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THE EFFECT OF A MONOAMINE OXIDASE (MAO)-B INHIBITOR; PARGYLINE ON OXIDANT STRESS/ ANTIOXIDANT STATUS IN AGING RAT TISSUES

YAŞLANAN SIÇAN DOKULARINDA OKSİDAN STRES VE ANTİOKSİDAN SAVUNMA DURUMUNA MONOAMİNOKSİDAZ (MAO)-B İNHİBİTÖRÜ PARJİLİNİN ETKİSİ

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Anahtar sözcükler: MAO, yaşlanma, SOD, katalaz, MDA, dien konjugatları, lipid peroksidasyonu, antioksidant enzimler, oxidan stres

Key words: MAO, aging, SOD, catalase, MDA, diene conjugates, lipid peroxidation, antioxidant enzymes, oxidant stress

SUMMARY

Aging is the accumulation of changes responsible for the progressive increases in the chance of disease and death. Among the theories proposed to account for this process; “the free radical theory of aging” has been widely accepted. Meanwhile, the increase in catecholamine metabolism is another physiological alteration related to this process, predisposing the organism to enhanced H2O2 production. The aim of this study was to assess the role of oxidant stress and antioxidant status in the aging process by determining MAO activity, lipid peroxidation products (LPPs) – diene conjugates and MDA levels, antioxidant enzymes – SOD and CAT in young and aging rat tissues and to investigate the effect of pargyline, a MAO-B inhibitor in reducing oxidant stress via inhibition of MAO. In general, significant increases in tissue MAO activitiy and LPPs were noted in aging rats, positively correlated wıth each other and significantly decreased after pargyline administration. In conclusion, the inhibition of MAO activity may attenuate the process of aging by reducing increased lipid peroxidation and concomitant oxidant stress.

ÖZET

Yaşlanma, hastalık ve ölüm riskinde ilerleyen artıştan sorumlu değişikliklerin bir toplamıdır. Bu olguyu açıklamaya çalışan teoriler içinde ‘yaşlanmanın serbest radikal teorisi’ oldukça çok kabul görmüştür. Kateşolamin metabolizmasında artış bu olguya eşlik eden fizyolojik bir değişimdir ve organizmada artmış hidrojen peroksit (H2O2) üretimine neden olur. Bu çalışmanın amacı; oksidan stres ve antioksidan savunma durumunun yaşlanma üzerindeki etkisini; genç ve yaşlı sıçan dokularında MAO aktivitesi, lipid peroksidasyon ürünleri (LPPs) – dien konjugatları ve MDAdüzeyleri, antioksidan enzimler – SOD ve CAT enzimlerinin ölçümü yoluyla belirlemek ve MAO-B inhibitörü olan parjilinin MAO inhibisyonu yolu ile oksidan stressi azaltıcı etkisini araştırmaktır. Genel olarak yaşlı sıçanlarda doku MAO aktivitelerinde ve LPP’lerinde birbirleri ile korele bir artma ve parjilin uygulaması sonrası belirgin bir düşme görülmüştür. Sonuç olarak MAO aktivitesinin inhibisyonu, artmış lipid peroksidasyonu ve oksidan stresi azaltarak yaşlanma sürecini yavaşlatabilmektedir.

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INTRODUCTION

With advancing age, sequential alterations leading to a progressive increase in the chance of disease and death occur and hence, this is defined as the process of aging. Till today, many theories have been put forward to account for this process (1, 2). Tissue damage due to free radical reactions has been considered as "the free radical theory of aging" and widely accepted as one of the most popular theories (3).

The ultimate goal of geriatric research is to identify the mechanisms underlying the aging process and to increase the average life span and its quality (4, 5). An abundant number of reports imply the involvement of oxidative stress where increased formation of free radicals lead to random deleterious effects in aging tissues. The increase noted in lipid peroxidation parallel to the concomitant decrease in antioxidant status supports this theory (3, 6, 7).

With respect to the process of aging, increased catecholamine metabolism and altered tissue distribution of monoamine oxidase (MAO [EC 1.4.3.4.;amine: oxygen oxidoreductase; deaminating, flavin containing]); a key enzyme in this metabolism have been extensively investigated and reported to accompany this process (8, 9, 10, 11). MAO is an enzyme responsible for the metabolism of biologically active amines and oxidative deamination of these amines produce ammonia (NH_3) and hydrogen peroxide (H_2O_2) with established or potential toxicity (5,12). The importance of H_2O_2 is due to the fact that it is a mediator in the production of the most potent free oxygen radical, namely the hydroxyl radical ($OH^{\bullet}$) (6). Untill now most studies have focused mainly on the role of oxidant stress and antioxidant status related to the pathogenesis of aging (13, 14). Only few studies have investigated the role of MAO related to oxidant stress and antioxidant status and the possible beneficial effects of MAO inhibitory agents during the aging process (5, 15, 16).

Zhang and Piantadosi showed that inhibition of MAO with pargyline protected rats from convulsions by decreasing H_2O_2 production in the brain (12). Recently, Vindis et al. and Bianchi et al.also showed that dopamine induces oxidative stress, apoptotic cascade and cell apoptosis exclusively by mechanisms involving H_2O_2 production by MAO and pargyline prevented apoptosis of proximal tubule cells (16, 17) Based on these findings, we believed that there is a need to investigate pargyline's effects on various tissues influenced by aging in vivo by using a rodent model.

Therefore, the objective of this study was to assess the role of MAO with respect to alterations related to oxidant stress and antioxidant status during aging and to investigate the role of pargyline, a selective irreversible MAO-B inhibitor in the prevention or amelioration of aging in various rat tissues.

MATERIAL AND METHODS

Reagents

All chemicals were analytical grade. Kynuramine, thiobarbituric acid, epinephrine, bovine erythrocyte SOD standard and bovine serum albumin standard were purchased from Sigma, cyclohexane from Riedel de Haens, and the remaining chemicals (KH_2PO_4, K_2HPO_4, Na_2HPO_4, 4-hydroxyquinoline, NaOH, TCA, methanol, chloroform, Na_2CO_3, $NaHCO_3$, Na-K tartarate, hydrogen peroxide , $CuSO_4$ and reactives of Folin-Ciocalteau) from Merck Darmstadt.

Animals

As shown in Table I, four groups, each consisting of young (3 months old) and aging (16-18 months old) male Swiss Albino rats (n=10 in each group) were formed. Animals were housed in a temperature controlled room (20-25˚C), on a 12 h dark / 12 h light cycle and fed with standard laboratory animal chow ad libitum and water. Rats were used in the study according to the permission and rules of the Ege University Animal Research Ethics Committee.

Table 1. Animal properties and treatment protocol of the experimental study groups (n=10 animals in each group) (the experimental procedure used in the study) (IP=intraperitoneal)

Young Control	2-3 months old	1 ml isotonic saline, IP, 90 minutes before decapitation
Young Pargyline	2-3 months old	25 mg/kg pargyline in 1 ml isotonic saline, IP, 90 minutes before decapitation
Aging Control	16-18 months old	2 ml isotonic saline, IP, 90 minutes before decapitation
Aging Pargyline	16-18 months old	25 mg/kg pargyline in 2 ml isotonic saline, IP, 90 minutes before decapitation

Preparation of the tissues

The animals were decapitated; their livers, hearts, brains and kidneys immediately excised and washed thoroughly in ice cold saline. Brain tissue specimens were prepared according to the method in our previous study (5). The brain as a whole was homogenized in phosphate buffer (KH2PO4, Na2HPO4.2 H2O, 50 mM, pH=7.0) (1/10, w/v). The other tissues, namely the livers, hearts and kidneys were minced to three fractions; for MAO activity a homogenate with EDTA/saccharose (1/10, w/v), for diene conjugates, superoxide dismutase (SOD) and catalase (CAT) a homogenate with phosphate buffer (KH2PO4, Na2HPO4.2 H2O, 50 mM, pH=7.0) (1/10, w/v) were prepared. For malondialdehyde (MDA) analysis, the remaining tissue was homogenized in distilled water (2/1, w/v) followed by 5% TCA precipitation. Supernatants were obtained from all homogenates, after centrifugation at 1500 g for 10 minutes. All procedures were carried on ice , the analyses being performed on the day of decapitation.

MAO activity was determined fluorometrically (Aminco-Bowman spectrofluorometer) by the modified method of Kraml (18); results were expressed as nmol/mg protein/hour. For diene conjugates, Bueger and Yust's spectrophotometric method was used (19); results were given as nmol hydroperoxide/mg protein. MDA determinations were conducted by Omar's method; the concentration of MDA being calculated applying an extinction coefficient of 153 000/Mol/cm and expressed as nmol/g ww (gram wet weıght) (20). SOD activities were determined by the method of Misra and Fridovich (21); results expressed as U/mg protein. CAT activities were determined by a modified method of Aebi (22) and Luck (23); results given as U/mg protein. Protein determinations were performed by the method of Lowry et al (24).

Statistical analysis

Statistical analysis was performed by MICROSTA and ANOVA programs. For comparison of the means, the student's t test, for intergroup comparisons, analysis of variance followed by the Newman Keuls multiple range test were used. The correlations between the parameters were determined by paired correlation tests.

RESULTS

MAO Activity:

Except renal tissue, MAO activities manifested statistically significant increases in all aging tissues ($p<0.01$). As presented in Figure 1, acute pargyline administration caused a significant decrease ($p<0.01$) in the MAO activities of both young and aging rat tissues.

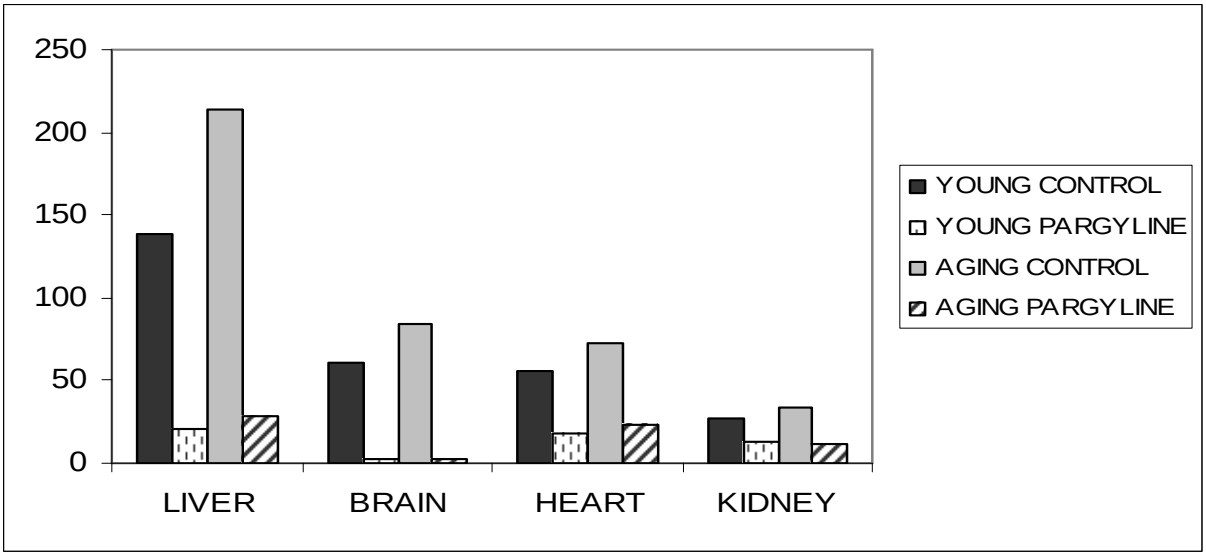

Figure 1. MAO enzyme activities (nmol/mg protein/hour) of the study groups for all tissues

Lipid Peroxidation Parameters (LPPs):

Except for brain , MDA levels were found to be significantly elevated ($p<0.01$) in all the tissues of aging control and pargyline groups as compared to young controls. Similar results ($p<0.01$ for liver, $p<0.05$ for kidney) were also noted for diene conjugates. In addition, both MDA and conjugated diene levels showed a significant correlation with MAO activity ($r=0.735$, $p<0.05$, $r=0.894$, $p<0.01$ for liver, and $r=0.8732$, $p<0.05$, $r=0.8804$, $p<0.05$ for kidney respectively) in the aging controls (Figure 2.). After Pargyline administration, only

an insignificant decrease was determined in MDA and diene conjugates in all young tissues. On the contrary a significant decrease in MDA levels was noted in all aging tissues ($p<0.01$) except brain . As to diene conjugates, pargyline also caused a significant decrease in aging liver, kidney and brain ($p<0.01$), and an insignificant decrease in cardiac tissue.

The decreases in LPPs after administration of pargyline correlated positively with the decreases in MAO activity in liver ($r=0.8750$, $p<0.05$ and $r=0.9299$, $p<0.01$for MDA; $r=0.8044$, $p<0.05$ and $r=0.8268$, $p<0.05$ for diene conjugates in young and aging groups, respectively) and in kidney ($r=0.8967$, $p<0.05$ and $r=0.9384$, $p<0.01$for MDA; $r=0.9021$, $p<0.05$ and $r=0.9120$, $p<0.01$ for diene conjugates in young and aging rats, respectively).

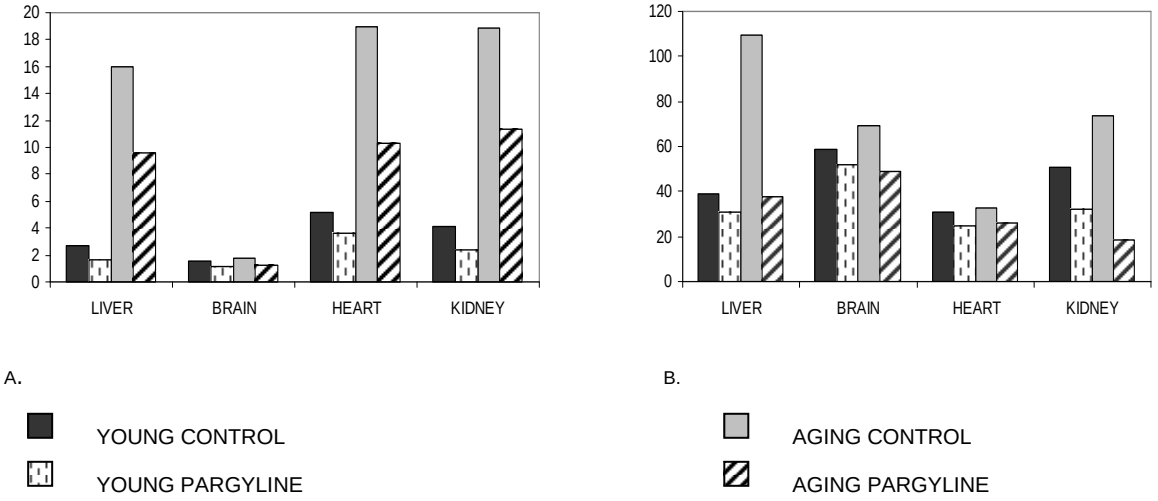

Figure 2. Lipid peroxidation parameters of the study groups for all tissues A: MDA levels B: Diene conjugate levels

Antioxidant Parameters

SOD and CAT activities showed a statistically insignificant decrease in all the tissues of aging groups compared to young controls. Except for a significant decrease in SOD activity in young brain tissue ($p<0.01$), pargyline did not induce any significant alteration in antioxidant enzyme activities either in young or aging rat liver, heart and kidney tissues. Data for antioxidant enzyme activities are presented in figure 3.

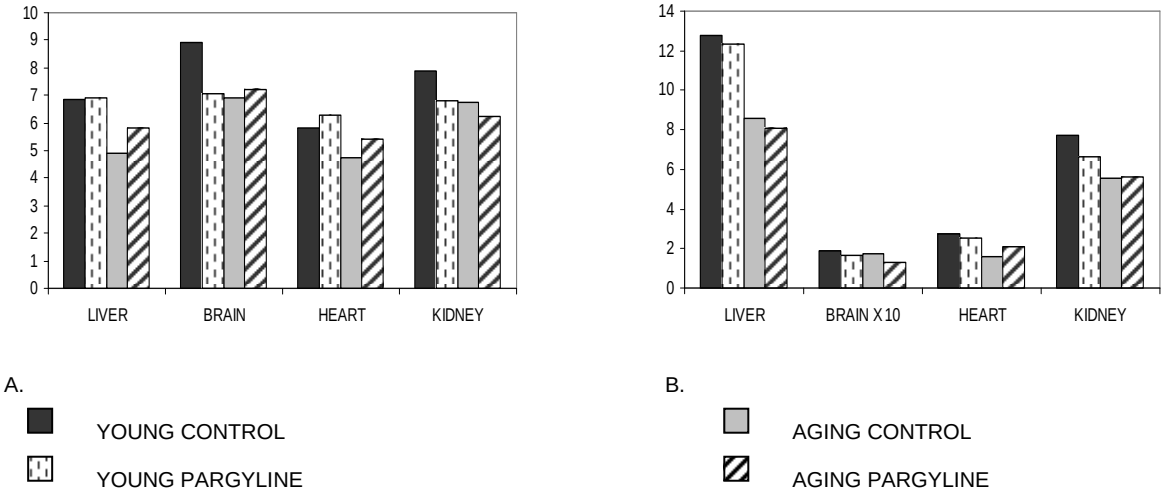

Figure 3. Antioxidant enzyme activities of different tissues in the study groups A: SOD activities B: CAT activities

DISCUSSION

Recently the process of aging has drawn attention as an important field of research. Since every species follows its own constant intrinsic pathway; control of aging only seems possible on extrinsic factors influencing life span and quality (1, 25). Free radical damage is considered as one of the extrinsic factors decreasing the organism's ability in maintaining homeostasis by causing cumulative damage. Our data prove that in the aging rat, the elevations in hepatic and renal LPPs are associated with concomitant increases in MAO activity, also accompanied by decreases in the activities of antioxidant enzymes. These data indicate that increases in MAO contributing to free radical-mediated lipid peroxidation, along with diminished cellular defence might play a key role in the pathogenetic mechanism related to the chain of events eventually leading to hepatic and renal cellular damage during aging.

Up to date, a number of studies indicate that among all tissues, hepatic tissue manifests the highest MAO content both in rodents and humans (26, 27). While Lai et al. state that hepatic and cardiac MAO activities increase and renal MAO activity decreases with aging; Benedetti et al. declare that they could not find any significant changes in the activities of either form of MAO with age in renal tissue (11, 28). Our findings are in concordance with the other investigators with respect to the highest MAO content of liver tissue, as well as age-related increases in liver, brain and heart MAO activity (26, 27). Our results support Benedetti et al. who could not show any significant changes and are different from those of Lai et al. who pointed out to significant decrease in renal tissue MAO activity with aging (11, 28) (Figure 2). These conflicting results may be due to differences in the age, gender and species of the rodents used in each experimental study.

H202; the potentially deleterious by product of MAO, accumulates parallel to increased MAO activity in aging tissues. H202 may be toxic due to the fact that it is easily reduced in the presence of ferrous iron (Fe+2) (via Fenton reaction) forming the hydroxyl radical (OH•), the most dangereous free radical for living cells. As a result of accelerated OH• production, increased lipid peroxidation also occurs in aging tissues (29).

In order to assess lipid peroxidation, one of the most important organic expressions of oxidative stress induced by reactive oxygen species (ROS), various analytical methods have been developed such as determining MDA and diene conjugates, reliable markers of lipid peroxidation. MDA, a stable end-product of lipid peroxidation as well as conjugated dienes, forming a transition state between lipid radicals and peroxy radicals are both used to assess lipid peroxidation (5).

Our results reflect increased lipid peroxidation and reduced cellular antioxidant status similar to the findings of previous studies on the aging process (10, 30, 31). Hence, it can be once more stated that, increased oxidant stress followed by antioxidant enzyme depletion may predispose the aging organism to degenerative changes.

Pargyline, a selective and irreversible MAO-B inhibitor, has proved to be an effective antidepressant not causing to the unpleasant side effects of other MAO inhibitors (32). Hermida-Ameijeiras and coworkers state that this agent leads to a significant reduction in OH• generation in the mitochondrial extracts of rat brains (29). Furthermore, pargyline administration also results in a reduced H2O2 production in vivo in rat brain tissue (12). In accordance with these findings, our data point to the beneficial effect of pargyline in preventing lipid peroxidation by inhibiting MAO activity in vivo. Administered as a single dose, pargyline had no prominent effect on antioxidant status. However in the future, the chronic administration of this agent may improve antioxidant status as well. Investigating the long lasting effects of MAO inhibitors, such as pargyline on oxidant/antioxidant status deserves futher future research.

CONCLUSION

In the present study, the effect of MAO inhibition in kidney, heart, brain and liver tissues, with respect to the initiation and/or propagation of aging via limiting free radical production is investigated. It is concluded that the data available so far support the fact that free radicals seem to contribute extensively to the deleterious changes in aging tissues. Increased MAO activity is a rich source of oxidant stress during aging. Inhibition of MAO by agents such as Pargyline may be proposed as a hopeful treatment strategy attenuating the process of aging by preventing oxidant stress.

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