction of the tissue was homogenized in distilled water (2/1, w/v) and later with TCA, 5%. Supernatants were obtained from all homogenates, after being centrifuged at 1500 g for 10 minutes. All procedures were conducted on ice, analysis being performed on the same day.

Methods

MAO activity was determined fluorometrically (by Aminco-Bowman spectrofluorometer) by the modified method of Kraml (9), results being given as nmol/mg prot/hr.

For the determinations of the diene conjugates, Bueger's spectrometric method was used (10), results being given as nmol hydroperoxide/mg protein.

MDA determinations were conducted by Slater and Sawyer's modification of the MDA-TBA test (11) originally proposed by Yagi, the concentration of MDA (in nmol/gr. tissue) being calculated using an extinction coefficient of 153 000/Mol/cm.

SOD and catalase activities were determined by the methods of Misra of Fridovich (12) and Aebi Luck (13) respectively, the results being given as U/mg protein.

Protein determinations were performed by the method of Lowry (14).

Statistical analysis

Statistical analysis was performed by MICROSTA and ANOVA programs. For comparison of the means, the student's t test was used, and for intergroup

Physiologic young group	n=10	2-3 months (150-200 gr)	no agent was given
Physiologic aging group	n=10	16-18 months (250-300 gr)	no agent was given
Young placebo group	n=10	2-3 months (150-200 gr)	2.5 cc isotonic saline given intraperitoneally 1 1/2 hours before decapitation
Aging placebo group	n=10	16-18 months (250-300 gr)	2.5 cc isotonic saline given intraperitoneally 1 1/2 hours before decapitation
Young deprenyl group	n=10	2-3 months (150-200 gr)	25 mg/kg deprenyl dissolved in 2.5 cc isotonic saline given intraperitoneally 1 1/2 hours before decapitation
Aging deprenyl group	n=10	16-18 months (250-300 gr)	25 mg/kg deprenyl dissolved in 2.5 cc isotonic saline given intraperitoneally 1 1/2 hours before decapitation
Young pargyline group	n=10	2-3 months (150-200 gr)	25 mg/kg pargyline dissolved in 2.5 cc isotonic saline given intraperitoneally 1 1/2 hours before decapitation
Aging pargyline group	n=10	16-18 months (250-300 gr)	25 mg/kg pargyline dissolved in 2.5 cc isotonic saline given intraperitoneally 1 1/2 hours before decapitation

Table 1. The characteristics and treatment protocols of the experimental groups.

comparisons, analysis of variance was used, followed by the Newman Keuls multiple. The correlations between the parameters were determined by paired correlation tests.

Results

In comparison to young control tissue, aging cardiac tissue showed significant increases ($p < 0.005$) in MAO enzyme activities. Acute administration of MAO inhibitors caused a significant reduction in these activities in both the young ($p < 0.0005$ with deprenyl and $p < 0.0001$ with pargyline) and aging ($p < 0.0001$ with deprenyl and $p < 0.00001$ with pargyline) groups. The overall findings for MAO enzyme activities are presented in Table 2.

In the second phase of the study where lipid peroxidation parameters were investigated, a significantly

higher increase was observed in the aging group ($p < 0.0001$ for diene conjugates and $p < 0.005$ for MDA) than in the young control group (Table 3).

When the correlation between lipid peroxidation and MAO activities was investigated, it was found that MDA showed a significant correlation ($r = 0.9358$) with MAO in the aging control group. Similarly, the correlation between diene conjugates and MAO was also significant ($r = 0.9668$) in this group.

The administration of acute doses of deprenyl resulted in significant decreases in diene conjugates and MDA levels ($p < 0.005$ in both young and aging groups as compared to controls); however, a more significant reduction in the MDA levels of the aging group ($p < 0.0005$ in both) was detected with pargyline. These decreases in MDA and diene conjugate levels after

Groups			MAO (nmol/mg protein)	SD	p
Physiologic control	Young	n=10	53.35	20.35	p<0.005
	Aging	n=10	76.19	12.93	
Placebo control	Young	n=10	55.72	18.56	p<0.005
	Aging	n=10	72.96	13.05	
Deprenyl	Young	n=10	21.54	4.94	p<0.0005
	Aging	n=10	21.61	3.86	
Pargyline	Young	n=10	18.12	6.35	p<0.0001
	Aging	n=10	23.31	4.96	

Table 2. Cardiac tissue MAO activities of all groups.

Groups	MDA*	SD	Diene Conj.**	SD		
Physiologic control	Young	n=10	5.35	1.53	31.98	4.20
	Aging	n=10	20.72	6.92	46.56	17.93
Placebo Control	Young	n=10	5.17	1.65	30.96	5.46
	Aging	n=10	18.97	5.43	32.76	6.53
Deprenyl	Young	n=10	3.72	0.57	25.19	5.70
	Aging	n=10	11.86	3.74	25.47	6.74
Pargyline	Young	n=10	3.65	0.87	24.97	2.47
	Aging	n=10	10.32	2.68	25.96	6.97

Table 3. Diene conjugate and MDA levels of all groups.

*MDA expressed in nmol/g tissue.

**Diene conjugates expressed in nmol/mg protein.

administration of MAO inhibitors correlated with the decreases in MAO levels, as exemplified in Figures 1 and 2.

As to antioxidant enzyme activities, SOD and catalase activities showed a decrease ($p<0.05$) in the aging group as compared to the young controls. Administration of MAO inhibitors induced only statistically insignificant increases in these antioxidant enzymes.

Discussion

This study provides evidence of increased cardiac tissue MAO activities in the aging organism, parallel to the increases observed in MDA and diene conjugates, which are reliable lipid peroxidation parameters. Inhibition of

the MAO activity in rat cardiac tissue by acute administration of MAO inhibitors not only limited the activity of this enzyme, but also the increase in oxidative stress. These results confirm the role of MAO activity as an important source in the free radical theory of aging.

Recently, pathological alterations in the cardiac function of the aging organism have received much attention, and these changes have been partly ascribed to the presence of increased ROS. Reduced effectiveness of β adrenergic stimulation in relaxing vascular muscle leads to increased ischemia-reperfusion periods and oxidant stress in cardiac tissue, the overall effects being reflected in sarcolemmal and mitochondrial membrane changes, alterations in carbohydrate metabolism and myofibrillary creatine kinase activity. These effects could explain the development of heart dysfunction, which seems to be an

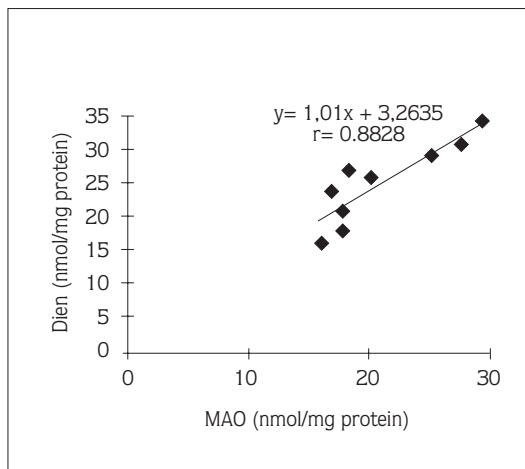


Figure 1. The correlation between MAO and diene conjugates in young groups given deprenyl.

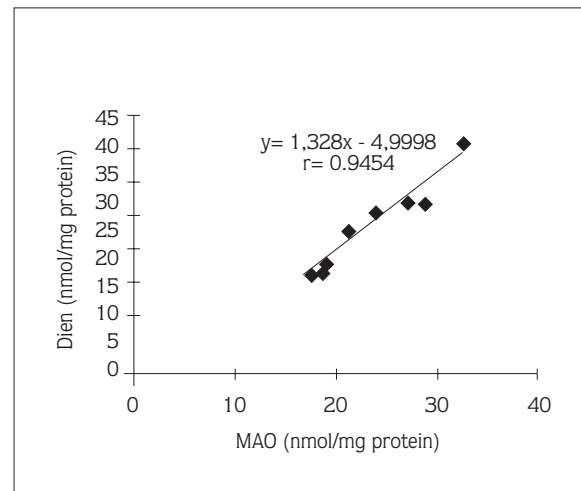


Figure 2. The correlation between MAO and diene conjugates in the aging group given pargyline.

inevitable progressive change in physiological aging (15, 16).

Since lipid peroxidation is one of the most important organic expressions of oxidative stress induced by ROS, various analytic methods have been tried in recent decades. Of these, MDA and diene conjugates appear to be reliable markers. The peroxidation of polyunsaturated fatty acids (PUFAs) is the major source in MDA formation, since MDA is formed during the last stages of the breakdown of endoperoxides (17). Conjugated dienes, on the other hand, are other lipid peroxidation markers which form a sort of transition state between lipid radicals and peroxy radicals.

The possible sources of free radicals in cardiac tissue are xanthine oxidase, mitochondrial respiration, metabolism of arachidonic acid and invading leucocytes, as well as catecholamine oxidation. Of these, one enzymatic reaction that may be enhanced in cardiac tissue upon aging is the deamination of catecholamines catalyzed by MAO. In the aging organism, the increased catecholamine availability during ischemia-reperfusion and the subsequent hyperoxia may lead to supranormal hydrogen peroxide production. MAO activity becomes oxygen-dependent during ischemia when catecholamine concentrations increase. Thus, when oxygen is reintroduced on reperfusion, a burst of H_2O_2 production may occur.

Increased H_2O_2 production and the accompanying oxidative stress could have various pathological changes in the cardiac tissue, and H_2O_2 may produce cellular damage directly or indirectly. Direct damage may result from inactivation of enzymes (ATP synthetase

mitochondrial way and glyceraldehyde 3 phosphate dehydrogenase glycolytic way) or the oxidation of NADH or sulfhydryl groups. On the other hand, H_2O_2 also can exert cytotoxicity indirectly via formation of the highly reactive OH^\bullet catalyzed by transition metals (7, 18, 19). In periods of increased oxidative stress, ATP reduction, functional deterioration, lipid peroxidation and myocardial tissue damage finally results. The injury potential of H_2O_2 -induced oxidative stress on heart muscle carbohydrate metabolism has been shown. H_2O_2 may also disturb heart muscle energy balance. It must also be stressed that H_2O_2 is an important mediator of the tissue injury associated with myocardial ischemia-reperfusion (9).

MAO, as a source of H_2O_2 was evaluated during ischemia-reperfusion in vivo in the rat brain by Simonson et al. who have concluded that MAO is an important source of H_2O_2 generation early in brain reperfusion (18). Going one step further, Zhang and Piantadosi have shown that inhibition of MAO with pargyline protected rats from convulsions and led to decreased H_2O_2 production in the brain (20).

Although our current work does not directly investigate H_2O_2 levels which would be the by-product of MAO metabolism, lipid peroxidation parameters were determined to prove the increase in oxidant stress. Our results, which manifested increased MAO activity with correlated increases in lipid peroxidation in the aging rat heart, support the hypothesis that catecholamine oxidation is an important source of increased oxidant stress. The aging organism also manifested lower levels of antioxidant capacity, another altered metabolism that

aids in the manifestation of oxidative stress. Although the acute administration of MAO inhibitors caused a reduction in MAO activities in heart tissue, which paralleled the reduction in lipid peroxidation parameters, no significant change in antioxidant status was determined. This observation was attributed to the acute administration of the agents. Long-term administration of the MAO inhibitors and its effects on oxidant and antioxidant status is still a challenging area of investigation in which our current work is now being carried out.

Elucidating the presence and nature of the aging process in the cardiovascular system is a formidable task. By this study, it has been shown that aging rat heart

tissue has increased MAO activity, which might be a crucial source of the increased lipid peroxidation and oxidative stress. Inhibition of MAO activity in the rat cardiac tissue has proved to be a way of decreasing the supranormal oxidative stress in the aging organism and perhaps exerting protective effects on survival. In conclusion, our experimental data suggests that catecholamine metabolism and MAO activity may play a role in the pathological alterations in aging rat cardiac tissue, and that inhibition of this activity may have a role in limiting the clinical sequelae of these alterations.

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