Investigating the Sulfhydryl Redox Status in the Hypotensive Acute Effects of Peroxyl Radicals

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Abstract  
Post-prandial hypotension is a well-known phenomenon. We aimed to investigate, in the absence of immune and metabolic interfering factors, the correlation of the plasma antioxidant capacity with the early alterations of blood pressure induced by peroxyl radicals. We previously suggested that the hypotensive effect of acute infusion of peroxyl radical generators in rats could be mediated by sulfhydryl (SH) redox status. Therefore, in this study we evaluated the plasma SH, peroxides (ROOH) and both lipophilic (LAC) and hydrophilic (HAC) antioxidant capacity, before, during and after the HAC induction caused by 1,5-diamino-2,4-di-methylvaleronitrile (AMVN). As a result AAPH infusion induced a rapid decrease of HAC and SH, these effects were followed by a strong increase of both markers, whereas AMVN treatment was unable to change the HAC and SH values. On the other hand AMVN reduced plasma LAC and increased ROOH, while, in the animals treated with AAPH, ROOH and LAC were decreased and unchanged, respectively. Therefore, SH redox status seems to be not involved in the hypotensive acute effects of peroxyl radicals. Further studies are needed to better understand the mechanisms responsible for paradoxical effects of acute oxidative stress.

Keywords — Antioxidant capacity, hypotension, peroxyl radicals, rats, sulfhydryl groups.


I. INTRODUCTION

Our previous findings in vivo¹ suggested that the early cardiovascular effects of free radicals may not involve a quenching activity on nitric oxide, but a thiol-regulated vasodilator mechanism as previously proposed². Iesaki and Wolin³ observed that the thiol oxidant diamide caused a reversible concentration-dependent relaxation of endothelium-denuded bovine coronary arteries and that this effect was reversed by the thiol reductant dithiothreitol (DTT) or by diamide removal, whereas the relaxation was enhanced by the inhibitor of the glutathione reductase 1,3-bis(2-chloroethyl)-1-nitrosourea⁴. Accordingly, after bolus infusion in single doses, the thiol oxidizing agent 5,5'-Dithiobis-(2-nitrobenzoic) (DTNB) and the chelating agent N-ethylmaleimide (NEM) both induced a dose-dependent decrease in rat blood pressure, whereas glutathione and DTT inhibited the decrease in blood pressure induced by peroxyl radicals⁵. This suggest that in humans the after meal increase of plasma sulfhydryl groups (SH)³,⁶, which was accompanied by increases of metabolic and inflammatory markers³, could be a response to the oxidative stress-induced postprandial hypotension. Indeed, despite after meal hyperglycaemia⁴ and lipaemia⁷ induced endothelial dysfunction, postprandial hypotension is a known phenomenon, influenced by baseline pressure and age⁸. Furthermore, in the early postprandial period has been observed, in individuals with the metabolic syndrome, a fast decrease of plasma adrenaline⁹.

After a high-fat meal (HFM) it has been observed an increased oxidative burst of leukocytes², responsible for the oxidation of low-density lipoprotein (LDL)¹⁰. The latter phenomenon contributes to the pathogenesis of atherosclerosis at the level of the vascular associated lymphoid tissue (VALT)¹⁰. Also perivascular adipose tissue (PVAT) has been involved in the early alterations of the vascular contractility associated to the changes in fatty acid composition and oxidative stress¹¹. PVAT has been also involved in the HFM induced inflammation¹². Besides, it has been suggested that the fast inflammatory response to HFM could be due to a fat-induced cytokines release from the gut associated lymphoid tissues (GALT)¹³.

On the other hand, the oxidative burst inducer peptide N-formyl-Met-Leu-Phe (fMLP) showed spasmodic actions on guinea-pig intestine partly through the release of acetylcholine from myenteric motorneurons and through prostanoïds, probably from the inflammatory cells of the enteric immune system¹⁴.
Therefore, dysfunctions of GALT, VALT, PVAT, adrenergic and cholinergic responses could have multiple site of interaction in the development of cardiovascular disease (CVD).

The aim of this study was to evaluate, in the absence of immune and metabolic interfering factors, the correlation of the plasma antioxidant capacity with the early alterations of vascular contractility induced by peroxyl radicals.

II. METHODS

A. Animals and chemicals

Male Wistar rats, weighing 0.3-0.35 kg were purchased from Charles River (Calco, Italy). All animals were acclimatized to a 12-h light/dark cycle at 23°C with food and water available ad lib for 1 week before starting the experiments.

2,2'-azobis-(2-aminopropane) dihydrochloride (AAPH) and 2,2'-Azobis 2,4-di-methylvaleronitrile (AMVN) were provided by Wako Chemical (Germany). All other chemicals were from Sigma Chemical (St. Louis, MO).

B. Ethics Statement

All procedures involving animal care or treatments were approved by the Italian Ministry of Health (Rome, Italy) and performed in compliance with the guidelines of the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize animal suffering and to reduce the number of animals used.

C. Surgical procedures in anesthetized rats

Rats were anesthetized with an intraperitoneal injection of sodium pentobarbitone (60 mg/kg). The trachea was exposed and cannulated to facilitate spontaneous respiration. Arterial blood pressure was measured from the carotid artery through a heparinized polyethylene cannula PE-50 connected to a pressure transducer calibrator coupled with a pen-recorder (Ugo Basile, Italy). Drugs were given as a bolus injection (0.1 mL) through a cannula inserted into the external jugular vein and the vein was flushed with saline (0.2 mL). Venous blood samples (0.1 mL) were collected through a cannula inserted into the femoral vein.

D. Antioxidants and ROS evaluations

In order to have comparable results, both plasma lipophilic (LAC) and hydrophilic (HAC) antioxidant capacity were evaluated using the crocin bleaching method\textsuperscript{15, 16}. The information produced by this test is similar to the popular "total radical trapping antioxidant potential" (TRAP)\textsuperscript{17} analysis, but it can be performed also in lipophilic condition\textsuperscript{16}. SH were measured using 5,5'-dithiobis-(2-nitrobenzoic)\textsuperscript{18}. The reactive oxygen species were assayed spectrophotometrically measuring the increase of the absorbance signal of 2',7'-dichlorofluorescein (DCF) produced by oxidation of 2',7'-dichlorofluorescein (DCFH) due to the plasma peroxides (ROOH) without free radical inducers\textsuperscript{19, 20}.

E. Statistics

Fig. 1. (A) Typical blood pressure tracing recorded during bolus infusion of 2,2'-azobis- (2-aminopropane) dihydrochloride (AAPH) (10 mg/kg) and venous blood sampling. (B) Typical blood pressure tracing recorded during bolus infusion of AAPH (10 mg/kg), before and after infusion of norepinephrine (NA) (1 μg/kg). (C) Hydrophilic (HAC) and lipophilic (LAC) antioxidant capacity time course in sham operated rats. ***p<0.001 HAC versus LAC.
All data are presented as the mean ± SEM. Statistical analysis was carried out with analysis of variance (ANOVA). Student–Newman–Keuls post hoc analysis was used to isolate differences between groups. Statistical evaluations were performed using Sigmastat software (Jandel Scientific Inc., San Rafael, CA).

III. RESULTS

The animals were allowed to equilibrate for at least 30 minutes before administration of two identical control doses of norepinephrine (NA, 1 μg/kg) or test substances. AMVN was dissolved in ethylene glycol and diluted with fresh saline (0.9% sodium chloride) at a final concentration (of 0.3%) that left blood pressure unchanged.

AAPH 10mg/Kg and AMVN 0.1 mg/Kg induced hypotension as previously described¹. Venous blood samples were collected 15m before treatment for baseline determinations (I), during the infusion (II) of AAPH (10mg/Kg) (Figure 1A), AMVN (0.1 mg/Kg), or saline and 15 min after the treatment (III).

Then, animals were allowed to equilibrate before administration of control (NA) and we confirmed the previous observation¹ that AAPH (Figure 1B) or AMVN left NA control doses unchanged. The HAC was significantly higher than the LAC in the sham operated rats and no variations have been observed during the control (saline) test (Figure 1C).

AAPH infusion induced a rapid decrease, followed by a strong increase, of HAC. (Figure 2A). On the contrary, the AMVN treatment was unable to change the HAC (Figure 2A), however it reduced plasma LAC, whereas AAPH did not change the lipophylic antioxidant compartment (Figure 2B).

In agreement with these results, we can observe that the total plasma SH after AAPH infusion also followed the same trend of HAC (Figure 2C). On the other hand, the ROOH values decreased in the animals treated with AAPH (Figure 2D). Contrarily, AMVN did not have any effect on plasma SH groups (Figure 2C), but increased the ROOH levels (Figure 2D).

Fig. 2. Hydrophilic antioxidant capacity (HAC) (A), Lipophilic (LAC) antioxidant capacity (B), sulfhydryl (SH) (C) and peroxides (ROOH) (D) levels 15min before (I), immediately after (II) and 15min after (III) 2,2'-azobis- (2-amidinopropane) dihydrochloride (AAPH) (10 mg/kg) or 2,2'-Azobis 2,4-di-methylvaleronitrile (AMVN) (0.1 mg/kg) infusion (n=4). *p<0.05, ** p<0.01, ***p<0.001 versus baseline levels.
IV. Discussion

The free radicals mediated modulation of plasma redox status during acute hypotension was investigated by treatment of anaesthetized rats with AAPH and with its lipo-soluble analog AMVN. AAPH infusion induced a rapid decrease, followed by a strong increase, of HAC. In agreement with these results, we observed that the total plasma SH also followed the same trend, in agreement with other authors, found that a consumption and regeneration of GSH in rat blood, in the time range 0-120 min, after treatment with 100 and 200 mg/kg i.p. of diamide.

On the other hand, the ROOH values decreased in the animals treated with AAPH, this reduction in peroxides is accompanied by a remarkable increase of SH. Others reported that during reperfusion following sub lethal ischemia the analysis for antioxidant enzymes in rats revealed increased levels of catalase and glutathione peroxidase due to a rapid expression of genes which may enhance reactive oxygen intermediate scavenging. This could be coupled, during postprandial hypotension, to the activation of the Nuclear Factor-kappa B inflammatory pathway. This transcription factor could regulate both antioxidant and pro-oxidant gene expression.

On the contrary, the AMVN treatment was unable to change the HAC and did not have any effect on plasma SH groups, however it reduced plasma LAC, whereas AAPH did not. The ROOH levels increased after AMVN infusion and these results were inversely related with LAC of rats treated with AMVN. Probably lypophilic ROS generator AMVN mimics the peroxide production due to the post-prandial oxidative burst-induction reported by Ghanim, whereas hydrophilic ROS generator AAPH simulate the post-prandial increase of SH. However, our results show that the hydrophilic/lipophilic free radicals determine different responses against the free radicals injury, but have similar hypotensive effects. These findings suggest that the postprandial hypotension is not linked with redox balance but could be due to other mechanisms.

A role for voltage sensitive L-type Ca2+ channels and of Ca2+-activated K+ channels in the intestinal motility disorders induced by AAPH has been suggested. Moreover, AAPH modified the activity of several plasma membrane ion transport systems involved in calcium and pH homeostasis in rat aortic smooth muscle (RASM) cells. Pretreatment of RASM cells with atrial natriuretic peptide (ANP) attenuated the intracellular Ca2+ and pH fall induced by AAPH, suggesting a possible role of this hormone as a defensive effector against the early events of the oxidative stress. Recently, during post-exercise hypotension in pre-hypertensive subjects unchanged NO or S-nitrosothiols were observed, while the fall in blood pressure was accompanied by increase in lipid hydroperoxides and ANP.

The major limitation of this study is that it has been conducted on control rats and not on spontaneously hypertensive rats (SHR). In vitro, endothelial vasorelaxation to acetylcholine was blunted in carotids from SHR compared to normotensive rats. Excess of O2-• has been suggested to be responsible for the impaired endothelium dependent relaxation via a reduction in bioavailable nitric oxide (NO•) in SHR, whereas peroxynitrite (ONOO-) produces an endothelium independent relaxation of aortic ring.

Neutrophils caused relaxation of normotensive rat aorta with or without endothelium, whereas a contraction was observed in aorta with endothelium from SHR. Therefore, the postprandial increases of the neutrophils could have different effects on different subjects. In particular, the potential effects of genetic polymorphisms, including the calcium/calmodulin-dependent kinase IV polymorphism involved in hypertension, have not been evaluated in this study.

Also aging influenced the vascular reactivity to reactive oxygen species in rats. In sedentary older subjects, endothelium-dependent flow-mediated dilation (FMD) and plasma hydrophilic antioxidant capacity were lower compared to older athletes and young subjects, whereas the lipoperoxidation marker malondialdehyde (MDA) levels were higher. However, in hypercholesterolemic subjects, dietary supplementation with coenzyme Q decreased low-density lipoprotein oxidizability but had no significant effect on FMD. These findings suggest that lipoperoxidation has not a primary role in endothelial dysfunction and hypertension. Accordingly, the renin-angiotensin-aldosterone system has been recognized to have a crucial role in endothelial dysfunction and arterial stiffness. Furthermore, SHR had sympathetic dysfunction with a greater plasma noradrenaline concentration, which was normalized by a 4 weeks treatment with N-acetylcysteine.

In conclusion, SH redox status seems to be not involved in the hypotensive acute effects of peroxyl radicals. Further studies are needed to better understand the mechanisms responsible for the paradoxical effect of acute oxidative stress on blood pressure.

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References


