Pharmacodynamic characterization of the interaction between abciximab or tirofiban with unfractionated or a low molecular weight heparin in healthy subjects

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Aims The objective of our study was to define the interaction between either unfractionated heparin (UFH) or a low molecular weight heparin, reviparin (REV), and the pharmacodynamic profile of the GPIIb/IIIa-antagonists abciximab (ABC) or tirofiban (T).

Methods Two studies each containing 18 healthy subjects were performed, and all were pretreated with aspirin (ASA) for 3 days. Volunteers then received UFH (5000 IU bolus/infusion $7 \text{ IU kg}^{-1} \text{ h}^{-1}$ for 7 h, $n=6$), REV (4200-anti-Xa-IU s.c., $n=6$) or placebo ($n=6$). One hour later, ABC (study I) or T (study II) were given by i.v. infusion for 6 h. The pharmacodynamic effects measured were bleeding time (BT), fibrinogen-binding at the GPIIb/IIIa-receptor (FIB), expression of the platelet secretion marker CD62, and ADP ($20 \mu\text{M}$)- and collagen ($5 \mu\text{g ml}^{-1}$)-induced platelet aggregation.

Results After treatment with both GPIIb/IIIa-antagonists, prolongation of BT occurred to a similar magnitude (approximately 25–30 min) and was not affected by UFH or REV-comedication. ABC or T with ASA alone resulted in nearly the same magnitude of reduction in FIB and platelet aggregation. After coadministration with UFH, FIB was significantly higher (thus less inhibited) than after T + ASA alone ($19 \pm 16\%$ vs $55 \pm 36\%$) or ABC + ASA alone ($8 \pm 9\%$ vs $32 \pm 11\%$). This attenuation of FIB was not seen with REV. Inhibition of ADP- and collagen-induced aggregation tended to be attenuated by treatment with UFH (e.g. ADP-induced aggregation at 0.25 h after ABC + ASA alone = $13 \pm 4\%$; after coadministration with UFH = $40 \pm 26\%$). No such changes were noted with REV. Minor reductions in CD62-expression were seen in subjects given ABC or T alone, but expression was not affected by UFH or REV.

Conclusions Co-medication with UFH attenuated platelet inhibition during treatment with GPIIb/IIIa-antagonists, but these effects were not seen with the low molecular weight heparin reviparin. The results show that administration of reviparin together with abciximab or tirofiban did not adversely affect the pharmacodynamic profile of these GPIIb/IIIa-antagonists.

Keywords: aspirin, GPIIb/IIIa-antagonists, heparin, interaction

Introduction

The clinical efficacy of the GPIIb/IIIa receptor antagonists abciximab, tirofiban and eptifibatide in combination with unfractionated heparin (UFH) and aspirin (ASA) has been proven [1–5]. Although heparin seems to be a pivotal component of this antithrombotic regimen, it also

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contributes to its adverse effect profile, i.e. bleeding and thrombocytopenia. Furthermore, UFH might provoke platelet activation *in vitro* and *in vivo* [6–9], which may counteract the antiaggregatory effects of antiplatelet drugs. On the other hand, it has been shown for several GPIIb/IIIa-antagonist that owing to their potent inhibitory effects on platelets, formation of thrombin on the platelet membrane is considerably decreased [10–13], thus possibly reinforcing the anticoagulant effect of heparins.

Low molecular weight heparins (LMWH), given subcutaneously [14] or intravenously [15, 16] have gained an increasing role in the therapy of acute coronary syndromes. However, use of LMWHs together with a GPIIb/IIIa antagonist is not established in these conditions [14, 17, 18]. The first clinical report [19] in patients with unstable angina indicated that the treatment with the LMWH enoxaparin is as safe as UFH and did not affect the antiaggregatory potency of tirofiban. However, a comprehensive and controlled pharmacodynamic comparison of platelet aggregation and activation between a LMWH and UFH in subjects taking different classes of GPIIb/IIIa antagonists has not been reported. Accordingly, we compared the effects of the LMWH reviparin and conventional UFH-therapy given together with abciximab (a monoclonal antibody to the GPIIb/IIIa-receptor) or tirofiban (a nonpeptide analogue of the RGD-sequence on the GPIIb/IIIa-receptor) on underlying aspirin medication. A further comparison was made between these data and those from subjects taking the GPIIb/IIIa-antagonists with aspirin alone. Reviparin has a mean molecular weight of 3.900 Da and shows a ratio of anti-Xa/anti-IIa activity >3.6 [20]. The pharmacological profile of reviparin resembles that of enoxaparin [21]. Doses established for clinical indications (DVT prophylaxis, unstable angina) are between 4200 and 6300 U day⁻¹ i.v. or s.c. [16, 22].

Methods

The study was conducted in a randomized and placebo controlled, parallel group design. Laboratory personnel evaluating the pharmacodynamic parameters were blinded to the treatment allocation. After approval by the local Institutional Review Board and obtaining informed consent, 36 healthy male volunteers (age 18–40 years, weight 52–98 kg) were recruited. None of the volunteers had taken drugs or medications during the last 14 days before the study. Subjects were randomized to two sub-studies (I, II), each consisting of three treatment groups: (I) abciximab + ASA ($n=6$) or abciximab + ASA + UFH ($n=6$) or abciximab + ASA + reviparin, and (II) tirofiban + ASA ($n=6$) or tirofiban + ASA + UFH ($n=6$) or tirofiban + ASA + reviparin (Figure 1). All subjects received ASA (Bayer AG, Germany) at a dose of 300 mg on day 1

and 100 mg on day 2 and 3. On day 3, immediately after ASA intake, subjects in treatment group 3 received UFH at a dose of 5000 IU bolus, followed by a weight-adjusted ($7 \text{ IU kg}^{-1} \text{ h}^{-1}$) infusion over 7 h. Subjects in treatment group 3 received a single s.c. injection of 4200 anti-Xa IU reviparin (Knoll AG, Germany). One hour after ASA intake on day 3, abciximab (Lilly GmbH, Germany) was given as a bolus of 0.25 mg kg^{-1} , followed by an i.v. infusion of $0.125 \text{ µg kg}^{-1} \text{ min}^{-1}$ over 6 h (substudy I), and tirofiban (MSD, Germany) was given as an accelerated i.v. infusion of $0.4 \text{ µg kg}^{-1} \text{ min}^{-1}$ over 30 min followed by an i.v. infusion of $0.1 \text{ µg kg}^{-1} \text{ min}^{-1}$ over 5.5 h (substudy II). A summary of these dosage schedules and of the pharmacodynamic measurements is shown in Figure 1.

Bleeding time

For the measurement of bleeding times a sphygmomanometer cuff was inflated to 40 mmHg in the upper arm. A standardized horizontal laceration was created on the volar side of the forearm by using Simplate devices (Organon Teknika, Germany). Blood was removed from the cut with filter paper after 15 s and then at 15 s intervals until the bleeding had stopped (normal range 4–10 min).

Flow cytometric measurement of fibrinogen binding and expression of CD62

For assessment of fibrinogen binding to the GPIIb/IIIa-receptor and expression of the activation marker CD62 (P-selectin) on the platelet surface, blood samples were collected into sodium citrate (3.18%) tubes. For platelet activation, thrombin-receptor activating peptide (TRAP) was added at a final concentration of 10 µM . The antibodies (Ab) used were FITC-conjugated antihuman-fibrinogen chicken Ab (FITC-antih fibrinogen ex-chicken, WAK-Chemie, Germany), anti-CD62 Ab (CD62-FITC, IgG1 mouse, Cymbus Biotechnology, UK), and PE-conjugated anti-CD42b Ab (CD42b-PE, IgG1-mouse, Coulter-Immunotech, France). Flowcytometric measurement was carried out using a FACScanTM flowcytometer (Becton Dickinson, Germany). To establish resolution for measurement of platelet events only, the binding of PE-labelled platelet specific anti-CD42b was used to set a gate. The difference in fluorescence intensity at day 3, 1 h after aspirin intake (i.e. immediately before administration of the GPIIb/IIIa-antagonists) was set to 100% and the results were related to this maximal value and expressed as a percentage [23].

ADP- and collagen-induced platelet platelet aggregation

Citrated blood samples were centrifuged immediately for 10 min at 400 g at room temperature and the platelet

Figure 1 Schedule of dosing events and pharmacodynamic measurements. ABC = abciximab, T = tirofiban, ASA = acetylsalicylic acid, UFH = unfractionated heparin, REV = reviparin.

rich plasma (PRP) was removed. Platelet aggregation was assessed in PRP by a turbidimetric light-transmittance device (APACT aggregometer, Germany) within 1 h after collection. Inducers were ADP (DADE-Behring, Germany) at 20 µM final concentration and collagen at 5 µg ml⁻¹ final concentration. The aggregation response is reported as a percentage of maximal light transmission $A_{\max}$.

Anti-Xa activity, aPTT

Citrated blood was centrifuged within 20 min after sampling for 10 min at 2000 g at room temperature. Supernatant plasma was separated and immediately frozen

at -70°C until further analysis. Anti-Xa activity was determined using Coatest Heparin (Coatest, Germany). The results are expressed in anti-Xa units. Activated partial thromboplastin time (aPTT) was determined by an aPTT Ellagic Acid Device (Labour GmbH, Germany).

Statistical evaluation

The primary aim of this exploratory study was to characterize the influence of either UFH or reviparin on GPIIb/IIIa-inhibition in subjects on underlying (and clinically obligatory) ASA medication. Therefore the control group was treated with the GPIIb/IIIa-antagonist + ASA alone (treatment group 1). Bleeding time and

abciximab and tirofiban-related pharmacodynamic parameters (fibrinogen binding, CD62-expression, platelet aggregation) were subjected to ANOVA with factors for group and time. For each time point, a comparison was then made between treatment groups (1 *vs* 2, 1 *vs* 3, 2 *vs* 3) of each substudy using the *U*-test (Wilcoxon-Mann-Whitney). The method of Benferroni and Holms has been used to adjust for the multiple comparisons. For significant results, the 95% confidence intervals according to Moses and the Hodges-Lehmann point estimator were obtained. All statistical tests were performed using BIAS® for Windows (Version 7.05, epsilon Verlag Darmstadt, Germany).

Results

The study medication was tolerated well in all subjects and no side-effects (particularly bleeding or thrombocytopenia) occurred throughout the study.

Bleeding time

Bleeding time observed in the different treatment groups during treatment with ASA alone (day 3, 0 h) averaged between 7 and 11 min. Treatment with UFH and ASA led to a prolongation of bleeding time from approximately 9 min to between 16 and 18 min (Table 1). Treatment with either GPIIb/IIIa-antagonist alone prolonged bleeding time to 27 ± 10 min (abciximab) and 19 ± 13 min (tirofiban), respectively. In subjects treated with either GPIIb/IIIa-antagonist and UFH or reviparin, bleeding times at 7 h were not different from subjects taking the GPIIb/IIIa-antagonist alone, although in the tirofiban substudy a further, albeit nonsignificant prolongation of the bleeding time to approximately 28 min was observed with UFH as well as with reviparin. At 24 h, bleeding time was still prolonged (> 10 min) in the abciximab-substudy

Table 1 Bleeding time (min) observed in subjects treated with abciximab (ABC) or tirofiban (T). ASA was given from day 1 to day 3, UFH (bolus + infusion) was given from day 3, 0 h to 7 h, reviparin (REV) was given s.c. on day 3, 0 h, GPIIb/IIIa-inhibitors were given from on day 3 from 1 h to 7 h. Values are given as mean (s.d.).

Treatment	n	Bleeding time (min)			
		day 3, 0 h	1 h	7 h*	24 h
ABC + ASA	6	11 (6)	8 (3)	27 (10)	17 (8)
ABC + ASA + UFH	6	9 (3)	18 (15)	22 (9)	13 (3)
ABC + ASA + REV	6	7 (1)	11 (2)	34 (17)	12 (4)
T + ASA	6	9 (2)	10 (2)	19 (13)	6 (1)
T + ASA + UFH	6	8 (4)	16 (10)	28 (13)	7 (3)
T + ASA + REV	6	7 (1)	7 (1)	29 (13)	6 (1)

*Measurement started at 6 h.

within all treatment groups, but returned to pretreatment values in the tirofiban-substudy (Table 1).

Fibrinogen binding and expression of CD62

Abciximab and tirofiban given with ASA alone resulted in nearly the same magnitude of inhibition of fibrinogen binding, although a wide variation was noted (Table 2). The reduction in fibrinogen binding by tirofiban at 7 h was less pronounced ($46 \pm 28\%$) than seen with abciximab ($16 \pm 18\%$), and binding returned to normal 17 h after termination of treatment. Compared with the GPIIb/IIIa-antagonist + ASA treatment alone, coadministration of UFH attenuated fibrinogen binding, which was significantly less at 1.5 h in the tirofiban-treated volunteers ($55 \pm 36\%$) and at 4 h in the abciximab group ($32 \pm 11\%$). This attenuation of platelet fibrinogen binding was not seen with reviparin (Figure 2). However, when both heparins were compared directly (group 2 *vs* 3), no significant difference could be detected. Expression of the platelet activation marker CD62 is reported in Table 2. Briefly, we could not ascertain significant changes by or even differences between the therapy regimen on CD62-expression.

Platelet aggregation

ADP ($20 \mu\text{M}$) induced aggregation was inhibited to less than 20% of the pretreatment values immediately after starting abciximab therapy and remained reduced to approximately 35% over the remaining infusion period (Table 3). Collagen ($5 \mu\text{g ml}^{-1}$)-induced aggregation was reduced to less than 20% over the whole infusion period. After UFH coadministration, the inhibitory effects of abciximab on platelet aggregation seemed to be blunted, and at various timepoints during the infusion, inhibition of aggregation was significantly less than observed in subjects treated with abciximab and ASA alone. No such difference was seen with reviparin (Table 3, Figure 2). Tirofiban, given with ASA alone, reduced aggregation induced by $20 \mu\text{M}$ ADP or $5 \mu\text{g ml}^{-1}$ collagen to approximately 20–30% over the whole infusion period, with a rapid recovery of aggregation after termination of the infusion. UFH but also reviparin seems to attenuate the inhibitory effects of tirofiban on platelet aggregation (Figure 3, Table 3), but differences between treatment group 1 and 2 as well as between 1 and 3 seen during the tirofiban infusion did not reach statistical significance.

Activated thromboplastin time (aPTT) and anti-Xa-activity

No effect on aPTT or anti-Xa-activity was seen after treatment with abciximab or tirofiban alone (Table 4). After the bolus of UFH and immediately before dosing

Table 2 Fibrinogen binding and CD62-expression observed in subjects treated with abciximab (ABC) or tirofiban (T). ASA was given from day 1 to day 3, UFH (bolus + infusion) was given from day 3, 0 h to 7 h, reviparin (REV) was given s.c. on day 3, 0 h, GPIIb/IIIa-inhibitors were given from on day 3 from 1 h to 7 h. Values are given as mean (s.d.). Significant differences are indicated and 95% confidence interval (CI) and point estimator (PE) are given.

Treatment	n	day 3, 1 h	Fibrinogen binding (%)			
			1.25 h*	4 h	7 h*	24 h
ABC + ASA	6	100	1 (1)	8 (9)	16 (18)	48 (27)
ABC + ASA + UFH	6	100	5 (9)	32 (11) ⁺	33 (17)	54 (6)
ABC + ASA + REV	6	100	12 (9)	23 (10)	22 (9)	50 (21)
T + ASA	6	100	19 (16)	30 (26)	46 (28)	117 (16)
T + ASA + UFH	6	100	55 (36) [#]	54 (26)	50 (34)	85 (21)
T + ASA + REV	6	100	25 (30)	49 (22)	54 (18)	105 (20)

⁺ $P < 0.01$ treatment 1 vs 2 (95% CI -36; -13, PE -13), [#] $P < 0.01$ treatment 1 vs 2 (95% CI -65; -6, PE -45).

Treatment	n	day 3, 1 h	CD62-expression (%)			
			1.25 h*	4 h	7 h*	24 h
ABC + ASA	6	100	80 (9)	80 (13)	89 (6)	81 (7)
ABC + ASA + UFH	6	100	81 (23)	88 (15)	88 (11)	87 (12)
ABC + ASA + REV	6	100	87 (12)	84 (13)	84 (19)	76 (17)
T + ASA	6	100	72 (49)	55 (34)	79 (39)	53 (32)
T + ASA + UFH	6	100	86 (39)	98 (38)	110 (34)	89 (7)
T + ASA + REV	6	100	71 (15)	71 (23)	88 (10)	91 (3)

*In subjects treated with tirofiban, measurement time was 1.5 h (end of accelerated infusion).

Table 3 Aggregation response observed in subjects treated with abciximab (ABC) or tirofiban (T). ASA was given from day 1 to day 3, UFH (bolus + infusion) was given from day 3, 0 h to 7 h, reviparin (REV) was given s.c. on day 3, 0 h, GPIIb/IIIa-inhibitors were given from on day 3 from 1 h to 7 h. Values are given as mean (s.d.). Significant differences are indicated and 95% confidence interval (CI) and point estimator (PE) are given.

Treatment	n	day 3, 0 h	1 h	Aggregation to ADP $20 \mu\text{M ml}^{-1}$ (% A_{max})				
				1.25 h*	2 h	4 h	7 h*	24 h
ABC + ASA	6	82 (4)	83 (5)	13 (4)	31 (16)	32 (12)	41 (13)	67 (15)
ABC + ASA + UFH	6	78 (5)	85 (7)	40 (26) ⁺	53 (24)	55 (30) [#]	50 (24)	71 (14)
ABC + ASA + REV	6	82 (3)	81 (6)	25 (16)	42 (12)	41 (18)	50 (11)	74 (7)
T + ASA	6	85 (6)	83 (10)	14 (13)	26 (22)	22 (23)	27 (24)	76 (13)
T + ASA + UFH	6	76 (9)	82 (9)	25 (13)	46 (17)	33 (15)	40 (17)	74 (7)
T + ASA + REV	6	82 (9)	86 (8)	18 (16)	31 (21)	27 (24)	38 (25)	81 (7)

⁺ $P < 0.01$ treatment 1 vs 2 (95% CI -60; -3, PE -30), [#] $P < 0.01$ treatment 1 vs 2 (95% CI -54; -4, PE -21).

Treatment	n	day 3, 0 h	1 h	Aggregation to collagen $5 \mu\text{g ml}^{-1}$ (% A_{max})				
				1.25 h*	2 h	4 h	7 h*	24 h
ABC + ASA	6	82 (3)	73 (9)	6 (3)	9 (7)	14 (6)	18 (10)	63 (11)
ABC + ASA + UFH	6	79 (6)	84 (8)	22 (22)	37 (29)	36 (28) ⁺	40 (31)	66 (17)
ABC + ASA + REV	6	77 (11)	70 (9)	12 (11)	22 (14)	20 (7)	29 (17)	62 (14)
T + ASA	6	72 (13)	74 (11)	16 (5)	23 (16)	19 (11)	22 (18)	70 (11)
T + ASA + UFH	6	72 (12)	76 (8)	24 (16)	36 (21)	28 (17)	34 (21)	73 (10)
T + ASA + REV	6	79 (8)	80 (6)	18 (12)	30 (20)	29 (19)	35 (19)	74 (8)

⁺ $P < 0.01$ treatment 1 vs 2 (95% CI -58; -3, PE -19), *In subjects treated with tirofiban, measurement time was 1.5 h (end of accelerated infusion).

Figure 2 a) Fibrinogen-binding (mean values $\pm$ s.e. mean, $n=6$) by platelets from subjects taking abciximab, given alone (●), with UFH (◆) or with the LMWH reviparin (■). b) Fibrinogen-binding by platelets in subjects taking tirofiban, given alone (●), with UFH (◆) or with the LMWH reviparin (■). All subjects were pretreated with ASA for 3 days before administration of UFH or reviparin. ★ $P<0.01$ from corresponding value of the control treatment (abciximab or tirofiban + ASA alone).

with the GPIIb/IIIa-antagonists, aPTT values were prolonged to more than 250 s, and decreased to about 50 s 4 h after the UFH-infusion. In both substudies, reviparin caused a slight but significant prolongation of aPTT to approximately 42 s at 4 h. Anti-Xa activity 4 h after reviparin injection averaged 0.25 IE ml⁻¹ in both substudies, and at the same time point after the UFH-infusion anti-Xa activity was 0.11 IE ml⁻¹ (subjects treated with abciximab) and 0.22 IE ml⁻¹ (subjects treated with tirofiban), respectively.

Figure 3 a) Aggregation response (mean values $\pm$ s.e. mean, $n=6$) to 20 μ M ADP in subjects taking abciximab, given alone (●), with UFH (◆) or with the LMWH reviparin (■). b) Aggregation response to 20 μ M ADP in subjects taking abciximab, given alone (●), with UFH (◆) or with the LMWH reviparin (■). All subjects were pretreated with ASA for 3 days before administration of UFH or reviparin. ★ $P<0.01$ from corresponding value of the control treatment (abciximab or tirofiban + ASA alone).

Discussion

The key findings of this study were (1) following treatment with both GPIIb/IIIa-antagonists, prolongation of bleeding time occurred to the same degree and was not further enhanced by UFH or reviparin, and (2) inhibition of fibrinogen binding and platelet aggregation induced by the GPIIb/IIIa-antagonists abciximab and tirofiban was attenuated after coadministration of UFH. Expression of CD62, a marker of platelet secretory function was not affected by any treatment. Furthermore our results suggest

Table 4 Coagulation parameters observed in subjects treated with abciximab (ABC) or tirofiban (T). ASA was given from day 1 to day 3, UFH (bolus + infusion) was given from day 3, 0 h to 7 h, reviparin (REV) was given s.c. on day 3, 0 h, GPIIb/IIIa-inhibitors were given from on day 3 from 1 h to 7 h. Values are given as mean (s.d.).

Treatment	n	day 3, 0 h	Activated partial thromboplastin time aPTT (s)			
			1 h	4 h	7 h	24 h
ABC + ASA	6	33 (3)	34 (2)	33 (3)	32 (3)	32 (4)
ABC + ASA + UFH	6	32 (4)	251 (52)	51 (7)	35 (4)	31 (3)
ABC + ASA + REV	6	34 (6)	38 (7)	42 (7)	34 (10)	33 (5)
T + ASA	6	32 (4)	34 (3)	33 (2)	34 (2)	32 (3)
T + ASA + UFH	6	35 (3)	268 (50)	53 (14)	55 (33)	33 (3)
T + ASA + REV	6	33 (4)	36 (5)	41 (5)	38 (5)	31 (3)

Treatment	n	day 3, 0 h	Anti-Xa-activity (IE ml ⁻¹)			
			1 h	4 h	7 h	24 h
ABC + ASA	6	<0.04	<0.04	<0.04	<0.04	<0.04
ABC + ASA + UFH	6	<0.04	0.50 (0.24)	0.11 (0.02)	0.07 (0.03)	<0.04
ABC + ASA + REV	6	<0.04	0.11 (0.06)	0.24 (0.08)	0.12 (0.05)	<0.04
T + ASA	6	<0.04	<0.04	<0.04	<0.04	<0.04
T + ASA + UFH	6	<0.04	0.73 (0.08)	0.22 (0.14)	0.13 (0.14)	<0.04
T + ASA + REV	6	<0.04	0.08 (0.02)	0.25 (0.03)	0.13 (0.01)	<0.04

that the LMWH reviparin does not affect the pharmacodynamic profile of the GPIIb/IIIa antagonists abciximab and tirofiban. However, and perhaps because of the relatively small study population, no differences were detected between the UFH and LMWH-treated subjects, and a larger trial with greater restrictions on time frame and target parameters might confirm these findings.

The issue of safety with respect to combinations of LMWHs and GPIIb/IIIa-antagonists and the clinical relevance of data characterizing their interaction has been previously emphasized [24]. This is the first study which provides a direct comparison between different GPIIb/IIIa-antagonists and UFH and LMWH and which is devoid of possible confounders (e.g. invasive procedures, contrast media application during acute events, presence of other drugs interfering with haemostatic or platelet function). The study was carried out in healthy subjects, but all volunteers followed a clinically relevant dosing regimen. It should be noted that LMWH are effective in acute coronary events after both subcutaneous injections used in the present study [14] and intravenous injection [15, 16]. The biological profile of 4200 IU reviparin s.c. was found to be similar to that of 40 mg enoxaparin s.c. [21], and the extent of anti-Xa-activity seen 4 h after a single dose of reviparin (approximately 0.25 IE ml⁻¹) is reasonably comparable with that after s.c. enoxaparin (0.24–0.69 IE ml⁻¹) [25] in a dose regimen which has been used in the ESSENCE trial [14].

Data on the effects of tirofiban and either s.c. enoxaparin or i.v. UFH in coronary patients have been

published [19], but assessments of platelet function were confined to bleeding time and platelet aggregation induced by 5 µM ADP only. In that study, a lesser degree of platelet inhibition was demonstrated with UFH than with the LMWH enoxaparin, and bleeding time was prolonged even longer with UFH (>30 min) than with enoxaparin (21 min). In our study, inhibition of fibrinogen binding and aggregation caused by the two GPIIb/IIIa-antagonists coadministered with UFH was less than that without UFH. The attenuating effect on platelet inhibition was not seen with the LMWH reviparin. It should be noted, however, that the aim of this study was the comparison of two concurrent dosing schemes, the commonly used i.v. UFH-regimen *vs* a s.c. regimen with the LMWH reviparin without a preceding bolus, as for example used for enoxaparin in the ESSENCE-trial. Clearly, the anti-coagulant profile of i.v. UFH differs from s.c. LMWH. In our study the combination of UFH and aspirin led to a prolongation of bleeding time to approximately 20 min even before administration of the GPIIb/IIIa-antagonists. Reviparin exhibits its peak anti-Xa-activity at 4 h. At this time, subjects being given UFH had less anti-Xa-activity than those who had reviparin, and their aPTT was prolonged to approximately two-fold, thereby being in the lower therapeutic range of UFH.

The mechanism and clinical relevance of the possible platelet activating effects of UFH have yet to be elucidated. Experimental studies with different sources and concentrations of heparin and different agonists have documented either platelet activation [6, 26, 27] or platelet

inhibition [28, 29]. In patients with unstable angina before and during treatment with UFH and enoxaparin, UFH increased the percentage of activated platelets, and platelets were hyperresponsive to stimulation with ADP and TRAP [6]. Furthermore, addition of UFH to blood from volunteers also resulted in activation of the GPIIb/IIIa receptors, increased CD62 expression and enhanced aggregation [6]. In contrast, enoxaparin had only minor effects on platelet activation *in vivo* and *ex vivo* in that study. Our findings are supported further by recent work from Mascelli *et al.* [29], confirming that therapeutic heparin concentrations augment platelet inhibition by abciximab *in vitro*. Considering the relatively small changes in the pharmacodynamics of GPIIb/IIIa-antagonists coadministered with UFH, the clinical relevance of these findings needs to be considered. The attenuation of fibrinogen binding seen in our study in subjects given UFH was reflected by smaller antiaggregatory effects. Based on results of the recently reported GOLD study [30], inhibition of receptor binding more than 70% is suggested as the lower limit of the therapeutic range for GPIIb/IIIa-antagonists, and values after UFH coadministration below this target were seen more frequently (24 of 36 measurements) than after treatment with the GPIIb/IIIa-antagonists alone (9 of 36) or with reviparin (12 of 36).

Platelet granular secretion and release of α -granule content like growth factors is associated with the appearance of CD62 (P-selectin), which acts as ligand to leucocytes, thereby initiating inflammatory responses [31]. CD62 expression is enhanced in patients with acute coronary syndromes [32] and has been described as a predictor of restenosis after PTCA [33]. In our study, CD62 expression was little reduced by any of the treatments and no differences could be observed between the different treatment groups. Furthermore, there are recent reports on a possible dissociation of antiaggregatory and antisecretory activity of GPIIb/IIIa-antagonists [34–36]. We showed recently, at concentrations of abciximab that inhibited 80–95% of platelet aggregation *in vitro*, only minor effects on CD62 expression and ATP-secretion [37].

In conclusion, the results of our study demonstrate that UFH attenuated platelet aggregation and fibrinogen binding induced by the GPIIb/IIIa antagonists abciximab and tirofiban, whereas the LMWH reviparin does not affect their pharmacodynamic profile. However, it remains to be elucidated whether these findings which might indicate an advantage of reviparin over UFH are reflected by an equivalent or better clinical outcome, as suggested by initial reports [19].

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