THE ANTIOXIDANT FACTORS REDUCED THE IMPAIRMENT OF ENDOTHELIAL-DEPENDENT VASODILATATION IN ISOLATED HUMAN MAMMARY ARTERIES PREINCUBATED WITH TRIGLYCERIDE-RICH REMNANT LIPOPROTEINS

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Abstract. The main changes of the plasma lipid profile in patients with endothelial dysfunction are the increased triglyceride content of the lipoprotein remnant particles, the presence of the small and dense LDL particles and the decreasing of the HDL-cholesterol level. Considering these observations, we performed *in vitro* experiments using human mammary artery rings, in order to examine the effect of the lipoprotein "remnants" on endothelium-dependent vasodilatation induced by cumulative doses (10^{-9}–10^{-4} M) of adenosine (ADP) and to study the effect on endothelial-independent vasodilatation induced by cumulative doses (10^{-9}–10^{-4} M) of sodium-nitropruside (SNP), respectively. Our results showed that 1 hour pre-incubation with triglyceride-rich lipoprotein remnants diminished the endothelial-dependent vasodilator response to ADP, but it has not modified the endothelial-independent vasodilator response to SNP. Vascular response was expressed as maximal vasodilatation from the 10^{-5} M phenylephrine (PE) induced pre-contraction, considered as reference. In the case of ADP, the maximal vasodilatation was ranged in 14.39% ± 5.80% interval, comparing with the control group that presented a maximal vasodilatation of 64.3% ± 15.80% ($p < 0.001$). In the case of SNP the maximal vasodilatation was ranged in 93.33% ± 7.36% interval ($p < 0.001$), comparing with the control that presented a maximal vasodilatation of 95.86% ± 3.48% ($p = 0.46$). One hour co-incubation of the rings with lipoprotein "remnants" 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 SNP. As a conclusion, the endothelial dysfunction induced by the triglyceride-rich lipoprotein "remnants" could contribute to the pathogenesis of

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atherosclerosis and the treatment with high doses of antioxidants could "protect" the endothelium against the pro-atherogenic action of the lipoprotein "remnants".

Key words: endothelial dysfunction, lipoproteic remnants, antioxidant factors.

INTRODUCTION

There is a growing body of evidence from epidemiologic, clinical, and experimental studies that indicates that elevated triglyceride levels are an independent risk factor for cardiovascular disease [1, 6].

Hypertriglyceridemic patients at risk for cardiovascular disease often develop a plasmatic lipoprotein profile characterized by: (i) triglyceride-rich remnant, (ii) small and dense LDL, and (iii) low level of HDL cholesterol [2].

If the atherogenic role of HDL cholesterol decrease as well as of LDL phenotype B, with recognized predisposition for peroxidation, is well established, the data concerning the atherogenity of triglyceride rich remnants are contradictory and scarce [2].

The present study was designed to investigate *in vitro* the effect of triglyceride rich remnant lipoproteins on vasomotor endothelial function in human mammary arterial rings.

MATERIALS AND METHODS

PREPARATION OF REMNANT LIPOPROTEINS

Venous blood from hypertriglyceridemic subjects (TG $\geq$ 200 mg %) was collected into specimen tubes containing EDTA. Lipoproteins have been separated from plasma by sequential density gradient ultracentrifugation [7]. First, chylomicrons (chy) and very low density lipoproteins (VLDL) were removed by centrifugation of plasma (non-protein density 1,006) at 50.000×g for 3.5 hours at 12–15 ºC. Then, remnant lipoproteins (IDL with density 1.006 to 1.019 g/ml) were concentrated in a layer at the top of mixture with 1.019 g/ml density after ultracentrifugation during 20 to 22 hours at a speed of 100.000×g.

Isolated native IDL was oxidatively modified by incubation with $CuSO_4$ 5 μM for 3 h at 37 ºC. Oxidized IDL (ox-IDL) was eluted with phosphate buffer solution (PBS) to 5 mg proteins/ml.

PREPARATION OF VASCULAR RINGS

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 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 ºC 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 minutes 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].

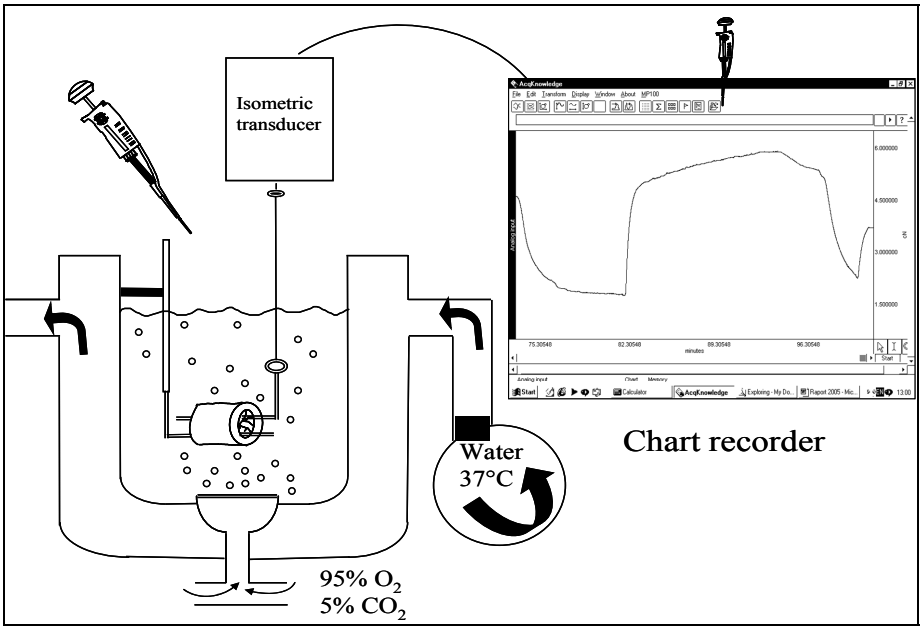

Fig. 1. BIOPAC organ bath.

EXPERIMENTAL PROTOCOL

After washout and return to baseline, the rings were incubated for 1 hour with a solution of 1% ox-IDL (100 µl proteins / ml Krebs buffer) (n = 6), or coincubated for 1 hour with 1% ox-IDL and GSH 10^{-3} M (n = 6) or with 1% ox-IDL and

ascorbic acid 10^{-3} M (n = 6). In the 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 returns 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 × [precontracted tension – measured tension]/[precontracted tension – baseline tension]). Maximal relaxation was the greatest reduction in tone to response to a vasodilator.

DATA ANALYSIS

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

The results of assessment of vascular reactivity in human mammary artery rings are presented in Table 1. Values are means $\pm$ SD. PE: phenylephrine; ADP: adenosine diphosphate; GSH-glutathione reduced, SNP: 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 ox-IDL (Fig. 2). 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. 3).

Table 1

Vascular reactivity of human mammary artery rings

	n	Control group	ox-IDL 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
SNP	6	95.86 ± 3.48	93.33 ± 7.36	0.46
	n	ox –IDL group	ox-IDL + 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
SNP	6	93.33 ± 7.36	99.77 ± 4.73	0.33
	n	ox –IDL group	ox-IDL + 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
SNP	6	93.33 ± 7.36	93 ± 6.18	0.52

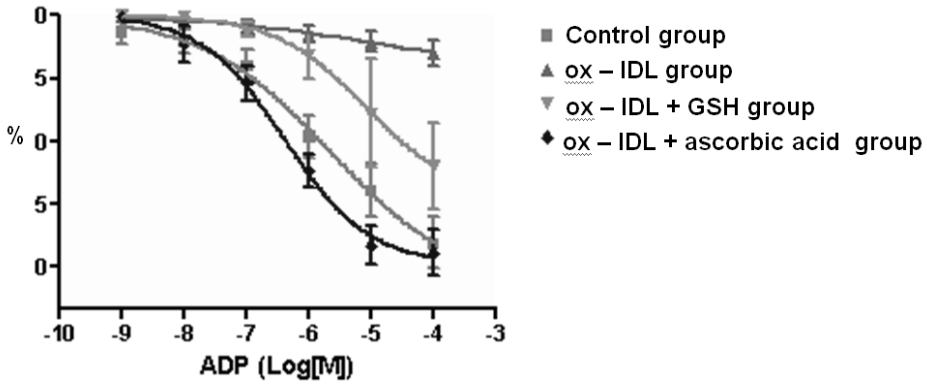

Fig. 2. The dose-response curves to ADP in all groups.

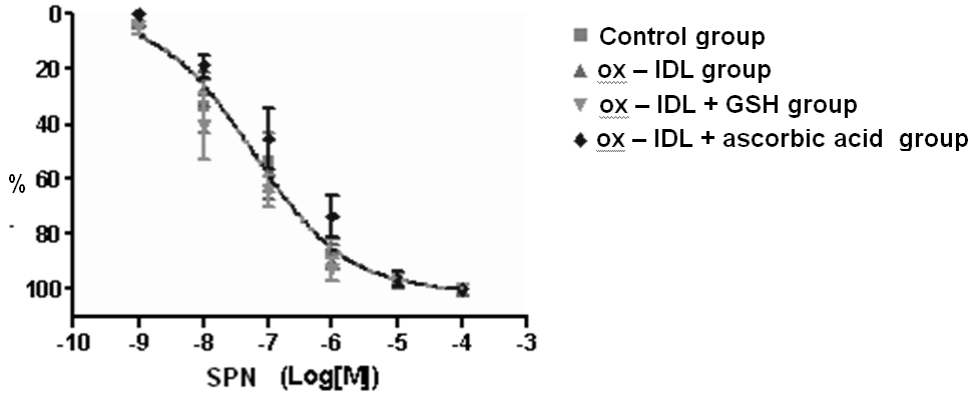

Fig. 3. The dose-response curves to SNP in all groups.

DISCUSSIONS

The present data indicate that in human mammary artery rings, ADP-mediated endothelium-dependent relaxation was significantly attenuated by preincubation with oxidized IDL (ox-IDL). 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 cyclooxygenase inhibitor indomethacin was present in all experiments, strongly suggests that remnant lipoproteins 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].

Recently it was revealed that increased serum triglyceride levels are closely related to atherosclerosis, independently of serum levels of high-density lipoproteins and low-density lipoproteins [8].

Among triglyceride-rich lipoproteins, remnant lipoproteins 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 remnant lipoprotein levels. Other molecular mechanisms for proatherogenic properties of remnant lipoproteins are the 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].

CONCLUSION

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

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

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