Reduced plasma high-density lipoprotein and increased platelet activity in arterial versus venous blood

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Summary: Plasma lipid and lipoprotein pattern and platelet activity were studied in blood samples derived from veins and arteries of 10 healthy male subjects. A significant reduction in plasma high-density lipoprotein (HDL) cholesterol, triglyceride and protein levels, as well as in plasma apolipoprotein A-I, was found when lipoproteins were derived from arterial blood in comparison to venous blood. All other lipoproteins were not significantly changed. Platelet activity measured as plasma beta-thromboglobulin levels and as collagen-induced platelet aggregation and ^{14}C -serotonin release in platelet-rich plasma was markedly elevated when platelets were derived from arterial blood. Since reduced plasma HDL concentration and platelet activation are known risk factors for atherosclerosis, our study may suggest a further explanation for the presence of atherosclerotic lesions in arteries but not in veins.

Introduction

Atherosclerosis is the result of degenerative disorders of the arteries, characterized by thickening of the arterial wall, when accumulation of lipids in smooth muscle cells and macrophages occurs.¹ The atherosclerotic plaque occurs in arteries, but not in veins, and this can be related to differences in blood pressure, pH and morphology. Plasma lipid and lipoprotein pattern is well related to the risk of coronary heart disease.² Platelet function has been shown to be elevated in patients with accelerated atherosclerosis.³

In the present study we analysed plasma lipid and lipoprotein pattern as well as platelet function in blood obtained from the arteries and veins of healthy subjects. Our purpose was to find out whether possible differences among the above parameters could explain the presence of atherosclerosis in arteries but not in veins.

Materials and methods

Blood (60 ml) was drawn at 0800 h, after 14 hours of fasting, from the antecubital vein and then from the radial artery of 10 healthy males who had taken no medication for at least 3 weeks preceding the study. The blood was drawn into EDTA (1 mmol/l) for lipid analysis and into 3.8% sodium citrate (vol/vol = 9:1)

for platelet studies. Plasma and lipoprotein cholesterol and triglycerides^{4,5} were determined by enzymatic methods, and protein concentration by the Folin reagent.⁶ Plasma lipoproteins were separated by discontinuous density gradient ultracentrifugation.⁷ The density of 4 ml of plasma was raised to 1.25 kg/l with potassium bromide. Four millilitres of sodium chloride solution ($d = 1.084 \text{ kg/l}$) were then carefully laid over the plasma sample, followed by 4 ml of sodium chloride ($d = 1.006 \text{ kg/l}$). Ultracentrifugation was performed in an SW-41 rotor in a Beckman L2-65B preparative ultracentrifuge at $35,000 \times g$ for 48 hours at 4°C . Lipoproteins were separated and analysed for purity by immunoelectrophoresis. Plasma apolipoproteins were determined by immunoelectrophoresis⁸ and platelet aggregation and serotonin release induced by $1 \mu\text{g/ml}$ of collagen (Hormon Chemie, Munchen, FRG) was studied within 1 hour of sampling, in platelet-rich plasma (PRP) preparation adjusted to $300,000/\mu\text{l}$ with platelet-poor plasma, according to the method of Born⁹ and Jerushalmy & Zucker,¹⁰ respectively. Plasma beta-thromboglobulin was analysed by radioimmunoassay kit (Radiochemical Centre Amersham, Buckinghamshire, UK). Special precautions were used in taking the blood, using a polystyrene syringe fitted with a 20-gauge needle, without vein compression, in order to prevent platelet activation during sampling. For apolipoprotein analysis, rocket immunoelectrophoresis was performed, using dilutions of 1:150 and 1:1000 for anti-A-I and anti-B, respectively. Plasma

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was diluted 1:25 with barbital buffer. The antibodies were prepared from purified apolipoproteins, which were also used for standardization.

The Wilcoxon rank test was used for analysis of the data. Results are expressed as mean $\pm$ standard deviation.

Results

Plasma cholesterol and triglycerides refers to the total levels present in all lipoprotein fractions (Table I). Plasma concentrations of cholesterol and triglyceride and very-low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) cholesterol and triglyceride concentrations and plasma apolipoprotein B concentrations were not significantly different when derived from venous or arterial blood (Table I). However, plasma high-density lipoprotein (HDL) cholesterol, triglyceride and protein components as well as plasma apolipoprotein A-I levels were significantly reduced when derived from arterial blood in comparison to venous blood (Table I), with reduction of 11, 21, 18 and 15%, respectively.

Platelet functions studied by three different parameters were significantly increased when derived from arterial blood in comparison to venous blood (Table II). Plasma beta-thromboglobulin, which is a measure of *in vivo* platelet activity (whereas collagen-induced aggregation and release measure activity *in vitro*), increased by 40%. Platelet aggregation and ^{14}C -serotonin release in PRP, induced by collagen, were also enhanced when obtained from arterial blood, by 11 and 13%, respectively. Plasma beta-thromboglobulin was found to correlate positively ($r = 0.80$) with collagen-induced aggregation.

Discussion

Our study demonstrated increased platelet activity and reduced plasma HDL levels in blood samples derived from the arteries in comparison to those derived from veins. These results are associated with increased risk for atherosclerosis and thus may explain the phenomenon of atherosclerotic plaque in arteries but not veins. Platelet activation is associated with increased risk for atherosclerosis, as demonstrated in hyperlipidaemic patients and in patients with coronary heart disease.^{3,11,12} HDL has been shown to be an independent risk factor for atherosclerosis, and decreased plasma HDL concentration has been found in patients with accelerated atherosclerosis.^{13,14} In the present study, both increased platelet activity and reduced plasma HDL level were found, thus favouring increased risk for atherosclerosis in blood preparations derived from arteries in comparison to blood

Table I Lipid, lipoprotein and apolipoprotein concentrations (mg/dl) in plasma derived from venous and arterial blood

Blood origin	Plasma			VLDL			LDL			HDL		
	Chol	TG	Apo A-I	Apo B	Chol	TG	Prot	Chol	TG	Prot	Chol	TG
Venous	198 $\pm$ 31	86 $\pm$ 11	130 $\pm$ 8	84 $\pm$ 11	35 $\pm$ 6	35 $\pm$ 5	16 $\pm$ 2	104 $\pm$ 9	21 $\pm$ 2	70 $\pm$ 8	62 $\pm$ 5	23 $\pm$ 3
Arterial	185 $\pm$ 39	89 $\pm$ 10	110 $\pm$ 9*	80 $\pm$ 7	31 $\pm$ 4	40 $\pm$ 6	13 $\pm$ 3	100 $\pm$ 10	18 $\pm$ 3	65 $\pm$ 5	55 $\pm$ 6†	18 $\pm$ 3†

Results represent mean ($n = 10$) $\pm$ s.d. Abbreviations: Chol, cholesterol; TG, triglycerides; Prot, protein; Apo, apolipoprotein. Statistically significant differences between venous and arterial blood are shown by: * $P < 0.02$; † $P < 0.05$.

Table II Plasma β -thromboglobulin and platelet aggregation and ^{14}C -serotonin release in PRP derived from venous and arterial blood

Blood origin	Plasma β -thromboglobulin (ng/ml)	Platelet aggregation (%)	Platelet ^{14}C -serotonin release (%)
Venous	25 $\pm$ 5	80 $\pm$ 6	70 $\pm$ 7
Arterial	35 $\pm$ 6*	89 $\pm$ 5†	79 $\pm$ 5†

Platelet aggregation (amplitude) and ^{14}C -serotonin release were determined in response to 1 $\mu\text{g}/\text{ml}$ collagen in PRP. Results represent mean ($n = 7$) $\pm$ s.d. Statistically significant differences between venous and arterial blood are shown by:

* $P < 0.01$; † $P < 0.05$.

derived from a vein. Increased platelet activity in arteries may result from increased shear stress in comparison to the venous blood. The increased pH level as well as elevated PO_2 in arterial blood can also contribute to increased platelet functions observed in PRP derived from arteries. Since plasma lipoproteins also affect platelet function^{15,16} and HDL has been shown to cause reduction in platelet activity *in vitro*,^{17,18} it might be that the reduced plasma HDL concentration in arteries is also responsible for the enhanced platelet activity. In fact, in several studies in humans, an inverse relationship between HDL levels and platelet function has been demonstrated.^{19,20}

We can only speculate about the lower plasma HDL concentration found in arterial versus venous blood,

and we may suggest increased HDL binding to the arterial wall; thus, plasma HDL would be reduced in comparison to the venous wall.

Our study thus demonstrates increased risk for atherosclerosis in arteries. The reduced HDL levels can be related to impaired cholesterol-removal ability from the peripheral cells to the liver, and increased platelet activity can be associated with enhanced thrombotic tendency which is related to blood platelets found in arteries.

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