BASIC SCIENCES

MONOAMINE OXIDASE - A INHIBITION REVERSES ENDOTHELIAL DYSFUNCTION IN HYPERTENSIVE RAT AORTIC RINGS

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MONOAMINE OXIDASE-A INHIBITION REVERSES ENDOTHELIAL DYSFUNCTION IN HYPERTENSIVE RAT AORTIC RINGS (Abstract). Aim. Monoamine oxidases (MAOs) are mitochondrial enzymes, with 2 isoforms, A and B that convert biogenic amines to their corresponding aldehydes via a reaction that produces hydrogen peroxide. Since MAO-A is the predominant form at vascular level we hypothesized that MAO-A-dependent H₂O₂ production may contribute to the development of endothelial dysfunction and, MAOs inhibition could improve the vascular function, respectively. Material and methods. To this aim aortic rings were isolated from female adult spontaneously hypertensive rats (SHR) and their corresponding (Wistar-Kyoto) controls. The effect of MAO-A inhibitor, clorgyline (10µmol/l) on endothelium-dependent relaxation (EDR) in response to acetylcholine and endothelium-independent relaxation in response to sodium nitroprusside, was studied in isolated phenylephrine-preconstricted aortic segments in the presence of indometacine (10µmol/l). Results. In hypertensive group EDR was significantly decreased – maximal relaxation (% of KCl, mean±SD) being 37±3.5 in SHR vs. 3.7±1.8 in controls. If experiments were done in the presence of clorgyline, EDR in control segments was unaffected. However, the compound restored normal EDR in aortic segments from hypertensive rats (maximal relaxation - % of KCl, 13.7±2.3). Conclusions. Inhibition of the MAO-A isoform might be useful in restoring endothelium-dependent relaxation in this experimental model of hypertension in rat. Keywords: ENDOTHELIAL DYSFUNCTION, EXPERIMENTAL HYPERTENSION, MONOAMINE OXIDIZE.

Cardiovascular disease is a leading cause of morbidity worldwide and endothelial dysfunction (ED) represents both a widely investigated underlying mechanism and a therapeutic target. Accumulated lines of evidence indicate that increased production of reactive oxygen species (ROS) significantly contributes to ED pathogenesis. Four enzymatic systems have been systematically investigated as major sources of ROS generation by vascular cells: NAD(P)H oxidases, xanthine oxidase, uncoupled eNOS and mitochondrial respiratory chain (1). However, recent data have incriminated monoaminooxidases (MAO) with two functional isoforms, A and B, as another important source of deleterious hydrogen peroxide (H₂O₂) production (2). In particular, increased activity of myocardial MAO-A has been reported to contrib-
ute to ventricular dysfunction associated with postischemic reperfusion, and also to the maladaptive hypertrophy within the evolution of heart failure (3). Moreover, with respect to vasculature, MAO-A mediated ROS production has been shown to induce mitogenic signaling in smooth muscle cells by a process that may involve the activation of matrix metalloproteinase 2, which likely contributes to vascular wall remodeling (4). Enhanced MAO-A protein expression and consecutive generation of H$_2$O$_2$ in the cerebral arteries have been also demonstrated to be responsible for the exaggerated *in vitro* tension development induced by serotonin, in the isolated basilar arteries of SHR (5).

The unfavorable effects of MAO activation are antagonized by a couple of available selective MAO inhibitors (MAOI) that can be divided in: i) irreversible MAOI, such as clorgyline for MAO-A and sele-giline for MAO-B and ii) reversible MAOI, such as moclobemide for MAO-A and laz-abemide for MAO-B, respectively (6).

In the light of these new findings that consider MAO an important source of H$_2$O$_2$ not only in the brain, as widely recognized (7) but also in the heart, the aim of the present study was to determine whether inhibition of MAO-A, the predominant isoform in the cardiovascular system, could improve the vascular relaxation in hypertensive and normal rat vessels. We hypothesized that, similarly to ischemia/reperfusion injury and hypertrophy, the activity of this enzyme is increased in pathological conditions associated with ED, such as experimental hypertension, with the subsequent generation of a large amount of H$_2$O$_2$, that might cause a decrease in nitric oxide (NO); in this respect, MAO inhibition might correct the endothelial dysfunction by improving the NO-dependent vasodilatation.

**MATERIAL AND METHODS**

All experimental procedures used in this study were conducted in accordance with the Directive 2010/63/EU on the protection of animals used for scientific purposes. The experimental protocol was approved by the Ethics Committee of the University for Medicine and Pharmacy of Timişoara, Romania.

Animals were fed *ad libitum* and housed under standard conditions (constant temperature and humidity of 22.5 ± 2° C and 55 ± 5%, 12-h light/dark cycle). Twenty-four hours prior to the experiment solid food was withdrawn with no limitation in water supply.

**Experiment protocol.** Experiments were performed in vascular preparations (thoracic aortas) isolated from SHR and control female rats weighing 250-350 g (n = 6/group). The arterial segments were carefully cleared of connective tissue to not damage the intimal surface and cut into 2-3 mm wide rings. The rings were suspended between two parallel stainless steel hooks in two individual jacketed organ baths containing Krebs-Henseleit buffer (composition in mM: NaCl 118, KCl 4.7, CaCl$_2$ 2.5, MgSO$_4$ 1.2, KH$_2$PO$_4$ 1.2, NaHCO$_3$ 25, and glucose 11.1), gassed with a mixture of 95% O$_2$ and 5% CO$_2$ and maintained at 37°C. The upper hook was connected to a force transducer for isometric tension recording. The amplified analog signal from the force transducer was digitized using a data acquisition system (Digi-data 1200B, Axoscope 10, and Molecular Devices Ltd).

The rings were equilibrated for 60 minutes at 37°C under 1.75 cN passive tension and the buffer was replaced every 15 min. To confirm the viability of vascular smooth muscle, vessel rings were contracted twice with KCl. A relaxation re-
response to acetylcholine, an endothelium-dependent vasodilator, of 15% or more from the stable tension induced by KCl was considered as functional endothelium. After washout and return to baseline the tissues were pre-contracted with 10-5 M phenylephrine (PE). Cumulative concentration-response curves (10^-9 to 10^-5 M) for: acetylcholine (ACh), as endothelial-dependent response, and sodium-nitroprusside (SNP), as endothelial-independent response, were recorded in the absence vs. the presence of MAO-A inhibitor (Clorgyline, 10^-5 M). Indomethacin (10^-5 M), as inhibitor of cyclooxygenase, was present in the organ baths throughout the experiments in order to eliminate the influence of prostaglandine synthesis on vasodilator response.

Relaxation response of each vascular ring was assessed by measuring the reduction in vascular tone at cumulative doses of the vasodilator agent and expressed as percentage change from the stable tension produced by PE.

**Statistical analysis.** Acquired data were statistically processed using GraphPad Prism 5 and Microsoft Office Excel 2003 software (Microsoft Corporation). Central tendencies of the variables obtained from n different rings were expressed as mean (M) and the dispersion as the standard deviation (SD). Statistical comparison between two groups of averages was performed using t test.

In order to assess overall vascular reactivity, we chose to calculate the following variables in the equation for a Hill-type sigmoidal relationship: i) the estimated maximum response, ii) the concentration required to achieve 50% of maximum response (EC50 (-log [M]) and the iii) dose-response slope. In order to compare the dose - response curves, F test was used.

**RESULTS**

The rings isolated from SHR develop a pronounced attenuation of endothelium dependent-relaxation (EDR). In control segments, EDR was unaffected in the presence of the MAO-A inhibitor, clorgyline (10µmol/l). In contrast, the irreversible MAOI partially restored normal EDR in aortic rings harvested from SHR rats (fig. 1).

![Fig.1. Acetylcholine dose-response curve of aortic rings in SHR vs. CONTROL in the presence vs. the absence of the MAO A inhibitor, clorgyline (10 µmol/l) (*, # p<0.0001)](image-url)
The relaxation induced by sodium nitroprusside was similar in both experimental groups, regardless the presence or the absence of clorgyline (fig. 2).

Preincubation with L-NAME, a potent eNOS inhibitor caused a significant reduction in relaxation response in SHR group as compared to control; also, relaxation in the rings from SHR group treated with clorgyline was improved as compared to the non-treated rings. This observation suggests that the mechanism underlying the improvement of relaxation could be NO-dependent (fig. 3).

**Fig. 2.** Sodium nitroprusside dose-response curve of aortic rings in SHR vs. CONTROL, +/− clorgyline(10 µmol/l) in the presence of indometacin (10 µmol/l).

**Fig. 3.** Acetylcholine dose-response curve in aortic rings from SHR vs. CONTROL, +/- clorgyline (10 µmol/l) in the presence of L-NAME (10 µmol/l) and indometacin (10 µmol/l). (*, # p<0.001)
DISCUSSION

Increased production of radical species has been constantly associated to various pathologies, including hypertension, atherosclerosis, diabetes (8, 10). ROS-induced endothelial dysfunction is a well established pathomechanism but currently this phenomenon was attributed to the increased formation of the highly reactive superoxide anion (11), not to H$_2$O$_2$ with the former being considered the only responsible for scavenging NO (with the generation of peroxynitrite required for eNOS uncoupling). However, since hydrogen peroxide is freely diffusible through cell membranes, it can further activate inflammation, induce apoptosis and promote endothelial dysfunction (12).

The roles of MAOs, in terminating the action of neurotransmitters in the central and peripheral nervous system and in the oxidation of dietary amines in extraneuronal tissues have been extensively studied (6,7). Thus, it has been shown that in vitro or in vivo application of L-deprenyl, an irreversible MAO-B inhibitor, produced vasodilatation via an increase in the amount of NO in brain tissue and cerebral blood vessels (13). NO inhibits mitochondrial monoamine oxidase activity and decreases outer mitochondria membrane fluidity, yet, the mechanism by which NO inhibits MAO remains unknown at present. However, it seems likely that the effect of NO on MAO activity is by a direct interaction of the compound or a metabolite with the protein (14, 15).

In the present study we focused on the role of MAO-A in the development of endothelial dysfunction in an experimental model of genetically induced hypertension. Ex vivo blockade of MAO A with clorgyline improved endothelium-dependent relaxation in vessels from hypertensive animals. Of note, this effect was not present in vessels isolated from the control animals. This observation strongly suggests that inhibition MAO activity in basal conditions do not interfere with vascular reactivity, most probably due to minimal generation of H$_2$O$_2$.

However, catabolism of monoamines results not only in H$_2$O$_2$, but also in production of ammonia, a much less investigated by-product (3). Generation of NH$_3$ could also explain the deleterious effects of MAO activation in vascular cells. NH$_3$ is known to be a mediator of cerebral vascular dysfunction in hepatic encephalopathy. Ammonia stimulated the formation of ROS by cerebral endothelial cells (16, 17) and reduction of ammonia levels in a liver failure model, improved endothelial function (18).

The present study has at least two limitations: first, MAO activity and H$_2$O$_2$ generation were not quantified and the experiments were restricted to the functional analysis of isolated vessels without addressing the effects of in vivo MAO inhibition. However, systemic MAO inhibition in this model is not suited to address the role of MAO in endothelial function. As MAOs are a main pathway of catecholamine degradation, the MAO inhibitor-induced accumulation of these vasoactive compounds may increase the already present hypertensive response and further promote vascular dysfunction (19). Also, it must be pointed out that information concerning the pharmacokinetics of MAO inhibitors is quite scarce. For instance, it is not clear whether the plasma concentrations achieved in patients treated for neurological disorders are in the range of the dosages tested in this study. Systematic pharmacokinetic studies
are undoubtedly necessary to establish the dose regimen necessary to achieve and maintain the concentrations of the circulating compounds in the micromolar range.

MAO inhibitors are currently used in psychiatry and neurology for the treatment of anxiety and depressive disorders, Parkinson’s and Alzheimer’s diseases, respectively (6). Therefore, no major problem should exist for their introduction in clinical studies concerning heart disease. Of note, MAO inhibition was already used in the late ‘60s in the treatment of angina pectoris (20, 21), although obviously the involvement of mitochondria and ROS generation was not even in its infancy. In a recent paper, Li et al demonstrated a therapeutic effect of an antidepressant medication (selective serotonin uptake inhibitor) in ameliorating experimental heart failure (22). If evidence will be provided for common novel pathways contributing to these co-morbid pathologies maybe, indeed, in the future antidepressants will be able to heal both minds and hearts (23). In this light, further studies addressing the potential role of MAO inhibitors in reversing an impaired vascular dysfunction are clearly warranted.

CONCLUSIONS
Inhibition of MAO-A improved the vasodilator response to Ach in hypertensive rat aortas whereas no effect was observed in control animals. Therefore, the MAO-A inhibitor, clorgyline, might be useful in restoring endothelium-dependent relaxation in situations of increased vascular oxidative stress and endothelial dysfunction.

ACKNOWLEDGEMENTS
The study was supported by the HU-RO/0901/137/2.2.2 project and the POSDRU fellowship nr. 88/1.5/S/63117.

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