Reduced platelet aggregation after fluvastatin therapy is associated with altered platelet lipid composition and drug binding to the platelets

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Aims High plasma cholesterol concentration and increased platelet activity are two major risk factors for atherosclerosis. Lovastatin, the lipophilic drug was shown to inhibit platelet aggregation whereas pravastatin, the hydrophilic drug had no such effect. Analysis of the effect of fluvastatin which is both a lipophilic and hydrophilic drug, on platelet aggregation was the goal of the present study.

Methods Fluvastatin 40 mg daily was administered to 25 patients with hypercholesterolaemia for up to 24 weeks. Normal subjects acted as controls. The influence of fluvastatin on plasma lipids and on platelet aggregation and fluidity was studied. The direct effect of fluvastatin on platelets was compared with that of other statins.

Results Fluvastatin therapy (40 mg day^{-1} for a period of 4 weeks) in hypercholesterolaemic patients resulted in a 23% and 29% reduction in plasma levels of total cholesterol and LDL-cholesterol respectively. Platelet cholesterol/phospholipids molar ratio was reduced by 26% and platelet aggregation was significantly ($P<0.02$) reduced by 10% after 4 weeks of fluvastatin treatment. On continuing fluvastatin therapy for additional 20 weeks, no further decrement in plasma LDL cholesterol levels or in platelet cholesterol/phospholipid ratio were noted. However, platelet aggregation was further significantly ($P<0.01$) reduced by up to 15%. Incubation of platelets with increasing concentrations of fluvastatin or lovastatin, demonstrated a dose-dependent reduction in platelet aggregation, whereas pravastatin showed no effect. This inhibitory effect of fluvastatin or lovastatin on platelet aggregation (up to 34% or 22% respectively at a concentration of 1 μg statin ml^{-1}) was found both in platelet rich plasma and in washed platelet suspensions. Fluvastatin and lovastatin (but not pravastatin), seem to share similar platelet binding sites, as non labelled fluvastatin or lovastatin were able to displace [^{3}H]-labeled-fluvastatin from its binding sites on platelets.

Conclusions Fluvastatin therapy reduces platelet aggregation via a dual effect which involves its *in vivo* hypocholesterolaemic action on platelet cholesterol content, and also a direct effect of the drug binding to the platelets. The antiatherogenicity of fluvastatin may be related, in addition to its plasma cholesterol lowering ability, to its inhibitory effect on platelet activation.

Keywords: fluvastatin, pravastatin, lovastatin, hypercholesterolaemia, platelet aggregation

Introduction

Atherosclerosis is a complicated process involving the interaction of plasma lipoproteins, blood platelets and arterial wall cells [1–4]. Blood platelets have been shown to be intimately involved in atherosclerosis [5–7]. Activated platelets can affect macrophage cholesterol accumulation, and foam cells formation, by altering the uptake of low density lipoprotein (LDL) by arterial wall macrophages [8–14]. Plasma LDL in turn, can activate platelets in a process which involves its binding to the platelets [1, 15, 16].

Activation of platelets occurs in hypercholesterolaemic patients, as enhanced platelet responsiveness was noted when exposed to aggregatory agonists *ex vivo* [1, 2, 5, 17, 18]. Several hypolipidaemic drugs as well as plasmapheresis were shown to reduce platelet tendency to aggregation [19–22]. Statins are most potent hypocholesterolaemic agents which inhibit cellular hydroxymethyl glutaryl coenzyme A (HMGCoA) reductase, the key enzyme in cholesterol biosynthesis [23, 24]. Although all statins share the inhibitory effect on HMGCoA reductase, the different formulations of the various statins are associated with differences in their effects on macrophage foam cell formation [25–27]. Administration of statins to hypercholesterolaemic patients also affects platelets function [28]. Recently, lovastatin (which is converted to its active form within the gastrointestinal tract) administration for 20 weeks to hypercholesterolaemic patients was shown to decrease significantly the extent of their platelet aggregation [28]. We thus hypothesized that the relatively new active drug fluvastatin can similarly affect platelet function. The purpose of the present study was to

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analyze the effect of another new statin, fluvastatin, which is an active drug, on platelet aggregation in hypercholesterolaemic patients, in relation to its effect on platelet cholesterol content, as well as to its binding characteristics to the platelets.

Methods

Patients

Thirty male patients were recruited for this study. Inclusion criteria were 40–70 years old, plasma total cholesterol levels above 6 mmol l^{-1} but less than 8 mmol l^{-1} despite dietary therapy, plasma triglycerides level below 3 mmol l^{-1} with no chronic or metabolic diseases, with no acute coronary event and without any hypolipidaemic treatment. Five patients did not complete the whole study: three patients stopped fluvastatin therapy because of severe abdominal pain or urticaria. One patient was excluded from the study because of acute myocardial infarction and another because of increased plasma creatine kinase (CK) activity twice the upper limit. Normal healthy subjects under no medication served as control group for analyses of the effect of statins on platelet aggregation. The patients were treated with fluvastatin (40 mg day^{-1}) for up to 24 weeks. Blood samples were taken before therapy, and 4, 8, 12 and 24 weeks after fluvastatin administration. Blood samples were taken for analyses of plasma lipid concentrations, as well as for determinations of platelet aggregation in response to collagen. The study was approved by the Helsinki Ethical Committee, Rambam Medical Center, Haifa, Israel.

Platelet separation

For platelet studies, venous blood (40 ml) was collected through siliconized syringes into sodium citrate, 3.8% at a ratio of 9 : 1 (v : v) for platelet-rich plasma (PRP) preparation or into acid citrate dextrose solution (1.4% citric acid, 2.5% sodium citrate, and 2% dextrose) at a ratio of 9 : 1 (v : v) for washed platelets (WP) preparation. PRP was prepared by low-speed centrifugation (100 *g* for 10 min) at 25 °C, and the remaining sample was recentrifuged at 1000 *g* for 10 min to obtain platelet-poor plasma (PPP). Platelets in PRP were counted and diluted with PPP to achieve a uniform concentration of 3×10^8 cells/ml. Washed platelets (WP) were prepared from PRP by centrifugation at 240 *g* for 20 min. The platelet pellet was washed twice in 5 mmol l^{-1} Hepes buffer, pH 7.4 (140 mmol l^{-1} NaCl, 2 mmol l^{-1} KCl, 1 mmol l^{-1} $MgCl_2$, 5 mmol l^{-1} Hepes, 12 mmol l^{-1} $NaHCO_3$, and 5.5 mmol l^{-1} of glucose). For the preparation of WP suspension, 15 μl of acetic acid (1 mol l^{-1}) were added to 1 ml of platelet suspension throughout WP preparation in order to ensure acidic conditions which are required for platelet resuspension. This procedure reduced the medium pH to 6.5 and it did not influence the aggregation response of the WP.

Platelet aggregation

Collagen (Nycomed Arzneimittel, Munchen, Germany) was used as the aggregating agent at a concentration of 4 μg ml^{-1} (this concentration caused up to 75% and 60% aggregation amplitude in PRP and WP respectively). Platelet aggregation was performed at 37 °C in a model PAP-4 computerized aggregometer using PPP or Hepes as a reference system for PRP or WP respectively [29]. In the *in vitro* studies, increasing concentrations of statins (0.01, 0.1, 1 and 10 μg ml^{-1}) were incubated for 30 min with 1 ml of PRP or WP before testing platelet aggregation. The fluvastatin salt which is hydrophilic, was dissolved in water whereas the lipophilic drugs lovastatin and pravastatin were dissolved in dimethyl sulphoxide (DMSO). There were no solvent effects on platelet aggregation. There was no significant effect of the statins on platelet viability as analyzed microscopically. Results were expressed as the extent of maximal aggregation (% of maximal amplitude) and also as the slope of the aggregation curve (cm min^{-1}). The inter- and intra-assay variabilities for platelet aggregation were 3–5%.

Statin binding to platelets

Labelled statins [([^{3}H]-fluvastatin sodium salt, 215 μCi/mg; Sandoz), ([butanoate-1-^{14}C] lovastatin, 26 μCi/mg; Merck-Sharp) and ([^{14}C] pravastatin; Bristol-Myers-Squibb)] were added at the indicated concentrations to 1 ml of PRP (3×10^8 platelets). After 30 min of incubation at 37 °C, the PRP was centrifuged at 240 *g* and the platelet pellet was washed twice in 5 mmol l^{-1} Hepes buffer, pH 7.4. Platelet-associated radioactive statin was then measured in scintillation fluid, using a β-counter and expressed as a percentage of the added labelled statin.

Platelet cholesterol and phospholipids content

Platelets from hypercholesterolaemic patients were washed three times with Hepes buffer, and then sonicated twice for 20 s at 80 watt. Platelet lipids were extracted with hexane: isopropanol (3 : 2, v : v). The cholesterol content was measured in the dried hexane phase by the method of Chiamori *et al.* [30]. Total platelet phospholipid content was also determined in the dried hexane phase by the method of Rouser *et al.* [31]. Platelet protein was determined using the method of Lowry *et al.* [32].

Statistical analysis

Data were analyzed for significance of the results by Student's *t*-test. Values are given as means ± s.d. Differences at a level of less than 0.05 were considered significant.

Results

Effect of fluvastatin therapy on plasma lipids

Fluvastatin therapy in 25 patients (40 mg day^{-1} for a period of 24 weeks) resulted in 23% and 29% decrement in the plasma levels of total cholesterol and LDL cholesterol, respectively (Figure 1). This hypercholesterolaemic effect was achieved after 4 weeks of therapy (Figure 1). Maximal decrement in plasma triglyceride levels (26%) was obtained after 8 weeks of treatment (Figure 1). Fluvastatin therapy however did not affect plasma high density lipoprotein

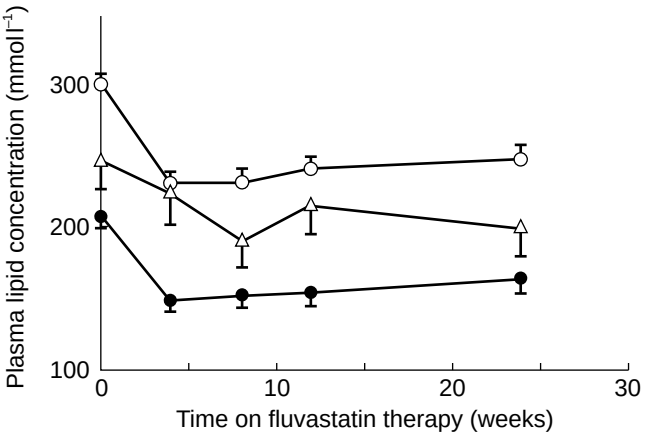

Figure 1 Effect of fluvastatin therapy on plasma lipid concentration. Fluvastatin (40 mg day^{-1}) was administered to 25 hypercholesterolaemic patients for a period of 24 weeks. Blood samples were taken at 0 time and after 4, 8, 12 and 24 weeks of drug therapy. Total cholesterol (○), triglycerides (△) and HDL-cholesterol (●) concentrations were determined in the plasma. Results represent mean ± s.d. ($n = 25$).

(HDL) levels (1.15 ± 0.11 and 1.18 ± 0.10 mmol l^{-1} before and after 24 weeks of fluvastatin therapy respectively).

Effect of fluvastatin therapy on platelet aggregation and fluidity

Platelet aggregation was significantly ($P < 0.02$) reduced after 4 weeks of fluvastatin therapy by 6% and 10% as analyzed by determination of the maximal aggregation amplitude and the aggregation curve slope respectively (Figure 2a,b). After 24 weeks of therapy a further significant reduction, by 11% and 15% respectively, was obtained (Figure 2a,b).

Maximal decrement in platelet cholesterol/phospholipid molar ratio (26%) was achieved after four weeks of fluvastatin therapy and this effect was not further changed on continuing fluvastatin therapy up to 24 weeks (Figure 2c). Platelet cholesterol/phospholipid molar ratio was decreased as a result of the decrement in platelet cholesterol content from 38 ± 2 μg of cholesterol per mg cell protein to 28 ± 2 μg of cholesterol per mg cell protein after 24 weeks of fluvastatin therapy, with no significant change in platelet phospholipid content (92 ± 3 *vs* 86 ± 3 μg of phospholipids mg^{-1} cell protein before and after 24 weeks of fluvastatin therapy respectively).

The in vitro *effect of fluvastatin on platelet aggregation*

All three studied statins, at a concentration of 0.01 μg ml^{-1}, had no effect on platelet aggregation (data not shown). At a concentration of 0.1 μg ml^{-1}, fluvastatin inhibited platelet aggregation (in PRP) by 26% as determined by analysis of the maximal aggregation amplitude (Figure 3a). Lovastatin and pravastatin had no effect on aggregation at this low concentration. At 1.0 μg ml^{-1}, both fluvastatin and lovastatin had their maximal inhibitory effect on platelet aggregation (Figure 3a). At this concentration, fluvastatin and lovastatin inhibited maximal aggregation amplitude by 34% and 22% respectively ($P < 0.01$). Pravastatin had no effect on platelet

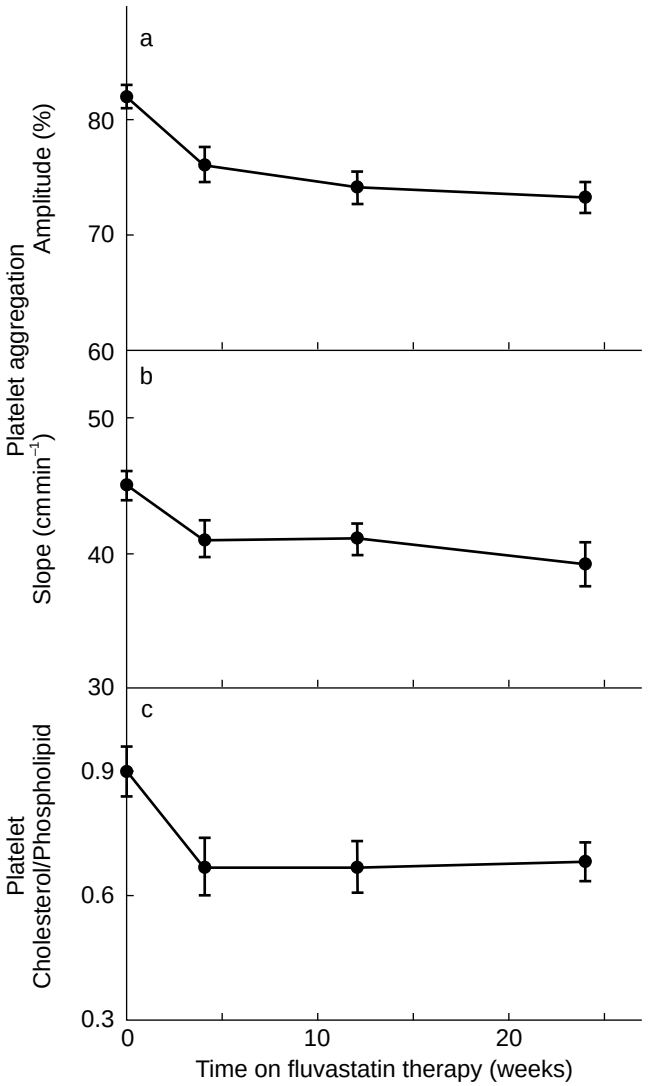

Figure 2 Effect of fluvastatin therapy on platelet aggregation and on platelet cholesterol/phospholipid molar ratio. Platelet aggregation (in PRP that was obtained from 25 hypercholesterolaemic patients) in response to 4 μg ml^{-1} of collagen, and platelet cholesterol/phospholipid molar ratio were determined at zero time, and after 4, 12 and 24 weeks of fluvastatin therapy (40 mg day^{-1}). Platelet aggregation maximal amplitude (a), and platelet aggregation curve slope (b). Platelet cholesterol/phospholipid molar ratio (c) was determined in the platelet lipids extract, as described under 'Methods'. Results represent mean ± s.d. ($n = 25$).

aggregation at all studied concentrations (Figure 3a). Upon using washed platelets (WP), free of plasma constituents, the maximal inhibitory effects of fluvastatin and lovastatin on platelet aggregation was also obtained at 1 μg ml^{-1} (Figure 3b).

Maximal aggregation amplitude was inhibited by 16% or 11% for fluvastatin or lovastatin respectively ($P < 0.01$) (Figure 3b). Platelet aggregation slope also decreased, by 24% and 14%, for fluvastatin and lovastatin respectively, whereas pravastatin, had no inhibitory effect on the platelet aggregation slope (data not shown).

PRP from hypercholesterolaemic patients exhibited a significant ($P < 0.01$) increment of 14% in the aggregation curve slope in comparison to normal volunteers (Figure 4).

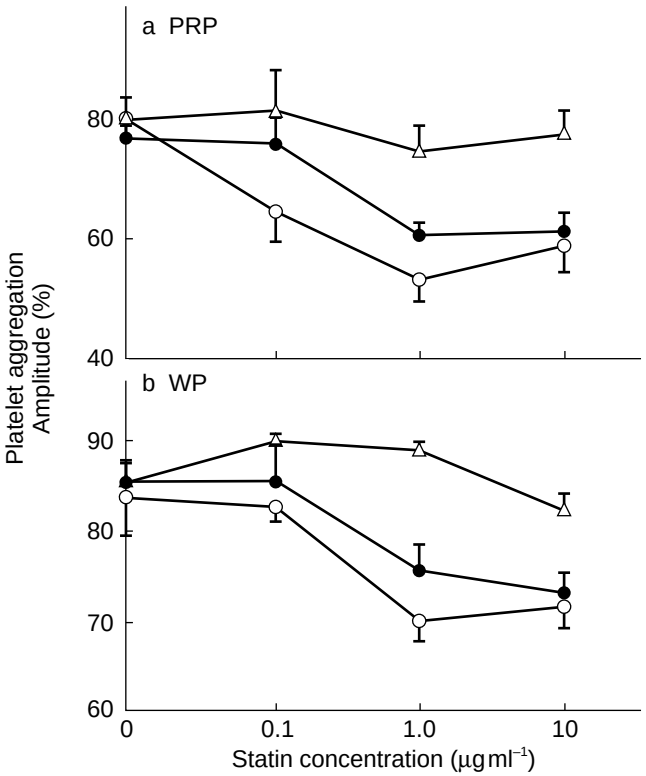

Figure 3 The *in vitro* effect of fluvastatin (○), lovastatin (●) and pravastatin (△) on platelet aggregation in platelet rich plasma (PRP) and in washed platelets (WP). PRP or WP (3×10^8 platelets ml^{-1}) were separately prepared from normal volunteers. Increasing concentrations (0–10 $\mu g\ ml^{-1}$) of fluvastatin, lovastatin or pravastatin were incubated with the platelets for 30 min at 37 °C. Then, platelet aggregation in response to 4 $\mu g\ ml^{-1}$ of collagen was analyzed in PRP (a) or in WP (b). Results represent mean ± s.d. ($n = 3$).

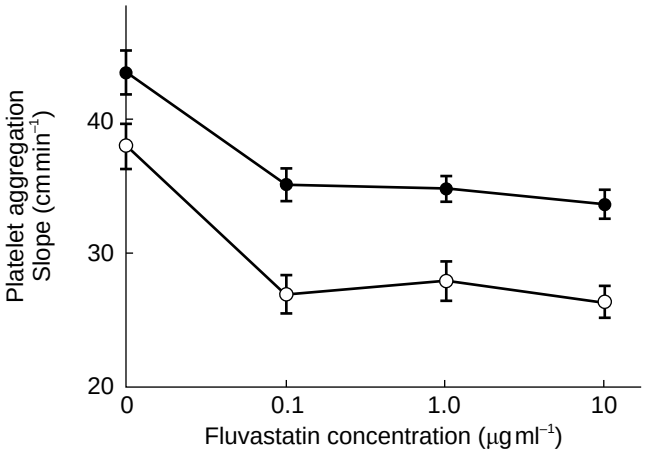

Figure 4 The *in vitro* effect of fluvastatin on platelet aggregation (in PRP) in platelets obtained from healthy subjects (○) or from hypercholesterolaemic patients (●). PRP (3×10^8 platelets ml^{-1}) was separately prepared from five normolipidemic subjects or from five hypercholesterolaemic patients. Fluvastatin (0–10 $\mu g\ ml^{-1}$) was then incubated with the platelets for 30 min at 37 °C. Platelet aggregation in response to 4 $\mu g\ ml^{-1}$ of collagen was then determined. Results represent mean ± s.d. ($n = 3$).

Upon *in vitro* incubation of PRP from hypercholesterolaemic patients or from healthy control subjects, with fluvastatin (0.1 $\mu g\ ml^{-1}$), the aggregation curve slope was decreased by 20% or by 30%, respectively (Figure 4).

Table 1 Fluvastatin binding to platelets derived from hypercholesterolaemic patients and from normolipidaemic subjects.

[^{3}H]-Fluvastatin concentration	Fluvastatin binding to platelets *(% of added statin)*	
	A	
($\mu g\ ml^{-1}$)	*Normals*	*Patients*
1	0.10 ± 0.02	0.09 ± 0.01
10	0.21 ± 0.03	0.22 ± 0.04

[^{3}H]-Fluvastatin, at increasing concentrations (0–10 $\mu g\ ml^{-1}$) was added to PRP (3×10^8 platelets) that was obtained separately from five healthy control subjects or from five hypercholesterolaemic patients. After an incubation period of 30 min at 37° C, washed platelets were prepared as described under Methods. The platelet-associated radioactivity was determined and the drug binding to platelets was expressed as percentage of the added statin. Results represent mean ± s.d. ($n = 5$).

Fluvastatin binding to platelets

Maximal binding of [^{3}H]-labeled fluvastatin, to the platelets was obtained at a concentration of 4 $\mu g\ ml^{-1}$ (0.22 + 0.02% of the added labelled statin) (Figure 5). Maximal binding of [^{14}C]-labelled lovastatin to the platelets was also at a concentration of 4 $\mu g\ ml^{-1}$ with an equilibrium dissociation constant (K_d) of 3.8 $\mu g\ ml^{-1}$, whereas ^{14}[C]-labelled pravastatin showed no binding to the platelets (data not shown). By plotting 1/B against 1/S, where S is the statin concentration and B is expressed as the percent of drug binding (for the added statin) to platelet (given as % of added labelled statin), the apparent K_d for fluvastatin was found to be 1.6 $\mu g\ ml^{-1}$ (Figure 5, inset). Fluvastatin binding to platelets from normal subjects or from hypercholesterolaemic patients showed a similar dose-dependent pattern (Table 1). Both nonlabelled fluvastatin and nonlabelled lovastatin substantially decreased the binding of the labelled fluvastatin to the platelets (by up to 80%), whereas pravastatin showed only minimal effect (Figure 6), suggesting that fluvastatin and lovastatin share similar platelet binding sites.

Discussion

Platelet activation is a risk factor for atherosclerosis, and thus reduction of the enhanced platelet aggregation in atherosclerotic patients is considered to be an important antiatherogenic intervention.

The present study demonstrates that fluvastatin therapy in addition to its hypercholesterolaemic effect on plasma LDL, significantly reduce platelet aggregation in hypercholesterolaemic patients. These results were shown in 25 male hypercholesterolaemic patients. The relevance of these results to women or to hypertriglyceridaemic patients, needs to be evaluated.

The inhibitory effect of fluvastatin on platelet aggregation could be associated with the decrement in platelet cholesterol/phospholipid molar ratio, which parallelled the decrement in plasma cholesterol concentration. Increased platelet cholesterol content contributes to platelet activation in hypercholesterolaemic patients [33], and this is related to

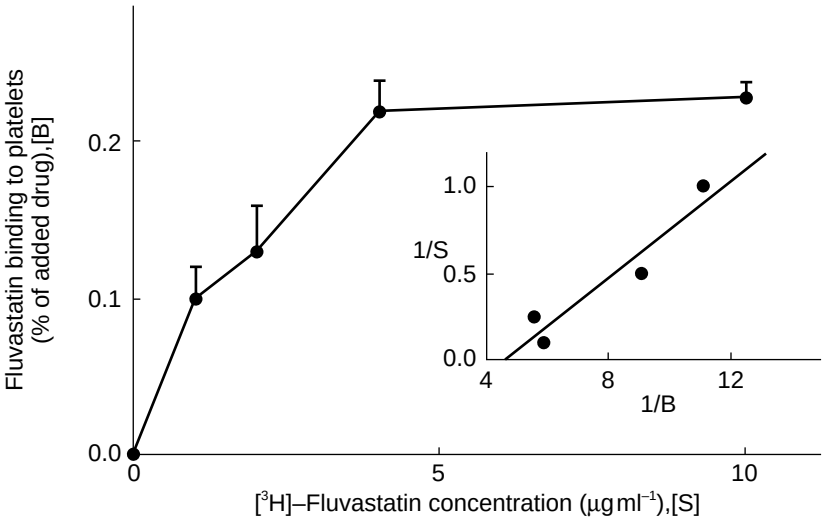

Figure 5 Binding characteristics of fluvastatin to platelets *in vitro*. [^{3}H]-Fluvastatin, at increasing concentrations (0–10 μg ml^{-1}) was added to PRP (3×10^8 platelets). After an incubation period of 30 min at 37 °C, washed platelets were prepared as described under Methods. The platelet-associated radioactivity was determined and the drug binding to platelets was expressed as percentage of the added labelled fluvastatin. *Inset*: S, fluvastatin concentration, B, fluvastatin binding to platelet (% of added labelled drug). Results represent mean ± s.d. ($n = 3$).

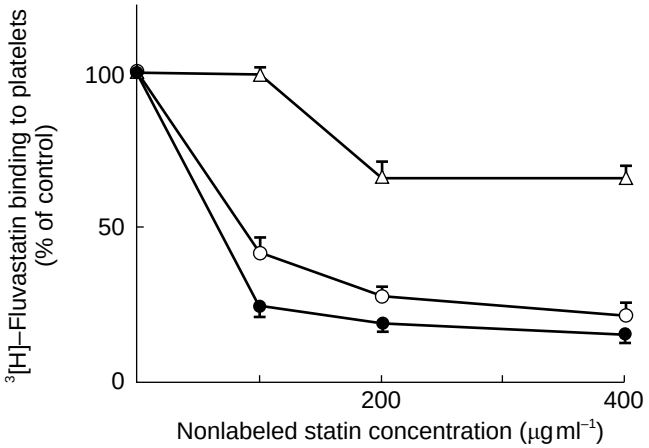

Figure 6 Specificity of fluvastatin binding to platelets. Increasing concentrations (0–400 μg ml^{-1}) of nonlabeled statins (fluvastatin (○), lovastatin (●), or pravastatin (△), were added to 1 ml of PRP (3×10^8 platelets), and incubated with 10 μg ml^{-1} of labeled [^{3}H]-fluvastatin for 30 min at 37 °C. The platelet-associated radioactivity was determined as described under Methods. Results are expressed as percentage of control (maximal binding of [^{3}H]-fluvastatin, without the addition of nonlabelled statin). Results represent mean ± s.d. ($n = 3$).

the effect of platelet cholesterol on the interaction between platelets and the aggregating agents. Hochgraf *et al.* [28] found that in hypercholesterolaemic patients, lovastatin therapy attenuates the increased platelet cholesterol/phospholipid molar ratio and reduced the increased platelet aggregatory response in these patients to normal values. It seems reasonable to postulate that fluvastatin increased platelet fluidity, which also affects platelet aggregation, by reducing platelet cholesterol/phospholipid molar ratio. Plasma LDL, and more so oxidatively modified LDL, triggers platelet activation and enhances platelet aggregation and secretion via specific binding sites for the lipoprotein on the platelet surface which differ from the classical apolipoprotein B/E receptors on fibroblasts [15, 34–36].

As plasma LDL has been shown to increase platelet aggregation [15], reducing plasma LDL levels by statins therapy can be expected to reduce the platelets' tendency to aggregate. Indeed lipid lowering treatment in hypercholesterolaemic patients resulted in a substantial reduction in platelet aggregation [37]. Recently, it was shown that cholesterol lowering therapy using pravastatin reduced platelet thrombus formation and hence the risk of acute thrombosis and coronary events in hypercholesterolaemic patients [38]. In the present study the inhibitory effect of fluvastatin on platelet aggregation increased with time beyond the first 4 weeks of therapy even though there was no further decrement in platelet cholesterol/phospholipids molar ratio. This may be the result of the reduction (by 26%) of plasma triglycerides levels after 8 weeks of drug therapy, as very low density lipoprotein (VLDL) has been shown to enhance platelet aggregation [15]. Fluvastatin can also reduce platelet aggregation by other mechanisms such as a direct drug interaction with the platelets which can affect platelet response to the aggregating agents. Fluvastatin was shown to be more potent than lovastatin or pravastatin as an inhibitor of platelet aggregation. Indeed, fluvastatin showed a pronounced *in vitro* inhibitory effect on platelet aggregation at concentrations of 0.1–1 μg ml^{-1}, which are comparable with the *in vivo* drug concentration in the plasma.

The peak concentration (C_{max}) of fluvastatin in the plasma after multiple doses is about 0.4 μg ml^{-1} [39]. Unlike fluvastatin, lovastatin was less potent at this concentration; the C_{max} of lovastatin in plasma is as low as 0.07 μg ml^{-1} [40].

Fluvastatin and pravastatin are used in their active hydroxy acid forms, whereas lovastatin is used as an inactive lactone. This inactive lactone form of lovastatin is converted to its active form (β-hydroxy acid) in the liver by the enzyme lactonase [41]. In the present study we compared fluvastatin to the inactive form of lovastatin and demonstrated a direct inhibitory effect *in vitro* of both drugs on platelet activation.

The inhibitory *in vitro* effects of fluvastatin and lovastatin on platelet aggregation was more prominent in PRP than in WP. This may be due to an effect of plasma constituents which can influence the interaction between the statin and

the platelets. Both fluvastatin and lovastatin inhibit platelet aggregation *in vitro* and this phenomenon may be explained by the binding capacities of these lipophilic statins to specific binding sites on the platelet surface. Although lovastatin is converted *in vivo* to an active metabolite, structural domains of this metabolite similar to that of its precursor and also to fluvastatin, may be responsible for their *in vitro* inhibitory effects on platelet aggregation. In contrast, pravastatin, a hydrophilic statin, neither binds to platelets nor inhibits platelet aggregation *in vitro*. In line with this observation is the finding that pravastatin did not affect platelet activation in patients with mild hypercholesterolaemia [42]. The maximal binding of fluvastatin and lovastatin to platelets was achieved at a concentration of 4 $\mu g\,ml^{-1}$ and the maximal inhibitory effect on platelet aggregation was achieved at concentrations as low as 1 $\mu g\,ml^{-1}$. This dissociation between the statin binding capacity to the platelets and its platelet inhibitory effect, may result from factors in addition to the number of platelet specific statin binding sites, such as a steric effect of the statin on platelet interaction with the aggregating agent (collagen). It is also possible that a maximal inhibitory effect of the statins on platelet aggregation can be reached by occupying only part of the binding sites on the platelet surface.

In conclusion, we have demonstrated that fluvastatin therapy significantly reduced platelet activation in hypercholesterolaemic patients. This effect may be secondary to its hypercholesterolaemic effect on platelet cholesterol content, as well as a direct effect of the drug binding to platelets. Thus, fluvastatin may be considered antiatherogenic [43] not only because of its plasma hypocholesterolaemic characteristics, but also as a result of its inhibitory effect on platelet aggregation.

References

1 Aviram M. Effect of lipoproteins and platelets on macrophage cholesterol metabolism. In *Blood Cell Biochemistry* ed Harris JR, New York, Plenum Publishing 1992; **2**: 179–207.

2 Surya II, Akkerman JW. The influence of lipoproteins on blood platelets. *Am Heart J* 1993; **125**: 272–275.

3 Ross R. The pathogenesis of atherosclerosis: an update. *New Engl J Med* 1986; **314**: 488–500.

4 Goldstein JL, Brown MS. The low density lipoprotein pathway and its relation to atherosclerosis. *Ann Rev Biochem* 1977; **46**: 897–930.

5 Aviram M. Platelets and the arterial wall lesion. *Curr Opin Lipidol* 1992; **3**: 344–348.

6 Sizinger H. Role of platelets in atherosclerosis. *Sem Thromb Haemost* 1986; **12**: 124–133.

7 Packham KA, Mustard JF. The role of platelets in the development and complications of atherosclerosis. *Semin Hematol* 1986; **23**: 8–26.

8 Folkman J, Klagsburn M. Angiogenic factors. *Science* 1987; **234**: 442–447.

9 Chait A, Ross R, Albers JJ, Bierman EL. Platelet-derived growth factor stimulates activity of low density lipoprotein receptors. *Proc Natl Acad Sci U.S.A.* 1980; **77**: 4084–4088.

10 Curtiss LK, Black AS, Takagi Y, Plow EF. New mechanism for foam cell generation in atherosclerotic lesions. *J Clin Invest* 1987; **80**: 367–373.

11 Mendelsohn ME, Loscalzo J. Role of platelets in cholesteryl ester formation by U-937 cells. *J Clin Invest* 1988; **81**: 62–68.

12 Fuhrman B, Brook JG, Aviram M. Activated platelets secrete a protein like factor that stimulates oxidized-LDL receptor activity in macrophages. *J Lipid Res* 1991; **32**: 1113–1123.

13 Aviram M. Platelet secretory products enhance LDL receptor activity and inhibit scavenger receptor activity in human monocyte derived macrophages. *Metabolism* 1989; **38**: 425–430.

14 Hayashi T, Naito M, Kuzuya M, *et al*. Role of platelet secretory products in modified lipoprotein metabolism in macrophages. *Artery* 1989; **16**: 248–262.

15 Aviram M, Brook JG. Platelet interaction with high and low density lipoproteins. *Atherosclerosis* 1983; **46**: 259–268.

16 Aviram M. LDL-platelet interaction under oxidative stress induced macrophage foam cell formation. *Thrombosis and Haemostasis* 1995; **74**: 560–564.

17 Aviram M, Brook JG. The effect of human plasma on platelet function in familial hypercholesterolaemia. *Thromb Res* 1982; **26**: 101–109.

18 Viener A, Brook JG, Aviram M. Abnormal plasma lipoprotein composition in hypercholesterolaemic patients induces platelet activation. *Eur J Clin Invest* 1984; **14**: 207–213.

19 Sirtori CR, Colli S. Influence of lipid-modifying agents on hemostasis. *Cardiovasc Drugs Ther* 1993; **7**: 817–823.

20 Miller GJ. Lipoproteins and thrombosis: effects of lipid lowering. *Curr Opin Lipidol* 1995; **6**: 38–42.

21 Brook JG, Winterstein G, Aviram M. Platelet function and lipoprotein levels after plasma exchange in patients with familial hypercholesterolaemia. *Clin Sci* 1983; **64**: 637–642.

22 Kaczmarek D, Hohlfeld T, Wambach G, Schror K. The actions of lovastatin on platelets function and platelet eicosanoid receptors in type II hypercholesterolaemia. A double-bind, placebo-controlled prospective study. *Eur J Clin Pharmacol* 1993; **45**: 451–457.

23 Levy RJ, Troendle AJ, Fattu MA. A quarter century of drug treatment of dyslipoproteinemia, with a focus on the new HMG-CoA reductase inhibitor fluvastatin. *Circulation* 1993; **87**(Suppl 4): 1145–1153.

24 Grundy SM. HMG-CoA reductase inhibitors for treatment of hypercholesterolemia. *N Engl J Med* 1988; **319**: 24–33.

25 Aviram M, Dankner G, Cogan U, Hochgraf E, Brook JG. Lovastatin inhibits low-density lipoprotein oxidation and alters its fluidity and uptake by macrophages: *in vitro* and *in vivo* studies. *Metabolism* 1992; **41**: 229–235.

26 Keidar S, Aviram M, Maor I, Oiknine J, Brook JG. Pravastatin inhibits cellular cholesterol synthesis and increases low density lipoprotein receptor activity in macrophages. *Br J Clin Pharmacol* 1994; **38**: 513–519.

27 Aviram M, Keidar S, Brook JG. Dual effect of lovastatin and simvastatin on LDL-macrophage interaction. *Eur J Clin Chem Clin Biochem* 1991; **29**: 657–664.

28 Hochgraf E, Levy Y, Aviram M, Brook JG, Cogan U. Lovastatin decreases plasma and platelet cholesterol levels and normalizes elevated platelet fluidity and aggregation in hypercholesterolaemic patients. *Metabolism* 1994; **43**: 7–11.

29 Aviram M, Rosenblat M, Brook JG. Secretory products from human monocyte-derived macrophages enhance platelet aggregation. *Metabolism* 1991; **40**: 270–274.

30 Chiamori N, Henry RJ. Study of the ferric chloride method for determination of total cholesterol and cholesterol esters. *Am J Clin Pathol* 1959; **31**: 305–309.

31 Rouser G, Fkeischer S, Yamamoto A. Two dimensional thin layer chromatography of polar lipids and determination of phospholipids by phosphorus analysis of spots. *Lipids* 1970; **5**: 494–496.

32 Lowry OH, Rosebrough NJ, Farr AL, Randall RS. Protein

measurement with the folin phenol reagent. *J Biol Chem* 1951; **193**: 265–275.
33 Shastri KM, Carvlho ACA, Lees RS. Platelet function and platelet lipid composition in the dyslipoproteinemias. *J Lipid Res* 1980; **21**: 467–478.
34 Ardlie NG, Selley ML, Simons LA. Platelet activation by oxidatively modified low density lipoprotein. *Atherosclerosis* 1989; **76**: 117–124.
35 Shmulewitz A, Brook JG, Aviram M. Native and modified low-density lipoprotein interactions with human platelets in normal and homozygous familial hypercholesterolaemic subjects. *Biochem J* 1984; **224**: 13–20.
36 Hassal DG, Bruckdorfer KR. Platelet low density lipoprotein receptors. *Biochem Soc Trans* 1985; **13**: 1189–1190.
37 Gasser JA, Cooper MB, Tan KCB, Saggerson ED, Betteridge DJ. Decreased sensitivity to adenosine in platelets from patients with familial hypercholesterolaemia—a change reversed by cholestyramine treatment. *Eur J Clin Invest* 1993; **23**: 803–811.
38 Lacoste L, Lam JYT, Hung J, Letchacovski G, Solymoss CB, Waters D. Hyperlipidemia and coronary disease. Correction of the increased thrombogenic potential with cholesterol reduction. *Circulation* 1995; **92**: 3172–3177.
39 Tse FL, Jaffe JM, Troendle A. Pharmacokinetics of fluvastatin after single and multiple doses in normal volunteers. *J Clin Pharmacol* 1992; **32**: 630–638.
40 Pan HY, DeVault AR, Wang-Inverson D, Ivashkiv E, Swanson BN, Sugerman A. Comparative pharmacokinetics and pharmacodynamics of pravastatin and lovastatin. *J Clin Pharmacol* 1990; **30**: 1128–1135.
41 Blum CB. Comparison of properties of four inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *Am J Cardiol* 1994; **73**: 3D–11D.
42 Barrow SE, Stratton PD, Benjamin N, Brassfield T, Ritter JM. Reduction of LDL cholesterol by pravastatin does not influence platelet activation in patients with mild hypercholesterolaemia at risk of coronary heart disease. *Br J Clin Pharmacol* 1991; **32**: 127–129.
43 Hussein O, Schlezinger S, Rosenblat M, Keidar S, Aviram M. Reduced susceptibility of LDL to lipid peroxidation after fluvastatin therapy is associated with the hypocholesterolaemic effect of the drug and its binding to the LDL. *Atherosclerosis* 1997; **128**: 11–18.

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