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CASE REPORT

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Platelet aggregation and lipoprotein levels in a patient with familial hypercholescholesterolemia after selective LDL-apheresis

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Platelet aggregation was studied in a patient with familial hypercholesterolemia immediately after aphereis selective for low-density lipoprotein (LDL), a lipid-lowering procedure. This treatment reduced plasmatic levels of total and LDL-cholesterol, apo B, and triglyceride. Increased platelet aggregation was reduced immediately after the apheresis in whole blood as well as in platelet-rich plasma. However, aggregation in washed platelets remained unchanged after LDL-apheresis. In conclusion, in this patient reduction of LDL-cholesterol improved platelet function in the very short term.

UNITERMS: Platelet aggregation. Familial hypercholesterolemia. LDL-apheresis.

INTRODUCTION

Platelets and lipoproteins are intimately involved in the pathogenesis of a wide variety of diseases, including atherosclerosis and thrombosis. Accumulating evidence suggests the possibility of a direct relationship between plasma lipoproteins and the hemostatic function of platelets. Reports of abnormal platelet function in patients with familial hypercholesterolemia (FH) are particularly pertinent in this regard. Platelets from patients with FH exhibit enhanced aggregation to a variety of physiological stimuli, as well as an increased cholesterol and phospholipid content.[1] Therefore, the lipid content may provide a basis for the abnormalities of platelet function observed in this disease.

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CASE REPORT

The patient was a 27-year-old female with a diagnosis of familial hypercholesterolemia based on clinical and biochemical criteria. She was on a low-lipid diet, and lipid-lowering drugs were stopped one month before the start of the study. The protocol of the study was approved by the Institutional Board, and the patient was fully informed. Plasma exchange was performed in this patient once a week when the plasma lipid values were high again, on an outpatient basis. Selective low-density lipoprotein (LDL)-apheresis was performed using DSC columns (LipoSorber, Kanegafushi, Chemical Industrial Co. Ltd., Osaka, Japan).

Plasma exchange had already been performed before the experiment: 114 procedures (3 times/week) followed by 31 procedures (once a week). Before the first LDL-apheresis procedure, plasma readings were as follows: total cholesterol (506 mg/dl), triglycerides (183 mg/dl), HDL-cholesterol (39 mg/dl), LDL-cholesterol (432 mg/dl), VLDL-cholesterol (33 mg/dl) and apo B (2.85 g/l).

Blood was drawn just before the plasmapheresis procedures, initiated after 14 hr of fasting, and then immediately afterwards. For lipid studies, blood samples were collected on EDTA (1mg/ml), and plasma obtained by low-speed centrifugation. Total cholesterol (TC) and triglycerides (TG) were measured by enzymatic methods. HDL-cholesterol (HDL-C) was measured with the same method after precipitation of LDL and VLDL with $MgCl_2$ and phosphotungstic acid. VLDL-cholesterol (VLDL-C) was calculated assuming the relation VLDL = TG/5, and LDL-cholesterol (LDL-C) was estimated according to Friedwald's equation. Plasma apolypoproteins A, A_1, and B were determined by radial immunodiffusion.

For platelet aggregation studies, blood was collected in 3.8 percent sodium citrate (1:10) just before and immediately after plasmapheresis procedures. Platelet aggregation was studied in whole blood using an impedance aggregometer. The curve amplitude was calculated 5 min after the aggregation. ADP(Sigma Chemical Co., St. Louis, MO, USA) was used as the aggregating agent.

Platelet-rich plasma (PRP) was obtained by centrifugation at 200g for 10 min at room temperature. Platelet aggregation in PRP was measured in a photometric aggregometer. Platelet concentrations were mainly in the range 4.20-4.45 x 10^5/ml. Adrenaline, ADP and thrombin (all from Sigma) were used as aggregating agents.

Washed platelets were obtained after adjusting the pH of the PRP to 6.5 with citric acid and platelet sedimentation by centrifugation at 1,600g for 10 min. The platelets were resuspended and washed in citrate wash buffer supplemented with 0.35 percent bovine serum albumin (fraction V, Sigma). After 3 washes, the platelets were resuspended in a modified Tyrode's buffer (136 mmol/l NaCl; 5.5 mmol/l glucose; 2.7 mmol/l KCl; 0.07 mmol/l Na_2HPO_4; 0.01 mmol/l $NaHCO_3$, pH7.4). Thrombin was used as aggregating agent. Washed platelet aggregation was performed as described for PRP.

The values for plasma lipids in the patient correspond with values previously obtained in defining this disease. Changes in plasma lipids, lipoproteins and apolipoproteins, immediately after LDL-apheresis, are reported in Table 1. All measured lipid classes, with the exception of HDL-C, decreased. The procedure was more selective to remove apo B- containing lipoproteins. The main decrements were found for total cholesterol, triglycerides, LDL-C, VLDL-C and apo B (around 71, 68, 79, 65, and 80 percent, respectively).

In Table 2, the effect of LDL-apheresis on whole blood platelet aggregation can be seen. Removal of lipids

Table 1
Plasma Lipid, Lipoprotein and Apolipoprotein Levels Immediately Before and After Ldl-Apheresis in a Patient with Familial Hypercholesterolemia

	Before treatment	After treatment
Total cholesterol (mg/dl)	384 ± 17	112 ± 5*
Triglycerides (mg/dl)	170 ± 13	54 ± 3*
HDL-Cholesterol (mg/dl)	32 ± 3	37 ± 6
LDL-Cholesterol (mg/dl)	318 ± 12	67 ± 3*
VLDL-Cholesterol (mg/dl)	32 ± 2	11 ± 0.7*
apo A (g/l)	0.93 ± 0.02	0.84 ± 0.02*
apo A1 (g/l)	0.46 ± 0.01	0.36 ± 0.01*
apo B (g/l)	1.96 ± 0.01	0.39 ± 0.01*

Results are expressed as means ± SEM from 6 different LDL-apheresis procedures
* $p < 0.05$

Table 2
Whole Blood Platelet Aggregation Immediately Before and After Ldl-Apheresis in a Patient with Familial Hypercholesterolemia

ADP (final concentration)	Maximum Amplitude (mm)	
	Before Treatment	After Treatment
0.75 µM	46.5 ± 11.5	18.5 ± 4.5*
1.50 µM	60.7 ± 10.3	41.2 ± 3.3*
3.00 µM	88.7 ± 6.3	64.5 ± 10.5*

Results are expressed as means ± SEM from 3 different LDL-apheresis procedures
* $p < 0.05$

decreased aggregation induced by ADP. The same results were obtained in PRP aggregation, induced by ADP (Table 3), as well as by adrenaline and thrombin (data not shown). Moreover, maximum aggregation was obtained, before LDL-apheresis procedure, with lower concentrations of aggregatory agents, when compared to post-apheresis platelet aggregation.

The effects of LDL-apheresis on the aggregation of washed platelets induced by thrombin are shown in Table 4. LDL-apheresis did not affect aggregation in washed platelets.

DISCUSSION

In this patient, selective LDL-apheresis allowed the removal of close to 70 percent of plasma cholesterol and triglycerides. The procedure also decreased LDL-C and VLDL-C by 80 and 65 percent, respectively, without changing the plasma content of HDL-C. Apo-B levels also dramatically dropped by 80 percent.

The effect of the lipoprotein plasma concentration on the function of circulating platelets becomes of importance in the light of accumulating evidence that hyperlipidemia, platelet function and thrombosis are interrelated. We have observed, in this patient, that platelet aggregation in whole blood as well as in PRP, decreased immediately after LDL-apheresis. Moreover, maximum aggregation levels were obtained, before the treatment, with lower concentrations of aggregating agents.

LDL is known to influence platelet reactivity. It was shown that platelets separated from their plasma milieu and incubated briefly with isolated LDL at concentrations within the physiological range are rapidly sensitized; at higher concentrations, LDL particles as well as cholesterol-rich liposomes act as agonists themselves, with stimulation of phosphatidylinositol and TXB2 formation, similarly to other platelet activators.[1] The possibility that LDL changes the reactivity of platelets by influencing the cholesterol/phospholipid ratio of the membrane is a strong explanation for the observed effects.

FH-derived platelets have been shown to contain more cholesterol than normal platelets, and it has been suggested that the platelet hyperactivity observed in FH patients is a consequence of their increased cholesterol content, which in turn is dependent on the cholesterol concentration of the platelet plasma environment. This is substantiated by studies in which labeled LDL binding to platelets was decreased when the platelets were suspended in plasma, as consequence of saturated non-labeled LDL.[2]

Platelets from FH patients have been described as having increased aggregation whether as PRP or as plasma-free-gel-filtered platelets, which has been taken to indicate a change in the platelets induced by abnormal plasma constituents. On the other hand, it was shown that aggregation of washed platelets from patients with FH decreased to the low normal range.[2]

Plasma exchange in patients with hypercholesterolemia was described to have a beneficial effect on platelet function in vitro;[3] however, in that study, the plasma exchange technique used was probably not selective for apo B-containing particles.[3] In contrast to these positive data, it has been shown that the enhanced in vitro sensitivity of platelets from FH patients to a variety of aggregating agents did not change after a single, as well as regular removal of plasma cholesterol by LDL-apheresis;[4] however, in these reports, platelet studies were performed two days after the apheresis procedure, when plasma cholesterol concentration was again at a high level.

Table 3
Platelet Aggregation in PRP Immediately Before and After Ldl-Apheresis in a Patient with Familial Hypercholesterolemia

ADP (final concentration)	% Light transmission	
	Before treatment	After treatment
2.50 µM	61 ± 5	38 ± 9*
5.00 µM	94 ± 3	61 ± 12*

Results are expressed as means ± SEM from 3 different LDL-apheresis procedures.
* $p < 0.05$

Table 4
Aggregation in Washed Platelets Immediately Before and After Ldl-Apheresis in a Patient with Familial Hypercholesterolemia

Thrombin (final concentration)	% Light Transmission	
	Before treatment	After treatment
0.12 U/ml	63 ± 15	71 ± 21
0.25 U/ml	86 ± 2	90 ± 7

Results are expressed as means ± SEM from 4 different LDL-apheresis procedures.

Although we were able to study only one patient, our results do not support the existence of an intrinsic platelet abnormality in hyperlipoproteinemia. This conclusion arises from:

1. Removal of cholesterol and LDL-cholesterol by LDL-apheresis immediately decreased platelet aggregation in PRP as well as in whole blood, and
2. The same degree of aggregation was seen in washed platelets, independently of the apheresis procedure. Under the circumstances, the cholesterol levels might still be supportive of these platelet abnormalities, although other plasma factors cannot be discarded.

In conclusion, although our data may reflect just the patient studied, they support the concept that increased platelet aggregation is not a problem of abnormality of the platelet itself in FH patients; but rather is a question of the amount of cholesterol present in the milieu, since the reduction of LDL-C decreases platelet aggregation at least in the very short term. Furthermore, LDL-apheresis was shown to be an useful model to study the effect of hypercholesterolemia on platelet function.

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RESUMO

A agregação plaquetária foi estudada em um paciente com hipercolesterolemia familiar imediatamente após aférese seletiva para lipoproteína de baixa densidade (LDL), para diminuição dos lípides plasmáticos. O tratamento reduziu os níveis plasmáticos de colesterol total, LDL-colesterol, apo B e triglicérides. A agregação plaquetária aumentada foi reduzida, imediatamente após aférese, em sangue total e em plasma rico em plaquetas, mas permanaceu sem modificações em plaquetas lavadas. Em conclusão, neste paciente, a redução dos níveis de LDL-colesterol melhorou a função plaquetária, pelo menos a muito curto prazo.

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