Hypercholesterolemia

Inhibition of Thrombin Generation by Simvastatin and Lack of Additive Effects of Aspirin in Patients With Marked Hypercholesterolemia

Andrzej Szczeklik, MD, PhD, Jacek Musiał, MD, PhD, Anetta Undas, MD, Piotr Gajewski, MD,
Paweł Góra, PhD, Jakub Swadźba, MD, Miłosz Jankowski, MD

Cracow, Poland

OBJECTIVES   To assess the effects of aspirin compared with simvastatin on thrombin generation in hypercholesterolemic men, and to establish whether the reduction of elevated blood cholesterol by simvastatin would affect the action of aspirin on thrombin formation.

BACKGROUND   Aspirin inhibits thrombin formation, but its performance is blunted in hypercholesterolemia. By virtue of altering lipid profile, statins could be expected to influence thrombin generation.

METHODS   Thirty-three men, aged 34 to 61 years, with minimal or no clinical symptoms, serum total cholesterol >6.5 mmol/liter and serum triglycerides <4.6 mmol/liter, completed the study consisting of three treatment phases. First, they received 300 mg of aspirin daily for two weeks (phase I), which was then replaced by simvastatin at the average dose of 24 mg/d for three months (phase II). In phase III, aspirin, 300 mg/day, was added for two weeks to simvastatin, the dose of which remained unchanged. Thrombin generation was assessed: 1) in vivo, by measuring levels of fibrinopeptide A (FPA) and prothrombin fragment 1+2 (F1+2) in venous blood; and 2) ex vivo, by monitoring the rates of increase of FPA and F1+2 in blood emerging from standardized skin incisions of a forearm. A mathematical model was used to describe the kinetics of thrombin formation at the site of microvascular injury.

RESULTS   Two-week treatment with aspirin had no effect on thrombin markers in vivo, while ex vivo it depressed the total amount of thrombin formed, though not the reaction rate. After simvastatin treatment, serum cholesterol decreased by 31% and LDL cholesterol by 42%, while thrombin generation became markedly depressed. In venous blood, FPA was significantly reduced. Concomitantly, the initial thrombin concentration and total amount of thrombin generated decreased significantly. Addition of aspirin to simvastatin (phase III) had no further effect on any of these parameters.

CONCLUSIONS   In men with hypercholesterolemia, lowering serum cholesterol level by a three-month simvastatin treatment is accompanied by a marked reduction of thrombin generation both at basal conditions in venous blood and after activation of hemostasis by microvascular injury. Once blood cholesterol became reduced, adding aspirin to simvastatin did not enhance dampening of thrombin formation. (J Am Coll Cardiol 1999;33:1286–93) © 1999 by the American College of Cardiology

The link between hypercholesterolemia and atherosclerosis is well established, and recent studies (1–3) demonstrated that lowering serum cholesterol, particularly with statins,

(3-hydroxy-3-methylglutaryl-CoA reductase inhibitors), decreases cardiovascular events in patients with and without

coronary artery disease. Hypercholesterolemia may predispose to coronary heart disease by several mechanisms, including hypercoagulability. Indeed, in hypercholesterolemic subjects, platelet aggregability is enhanced, thromboxane A2 production is increased, and markers of thrombin generation are elevated in venous blood (4–6). Aspirin effectively blocks platelet aggregation (7) and inhibits thrombin generation in the clotting blood (8), which explains its beneficial prophylactic and therapeutic effects in atherothrombotic disease. However, in hypercholesterolemia, the inhibitory effect of aspirin on thrombin generation is blunted (9).

Statins, by altering blood lipids profile, could modulate thrombin production and its activity. Therefore, in hyper-

Abbreviations and Acronyms

ALT	=	alanine aminotransferase
AP	=	alkaline phosphatase
AST	=	aspartate aminotransferase
CK	=	creatine phosphokinase
F1+2	=	prothrombin fragments 1+2
FPA	=	fibrinopeptide A
HDL	=	high-density lipoprotein
LDL	=	low-density lipoprotein
TAT	=	thrombin-antithrombin III complexes

cholesterolemic men, we compared the effects of simvastatin versus aspirin on thrombin generation. We also investigated whether reduction in elevated serum cholesterol would restore the inhibitory effect of aspirin on thrombin formation to its full potential. For this purpose, we evaluated the effects of a two-week treatment with aspirin on thrombin generation before and after lowering serum cholesterol levels with simvastatin.

METHODS

Patients. Patients were recruited through the outpatient lipid clinic, to which they had been referred for evaluation of elevated blood lipids or because of the history of hyperlipidemia in the family.

Male patients with serum total cholesterol >6.5 mmol/liter (250 mg/dL) and triglycerides <4.6 mmol/liter (400 mg/dL), not responding to dietary treatment alone, were eligible to enter the study. Exclusion criteria included: aged >65 or <30 years, unstable coronary heart disease, diabetes mellitus and other severe chronic illness (eg, cancer), active peptic ulcer, impaired hepatic or renal function, secondary hypercholesterolemia, history of alcoholism, systolic blood pressure greater than 180 mm Hg and/or a diastolic blood pressure greater than 100 mm Hg and use of other hypolipemic drugs. Participants did not take any medication that could possibly interfere with hemostatic parameters for one month before the start of the study. They were on a standard lipid-lowering diet [phase 1 according to the American Heart Association (10)] for at least 6 weeks before entering the trial and throughout it.

All subjects gave informed consent, and the protocol was approved by the University Ethical Committee.

Drug regimens. Study protocol comprised three treatment phases. First, the patients received 300 mg of aspirin daily for two weeks (week 0–2; phase I). Thereafter, they entered a 12-week active treatment with simvastatin (week 3–14; phase II), which was begun with the dose of 20 mg once a day with the evening meal. If total cholesterol level did not fall by at least 1 mmol/liter after four weeks of the drug administration, the dose was increased to 40 mg. After a three-month simvastatin treatment, aspirin (300 mg/d) was added for two weeks to simvastatin, the dose of which

remained unchanged (ie, 20 or 40 mg/d; week 15–16; phase III).

Laboratory investigations. Blood was sampled in the morning after 16 h of fasting. Smokers were requested not to smoke for at least 2 h before blood sampling. At entry (week 0), after six weeks (week six) and at the end of the study (week 16), the following estimations were performed: blood morphology and platelet count, serum creatinine, bilirubin, aspartate (AST) and alanine aminotransferases (ALT), alkaline phosphatase (AP), creatine phosphokinase (CK) and urinalysis.

Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were determined by standard procedures in serum. Serum low-density lipoprotein cholesterol (LDL-C) levels were calculated by the Friedewald formula. Lipid profile was determined five times: before starting therapy (week 0), after the first (week 6), the second (week 10), and the third month (week 14) of treatment with simvastatin and at the end of the study (week 16). The primary criterion used to evaluate the efficacy of treatment was TC. A posttreatment reduction by more than 1 mmol/L was chosen as the therapeutic goal. If such reduction was not obtained, the patient was excluded from the final analysis.

Safety assessments included vital signs, a 12-lead electrocardiogram (ECG) and laboratory tests (AST, ALT, and CK), performed monthly during simvastatin treatment. Notable increase was defined as more than threefold rise above the upper limit of normal range of ALT or AST, or more than 10-fold rise above the upper limit of normal range of CK on any occasion. The notable rise in one of these three enzymes levels was the reason for discontinuation of simvastatin administration.

Hemostatic variables measured included: fibrinogen, plasminogen, antithrombin III, prothrombin, platelet aggregation induced by arachidonic acid (AA) and thrombin generation in vivo and ex vivo. They were assessed at baseline (week 0), after two weeks of aspirin administration (phase I), after three months of simvastatin treatment (phase II) and after final two weeks of combined aspirin and simvastatin (phase III) therapy. Blood was collected into 3.8% sodium citrate (9:1, vol/vol).

Platelet aggregation by arachidonic acid (AA) was measured in platelet-rich plasma. If platelets did not respond to 1.2 μmol AA, the subjects were considered to be under the probable influence of platelet-inhibiting drugs and they did not enter the study. Platelet aggregability was also tested at week 14, after three months of simvastatin therapy.

Plasma fibrinogen, plasminogen, antithrombin III and prothrombin concentrations were determined nephelometrically (Dade Behring, Marburg, Germany).

Thrombin generation in vivo was measured in peripheral venous blood using two specific markers: fibrinopeptide A (FPA) (Asserachrom; Diagnostica Stago, Asnières, France)

and prothrombin fragment 1+2 (F1+2) (Enzygnost; Behring, Germany).

The thrombin generation ex vivo assay consisted of monitoring the rates of increase of FPA and F1+2 concentrations in blood emerging from standardized skin incisions of a forearm. The details of the method were described previously (9,11). Briefly, skin incisions were made on a forearm with a Simplate II device (Organon Teknika). Blood aliquots were collected into heparinized canules (Baxter) at 30-s intervals and immediately mixed with anticoagulant (Byk Gulden, Konstarz, Germany) in plastic tubes (1:10, vol/vol). They were centrifuged at 3,000 rpm for 20 min at 4°C and stored for two months at −80°C until assayed. Volumes of consecutive blood samples were also recorded. To minimize technical limitations of the method pertaining to sample collection and analysis, all the procedures were performed by the same investigators. The concentrations of FPA were expressed in ng/mL, and those of F1+2 in nmol/liter.

Data were analyzed by comparing FPA or F1+2 levels at the same time intervals, before and after the specific drug treatment. In addition, the kinetics of thrombin generation at sites of microvascular injury was calculated using a mathematical model, reported in detail elsewhere (9). According to the model, plasma concentrations of FPA or F1+2 at any time point can be determined using the equation:

$$c(t) = c_o \exp(\lambda t),$$

where c_o denotes the calculated concentration of the thrombin marker at the very beginning of bleeding, and λ represents the rate of its increase. Parameter S, calculated as the area under the curve, is a measure of the total amount of thrombin generated in 3 min (Fig. 1).

Statistical analysis. Two-way repeated measure analysis of variance (ANOVA) was used wherever its assumptions were fulfilled. The majority of variables did not meet ANOVA criteria, and Friedman's test was applied followed by paired analysis using Wilcoxon matched pairs test. Statistical significance was accepted at a level of $p < 0.05$. Calculations were performed on a PC computer with Statistica (StatSoft, Inc., Tulsa, Oklahoma).

RESULTS

Subjects and treatment. Thirty-eight men entered the study. Three presented with intermittent claudication (grade II according to Fontaine), five had mild arterial hypertension and one had coronary heart disease. No other clinical pathology was evident. None of the subjects had history of venous thromboembolism. Two received enalapril, one metoprolol and one molsidomine and isosorbide mononitrate. Twenty were current smokers.

Two subjects were withdrawn from the study: one because of nonfatal acute myocardial infarction that occurred
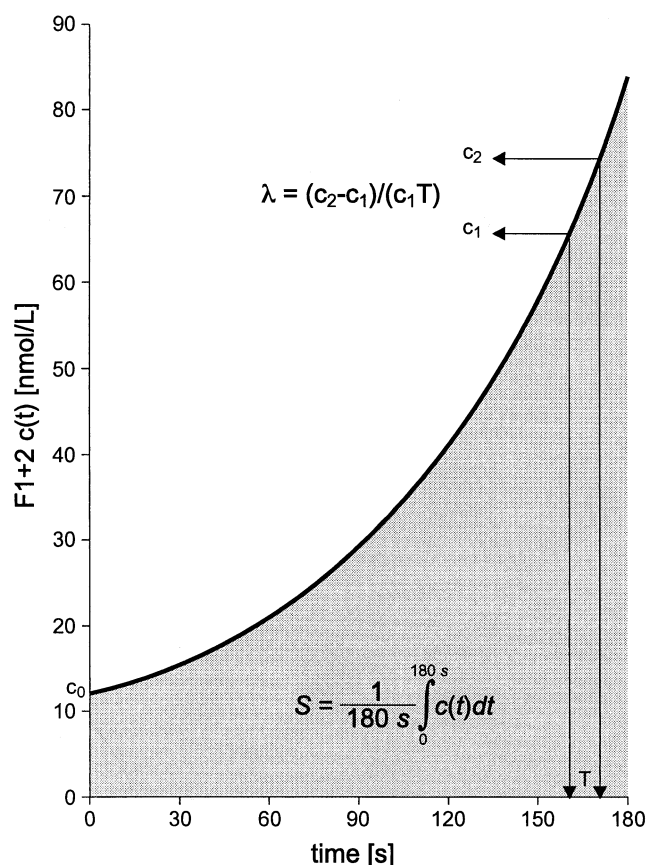
Figure 1. Thrombin generation as a function of time, c(t). c_o denotes the initial thrombin generation, λ determines the rate of increase of the curve and S denotes the total amount of thrombin generated in the first 180 s, reflected by the shaded area under the curve.

6 weeks after the onset of the study and another because of subarachnoid hemorrhage with short-lived neurological symptoms, which occurred 8 weeks after the start of the study. These two serious adverse events could not be definitely attributed to simvastatin therapy.

Nine patients received 40 mg simvastatin daily; the remaining received 20 mg a day. Overall, tolerability of simvastatin was good. Most laboratory safety values remained within the normal range. No notable increases in AST, ALT or CK levels were recorded during the study. There was no significant change in blood pressure, heart rate, clinical symptoms or ECG recordings during the study.

Three patients were excluded from the final analysis, because of insufficient reduction in cholesterol level (on average, 0.5 mmol/liter). Thus, the final analysis comprised data from 33 subjects, aged 34 to 61 (mean, 47.4) years.

Blood lipids and hemostatic parameters. Simvastatin treatment resulted in a significant decrease in total cholesterol, LDL cholesterol, and triglycerides (Table 1). Plasma fibrinogen, prothrombin, antithrombin III and plasminogen

Table 1. Serum Lipids and Hemostatic Parameters

Variables	Baseline	Phase I	Phase II	Phase III	p Aspirin	p Simvastatin	p Interaction
Cholesterol, total (mmol/liter)	8.03 ± 0.82	—	5.43 ± 0.49	5.53 ± 0.69	—	0.000001	—
LDL cholesterol (mmol/liter)	5.59 ± 0.87	—	3.19 ± 0.51	3.16 ± 0.60	—	0.000001	—
HDL cholesterol (mmol/liter)	1.31 ± 0.20	—	1.42 ± 0.27	1.41 ± 0.25	—	0.01	—
Triglycerides (mmol/liter)	2.46 ± 0.95	—	1.85 ± 0.76	2.07 ± 0.93	—	0.00001	—
Fibrinogen (g/liter)	2.77 ± 0.33	2.78 ± 0.45	2.81 ± 0.58	2.79 ± 0.41	NS	NS	NS
Prothrombin (g/liter)	0.104 (0.088; 0.112)	0.099 (0.088; 0.111)	0.095 (0.083; 0.106)	0.105 (0.092; 0.111)	NS	NS	NS
Antithrombin III (g/liter)	0.264 (0.250; 0.274)	0.267 (0.251; 0.275)	0.260 (0.246; 0.270)	0.264 (0.252; 0.276)	NS	NS	NS
Plasminogen (g/liter)	0.118 (0.112; 0.124)	0.117 (0.112; 0.124)	0.116 (0.106; 0.126)	0.121 (0.108; 0.125)	NS	NS	NS
F1+2 (nmol/liter)	0.84 (0.74; 1.05)	0.82 (0.69; 1.07)	0.71 (0.52; 1.01)	0.68 (0.59; 0.87)	NS	NS	NS
Fibrinopeptide A (ng/mL)	5.20 (3.90; 6.48)	4.76 (3.70; 6.57)	4.57 (2.81; 4.84)	4.28 (2.63; 5.26)	NS	0.046	NS
Bleeding time (s)	365 (310; 435)	480 (420; 540)	430 (375; 500)	522 (405; 562.5)	0.00003	NS	NS

Values are mean ± SD or median (quartile 1;3). Serum lipids after phase II were compared with baseline by Wilcoxon test. For the remaining parameters, two-way repeated measures ANOVA was used.

levels did not change during the study. Bleeding time became markedly prolonged only when aspirin was used.

Thrombin generation in vivo. Administration of aspirin for 2 weeks at a dose of 300 mg daily did not affect the levels of either FPA or F1+2. On the contrary, 12-week simvastatin treatment significantly reduced FPA concentration. Addition of aspirin to simvastatin for 2 weeks had no further effect (Table 1).

Thrombin generation ex vivo. In blood emerging from the standardized skin incisions, FPA and F1+2 levels increased exponentially and reached a plateau at approximately 4 min, when blood flow decreased. Six measurements of both FPA and F1+2 were performed in each subject before and after the drug.

1/FPA FORMATION. After two weeks of treatment with aspirin, FPA values became significantly decreased at 90, 150 and 180 s, but not at other time points of blood sampling. Simvastatin treatment significantly reduced FPA values at 90, 120 and 180 s. Addition of aspirin to simvastatin treatment for two weeks did not produce any further depression of FPA concentrations (Table 2). Volumes of blood samples did not affect FPA concentrations. Analysis of the kinetics of thrombin formation, using the mathematical model, revealed that both aspirin and simvastatin used separately depressed the total amount of thrombin generated (S). Addition of aspirin to simvastatin (phase III) had no further effect on this parameter (Table 3).

2/F1+2 FORMATION. Both drug regimens, administered separately, depressed significantly F1+2 formation at all time intervals studied as compared with the baseline values (Table 2, Fig. 2). There was no relationship between volumes of blood samples and F1+2 concentrations. After simvastatin, the average values were usually lower than after aspirin therapy, but the difference between the two regimens reached the significance only at time 30 s. Depression of thrombin generation remained at the same level when both drugs were administered in combination for the last two weeks. Of parameters describing the thrombin generation curve, both initial thrombin concentration (c_o), and total amount of thrombin generated (S) became depressed after treatment with either aspirin or simvastatin (Table 3).

DISCUSSION

Design of the study and methods used. We designed the study to compare the effects of aspirin on thrombin generation with those of simvastatin, and to evaluate the possibility that reduction of elevated blood cholesterol levels may improve aspirin-induced changes in thrombin formation. We, therefore, studied the generation of thrombin at baseline and after a two-week treatment with aspirin followed by administration of a potent cholesterol-lowering agent, simvastatin. At the end of this period, we assessed thrombin generation, added aspirin to simvastatin for two

Table 2. Prothrombin Fragment 1+2 (F1+2) and Fibrinopeptide A (FPA) in Blood Ex Vivo

Time (s)	Baseline	Phase I	Phase II	Phase III	p
		F1+2 (nmol/liter)			
30	15.91 (4.54; 31.09)	5.19 (3.47; 9.48)*	2.70 (1.70; 5.05)*‡	2.25 (1.32; 3.90)*‡	< 0.00001
60	19.35 (6.80; 51.60)	7.40 (4.22; 16.17)*	4.80 (2.00; 10.10)*	4.80 (2.49; 11.00)*	0.002
90	34.41 (16.35; 80.50)	11.70 (3.10; 28.32)*	7.60 (3.72; 21.60)†	8.65 (4.94; 15.45)*	0.0007
120	49.35 (18.50; 80.66)	16.40 (7.90; 66.08)*	17.20 (5.18; 38.40)*	11.31 (5.68; 32.72)*‡	0.002
150	72.90 (43.10; 17.70)	25.20 (10.60; 72.68)†	21.20 (10.80; 50.80)*	22.40 (11.66; 49.57)*	0.0003
180	97.30 (41.00; 224.48)	39.17 (16.90; 106.67)†	32.80 (13.60; 74.55)*	38.60 (16.20; 58.47)*	0.0003
		FPA (ng/mL)			
30	344.7 (193.6; 911.1)	267.7 (98.5; 404.6)	192.7 (119.1; 382.3)	243.4 (139.9; 361.2)	NS
60	623.8 (322.5; 976.8)	498.9 (292.1; 776.9)	427.5 (235.3; 734.2)	416.7 (240.9; 799.7)	NS
90	1053.1 (535.6; 1634.5)	585.0 (278.0; 934.5)*	547.1 (292.8; 991.6)*	569.8 (323.6; 926.6)*	0.0008
120	1070.7 (660.8; 1716.6)	845.0 (460.1; 1334.3)	817.4 (339.3; 1008.2)*‡	597.1 (366.6; 1048.1)*‡	0.0001
150	1593.8 (1161.9; 2773.9)	962.8 (632.1; 1629.3)*	1213.5 (887.7; 1878.2)†	1215.8 (859.4; 2338.1)	0.04
180	1854.8 (1264.5; 2750.4)	1031.8 (882.3; 1997.4)	2442.2 (1057.4; 2932.2)	1625.1 (1136.1; 2816.4)	NS

Values are median (quartile 1;3). Friedman's test was applied followed by paired analysis using Wilcoxon test:
*p ≤ 0.005 as compared with baseline, †p ≤ 0.05 as compared with baseline, ‡p < 0.05 as compared with phase I.

weeks and then repeated thrombin generation measurements.

To assess thrombin formation in vivo, we determined concentrations of two specific and sensitive markers in venous blood. Although they both reflect the appearance of thrombin, their origin is not the same (12). F1+2 is a polypeptide cleaved from prothrombin during its conversion to thrombin, and FPA is split off from fibrinogen by thrombin. Thus, F1+2 reflects the final stage in the process of thrombin formation, while FPA is a measure of thrombin activity. The same two markers were used in the ex vivo method, based on the sampling of blood coming out from standardized skin incisions. The technique has been introduced into coagulation studies by Thorngren et al. (13), and successfully applied by other investigators (14–16). A mathematical model describing the process of thrombin formation ex vivo has been developed (11).

Aspirin and thrombin formation. There is convincing evidence that aspirin inhibits effectively thrombin formation (11,14). After a two-week administration at a dose of 300 mg per day, it significantly reduced thrombin markers in venous blood and depressed thrombin formation in skin-bleeding-time blood (9). These effects were observed in subjects with average cholesterol levels, but were blunted in patients with marked hypercholesterolemia (9). The present study confirms the previous observations on reduced capacity of aspirin to inhibit thrombin formation in hypercholesterolemic men, particularly when assessed by changes in FPA concentrations. Thus, in vivo both thrombin markers remained unaffected by aspirin treatment, while the velocity of the reaction did not change at sites of the microvasculature injury. Furthermore, ex vivo aspirin did not depress significantly FPA concentrations at some blood sampling points, though the total amount of thrombin generated within 3 min was significantly reduced. Assessment of F1+2 curve showed more consistent inhibition by aspirin.

Simvastatin treatment. A 12-week simvastatin therapy led to a marked reduction in blood total and LDL cholesterol. This was accompanied by depression of thrombin generation, as evidenced by decreased concentrations of FPA in venous blood, unaffected by the preceding aspirin treatment. In skin-

Table 3. Kinetics of Thrombin Generation Based on Concentrations of Prothrombin Fragment 1+2 (F1+2) and Fibrinopeptide A (FPA) in Blood Ex Vivo

Parameter	Baseline	Phase I	Phase II	Phase III	p
		F1+2			
λ	0.011 (0.008; 0.017)	0.013 (0.010; 0.017)	0.015 (0.011; 0.020)†	0.016 (0.013; 0.018)†	0.004
c_o	11.56 (6.43; 23.25)	4.55 (2.55; 8.16)*	2.42 (1.12; 4.72)†	2.06 (1.08; 4.95)*	0.00008
S	43.44 (21.44; 85.01)	18.36 (9.30; 53.41)*	13.70 (7.67; 34.99)†	15.34 (7.99; 25.60)*	0.006
		FPA			
λ	0.009 (0.004; 0.013)	0.011 (0.006; 0.014)	0.011 (0.009; 0.015)	0.011 (0.008; 0.013)	NS
c_o	387.8 (216.6; 783.7)	239.7 (97.0; 479.5)	234.3 (114.1; 398.8)	223.1 (145.3; 407.6)	NS
S	989.7 (727.6; 1561.9)	714.4 (445.0; 1057.5)†	783.0 (562.3; 1122.6)†	790.8 (475.9; 1181.2)†	0.01

Values are median (quartile 1;3). Friedman's test was applied followed by paired analysis using Wilcoxon test:
*p ≤ 0.0005 as compared to baseline, †p ≤ 0.01 as compared with baseline, ‡p < 0.05 as compared with phase I.
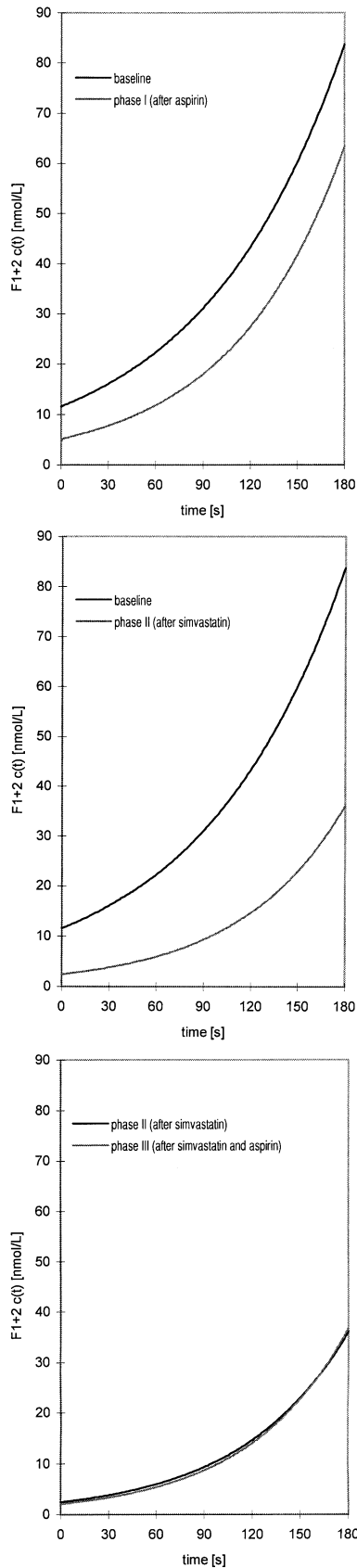
Figure 2. Thrombin generation expressed as concentrations of prothrombin fragment 1+2 (F1+2) as a function of time, at baseline and after phase I, II and III.

bleeding-time blood, the amount of thrombin formed also became significantly reduced. This effect was reflected by changes in two parameters describing the kinetics of thrombin formation: the initial thrombin concentration and total amount of thrombin generated in 3 min, which decreased significantly. This considerable decrease in thrombin generation by simvastatin was not enhanced by an additional supplementation of aspirin for two weeks.

While this work was in progress, three reports appeared suggesting that cholesterol reduction by statins might decrease the thrombogenic potential in hypercholesterolemia. Aoki and colleagues (17) observed that pravastatin, administered at a dose of 15 mg/day to patients with hypercholesterolemia for at least one month, reduced platelet-dependent thrombin formation, measured in vitro in recalcified plasma, though thrombin-antithrombin III (TAT) complexes in venous blood remained unchanged. Lacoste and colleagues (18) reported that pravastatin at a dose of 40 mg/day administered for 2.5 months to 16 hypercholesterolemic patients reduced platelet thrombus formation, assessed by the exposure of porcine aortic media to the patients' flowing venous blood in an ex vivo superfusion chamber. DiGarbo et al. (19) concluded that simvastatin inhibits initially augmented thrombin formation in hypercholesterolemia, as reflected by a significant posttreatment fall in plasma TAT and F1+2 concentrations. Both Aoki's and Lacoste's groups expressed the view that the effects they observed were not due to a direct action of pravastatin on hemostasis but to cholesterol lowering. Thus, pravastatin added to recalcified plasma did not affect the production of thrombin in test tubes (17), and thrombus formation was unchanged after 1 week of pravastatin treatment, when serum cholesterol levels were not reduced yet (18). Our results corroborate and extend the recent findings by DiGarbo et al. (19), indicating that statins not only decrease steady-state thrombin generation in peripheral venous blood, but also lower its formation when hemostatic system is activated by a microvascular injury.

Statins exert their beneficial effects on coronary events rate also in patients with average blood cholesterol levels (3,20). Thus, it remains to be established whether highly desirable clinical benefits from successful statin therapy depend solely on the lipid-lowering effect or on a different mechanism, e.g., an influence on the expression of tissue factor or endothelial function (*vide infra*). A reduction of thrombogenic potential might not be specific for statins. Gemfibrozil, a lipid-lowering drug with a different mechanism of action, has been reported to lower F1+2 and TAT levels in venous blood, which was associated with normalization of plasma lipids in men with combined hyperlipidemia (21).

Recently, it has been found that simvastatin reduces the expression of tissue factor in cultured human macrophages (22) and in monocytes of subjects with hypercholesterolemia (23). This effect could well explain a distinct depression of thrombin generation in the ex vivo model used in the present study, because increased expression of tissue factor

starts an exponential thrombin generation at sites of microvascular injury (16). Improvement in endothelial function, found after four weeks of simvastatin treatment (24), coupled with decreased adhesion of monocytes to endothelial cells (25), would also favor stricter control or even decreased thrombin formation. Simvastatin, by normalizing platelet lipid composition and membrane fluidity in hypercholesterolemic patients (26), may also attenuate platelet involvement in thrombin generation (27). The influence of simvastatin on platelet function in hypercholesterolemia has been, however, questioned (28).

Clinical relevance. Our results indicate that in hypercholesterolemia, lowering of total and LDL cholesterol with simvastatin is accompanied by a marked reduction in thrombin generation; the kinetics of thrombin formation in blood becomes considerably depressed. This effect of simvastatin on thrombin production is more pronounced than that of aspirin. Importantly, once serum cholesterol concentration became reduced, adding aspirin to simvastatin does not lead to any further suppression of thrombin formation.

When designing the study we anticipated that simvastatin, by altering lipid profile, would set the stage for aspirin to express its full capacity to inhibit thrombin generation. We did not expect that lipid-lowering treatment with simvastatin will be associated with such a profound reduction in thrombin formation. Aspirin was unable to suppress further thrombin generation, so effectively controlled by simvastatin; no additive or synergistic effects were observed. Perhaps, counterregulatory mechanisms oppose an additional dampening of thrombin generation, or simvastatin through poorly defined alterations in membrane lipid profile renders proteins less accessible for the acetylation induced by aspirin. It should not be concluded that there is no room for aspirin in hypercholesterolemic subjects whose cholesterol became successfully reduced by simvastatin, since aspirin has been proven to produce some of its beneficial effects through several mechanisms unrelated to thrombin generation (7).

In summary, our study shows that in hypercholesterolemia, lowering blood cholesterol by simvastatin treatment is associated with a reduction in thrombin generation both at basal conditions in venous blood and after activation of hemostasis by microvascular injury. The marked inhibitory effect of simvastatin on thrombin formation, observed as early as three months after the onset of therapy, might be of special clinical interest. It could also suggest that early clinical benefits observed during treatment with statins (3,20) might be due, at least in part, to a decrease in thrombogenic potential and might precede atherosclerotic plaque stabilization or regression.

Reprint requests and correspondence: Prof. Andrzej Szczeklik, Jagiellonian University, School of Medicine, Department of Medicine, 8 Skawinska Street, 31-066 Cracow, Poland. E-mail: mmszczek@cyf-kr.edu.pl.

REFERENCES

1. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 1994;344:1383–9.
2. Shepherd J, Cobbe SM, et al., for the West Scotland Coronary Prevention Study Group. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Engl J Med 1995;333:1301–7.
3. Sacks FM, Pfeffer MA, et al., for the Cholesterol and Recurrent Events Trial Investigators. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. N Engl J Med 1996;335:1001–9.
4. Davi G, Ganci A, Averna M, et al. Thromboxane biosynthesis, neutrophil and coagulation activation in type IIa hypercholesterolemia. Thromb Haemost 1995;74:1015–9.
5. Davi G, Averna M, Catalano I, et al. Increased thromboxane biosynthesis in type IIa hypercholesterolemia. Circulation 1992;85:1792–8.
6. Alessandri C, Basili S, Maurelli M, et al. Relationship between prothrombin activation fragment F1+2 and serum cholesterol. Haemostasis 1996;26:214–9.
7. Patrono C. Aspirin as an antiplatelet drug. N Engl J Med 1994;330:1287–94.
8. Szczeklik A. Thrombin generation in myocardial infarction and hypercholesterolemia: effects of aspirin. Thromb Haemost 1995;74:77–80.
9. Szczeklik A, Musial J, Undas A, et al. Inhibition of thrombin generation by aspirin is blunted in hypercholesterolemia. Arterioscl Thromb Vasc Biol 1996;16:948–54.
10. National Cholesterol Education Program. Second Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). Circulation 1994;89:1329–445.
11. Szczeklik A, Krzanowski M, Góra P, Radwan J. Antiplatelet drugs and generation of thrombin in clotting blood. Blood 1992;80:2006–11.
12. Rand MD, Lock JB, Van't Veer C, Gaffney DP, Mann KG. Blood clotting in minimally altered whole blood. Blood 1996;88:3432–45.
13. Thorngren M, Shafi S, Born GVR. Thromboxane A2 in skin-bleeding-time blood and in clotted venous blood before and after administration of acetyl-salicylic acid. Lancet 1983;1:1075–8.
14. Kyrle PA, Westwick J, Scully MF, Kakkar VV, Lewis GP. Investigation of the interaction of blood platelets with the coagulation system at the site of plug formation ex vivo in man—effect of low-dose aspirin. Thromb Haemost 1987;57:62–6.
15. Nowak J, FitzGerald GA. Redirection of prostaglandin endoperoxide metabolism at the platelet-vascular interface in man. J Clin Invest 1989;83:380–5.
16. Weiss HJ, Lages B. Evidence for tissue factor-dependent activation of the classic extrinsic coagulation mechanism in blood obtained from bleeding time wounds. Blood 1988;71:629–35.
17. Aoki I, Aoki N, Kawano K, et al. Platelet-dependent thrombin generation in patients with hyperlipidemia. J Am Coll Cardiol 1997;30:91–6.
18. 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;92:3172–7.
19. DiGarbo V, Cordova R, Avellone G. Increased thrombin

formation and complement activation in patients with type IIA hyperlipoproteinemia: effects of simvastatin treatment. Curr Therapeut Res 1997;58:706–23.

20. Gotto AM. Cholesterol management in theory and practice. Circulation 1997;96:4424–30.

21. Colli S, Eligini S, Lalli M, Camera M, Paoletti R, Tremoli E. Vastatins inhibit tissue factor in cultured human macrophages. A novel mechanism of protection against atherothrombosis. Arterioscl Thromb Vasc Biol 1997;17:265–72.

22. Ferro D, Basili S, Alessandri C, Mantovani B, Cordova C, Violi F. Simvastatin reduces monocyte-tissue-factor expression type IIa hypercholesterolemia. Lancet 1997;350:1222.

23. Broijersen A, Hamsten A, Silveira A, et al. Gemfibrozil reduces thrombin generation in patients with combined hyperlipidaemia without influencing plasma fibrinogen, fibrin gel structure or coagulation factor VII. Thromb Haemost 1996;76:171–6.

24. O'Driscoll G, Green D, Taylor RR. Simvastatin, an HMG-coenzyme A reductase inhibitor, improves endothelial function within 1 month. Circulation 1997;95:1126–31.

25. Weber C, Erl W, Weber KCS, Weber PC. HMG-CoA reductase inhibitors decrease CD11b expression and CD11b-dependent adhesion of monocytes to endothelium and reduce increased adhesiveness of monocytes isolated from patients with hypercholesterolemia. J Am Coll Cardiol 1997;30:1212–7.

26. 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 hypercholesterolemic patients. Metabolism 1994;43:11–7.

27. Musial J, Radwan J, Szczeklik A. Aspirin delays thrombin generation in vitro through interaction with platelet phospholipids. Thromb Res 1997;85:367–8.

28. Broijersen A, Eriksson M, Leijd B, Angelin B, Hjemdahl P. No influence of simvastatin treatment on platelet function in vivo in patients with hypercholesterolemia. Arterioscl Thromb Vasc Biol 1997;17:273–9.