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Protective Effect of Ischemic Preconditioning Plus Monoamine Oxidase (MAO) Inhibition on Ischemia-Reperfusion Injury in the Rat Pulmonary Vascular Bed

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Introduction

Ischemia-reperfusion (I/R) injury is a serious problem in clinical transplantation, angioplasty and coronary bypass surgery which is mostly associated with local injury during reperfusion and includes events such as reperfusion arrhythmias, microvascular damage, myocardial stunning and cell death[1-3]. I/R injury shows a complex pathophysiology with a number of contributing factors: energy degradation during ischemia, generation of reactive oxygen species (ROS) during reperfusion, "no-reflow phenomenon", and calcium overload reperfusion[4,5] It has been suggested that oxidative stress, associated with overproduction of ROS, is involved in the pathogenesis of I/R injury[6], since the increased ROS formation was known to modify lipids and proteins, to lead to lipid peroxidation and oxidation of thiol groups; to alter membrane permeability;

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to increase resistance in the pulmonary vascular system; and to cause a serious tissue damage[6-9]

Ischemic preconditioning (IP), an adaptive pathophysiological phenomenon which is decribed as a short cycle of I/R prior to a prolonged ischemic insult, has emerged as an important strategy with protection demostrated in cold and warm ischemia models[10-12]. Recent studies show that ischemic preconditioning has a protective effect against liver and lung injury induced by hepatic ischemia-reperfusion in the rat[13]. Although the mechanism of IP is not fully elucidated, it has been recently proposed that oxidative stress generated during IP triggers protective mechanisms such as natural antioxidant production against lethal I/R injury[14,15].

Monoamine oxidase (MAO) is a flavoenzyme which plays an essential role in the oxidative deamination of the biogenic amines both in the central nervous system and in peripheral tissues[16]. MAO is found in two different forms, designated as MAO-A and MAO-B, which are encoded by two different genes[17] and distinguished by different substrate specificities and sensitivities to the selective inhibitors[16,17]. MAO-A preferentially oxidizes serotonin and noradrenaline and is irreversibly inhibited by clorgyline, while MAO-B preferentially oxidizes benzylamine and phenylethylamine and is reversibly inactivated by pargyline[18]. It has been shown that MAO catalyzes the oxidative deamination of biogenic amines to their corresponding aldehydes, which is accompained by the reduction of molecular oxygen to hydrogen peroxide (H_2O_2)[19]. The toxicity of H_2O_2 is suggested to originate from its ability to induce oxidative damage to the proteins directly as H_2O_2 or through its conversion into hydroxyl radicals via Fenton reaction[20]. It was postulated that intramitochondrial hydroxyl radicals from H_2O_2 generated during MAO metabolism serves as a major contributor to I/R injury[21].

Although our previous studies demonstrated a significant ROS-mediated tissue injury in I/R, and IP significantly reduced the ROS production in I/R injury[22], it was also shown that IP could not fully protect the lung against I/R injury[23]. Since our studies revealed that MAO appeared as a potential source of excessive hydroxyl radical production in the reperfusion period after ischemia in the lung[24], it was thought that pre-treatment with specific MAO inhibitors prior to or together with IP may have a potential clinical relevance. Thus, the present study was

designed to investigate the possible protective effect of IP with and without MAO inhibition against ROS-mediated I/R injury in the pulmonary vascular bed of rat.

Materials and Methods

Chemicals

All chemicals were obtained from Sigma Chemical Co. (Germany).

Isolated buffer perfused lung (IBPR)

The experiment was performed in compliance with the "Principles of Laboratory Animal Care" formulated by the National Institutes of Health (National Institutes of Health publication no.96 to 23, revised 1996). The experiment and animal care protocol was approved by the Ethics Committee for animal care, established in our institute (# 2001/25-4) Male Wistar rats (200-300g) were anesthetized with thiopental (30 mg/kg, i.p.). After tracheal cannulation, the chest was opened and heparin (200 IU) was injected into the right ventricle. The main pulmonary artery was cannulated via the right ventricle and the vasculature was flushed with Krebs-Henseleit solution [(KHS, in mM): NaCl 118, KCl 4.7, CaCl2 2.5, KH2PO4 1.2, NaHCO3 25, MgSO4 1.2, glucose 10]. The left atrium was cut and the major part of the ventricles removed to allow free efflux of the perfusate. The lung was removed, suspended in a chamber and perfused with KHS (bubbled with 95% O_2 and 5% CO_2 at 37^{0}C) at a constant flow rate (0.03 ml.g^{-1}) by a peristaltic pump (Gilson Model M312). To inhibit the cyclo-oxygenase pathway, indomethacin (3 μM) was added to the perfusion solution. Mean perfusion pressure (PP) was measured via a pressure transducer attached to a side arm of the pulmonary artery cannula during experimental protocols. Changes in PP were recorded on a computer-based data acquisition system (TDA96).

In the I/R group, after 30 min of constant flow perfusion, the lungs were subjected to 2 h hypothermic ischemia at 4^{0}C in KHS. In the IP group, IP was performed by two successive cycles of 5 min ischemia, followed by 5 min of reperfusion prior to the 2 h of hypothermic ischemia (Fig. 1).

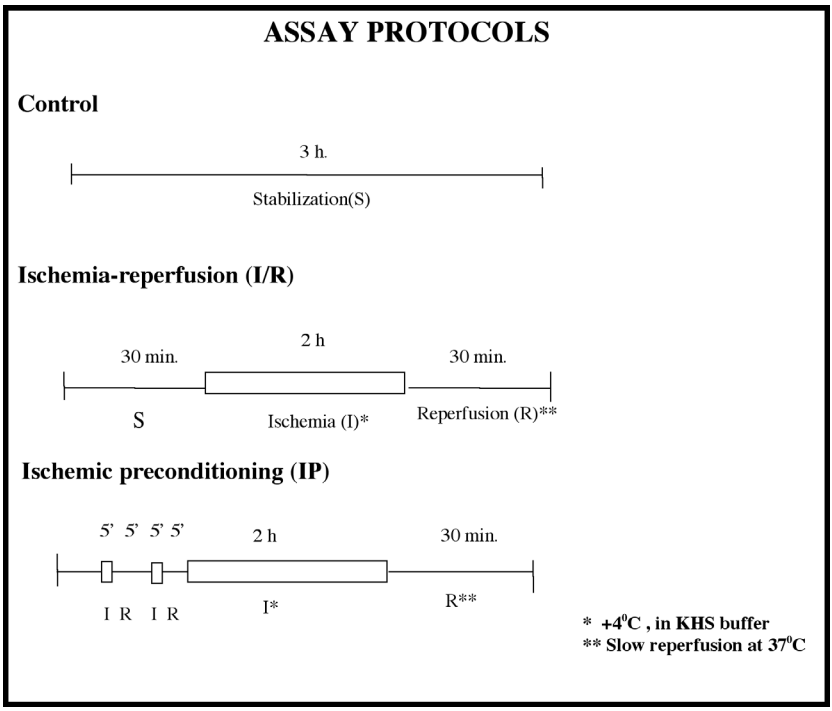

Figure 1

Experimental protocols

To investigate the effect of MAO inhibition on I/R injury in the rat lung, each group was divided into two subgroups as with and without MAO inhibition (MAOI). 2 mg/kg chlorgyline (clorgylinehydrochloride (N-methyl-N-propargyl-3-(2,4-dicholorophenoxy)- propylamine hydrochloride), a selective MAO-A inhibitor was dissolved in saline and injected intraperitonally (ip) as a volume of 100 µl/100 g of the body weigh to the rats of the "with MAOI group 1; 6 mg/kg pargyline (N-methyl-N-benzyl-2-propynylamine hydrochloride), ip as a volume of 100 µl/100 g of the body weight to the rats of the "with MAOI group 2, 15 min prior to experimental protocols. At the end of the protocols, the lungs were re-attached to the perfusion system, perfusion flow was gradually increased and the same flow rate was achieved as prior to ischemic protocol within 10 min. After the protocols, lungs were excised and weighed.

The lung tissue portions were homogenized in 50 mM potassium phosphate (KP) buffer, pH 7.4 for the measurements of tissue lipid peroxidation, glutathione, antioxidant enzyme and hydroxyl radical levels. Some tissue portions were kept for the determination of MAO activities.

Determination of lipid peroxidation

Lipid peroxidation in lung tissues was determined by the measurement of malondialdehyde (MDA) levels on the basis of MDA reacted with thiobarbituric acid (TBA) at 532 nm, according to a previous method[25]. The principle of the method was based on the spectrophotometric measurement of the colored complex generated by the reaction of TBA with MDA. MDA concentration was calculated using the molar extinction coefficient of the MDA-TBA complex, 1.56 x 105 M-1 cm^{-1}. Values were expressed as nmol. mg^{-1}.

Determination of total, reduced (GSH) and oxidized (GSSG) glutathione

Total glutathione content, as the sum of GSH and GSSG, and GSSG were determined in the lung according to the method described previously[26]. Total glutathione was determined using a kinetic assay in which amounts of GSH or GSSG and glutathione reductase brought about the continuous reduction of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) by nicotinamide adenine dinucleotide phosphate (NADPH). The formation of 5-thio-2-nitrobenzoate (TNB) was followed spectrophotometrically at 412 nm at 25^{0}C. Total glutathione and GSSG were expressed as µmol. mg $protein^{-1}$. GSH was calculated as [total glutathione]-2 x [GSSG] and expressed as µmol. mg^{-1}.

Determination of catalase (CAT) activity

CAT activity in lung tissue was determined according to the method of Ueda[27]. Decomposition of H_2O_2 was monitored by a decrease in absorbance at 240 nm. A molar extinction coefficient of 43.6 $M^{-1}cm^{-1}$ was used to determine the activity. Enzyme activity was expressed as $nmol.mg^{-1}$.

Determination of glutathione S-transferase (GST) activity

GST activity in lung tissue was determined spectrophotometrically at 340 nm by measuring the formation of the conjugate of GSH and 1-chloro-2,4-dinitrobenzene (CDNB)[28]. A molar extinction coefficient of 9.6

$mM^{-1}cm^{-1}$ was used to determine the activity. A correction for the spontaneous reaction was made by measuring and subtracting the rate in the absence of enzyme. One unit of enzyme is defined as the amount of enzyme that catalyzes the formation of 1 µmol of S-2,4-dinitrophenylglutathione per minute at 30°C using 1 mM of GSH and CDNB.

Determination of glutathione reductase (GR) activity

GR activity in lung tissue was determined by the method of Carlberg and Mannervik[29]. The reaction mixture contained 0.2 M KP buffer, pH 7.0, 1 mM EDTA, 0.3 mM NADPH in 10 mM Tris-HCl, pH 7.0, 2.5 mM GSSG in water, 1 mM β-mercapto-ethanol and the tissue sample. The decrease in absorbance at 340 nm was followed at 30°C. The extinction coefficient of 6.22 $mM^{-1}\,cm^{-1}$ was used to determine the activity. Enzyme activity was expressed as $nmol.mg^{-1}$.

Determination of glutathione peroxidase (GPx) activity

The GPx activity in lung tissue was measured according to a previous method[30]. The reaction mixture contained 50 mM KP buffer, pH 7.0, 4 mM NaN_3, 5 mM GSH, 0.3 mM NADPH and 1 unit of glutathione reductase. The reaction was initiated by adding 0.1 mM H_2O_2 and sample; the absorbance was measured at 340 nm. The activity was expressed as $U.mg^{-1}$. One unit was defined as the amount of enzyme which oxidizes 1 µmol of glutathione per minute.

Determination of superoxide dismutase (SOD) activity

SOD activity in lung tissue was determined according to the method of Misra and Fridovich[31]. 50 µl of sample was added to reaction mixture containing carbonate buffer (50 mM, pH 10.2, 0.1 mM EDTA), 200 µM epinephrine, 100 µM xanthine and 0.2 µM xanthine oxidase, and the absorbance was measured at 480 nm. Activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit of SOD. The activity was expressed as $U.mg^{-1}$.

Measurement of tissue MAO activity

Lung tissue (5-8 g) was homogenized 1:40 (w/v) in 0.3 M sucrose. Following centrifugation at 1,000 x g for 10 min., the supernatant was centrifuged at 10,000 x g for 30 min. to obtain crude mitochondrial pellet. The pellet was incubated with CHAPS of 1% at 37°C for 60 min. and centrifuged at 1,000 x g for 15 min. The pellet was resuspended in

0.3 M sucrose and layered onto 1.2 M sucrose, and then centrifuged at 53,000 x g for 2 h. Pellet was resuspended in KP buffer, pH 7.4, and kept at –70^{0}C until used. Total MAO activity was measured spectrophotometrically according to the method of Holt[32].

The chromogenic solution consisted of 1 mM vanillic acid, 500 µM 4-aminoantipyrine, and 4 U.ml^{-1} peroxidase in 0.2 M potassium phosphate buffer, pH 7.6. Assay mixture contained 167 µl chromogenic solution, 667 µl substrate solution (500 µM p-tyramine) and 133 µl potassium phosphate buffer, pH 7.6. The mixture was preincubated at 37^{0}C for 10 min. before the addition of enzyme. Reaction was initiated by adding the homogenate (100 µl), and increase in absorbance was monitored at 498 nm at 37^{0}C for 60 min. Molar absorption coefficient of 4654 M^{-1}. cm^{-1} was used to calculate the initial velocity of the reaction. Results were expressed as nmol.h^{-1}.mg^{-1}.

Selective measurement of MAO-A and MAO-B activities

Homogenates were incubated with the substrate p-tyramine (500 µM to measure MAO-A and 2.5 mM to measure MAO-B) following the inhibition of one of the MAO isoforms with selective inhibitors. Aqueous solutions of clorgyline or pargyline (50 µM), as selective MAO-A and –B inhibitor, were added to homogenates at the ratio of 1:100 (v/v), yielding the final inhibitor concentrations of 0.50 µM. Homogenates were incubated with inhibitors at 37^{0}C for 60 min. prior to activity measurement. After incubation of homogenates with selective inhibitors, remaining MAO activity was determined by the method described above.

Determination of hydroxyl radical (^{0}OH) release

The generation of ^{0}OH was measured fluorimetrically in fresh tissue samples according to a previously published method[33] in which terephtalic acid (THA) is used as a dosimeter of formation was monitored using 312 nm excitation and 426 nm emission wavelengths. Results were expressed as µmol.mg^{-1}.

Protein determination

Protein contents of lung tissues were determined according to the method of Bradford[34].

Statistical analysis

Results are expressed as mean ± S.E.M; n represents the number of animals and analyzed by SPSS (version 9.0). Mann-Whitney U test and one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls post hoc test were used for comparison of the groups of the variables. Correlations between variables were assessed with Pearson's correlation coefficients (r). $p<0.05$ was considered as statistically significant.

Results and Discussion

MDA concentration was found to be significantly increased in I/R group when compared to control responses obtained from a control group of experiments ($p<0.01$) and significantly decreased in IP groups when compared to of I/R group ($p<0.01$) (Table I). This result was in good agreement with the previous reports demonstrating that ROS production is increased in I/R and that IP protects the organs against ROS-mediated I/R injury[11-14]. However, in the present study, MDA level in the IP group in which no MAOI was applied was not reduced to its basal level as in control group ($p<0.01$), suggesting that IP alone can not be fully successful in protecting the lung against excess ROS production during I/R. Since the mean MDA content of the IP+MAOI (both MAO-AI and MAO-BI) subgroups were not found to be statistically different from that of control group, it was proposed that MAOI together with IP protects the lung against excess formation of ROS in I/R.

GSH content and the GSH/GSSG ratio were found to be significantly decreased and GSSG content significantly increased in the I/R group ($p<0.01$) (Table I), whereas GSH content and GSH/GSSG were found to be significantly increased and GSSG content significantly decreased in the IP group ($p<0.01$), suggesting that IP reduces the glutathione depletion in I/R and protects the lung against I/R injury. It was found that IP alone can not overcome the accumulation of GSSG in lung tissue during I/R. This finding is in good agreement with the previous reports suggesting that tissue lipid peroxidation is increased and GSH content is decreased in oxidative-stress-induced tissue injuries[23,35]. Since it was found that GSH and GSSG contents and GSH/GSSG in the IP+MAOI subgroups were found to be similar to that of the control subgroups, IP+MAOI seems

to be an effective treatment for inducing the non-enzymatic antioxidant systems against the ROS-mediated injury occurring during I/R.

The strong negative correlations between MDA and GSH levels (r=-0.58, -0.59; -0.63, -0.66; and -0.69, -0.67 in control, I/R and IP groups, respectively, $p<0.05$) and between MDA levels and GSH/GSSG (r=-0.56, -0.59; -0.63, -0.66; and -0.69, -0.72 in control, I/R and IP groups, respectively, $p<0.05$) as well as the strong positive correlations between MDA and GSSG levels (r=0.57, 0.56; 0.60, 0.62; and 0.69, 0.70 in control, I/R and IP groups, respectively, $p<0.05$) in all study groups supports our suggestion above.

Antioxidant enzyme activities were found to be significantly decreased in the I/R group ($p<0.01$) (Table I), possibly resulting from the depletion of the antioxidant pool which is used to remove excess ROS produced during I/R. Our data is in accordance with the previous reports which have indicated that antioxidant enzymes are overexpressed in IP[36]. Since the antioxidant enzyme activities in the IP+MAOI subgroups were found to be increased in comparison with the levels found in control subgroups, it was suggested that IP+MAOI may be an effective protocol for increasing the antioxidant capacity of the lung against ROS-mediated I/R injury.

Total MAO, MAO-A and -B activities of lung tissues in I/R group significantly elevated when compared to control group ($p<0.01$); decreased in IP group when compared to I/R group ($p<0.05$); and reached their basal levels in the IP+MAOI subgroups (Table II). Our data are supported with a recent study which proposed that MAO-A activity rapidly increased in the early phase of renal reperfusion, but gradually decreased during the in 24 hours[21]. Our results also suggested that IP+MAOI seems to gradually prevented the elevation of MAO increase in the early reperfusion period (Table II).

Strong positive correlations which were found between the tissue total MAO, MAO-A and -B activities and MDA levels in control (r= 0.60, 0.62 and 0.69, respectively, $p< 0.01$), I/R (r= 0.61, 0.63 and 0.68, respectively, $p<0.01$) and IP (r= 0.67, 0.69 and 0.72, respectively, $p<0.01$) subgroups suggest that increased MAO activity may be involved in the excessive ROS production in I/R of rat lung.

The present study indicated that hydroxyl radical release in lung tissue was significantly increased in I/R and decreased in IP groups, and

TABLE I
MDA, glutathione and antioxidant enzyme levels in lung tissues of the study groups.*

Parameters	Control group			I/R group			IP group		
	Without MAOI	With MAOI		Without MAOI	With MAOI		Without MAOI	With MAOI	
		Group 1	Group 2		Group 1	Group 2		Group 1	Group 2
MDA (nmol. mg^{-1})	41.22±3.02	40.76±3.53	41.12±4.04	97.22±8.23[a]	75.45±6.61[a,b]	77.38±6.05[a,b]	59.88±4.93[a,c]	41.02±3.70[c,d]	40.02±2.99[c,d]
GSH (µmol. mg^{-1})	77.22±6.31	76.49±6.45	76.88±7.03	41.34±5.15[a]	50.93±4.59[a,b]	51.33±4.00[a,b]	60.18±5.20[a,c]	76.98±6.07[c,d]	77.45±5.66[c,d]
GSSG (µmol. mg^{-1})	1.08±0.11	1.12±0.14	1.13±0.12	4.02±0.99[a]	3.00±0.18[a,b]	3.07±0.16[a,b]	2.10±0.19[a,c]	1.18±0.11[c,d]	1.10±0.10[c,d]
GSH/GSSG	72.97±6.06	73.02±6.11	72.77±6.51	10.77±1.02[a]	19.95±1.80[a,b]	20.24±1.77[a,b]	32.44±0.27[a,c]	71.95±6.65[c,d]	73.07±5.60[c,d]
CAT (nmol. mg^{-1})	66.23±5.91	67.54±5.84	65.90±6.02	39.65±3.22[a]	47.33±3.60[a,b]	48.60±3.55[a,b]	60.21±4.67[a,c]	67.80±5.08[c,d]	66.89±5.93[c,d]
GST ($U.mg^{-1}$)	3.16±0.27	3.11±0.23	3.20±0.30	1.54±0.29[a]	1.78±0.11[a,b]	1.79±0.13[a,b]	1.90±0.18[a,c]	3.20±0.20[c,d]	3.26±0.20[c,d]
GR (nmol. mg^{-1})	30.60±3.05	31.12±3.18	31.29±3.67	13.34±2.89[a]	18.20±1.04[a,b]	19.20±1.12[a,b]	24.12±2.16[a,c]	30.12±3.00[c,d]	31.20±2.98[c,d]
GPx ($U.mg^{-1}$)	0.78±0.05	0.77±0.05	0.78±0.05	0.23±0.08[a]	0.36±0.06[a,b]	0.37±0.10[a,b]	0.56±0.06[a,c]	0.78±0.06[c,d]	0.77±0.10[c,d]
SOD ($U.mg^{-1}$)	25.33±2.09	25.80±2.12	26.51±2.08	10.90±1.07[a]	14.12±1.48[ab]	14.99±1.47[ab]	19.90±1.87[a,c]	25.20±2.11[c,d]	26.03±2.88[c,d]

* Each group consisted of 10 rats. Values represent the mean±SD of three independent experiments.
Group 1 : 2mg/kg chlorgyline (MAO-A inhibitor) injected as a volume of 100 µl/100 g to the rats 15 min prior to protocol
Group 2: 6 mg/kg pargyline (MAO-B inhibitor) injected as a volume of 100 µl/100 g to the rats 15 min prior to protocol.
[a]$p<0.01$ versus the control subgroups with and without MAOI
[b]$p< 0.01$ versus the I/R group without MAOI
[c]$p< 0.05$ versus the I/R subgroups with and without MAOI
[d]$p<0.05$ versus the IP group without MAOI

TABLE II

MAO activities and ^{0}OH production in lung tissues of the study groups.*

Parameters	Control group			I/R group			IP group		
	Without MAOI	With MAOI		Without MAOI	With MAOI		Without MAOI	With MAOI	
		Group 1	Group 2		Group 1	Group 2		Group 1	Group 2
Total MAO ($nmol.h^{-1}.mg^{-1}$)	18.40±1.60	17.28±1.66	17.60±1.69	42.37±3.70[a]	30.21±3.02[a,b]	31.70±2.81[a,b]	21.42±1.98[a,c]	19.78±1.72[c,d]	18.99±1.56[c,d]
MAO-A ($nmol.h^{-1}.mg^{-1}$)	9.30±0.71	9.22±0.62	10.16±0.70	30.55±2.84[a]	24.78±2.07[a,b]	23.90±2.13[a,b]	18.67±1.65[a,c]	10.59±1.04[c,d]	10.02±1.03[c,d]
MAO-B ($nmol.h^{-1}.mg^{-1}$)	8.01±0.51	7.33±0.40	7.69±0.61	20.23±1.80[a]	14.93±1.46[a,b]	15.10±1.29[a,b]	10.55±1.03[a,c]	8.25±0.77[c,d]	7.95±0.60[c,d]
$^{\bullet}OH$ ($\mu mol.mg^{-1}$)	0.79±0.07	0.80±0.09	0.81±0.07	5.23±0.40[a]	3.71±0.22[a,b]	3.72±0.30[a,b]	2.66±0.28[a,c]	0.81±0.08[c,d]	0.82±0.08[c,d]

* Each group consisted of 10 rats. Values represent the mean±SD of three independent experiments.
Group 1 : 2mg/kg chlorgyline (MAO-A inhibitor) injected as a volume of 100 µl/100 g to the rats 15 min prior to protocol
Group 2: 6 mg/kg pargyline (MAO-B inhibitor) injected as a volume of 100 µl/100 g to the rats 15 min prior to protocol.
a $p<0.01$ versus the control subgroups with and without MAOI
b $p< 0.01$ versus the I/R group without MAOI
c $p< 0.05$ versus the I/R subgroups with and without MAOI
d $p<0.05$ versus the IP group without MAOI

declined to its basal level in the IP+MAOI subgroups ($p<0.01$) (Table II). Tissue total, MAO-A and –B activities of the lung were positively correlated with hydroxyl radical release in control ($r=0.60$, 0.61 and 0.63, respectively, $p<0.05$), I/R ($r=0.63$, 0.64 and 0.62, respectively, $p<0.05$), and IP ($r=0.69$, 0.72 and 0.73, respectively, $p<0.01$) subgroups, demonstrating that elevated MAO activity, which is possibly induced by increased amines in damaged tissue, caused excess hydroxyl radical release in I/R. Our data are in accordance with a previous report indicating that monoamine oxidases are a potential source of hydroxyl radical generation in the early reperfusion period[21].

Since it has been shown that H_2O_2 is removed mainly by catalase and its release is regulated by the glutathione cycle (37), significant depletion of these systems may cause an increase in H_2O_2 and $^{\cdot}OH$ accumulation in the tissues. It was recently reported that both glutathione and the catalase system were enhanced by low doses of H_2O_2 and that this stimulation was operative in cytoprotection against lethal H_2O_2 stress[38]. The present study demonstrated that lowered glutathione and catalase levels and increased hydroxyl radical production in I/R can be almost fully reversed by IP+MAOI, and we suggest that enhancement of antioxidant content by the process of IP+MAOI may have a protective effect against hydroxyl radical-mediated tissue injury.

In summary, we have demonstrated that the excess ROS formed during I/R may cause direct tissue damage in lung and initiate cell death and organ failure. Although our data showed that IP significantly reduced the ROS production in I/R, it could not totally reverse the impaired oxidant-antioxidant balance and it could only partly protect the lung against I/R injury. Our results also indicated that IP and MAOI together caused a more significant reduction in ROS levels and a marked elevation in antioxidant capacity; thus it was concluded that I/R injury may be a result of multiple interactions between different mechanisms. Further studies with MAOI will provide much data about the mechanisms involved in I/R injury of lung.

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Summary

Since it has been previously described that monoamine oxidase (MAO) is a potential source of hydrogen peroxide (H_2O_2) generation in ischemia/

reperfusion (I/R), the present study aimed to investigate the possible protective effect of ischemic preconditioning (IP) plus MAO inhibitors (MAOI) in the early phase of reperfusion injury of lung.

Male Wistar rats were randomized into 3 main groups: control lungs were subjected to 3 h of perfusion in absence and presence of selective MAO inhibitors, whereas the lungs of the I/R group were subjected to 2 h of cold ischemia following the 30 min of perfusion in absence and presence of MAO inhibitors. IP was performed by two cycles of 5 min ischemia followed by 5 min of reperfusion prior to 2 h of hypothermic ischemia in absence and presence of MAO inhibitors.

Lipid peroxidation, oxidized (GSSG) glutathione content, MAO activities and H_2O_2 release were increased in the I/R group, whereas reduced (GSH) glutathione content, GSH/GSSG ratio and antioxidant enzyme activities were decreased. MAO activities, $^{\cdot}OH$ release, GSSG content and lipid peroxidation were markedly decreased in the IP group, whereas GSH content, GSH/GSSG ratio and anti-oxidant enzyme activities were significantly increased. MAO activities were found to be positively correlated with H_2O_2 production in all study groups. All parameters changed in I/R returned to their basal levels when IP and MAO inhibition were applied together.

The present study suggests that application of IP and MAO inhibition together may be more effective than IP alone against I/R injury in the lung.

Key words: Monoamine oxidase, ischemia-reperfusion injury, ischemic preconditioning, reactive oxygen species, hydrogen peroxide, antioxidant enzymes.

Özet

Sıçan Pulmoner Damar Yatağında Oluşan İskemi Reperfüzyon Hasarında İskemik Önkoşullama ve Monoamin Oksidaz (MAO) İnhibisyonunun Koruyucu Rolü

Yapılan araştırmalar iskemi/reperfüzyon (I/R) da ortaya çıkan hidrojen peroksit (H_2O_2) in potansiyel kaynağının monoamin oksidaz (MAO) olduğunu bildirdiğinden, bu çalışma akciğerdeki erken reperfüzyon fazı hasarında iskemik önkoşullama (IP) ve MAO inhibitörleri (MAOI) nin birlikte uygulanmasının olası koruyucu rolünü araştırmayı amaçladı.

Erkek Wistar sıçanlar 3 ana gruba ayrıldı: kontrol akciğerler MAOI varlığı ve yokluğunda 3 saatlik perfüzyona tabi tutuldu; I/R grubunda

sıçanlara MAOI varlığı ve yokluğunda 2 saatlik soğuk iskemi ve 30 dk reperfüzyon uygulandı; IP grubunda MAOI varlığı ve yokluğunda iskemi öncesi iki döngü halinde 5 dakikalık iskemi, 5 dakikalık reperfüzyon ve 30 dakika perfüzyon uygulandı.

I/R grubunda lipid peroksidasyonu, okside (GSSG) glutatyon içeriği, MAO aktivitesi ve H_2O_2 salınımı artmış; redükte (GSH) glutatyon içeriği, GSH/GSSG oranı ve antioksidan enzim düzeyleri azalmış olarak bulundu. IP grubunda MAO aktivitesi, H_2O_2 salınımı, GSSG içeriği ve lipid peroksidasyonu azalırken GSH içeriği, GSH/GSSG oranı ve antioksidan enzim düzeyleri arttı. Tüm gruplarda MAO aktiviteleri ile H_2O_2 düzeyleri arasında pozitif bir ilişki bulundu. Ölçülen değerlerin IP ve MAOI'nin birlikte uygulandığı gruplarda bazal düzeylere yaklaştığı gözlendi.

Elde edilen veriler, akciğerdeki I/R hasarında IP ve MAOI'nin birlikte uygulamasının etkin koruyucu bir rolü olabileceğini düşündürdü.

Anahtar kelimeler: Monoamin oksidaz, iskemi-reperfüzyon hasarı, iskemik önkoşullama, reaktif oksijen türleri, hidrojen peroksit, antioksidan enzimler

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