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Scientific paper

Standardized ultrasound as a new method to induce platelet aggregation Evaluation, influence of lipoproteins and of glycoprotein IIb/IIIa antagonist tirofiban

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Abstract

Most of the published studies concerning platelet aggregation were performed with chemical stimulation procedures, however, mechanical stimulation might be a better simulation of physiological activation of platelets. In order to evaluate the influence of ultrasound on platelet aggregation in vitro, we developed an ultrasound device in a standardized set-up, and we evaluated the influence of lipoproteins and the glycoprotein IIb/IIIa inhibitor tirofiban on ultrasound induced platelet aggregation. A cylindrical shaped plastic test tube with 1 ml of platelet-rich plasma was placed in an ultrasound bath (35kHz) for 5 s. The ultrasound energy transfer into the sample ($\Delta W = 3.77$ J) was calculated using the average temperature increase (averaged by 0.935 °C) of the sample. Platelet aggregation was quantified immediately after stimulation with ultrasound or adenosine diphosphate (ADP 2.1 and 4.2 μM) by the Myrenne Aggregometer PA2 at low (40 s^{-1}) and afterwards at high (2500 s^{-1}) shear. To evaluate the influence of lipoproteins, seven healthy male volunteers were investigated before and after a fat load (50 g fat per m^2 body surface), and 11 patients suffering from hypercholesterolemia and atherosclerotic disease before and after a single low-density lipoprotein (LDL) apheresis. Platelet aggregation after ultrasound stimulation was well correlated with platelet aggregation after ADP (r between 0.50 and 0.95). However, when exposed to high shear, the low shear-induced platelet aggregates were more stable after ultrasound stimulation compared with ADP stimulation either with or without tirofiban. After the fat load triglyceride concentration increased from 0.86 ± 0.39 to 2.10 ± 1.10 mmol l^{-1} ($P < 0.05$) resulting in a reduced formation of platelet aggregates after weak (ADP 2.1 μM) but not after

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strong (ADP 4.2 μM or ultrasound) stimuli. After a single LDL apheresis LDL cholesterol dropped from 3.99 ± 0.90 to 1.06 ± 0.55 mmol l^{-1} ($P < 0.005$). No changes in platelet aggregation were observed with the exception of a lower aggregation when exposed to high shear after stimulation with 2.1 μM ADP. In conclusion, we found the ultrasound stimulation of platelet-rich plasma easy to perform. The platelet aggregation after ultrasound stimulation correlated well with stimulation after ADP. While a reduction in LDL cholesterol concentration had only slight effects on platelet aggregation, an increase in triglyceride concentration resulted in a reduced formation of platelet aggregates after weak stimulation.

Keywords: Ultrasound; Platelet aggregation; Hyperlipidemia; Tirofiban

1. Introduction

A promoting effect of ultrasound application on the biphasic process of activation (primary shape change) and aggregation (secondary deposition onto other platelets) of platelets has been known for about 20 years (Chater and Williams, 1977; Williams et al., 1978; Miller et al., 1979). In their experiments in vivo, Chater and Williams showed that the extent of platelet aggregation was increased as the ultrasound frequency decreased (from 3.0 to 0.75 MHz) or as the ultrasound intensity increased (Chater and Williams, 1977). Since these early studies no more further investigations concerning ultrasound-induced platelet aggregation have been published.

However, there is increasing evidence that in the circulating blood in vivo, platelets are not only activated by chemical mediators (e.g. adenosine diphosphate (ADP), thromboxan A_2, thrombin, collagen) but also, and perhaps primarily, by mechanical stimuli. It was shown that mechanical shear stress (Miyazaki et al., 1996) or mechanical irritation (Williams et al., 1998) could enhance platelet aggregation without an additional stimulating chemical agonist. The physical details of mechanically induced aggregation are incompletely understood as yet, but the platelet glycoprotein Ib has been implicated in chemically mediating the shear-induced aggregation through binding of large multimeric forms of von Willebrand factor (vWF), which also results in an opening of membranal calcium channels. The transmembrane influx of calcium ions is followed by the binding of vWF to the glycoprotein IIb/IIIa, which is assumed to be obligatory for the aggregation process of platelets (Miyazaki et al., 1996; Moake et al., 1996; Goto et al., 1995).

Therefore, it seems reasonable to investigate platelet aggregation not only in the presence of chemical stimuli, but also following mechanical stress which we performed by the exposure of platelet-rich plasma (PRP) to ultrasound in vitro. In contrast to previously published studies, we applied ultrasound in a standardized model, and compared the ultrasound-induced effects with the chemical stimulation (ADP).

Elevated concentrations of low-density lipoprotein (LDL) cholesterol and of triglycerides are associated with an increased risk of atherosclerosis. However, the mechanisms of the atherosclerotic process are by no means completely understood so far. It was suggested that enhanced platelet aggregation might be involved in the proatherogenic effect of elevated lipoprotein concentrations. In order to evaluate the influence of elevated lipoprotein concentrations on ultrasound-induced platelet aggregation, we performed investigations before and after a fat load as well as before and after a LDL apheresis.

2. Materials and methods

2.1. Determination of platelet aggregation

For the measurement of platelet aggregation, the collection of blood and all subsequent steps were performed under normal thermal conditions (37 °C). Blood was collected in tubes containing sodium citrate (Boehringer Mannheim, Mannheim, Germany) in a final concentration of 0.68% and blood was centrifuged ($300 \times g$, 3.5 min, EBA III, Hettich, Tuttlingen, Germany) at 37 °C. After centrifugation PRP was removed from the supernatant. Platelet-poor plasma was

obtained subsequently by centrifuging at $1750 \times g$ for 10 min. PRP was stored for 60 min at 37 °C until used in the experiments.

Platelet aggregation was measured at 37 °C using the Myrenne Aggregometer PA2 (Myrenne, Roetgen, Germany) (Blasberg et al., 1981), a device employing a uniform shear regime representative for closed cortex flow. Increasing platelet aggregation is quantified by a greater light transmission (helium neon laser, wave length 628 nm), since the aggregates reduce light scattering and settle towards the bottom of the sample tube. The PRP was first exposed to low shear (40 s^{-1}) for 7 min and immediately afterwards to high shear (2500 s^{-1}) for 3 min. A total of four platelet aggregation parameters were measured, (1) aggregation after 7 min of low shear (%) (LS); (2) area under the aggregation curve of 7 min low shear (% min) (AUC); (3) aggregation after 3 min of high shear (%) (HS); (4) fractional desaggregation index at high shear (FDI), calculated as $(LS - HS)/LS$.

Platelet aggregation parameters were measured without stimulation as well as after stimulation with ultrasound or with ADP (Sigma Co., St. Louis, MO, USA) in two concentrations (4.2 and 2.1 μM). After adding the glycoprotein IIb/IIIa receptor antagonist tirofiban (Aggrastat, MSD Sharp & Dohme GmbH, Haar, Germany) platelet aggregation was again measured after stimulation with ultrasound or ADP (4.2 μM). The concentration of tirofiban (12.5 ng ml^{-1}) was chosen, because this concentration was reported to induce a 50% inhibition of platelet aggregation in vivo (13.6 ng ml^{-1}) and in vitro (11.6 ng ml^{-1}) (Barrett et al., 1994). The ultrasound stimulation measurements were conducted in triplicate, single measurements were performed for other stimulation procedures.

2.2. Mechanical stimulation by ultrasound exposure

Ultrasound stimulation was applied for 5 s by an ultrasound bath (ultrasound frequency 35 kHz, Sonorex RK 102H, Bandelin, Berlin, Germany) which was filled with distilled water (Fig. 1). The cylindrical shaped plastic test tube was immersed 4-mm deep into the water. It was fixed to steel grips mounted on a slide and thus its location in the bath was reproducible within a range of 1 mm.

In the waterbath no single beams, but standing waves with undefined wave shapes were produced. Since additionally an incipient cavitation was observed in the bath, the ultrasound intensity cannot be specified in more detail. However, a very rough approximation for the ultrasonic sound pressure, which is needed to start cavitation can be given, if the setup is compared with a cavitation experiment in water. For very clean degassed water one can approximate the starting (lower) pressure limit for cavitation at about 10^8 Pa (Kuttruff, 1988). Within 'normal' water, it can be assumed that at least a magnitude of about 10^5 Pa is needed to initiate cavitation. For biological fluid sample the limit is lower since the sample is not as homogeneous as water.

To quantify the strength of the applied acoustic sound field, one can calculate the part of the ultrasound energy W_{abs} that is transferred into the sample by adsorption of the sound within the sample fluid.

The sample temperature was measured with a $2 \times 0.5 \times 4$ mm Pt100 sensor (Conrad, Hirschau, Germany), which because of its small size had a

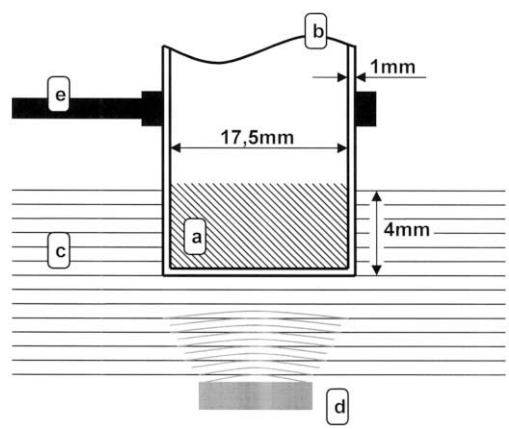

Fig. 1. Cross-section detail of the experimental setup. (a) Platelet-rich plasma sample (1 ml); (b) plastic test tube; (c) distilled water used to transfer ultrasonic waves emitted from the (d) ultrasound generator; (e) steel grips hold every test tube in reproducible position. Not drawn to scale.

negligible effect upon the sample temperature. The part of the ultrasound energy ΔW that is transferred into the sample by adsorption can be calculated via the temperature increase ΔT of the sample via

$$\Delta W = \Delta T \cdot c \cdot \rho \cdot V \quad (1)$$

with $c = 3.93$ kJ kg^{-1} K^{-1} being the heat capacity of the plasma sample, $\rho = 1.0269$ g cm^{-3} its mass density (Wissenschaftliche Tabellen, 1979) and V the sample volume of 1 ml. The temperature increase was averaged to 0.935 K in 5 s, giving an average energy increase of $\Delta W = 3.773$ J in the sample. The heat irradiation ΔW_{irr} of the sample during ultrasound exposition time was calculated using

$$\Delta W_{\text{irr}} = \alpha \cdot A \cdot t \cdot \Delta T \quad (2)$$

where $\alpha = 5.6$ W K^{-1} m^{-2} is the heat transfer coefficient between a plain surface and air (Kuchling, 1989); A the surface area and t the transfer time. Since ΔW_{irr} is less than 0.2% of the transferred ultrasound energy ΔW, its influence has been neglected in this study.

In five samples of PRP the lactate dehydrogenase (LDH) was measured before and after ultrasound stimulation. In these samples, LDH levels rose from 78 ± 7 before to 125 ± 52 U l^{-1} after stimulation.

2.3. Determination of lipids and fibrinogen

For the determination of the concentrations of lipoproteins, fibrinogen, and platelets blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes (1.6 mg EDTA per ml blood, Sarstedt, Nümbrecht, Germany), plasma was obtained by centrifugation ($1750 \times g$, 10 min). Cholesterol and triglyceride concentration in plasma were determined enzymatically (EPOS, Eppendorf, Hamburg, Germany) using reagents from Boehringer, high-density lipoprotein (HDL) cholesterol concentration was determined in the supernatant after the precipitation of apolipoprotein B containing particles by adding 20 μl (100 IE) heparin (Braun, Melsungen, Germany) and 20 μl of 2 M manganese chloride solution to 500 μl plasma (Warnick and Albers, 1978). LDL cholesterol concentration was calculated by the formula of Friedewald (Friedewald et al., 1972). Plasma for fibrinogen concentration was stored at -20 °C (for a maximum of 6 weeks) immediately after centrifugation. Fibrinogen concentration in all samples was measured in one assay at the end of the study by immunonephelometry (Laser Nephelometer, Behringwerke, Marburg, Germany) using specific antibodies against human fibrinogen (OSCA 08/09, Behringwerke).

2.4. Subjects and study design

We investigated eight healthy young men, who were free of clinically evident atherosclerosis. Inclusion criteria were fasting concentration of LDL cholesterol < 4.14 mmol l^{-1}, and of triglycerides < 1.82 mmol l^{-1}). One subject had fasting triglycerides of 3.61 mmol l^{-1} and was, therefore, excluded from the study, thus seven men were included in the statistical analysis (age 31.0 ± 1.9 years, body-mass-index 20.9 ± 1.1 kg m^{-2}). Within 7 days before the study day, all inhibitory drugs of platelet aggregation (especially aspirin) were forbidden. Blood was collected after a 12-h overnight fast as described above. A quantity of cream containing 50 g fat per m^2 body surface was then ingested within 5 min. Measurements were again performed 3 h later.

Additionally 11 patients (six women, five men, age 59.7 ± 3.5 years, body-mass-index 26.7 ± 2.3 kg m^{-2}) were investigated before and after performance of a single LDL apheresis. All patients suffered from drug-resistant hypercholesterolemia and from atherosclerotic complications (ten had coronary artery disease, one had peripheral atherosclerotic occlusive disease). Patients were treated regularly with weekly apheresis; six patients with dextran sulfate adsorption (Liposorber, KANEKA, Osaka, Japan), four with immunoadsorption (LDL-Lipopak, Pocard, Moscow, Russia) and one with a plasma filter (600 kDa). Measurements were performed immediately before and after apheresis. Informed consent was obtained from all subjects before they participated in the study.

2.5. Statistical analysis

Statistical analysis was performed with SPSS for Windows 7.5.2G (SPSS GmbH Software, Munich, Germany). The Wilcoxon signed rank test for paired samples was used to compare results (before and after apheresis, before and after fat load and different stimulation procedures). Due to a limited sample number in the fat load study, Pearson correlation coefficients were only calculated in apheresis patients (after Gauss distribution was proven) to determine whether there was an association between the results with different stimulation procedures (two-tailed probability is given). Results are reported as mean $\pm$ S.D., $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Ultrasound stimulation

Taken together all measurements of the study, in each of these 36 measurements ultrasound stimulation resulted in a more pronounced platelet aggregation concerning the parameters LS, HS, and AUC, compared with platelet aggregation parameters without stimulation. In apheresis patients, AUC after ultrasound stimulation was smaller than after ADP (2.1 and 4.2 μM) both before and after apheresis treatment ($P < 0.05$, respectively). However, FDI in these patients was significantly higher after ADP than after ultrasound application, indicating a greater 'looseness' of chemically induced platelet bonds in aggregates compared with mechanically induced platelet bonds. This behavior was also observed in healthy subjects both before and after fat load but significance was not reached in all comparisons ($0.028 < P < 0.176$), probably due to the limited case numbers. A representative measurement of platelet aggregation in a healthy volunteer with a higher FDI after ADP compared with ultrasound is shown in Fig. 2.

Platelet aggregation parameters after ultrasound stimulation and after ADP stimulation were correlated with each other. In apheresis patients AUC after ADP stimulation (2.1 and 4.2 μM) correlated with AUC after ultrasound stimulation before ($r = 0.77$, $P < 0.01$ and $r = 0.50$, $P = 0.12$) and after ($r = 0.58$, $P = 0.06$ and $r = 0.69$, $P < 0.05$) apheresis. Also HS before apheresis ($r = 0.95$, $P < 0.001$ and $r = 0.85$, $P < 0.005$) as well as LS before ($r = 0.75$, $P < 0.01$ and $r = 0.52$, $P = 0{,}10$) and after ($r = 0.60$, $P < 0.05$ and $r = 0.77$, $P < 0.01$) apheresis were associated with each other.

Adding of the glycoprotein IIb/IIIa receptor antagonist tirofiban depressed ultrasound-induced aggregation parameters LS, HS, and AUC in each measurement. However, no statistically significant differences were found concerning LS or AUC when the effect of tirofiban was compared between ADP and ultrasound stimulation. Interestingly, platelet aggregation at high shear was inhibited by tirofiban more effectively after chemical stimulation compared with ultrasound stimulation ($0.008 < P < 0.018$). This was due to a significantly higher FDI after ADP compared with ultrasound stimulation. No correlation was found between ultrasound stimulation and ADP stimulation for AUC, LS, or HS when tirofiban had been added.

3.2. Fat load

After the fat load in young men triglycerides increased from 0.86 ± 0.39 mmol l^{-1} at baseline to 2.10 ± 1.10 mmol l^{-1} at 3 h, total and LDL cholesterol mildly increased from 4.30 ± 0.64 and 2.90 ± 0.44 to 4.54 ± 0.57 and 3.21 ± 0.52 mmol l^{-1} ($P < 0.05$, respectively). Platelet count (229.1 ± 56.0 G l^{-1} at baseline, 239.9 ± 56.0 G l^{-1} at 3 h) and fibrinogen concentration (2.37 ± 0.24 at baseline, 2.40 ± 0.34 g l^{-1} at 3 h) remained unchanged after fat load.

Aggregation parameters before and after the fat load are presented in Table 1. No statistically significant differences in aggregation parameters were seen when ADP (4.2 μM) or ultrasound stimulation were compared before and after the fat load. However, when platelets were stimulated with the lower concentration of ADP (2.1 μM) or when the stimulation with ADP (4.2 μM) was inhibited by tirofiban aggregation parameters LS

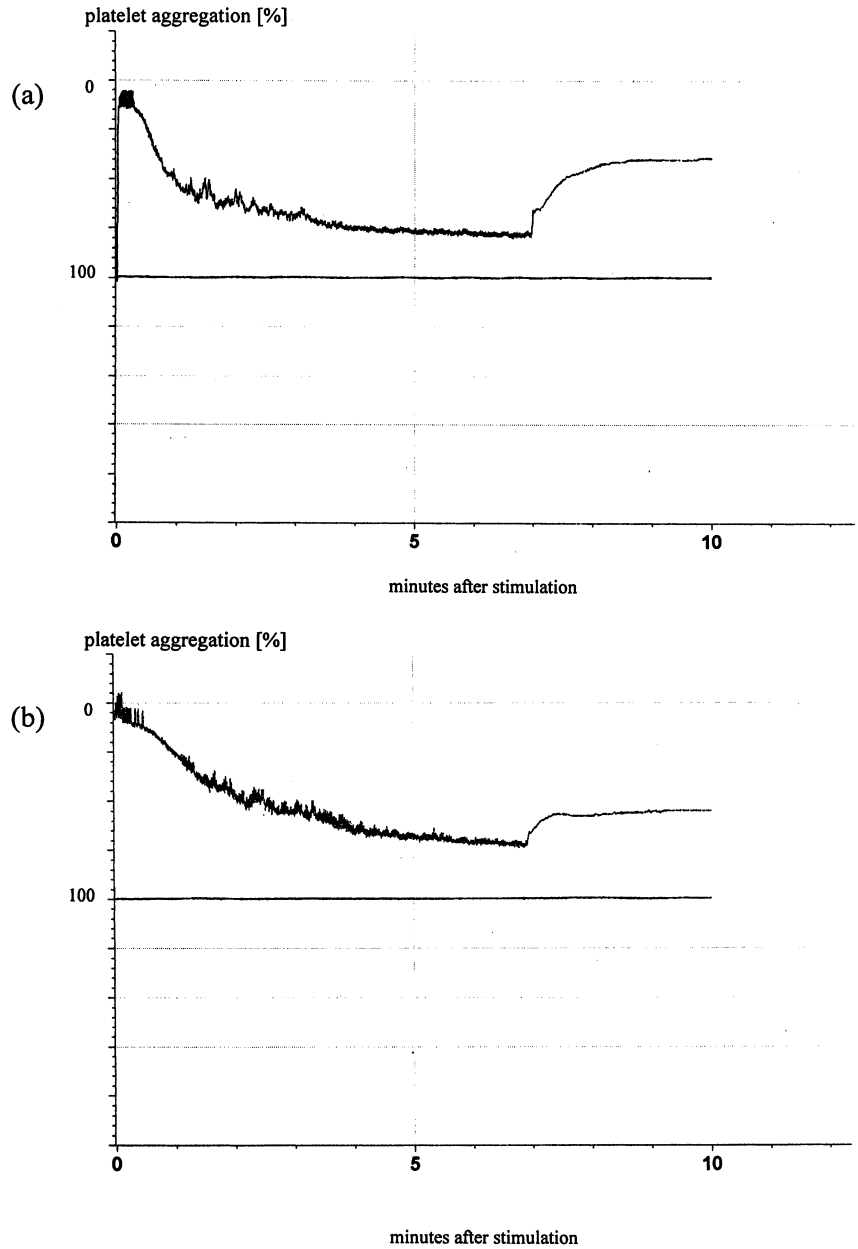

Fig. 2. Graphs for platelet aggregation in a healthy volunteer (7 min low shear, afterwards 3 min high shear). A fall of the graph represents an increasing aggregation, a rise of the graph represents a decreasing aggregation. Note the higher FDI at high shear after ADP stimulation compared with ultrasound application. (a) Platelet aggregation after ADP 4.2 μM (LS 80%, HS 33%, AUC 395% min, FDI 0.59). (b) Platelet aggregation after ultrasound (LS 67%, HS 50%, AUC 305% min, FDI 0.25).

and AUC were significantly lower after fat load than before ($P < 0.05$). Compared with fasting conditions, ultrasound application in the presence of tirofiban resulted in a significantly lower HS and higher FDI after the fat load ($P < 0.05$).

3.3. Apheresis treatment

In patients regularly treated with LDL apheresis, total cholesterol could be reduced from 6.11 ± 1.27 mmol l^{-1} before apheresis to 2.42 ± 0.65 mmol l^{-1} after apheresis procedure, LDL cholesterol from 3.99 ± 0.90 to 1.06 ± 0.55 mmol l^{-1} ($P < 0.005$). Triglyceride concentration (from 2.50 ± 1.36 to 1.25 ± 0.80 mmol l^{-1}) and HDL cholesterol concentration (from 0.98 ± 0.27 to 0.79 ± 0.23 mmol l^{-1}) were reduced significantly as it was fibrinogen concentration from 2.84 ± 0.64 to 2.14 ± 0.59 g l^{-1} by this single apheresis ($P < 0.005$, respectively). Despite these very marked reductions in total and LDL cholesterol after apheresis, no differences in platelet aggregation were observed when pre- and post-treatment values were compared (Table 2). There is one exception; there was a decrease in HS after stimulation with ADP (2.1 μM), associated with a fall in FDI after chemical stimulation.

Table 1
Platelet aggregation parameters after fat load compared with baseline levels (*$P < 0.05$), $n = 7$ (mean ± S.D.)

	Before fat load	After fat load
ADP 2, 1 μM		
Low shear LS (%)	44.9 ± 18.2	24.0 ± 19.9*
High shear HS (%)	25.3 ± 20.7	13.6 ± 15.8
Integral AUC (% min)	218.1 ± 81.6	122.1 ± 102.4*
FDI	0.51 ± 0.32	0.43 ± 0.39
ADP 4.2 μM		
Low shear LS (%)	59.2 ± 5.6	58.3 ± 12.8
High shear HS (%)	36.7 ± 15.5	40.2 ± 20.3
Integral AUC (% min)	315.2 ± 38.0	307.3 ± 74.5
FDI	0.38 ± 0.25	0.34 ± 0.30
ADP 4.2 μM + tirofiban		
Low shear LS (%)	33.9 ± 7.3	19.3 ± 9.6*
High shear HS (%)	2.0 ± 2.0	0.4 ± 1.1
Integral AUC (% min)	163.3 ± 39.0	88.3 ± 36.3*
FDI	0.94 ± 0.07	0.98 ± 0.07
Ultrasound		
Low shear LS (%)	55.0 ± 8.5	50.4 ± 9.2
High shear HS (%)	45.4 ± 7.3	42.6 ± 13.0
Integral AUC (% min)	269.3 ± 33.7	249.6 ± 44.6
FDI	0.17 ± 0.11	0.18 ± 0.16
Ultrasound + tirofiban		
Low shear LS (%)	25.7 ± 11.9	29.7 ± 10.9
High shear HS (%)	24.0 ± 13.3	15.4 ± 8.3*
Integral AUC (% min)	128.4 ± 51.7	110.0 ± 37.5
FDI	0.14 ± 0.19	0.47 ± 0.25*
Spontaneous		
Low shear LS (%)	4.4 ± 2.1	4.9 ± 4.1
High shear HS (%)	2.0 ± 5.3	1.9 ± 2.7
Integral AUC (% min)	19.0 ± 10.6	27.3 ± 15.3
FDI	0.86 ± 0.38	0.55 ± 0.48

FDI = (low shear aggregation value − high shear aggregation value)/low shear aggregation value.

4. Discussion

In the present study platelet aggregation was induced by standardized ultrasound resulting in higher aggregation rates compared with each non-stimulated sample.

In those studies in which platelet aggregation was induced mechanically, mechanical stress was either difficult to standardize (in one investigation, the platelet sample was agitated gently for 40 s (Saniabadi et al., 1997) or the study construction was very complex (Williams et al., 1998). In our study, however, ultrasound was quickly (5 s), easily and reproducibly applied with the new developed construction combined with the stratagems of normal thermal PRP preparation. Within 5 s of ultrasound exposure, slight cavitation was observed in the ultrasound bath and in the sample fluid. This might result in a higher applied energy than calculated as cavitation induces not only a global temperature increase (which can be measured), but may also induce a local temperature increase (which can not be quantified). This local temperature increase might have an additional pro-aggregating effect on platelets.

Platelet aggregation after ultrasound stimulation was correlated with that after ADP stimulation suggesting that there seems to be a certain platelet aggregability in one patient irrespectively

Table 2
Platelet aggregation parameters after apheresis compared with baseline levels before apheresis (*, $P<0.05$), $n=11$ (mean ± S.D.)

	Before apheresis	After apheresis
ADP 2, 1 μM		
Low shear LS (%)	48.8 ± 27.5	47.4 ± 23.8
High shear HS (%)	19.1 ± 25.2	10.2 ± 16.9*
Integral AUC (% min)	242.7 ± 131.6	237.2 ± 133.6
FDI	0.56 ± 0.43	0.81 ± 0.23*
ADP 4.2 μM		
Low shear LS (%)	64.9 ± 26.5	60.0 ± 20.1
High shear HS (%)	27.8 ± 26.7	27.0 ± 22.2
Integral AUC (% min)	315.2 ± 116.6	316.4 ± 107.8
FDI	0.57 ± 0.37	0.62 ± 0.29
ADP 4.2 μM + tirofiban		
Low shear LS (%)	28.0 ± 18.1	24.3 ± 19.2
High shear HS (%)	4.4 ± 6.6	5.3 ± 6.0
Integral AUC (% min)	136.6 ± 99.6	110.9 ± 93.9
FDI	0.83 ± 0.23	0.72 ± 0.38
Ultrasound		
Low shear LS (%)	33.6 ± 19.1	35.1 ± 16.4
High shear HS (%)	25.7 ± 16.6	27.4 ± 22.4
Integral AUC (% min)	171.0 ± 99.4	168.2 ± 85.7
FDI	0.26 ± 0.22	0.33 ± 0.35
Ultrasound + tirofiban		
Low shear LS (%)	19.8 ± 15.3	13.3 ± 7.4
High shear HS (%)	18.4 ± 11.2	15.4 ± 8.7
Integral AUC (% min)	99.6 ± 61.6	62.9 ± 24.9
FDI	0.19 ± 0.29	0.16 ± 0.26
Spontaneous		
Low shear LS (%)	9.4 ± 7.5	8.2 ± 3.9
High shear HS (%)	4.3 ± 6.4	5.8 ± 5.7
Integral AUC (% min)	47.6 ± 41.7	35.8 ± 13.2
FDI	0.69 ± 0.38	0.44 ± 0.46

FDI = (low shear aggregation value − high shear aggregation value)/low shear aggregation value.

from the stimulation method. This confirms early studies from Chater and Williams where the platelet sensitivity to ultrasound was suggested to be related to that to ADP, although no statistical correlation was found (Chater and Williams, 1977). One possible explanation for this close association might be the hypothesis that ultrasound-induced platelet lysis (indicated by an increase in LDH levels) liberated the same pro-aggregatory mediators from platelets as ADP stimulation did (e.g. thromboglobulin). It was shown recently that not only ADP-induced or thrombin-induced platelet aggregation but also ultrasound-induced platelet aggregation is calcium-dependent (Samal et al., 2000). Thus, the intracellular calcium concentration increased markedly after ultrasound stimulation probably due to an increased influx of calcium ions through membranal calcium channels (Samal et al., 2000).

When normal thermal preparation was strictly enforced, ultrasound induced a smaller aggregation compared with ADP in our study, but one of the principal findings was that the ultrasound-induced aggregates were more stable when exposed to high shear stress compared with ADP-induced aggregates, since fractional desaggregation indices were consistently smaller in ultrasound-induced aggregates compared with ADP-induced aggregates. This phenomenon was observed with and without the glycoprotein IIb/IIIa receptor antagonist tirofiban. The reason for this observation remains to be clarified; there is evidence that it might be related to intraaggregate fibrin formation (Blasberg et al., 1981). However, it might have a significant impact for the patient; it might be speculated that glycoprotein IIb/IIIa receptor antagonists are less effective in inhibiting platelet aggregation in the normal circulation than was suggested from in vitro data of ADP-induced platelet aggregation.

Both platelets and lipoproteins are known to be involved in the process of atheromatosis (Ross, 1993). Platelet function itself was shown to be influenced by lipoproteins, although conflicting results concerning the effects of lipids have been published.

During postprandial lipemia, platelet aggregation was reported to be enhanced (Fuhrmann et al., 1986; Belch et al., 1987), to be unchanged (Jakubowski et al., 1985; Bröijersén et al., 1998), or to be reduced (Johnston et al., 1979; Nimpf et al., 1989). And also when the effects of postprandial lipoproteins on platelet aggregation were in-

vestigated in vitro, some authors found an enhancement of aggregation (Saniabadi et al., 1997; Knöfler et al., 1995; Mochizuki et al., 1996) while others reported an inhibition of aggregation (Orth et al., 1995) when postprandial lipoproteins (chylomicrons, chylomicron remnants, or VLDL remnants) were added. If the techniques used to induce platelet aggregation are not standardized, contradictory results are not surprising; the high interindividual variation in platelet function might be another explanation. In addition, another possibility might be the fact, that platelet activation was induced by only one agonist in some studies, making it impossible to compare different studies.

In the light of this situation, we used two different stimulation procedures, which resulted in slight, but significantly different aggregation rates between the chemical and the physical stimulation procedures. When platelets were markedly stimulated (ADP 4.2 μM or ultrasound) no changes were seen in platelet aggregation parameters during postprandial lipemia compared with baseline. However, the use of weaker stimuli (ADP 2.1 μM) or inhibition of marked stimuli (tirofiban/ADP 4.2 μM or tirofiban/ultrasound) did reveal a reduced formation of platelet aggregates in the postprandial state. These results favor the hypothesis that postprandial changes can only be observed when a moderate stimulus intensity is applied, which can only be investigated when different stimuli are compared in one standardized study.

Cholesterol-rich lipoproteins LDL and HDL are well known to have effects on platelet function. HDL particles were shown to enhance the Na^+/H^+ antiport resulting in reduced aggregability of platelets (Nofer et al., 1996), on the other hand LDL particles have inhibitory effects on the function of this antiport (Nofer et al., 1997). Generally, in patients with type II hyperlipoproteinemia platelet aggregation is enhanced (Carvalho et al., 1974). But while the elimination of LDL particles by a single apheresis revealed no effect on platelet aggregation (Bröijersén et al., 1994), patients had a slight, non-significant reduction in platelet aggregation 2 weeks after initiating weekly apheresis, which became significant after 24 weeks of regularly administered apheresis treatment (Sinzinger et al., 1996). Therefore, the long-term effects of apheresis seem to be more pronounced than the effects of a single apheresis, which might explain that only minimal changes were observed in regularly treated patients after one apheresis compared with before apheresis values in our study. This is most likely caused by effects already operational at the level of thrombocytopoesis.

In conclusion, we found the application of ultrasound in a standardized set-up easy to perform. Ultrasound-induced platelet aggregation was well correlated with ADP-induced platelet aggregation suggesting that the pro-aggregatory effects of these stimulation procedures are mediated by similar pathways. The main difference between ultrasound and ADP induction, however, were the more stable aggregates when exposed to high shear after ultrasound stimulation compared with ADP stimulation, which was observed either with or without the glycoprotein IIb/IIIa inhibitor tirofiban.

Since ultrasound stimulation of platelets is easy to perform and mechanical stimulation might better reflect physicochemical conditions than chemical stimulation does, ultrasound stimulation of platelets should be considered to be a routine measurement.

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