Endogenous and Exogenous Antioxidant Protection for Endothelial Dysfunction

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We performed „in vitro" experiments using human mammary artery rings, in order to examine the effect of endogenous and exogenous antioxidants on endothelial-dependent vasodilatation induced by cumulative doses (10^{-9} M – 10^{-4} M) of adenosine (ADP) and to study the effect on endothelial – independent vasodilatation induced by cumulative doses (10^{-9} M – 10^{-4}M) of sodium-nitroprusside (NSP), respectively. Our results showed that 1 hour pre-incubation with triglyceride diminished the endothelial-dependent vasodilator response to ADP, but it has not modified the endothelial -independent vasodilator response to NSP. Vascular response was expressed as maximal vasodilatation from the 10^{-5} M phenilephrine (PE) induced pre-contraction, considered as reference. One hour co-incubation of the rings with triglyceride and antioxidant factors, such as 10^{-3} M reduced glutathione (GSH) or 10^{-3} M ascorbic acid significantly reduced the impairment of the vasodilatation response to ADP ($p = 0.02$ for GSH, and $p = 0.008$ for ascorbic acid) but has not modified the vasodilatation response to NSP. As a conclusion, the endothelial dysfunction induced by the triglyceride could contribute to the pathogenesis of atherosclerosis and the treatment with high doses of antioxidants could „protect" the endothelium against the pro-atherogenic action of the triglyceride.

Keywords: antioxidants, endothelial dysfunction, sodium nitroprusside

Antioxidants are substances that may protect the cells against the effects of free radicals. Free radicals are molecules produced when our body breaks down food, or by environmental exposures like tobacco smoke and radiation. Free radicals can damage cells, and may play a role in heart disease, cancer and other diseases.

Endothelial dysfunction is characterized by a shift of the actions of the endothelium toward reduced vasodilatation, a proinflammatory state, and prothrombic properties. It is associated with most forms of cardiovascular disease, such as hypertension, coronary artery disease, chronic heart failure, peripheral artery disease, diabetes, and chronic renal failure. Mechanisms that participate in the reduced vasodilator responses in endothelial dysfunction include reduced nitric oxide generation, oxidative excess, and reduced production of hyperpolarizing factor. In present work we have studied the effect of glutathione (GSH) as endogenous antioxidant and ascorbic acid (Vitamin C) as exogenous antioxidant on endothelial dysfunction caused by triglyceridemia.

Glutathione (GSH) is a tripeptide which contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side chain (fig 1). GSH is the major antioxidant produced by the cells, detoxifies many xenobiotics and carcinogens and plays an important role in metabolic and biochemical processes. Also it is essential for the modulatory role of immune response.

Ascorbic acid (vitamin C) is a sugar acid with antioxidant properties (fig 2). Ascorbate acts as an antioxidant by being available for energetically favourable oxidation. Many oxidants such as the hydroxyl radical (formed from hydrogen peroxide), contain an unpaired electron, and, thus, are highly reactive and damaging to humans and plants at the molecular level. This is due to their interaction
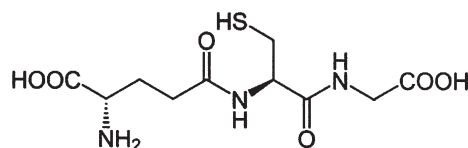

Fig. 1. Glutathione (2*S*)-2-amino-4-{[(1*R*)-1 [(carboxymethyl)carbamoyl]-2-sulfanylethyl]carbamoyl}butanoic acid
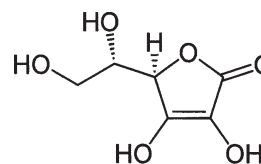

Fig. 2. Ascorbic acid
(5*R*)-[(1*S*)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5*H*)-one

with nucleic acid, proteins, and lipids. Reactive oxygen species oxidize (take electrons from) ascorbate first to monodehydroascorbate and then dehydroascorbate. The reactive oxygen species are reduced to water, while the oxidized forms of ascorbate are relatively stable and unreactive, and do not cause cellular damage.

Vitamin C accumulates in mitochondria, where most of the free radicals are produced. Ascorbic acid protects the mitochondrial genome and membrane.

Experimental part

Venous blood from hypertriglyceridemic subjects (TG ≥ 200 mg %) was collected.

Experiments were performed on human mammary arteries from patients undergoing coronary by-pass intervention. The segments of artery were cleared of connective tissue with care taken not to damage the intimal
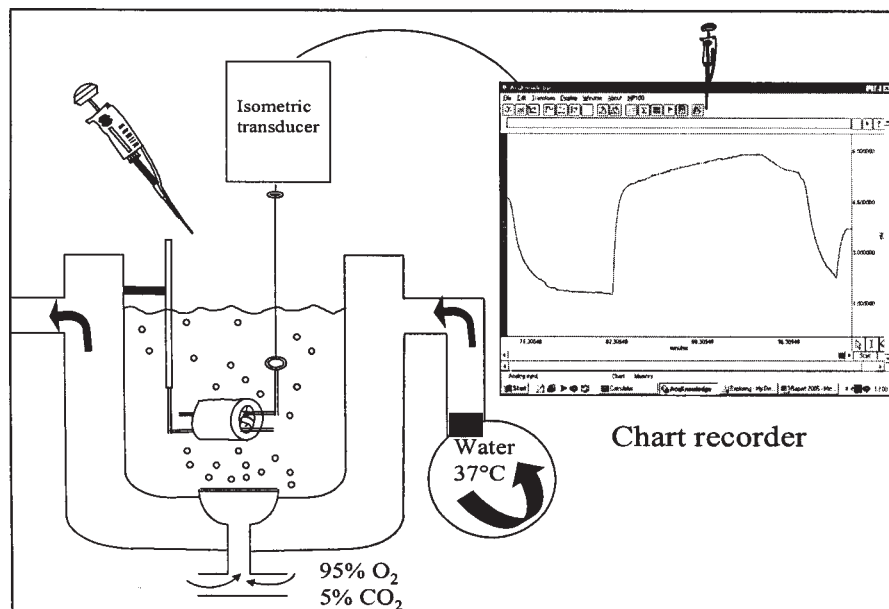
Fig. 3. BIOPAC organ bath

surface, and cut into 2 to 3 mm wide rings. The rings were suspended between two parallel stainless steel hooks in a 10 ml organ bath (BIOPAC System Inc., USA) containing modified Krebs-Henseleit buffer (composition: NaCl 118 mmol/L; $NaHCO_3$ 25 mmol/L; KCl 4.7 mmol/L; $CaCl_2$ 1.6 mmol/L; KH_2PO_4 1.2 mmol/L; $MgSO_4$ 1.2 mmol/L; glucose 11.1 mmol/L) maintained at 37°C and continuously ventilated with a mixture of 95% O_2 and 5% CO_2 (fig.1). One of the hooks was connected to a FORT 10 force transducer (World Precision Instruments Inc.) for isometric tension recording. For data acquisition we used a MP100 hardware and AcqKnowledge software, version 3.7.2 (BIOPAC System Inc., USA).

The rings were equilibrated for 60 min under a resting tension of 1.75 cN and the buffer was replaced every 15 min. To confirm viability of vascular smooth muscle vessel rings were contracted twice with KCl 70 mmol/L. A relaxation response to adenosine diphosphate (ADP), an endothelium-dependent vasodilator, of 15% or more from the stable tension induced by KCl triggered depolarization was considered as functioning endothelium [5] (fig 3).

After washout and return to baseline, the rings were incubated for 1 hour with a solution of 1% triglyceride (n = 6) or coincubated for 1 hour with 1% triglyceride and GSH 10^{-3} M (n = 6) or with 1% triglyceride and ascorbic acid 10^{-3} M (n = 6). In control group (n = 6) the vascular rings were exposed to the same volume of PBS. After incubation and repeated washing, indomethacin (10^{-5} M) was added to organ bath and left throughout the experiment to eliminate the influence of prostaglandin synthesis. To study endothelium-dependent relaxation, we preconstricted rings with phenylephrine (PE; 10^{-5} M) and a cumulative concentration-response curve to ADP (10^{-9} – 10^{-4} M) was obtained in all rings. After this first step, the baths were washed out three times with fresh Krebs solution and the rings were allowed to stabilize until the tension return to baseline. Finally, to examine endothelium-independent relaxation, vascular rings were exposed to sodium nitroprusside in increasing doses (from 10^{-9} to 10^{-4} M) following precontraction by PE and in the presence of indomethacin. All chemicals were purchased from Sigma Chemicals Co.

Relaxation response of each human mammary artery ring was assessed by measuring the reduction in vascular tone at cumulative dose of the vasodilator agent and expressed as percentage change from the stable tension produced by PE (100 x [precontracted tension – measured tension]/[precontracted tension – baseline tension]). Maximal relaxation was the greatest reduction in tone to response to a vasodilator.

All data in the text and figure are expressed as mean ± SD of experiments from n vascular rings. Statistical comparison was performed using 2-tailed Student's test. The differences were considered to be significant at a level of $P < 0.05$.

Results and discussions

The results of assessment of vascular reactivity in human mammary artery rings are presented in table 1. Values are means ± SD. PE: phenylephrine; ADP: adenosine dyphosphate; GSH - glutathione reduced, NSP: sodium nitroprusside.

The values of preconstriction with PE were not significantly different between groups. In PE-preconstricted vascular rings, ADP elicited a concentration-dependent relaxation that was diminished in human mammary arteries incubated with triglyceride (fig.4). Even if the reduction was observed for all the used concentrations, a significant decrease was recorded only for the 10^{-4} M concentration (p < 0.001). Endothelium-independent relaxation induced by SNP was similar in all groups (fig.5). The antioxidant agents administration may protect the endothelium against the atherogenic effects of triglyceride (fig 4, 5).

The present data indicate that in human mammary artery rings, ADP-mediated endothelium-dependent relaxation was significantly attenuated by preincubation with triglyceride. A nonspecific attenuation of vasodilator capacity is not very likely, since the endothelium-independent vasodilatation induced by sodium nitroprusside was preserved. The fact, that the cyclooxigenase inhibitor indomethacin was present in all experiments, strongly suggests that triglyceride may induce primary impairment of endothelial vasomotor function by decreasing the release and/or activity of nitric oxide (NO) in vascular endothelial cells. Our results are in agreement with the findings reported by the team of H. Yasue, in isolated rabbit aortas [4] and in human coronary arteries [9].

Recent it was revealed that increased serum triglyceride levels are closely related to atherosclerosis, independently

	n	Control group	triglyceride group	p
Contraction to PE (cN)	12	3.63 ± 1.14	3.23 ± 0.85	0.34
Maximal relaxation (%)				
ADP	6	64.30 ± 15.80	14.39 ± 5.80	< 0.001
NSP	6	95.86 ± 3.48	93.33 ± 7.36	0.46

	n	Triglyceride group	triglyceride GSH group	p
Contraction to PE (cN)	12	3.23 ± 0.85	3.33 ± 1.24	0.35
Maximal relaxation (%)				
ADP	6	14.39 ± 5.80	44.12 ± 25.08	0.02
NSP	6	93.33 ± 7.36	99.77 ± 4.73	0.33

	n	Triglyceride group	triglyceride ascorbic acid group	p
Contraction to PE (cN)	12	3.23 ± 0.85	3.56 ± 1.76	0.50
Maximal relaxation (%)				
ADP	6	14.39 ± 5.80	59.61 ± 14.58	0.008
NSP	6	93.33 ± 7.36	93 ± 6.18	0.52

Table 1
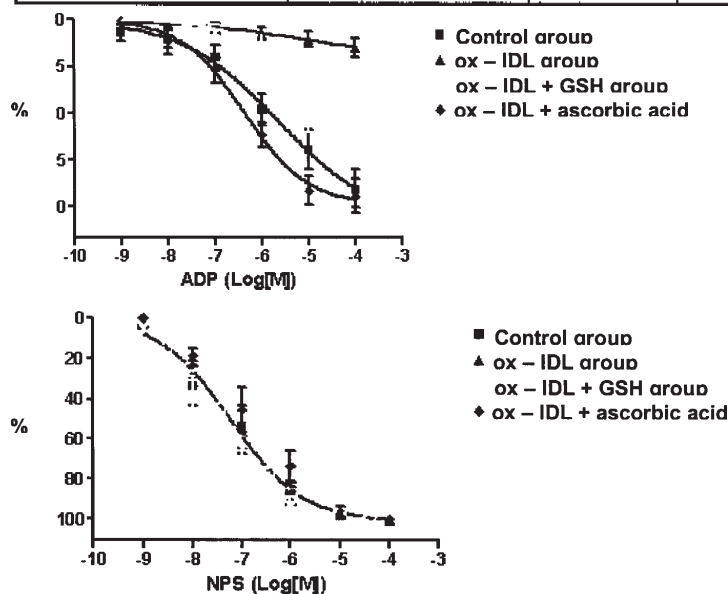VASCULAR REACTIVITY OF HUMAN MAMMARY ARTERY RINGS

Fig. 4. The dose –response curves to ADP in all groups

Fig. 5. The dose-response curves to NPS in all groups

of serum levels of high-density lipoproteins and low-density lipoproteins [8].

Among triglyceride may be oxidatively modified in the arterial intima and cause an increase in the susceptibility of vascular endothelium to oxidative stress, via lectin-like oxidized LDL receptor-1 (LOX-1) [10]. This mechanism may play a role in the genesis of endothelial dysfunction in subjects with high triglyceride levels. Other molecular mechanisms for proatherogenic properties of triglyceride are following: increased adhesion of monocytes to vascular endothelial cells monocytes; increased smooth muscle cell proliferation, independently of oxidative stress, via epidermal growth factor receptor transactivation; and induced foam cell formation in macrophages via apoB48 receptor [8].

Conclusions

Triglycerides impair vasomotor function of endothelium in isolated human mammary arteries. Endothelial dysfunction induced by these triglycerides could contribute to the pathogenesis of atherosclerosis associated with hypertriglyceridemia. Therefore, the antioxidant agents administration may protect the endothelium against the triglycerides.

This conclusion supports the concept that each of the changes in the plasma lipoproteins associate with elevated triglyceride plasma level contribute to the increased risk of premature cardiovascular disease.

References
1.AUSTIN, M.A. Epidemiology of hypertriglyceridemia and cardiovascular disease. Am J Cardiol, 1999, **83**, 13F–16F
2.BREWER, B.H., Hypertriglyceridemia: changes in the plasma lipoproteins associated with an increased risk of cardiovascular disease. Am J Cardiol 1999, **83**, 3F–12F
3.CARMENA, R., DURIEZ, P., FRUCHART, J.C., Atherogenic lipoprotein particles in atherosclerosis, Circulation, 2004, **109**, III2–7
4.DOI H., KUGIYAMA K., OHGUSHI M., et al., Membrane active lipids in remnant lipoproteins cause impairment of endothelium-dependent vasorelaxation, Arterioscler Thromb Vasc Biol, 1999, **19**, 1918–1924
5.GOTTO, A., Triglyceride as a risk factor for coronary artery disease. Am J Cardiol 1998, **82**, 22Q–25Q

6.HAVEL, R.J., EDER, H.A., BRAGDON, J.H., The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum, J Clin Invest, 1955, **34**, 1345–1353
7.KAWAKAMI, A.., YOSHIDA, M., Remnant lipoproteins and atherogenesis, J Atheroscler Thromb, 2005, **12**, 73–76
8.KUGIYAMA, K., DOI, H., MOTOYAMA, T. et al., Association of remnant lipoprotein levels with impairment of endothelium-dependent vasomotor function in human coronary arteries, Circulation, 1998, **97**, 2519–2526
9.NAKAJIMA, K., NAKANO, T., TANAKA, A., The oxidative modification hypothesis of atherosclerosis: the comparison of atherogenic effects on oxidized LDL and remnant lipoproteins in plasma, Clin Chim Acta, 2006, **367**, 36–47