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EXPERIMENTAL STUDIES

Reduced Acute Thrombus Formation Results in Decreased Neointimal Proliferation After Coronary Angioplasty

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Objectives. **We tested the hypothesis that reduced acute platelet deposition after angioplasty results in reduced late neointimal proliferation.**

Background. **Platelet-mediated mechanisms contribute to smooth muscle cell proliferation and migration.**

Methods. **Indium-111–labeled platelets were injected 16 h before coronary stent angioplasty in 10 Göttinger minipigs: *group 1* (n = 5) = heparin (100-U/kg bolus) before angioplasty; *group 2* (n = 5) = recombinant hirudin (CGP 39393, 1.0-mg/kg body weight bolus intravenously), followed by subcutaneous doses of 6 to 10 mg/kg every 8 h. Furthermore, stent angioplasty was performed in coronary arteries of 16 minipigs: *group 3* (n = 5, nine stents) = 100 U/kg heparin only; *group 4* (n = 5, 10 stents) = 1-mg/kg bolus hirudin before and 45 min after angioplasty; *group 5* (n = 6, 11 stents) = hirudin (1-mg/kg intravenous bolus) before and 45 min after angioplasty, followed by 6 to 10 mg/kg subcutaneously every 8 h.**

Results. **In segments with deep arterial injury, the number of platelets/angioplasty segment in group 2 after 72 h (mean 21, range 9.7 to 39.7 × 10^6) was significantly less than that in group 1 (mean 375, range 72 to 787 × 10^6). Morphometric analysis after 4 weeks showed no difference between groups in degree of vessel wall injury. Mean (±SD) neointimal thickness was 0.70 ± 0.06 mm in group 3 and was significantly reduced in both group 4 (0.46 ± 0.11 mm) and group 5 (0.48 ± 0.21 mm).**

Conclusions. **The direct thrombin inhibitor hirudin significantly reduces platelet deposition up to 72 h after coronary stent angioplasty. A hirudin bolus alone as well as continued subcutaneous administration for 14 days substantially reduced neointimal proliferation compared with heparin 4 weeks after coronary stent angioplasty in minipigs.**

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Since its introduction in 1978 (1), coronary angioplasty can now be performed in more and more complex lesions with a primary success rate >90%. Despite enormous technical advances and the use of new interventional techniques and devices, such as directional coronary atherectomy (2), excimer laser coronary angioplasty (3), stent implantation (4,5) or rotational ablation (6,7), restenosis remains the major limitation of long-term success. Thus, 30% to 50% of patients develop restenosis in the first 6 months after balloon angioplasty. Mechanisms underlying this restenosis include passive elastic recoil, which may result in diminution of the maximal achieved lesion diameter, depending on the composition of the lesion (i.e., fibrocellular, sclerotic or calcified) (8–10). Balloon-induced vascular injury induces neointimal proliferation by several mechanisms: Balloon inflation results in plaque rupture, commonly producing dissection of the intima and media. Thrombi form, and platelets are deposited and provide a potent nidus on which mural thrombus organization and collagen matrix formation can occur (11). In addition, mitogenic factors are released by platelets, endothelial cells and stretched smooth muscle cells, resulting in migration and proliferation of smooth muscle cells (12). Several growth factors (partly stored in the alpha-granules of platelets) are involved in smooth muscle cell proliferation: platelet-derived-growth factor (13), transforming growth factor-beta (14) and epidermal growth factor (15). A stimulatory effect on smooth muscle cell proliferation has also been demonstrated for thrombin (16).

Hirudin, a specific thrombin inhibitor, has been shown to reduce fibrin deposition and platelet deposition after balloon-induced arterial injury in a carotid angioplasty model (17) as well as after coronary stent angioplasty in minipigs (18).

We hypothesized that a reduction of platelet and fibrin deposition might result in decreased neointimal proliferation in a minipig coronary angioplasty model. We studied 1) the effectiveness of subcutaneous hirudin in maintaining reduced thrombus formation for 3 days after angioplasty; and 2) neointimal proliferation after angioplasty in minipigs treated with subcutaneous hirudin for 14 days.

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Methods

Animal model. Minipigs of the Göttingen strain (25 to 40 kg, 12 to 24 months old) were studied. After intramuscular sedation with azaperone (10 mg/kg body weight), a central venous line was inserted through an ear vein. Sixty milliliters of blood was withdrawn from animals; platelets were separated and were reinjected together with iodine-125–labeled fibrinogen. Animals were placed under general anesthesia for the angioplasty procedure.

Angioplasty. Animals were sedated by an intramuscular injection of azaperone (10 mg/kg) and intravenous piritramide (Dipidolor) (7 mg). Anesthesia was induced after orotracheal intubation and ventilation by a mixture of halothane (0.8% to 1.5%), nitrous oxide (69%) and oxygen (30%) (Sulla 19 respirator, Dräger, Germany). Blood gases were regularly monitored, and ventilation was adjusted to maintain blood gases in the physiologic range.

The right carotid artery was surgically exposed, and an 8F coronary guide catheter was advanced into the ascending aorta. Tantalum stents (Wiktor-stents, Medtronic, Düsseldorf, Germany), premounted on a balloon with a nominal inflated diameter of 3, 3.5 or 4 mm, were placed into coronary artery segments of slightly smaller diameter (0.3 to 0.5 mm less) under fluoroscopic guidance. Stents were inflated twice at 8 to 10 atm for a total of 30 s. Balloons were then deflated and withdrawn.

Thereafter the carotid artery was ligated, and the animals were returned to their cages after recovery from anesthesia. After 12 or 72 h (short term) or 4 weeks (long term), angiography was repeated to confirm patency of the stented artery. Thereafter, the hearts were excised, immediately perfused with phosphate-buffered saline solution at a pressure of 100 mm Hg and then perfusion fixed with buffered 4% formalin.

Labeled platelets and fibrinogen. Autologous platelets were labeled according to a method modified from Laue et al. (19). Blood was drawn into acid citrate dextrose (5:1), and the platelets were separated from 60 ml. Finally, 3.7 MBq (100 μCi) of indium-111 oxine was added, resulting in a labeling efficiency of 76 ± 4% (mean ± SD) and platelet recovery of 51 ± 3%.

Sterile water (1 ml) was added to lyophilized human fibrinogen (1.2 mg), which was labeled with 4.07 MBq (110 μCi) of iodine-125 30 min before intravenous injection. Equal amounts of activity were injected in each study: 3 MBq of indium-111–labeled platelets and 3 MBq of iodine-125–labeled human fibrinogen.

Blood samples drawn after 15 min and 2, 4, 6, 12, 18 and 24 h after injection as well as at the end of the 12- or 72-h short-term protocol were counted in a gamma well counter. A double-window technique was used with spilldown correction (30% from the 247-keV indium-111 window into the 35-keV iodine-125 window). A symmetric 50-keV window centered around the 247-keV peak was used for indium-111, with a symmetric 20-keV window centered around 35 keV for iodine-125. Typical (background-corrected) count rates 24 and 90 h after injection were between 1,000 and 2,000 and between 500 and 1,000 counts/min, respectively. Stent angioplasty was performed after platelet-bound indium-111 activity and plasma iodine-125 activity had reached steady-state conditions, at a mean of 16 h (range 14 to 18) after injection. Radiochemicals were obtained from Amersham Buchler, Braunschweig, Germany.

Anticoagulation. All animals received a single bolus of 250 mg of acetylsalicylic acid before insertion of the arterial catheter. Starting on the day after operation, they were given 100 mg of acetylsalicylic acid/day in tablet form.

Short-term protocol. In five minipigs (group 1), anticoagulation consisted of a bolus injection of unfractionated heparin (100 U/kg) given before insertion of the guiding catheter. Five animals in group 2 received a bolus injection of hirudin (1 mg/kg, CGP 39393, recombinant desulfatohirudin, Ciba Geigy, Basel, Switzerland) instead. This intravenous bolus was repeated 45 to 60 min later when the activated partial thrombin time had decreased below 50 s. In two group 2 animals, a subcutaneous bolus of 6 mg/kg of hirudin was given 3 h later, and anesthesia was maintained until the animals were killed at 12 h after angioplasty. Two group 1 animals were also killed after 12 h.

After recovery from anesthesia, three group 2 animals were given hirudin subcutaneously (6 to 10 mg/kg) and then every 8 h for 72 h at doses adjusted to maintain activated partial thromboplastin time >1.8 times control levels. In group 1 the remaining three animals were given subcutaneous saline injections. Animals were randomly allocated to either anticoagulation regimen.

At the end of each experiment, stent angioplasty segments were excised (exactly at the proximal and distal borders of the stent) from the hearts after perfusion fixation in 4% buffered formalin at a pressure of 100 mm Hg. The segments thus obtained were of comparable lengths of 15 to 17 mm and were counted for radioactivity, as described for the blood samples.

Long-term protocol. Minipigs were randomly assigned to receive either unfractionated heparin as a bolus (100 U/kg intravenously, group 3, n = 5) or recombinant hirudin (1 mg/kg intravenously, group 4, n = 5), followed by subcutaneous injections of 0.9% saline solution every 8 h for 2 weeks. Animals in group 5 (n = 6) received an intravenous bolus injection of hirudin before angioplasty and a second bolus (1 mg/kg) 45 to 60 min later. After angioplasty, subcutaneous injections of 6 to 10 mg/kg were given and repeated every 8 h to maintain the activated partial thromboplastin time prolonged to >1.8 times above control levels for 2 weeks. Repeat angiography and excision of the hearts were performed after 4 weeks.

Histologic studies. All stents were further processed as previously described (20). In brief, after dehydration in graded alcohol concentrations, they were embedded in methylmethacrylate. Six sections/stent ~8 μm thick were prepared without prior removal of the stent wire, and elastica van Gieson

Table 1. Laboratory Variables in Heparin-Treated (group 1) and Hirudin-Treated (group 2) Minipigs Before and 72 h After Angioplasty

	Group 1 (heparin, n = 3)		Group 2 (hirudin, n = 3)	
	Before Angioplasty	72 h After Angioplasty	Before Angioplasty	72 h After Angioplasty
Hemoglobin (g/liter)	121 ± 14	112 ± 20	109 ± 22	87 ± 22
Hematocrit	0.35 ± 0.05	0.34 ± 0.05	0.29 ± 0.07	0.25 ± 0.08
Fibrinogen (μmol/liter)	7.74 ± 2.62	9.88 ± 2.92	7.06 ± 2.76	8.77 ± 1.49
Platelet count ($\times 10^9$/liter)	437 ± 135	499 ± 108	411 ± 108	451 ± 98.8

Hemoglobin and hematocrit showed a tendency to decrease in the hirudin group after 72 h. Fibrinogen concentration and platelet counts were not different in the two groups. Data presented are mean value ± SD.

staining was performed. This procedure allowed microscopic analysis of damage limited to the intima or extending into the media. Morphometric analysis of the free lumen and vessel areas (lumen plus intima plus media) was performed in six slices/stent. Vessel wall area was calculated as the difference between vessel area and free lumen area. Injury was evaluated according to a score proposed by Schwartz et al. (21). Each stent strut/slice was assigned a numerical value: 0 = intact internal elastic lamina; 1 = lacerated internal elastic lamina, media compressed but not lacerated; 2 = lacerated internal elastic lamina and media, intact external lamina; 3 = large laceration of the media, stent strut in the adventitia. Mean injury scores were calculated as the quotient of the sum of weights for each wire and the number of wires present.

Laboratory testing. Blood samples for hemoglobin, hematocrit, fibrinogen and platelet counts were taken before angioplasty and 12 or 72 h later in the short-term protocol groups 1 and 2. The same variables were measured in the long-term groups 3 days and 4 weeks after angioplasty. Activated partial thromboplastin time was measured before anticoagulant drugs were given and 20 min and 1 and 3 h later, before each subcutaneous dose in the first 4 postoperative days and at the end of the study period.

Statistics. The Kruskal–Wallis test was used for comparison of the laboratory variables in the three long-term protocol groups. Within-group values at different times were compared by the Wilcoxon test. A p value <0.05 was considered to be statistically significant. Platelet and fibrin deposition in angioplasty segments from minipigs after 72 h as well as neointimal thickness in the long-term protocol groups 3, 4 and 5 were analyzed using a multivariate mixed linear model (22). Covariables were 1) anticoagulation, 2) target vessel (right, left circumflex or left anterior descending coronary artery), 3) balloon size, and 4) injury of the vessel wall. Data were analyzed for equality at the 5% level by the multiple test procedure of Scheffé (23). Anticoagulation was considered as a fixed factor; all other covariables were considered to be random. The square of the stent size was modeled as a fixed factor to account for differences in stent surface. The multiple 5% level was taken to indicate statistical significance.

Results

Platelet and fibrin deposition 12 and 72 h after coronary stent implantation. In 10 animals stent angioplasty was performed in 26 coronary arteries. Two additional animals, one in each group, died 30 min and 6 h after angioplasty. In one of these animals ventricular fibrillation was observed during repair of the neck wound. External defibrillation was ineffective. In the second animal the same mechanism most likely resulted in sudden death because both animals had occlusive spasm during implantation that could not be stopped by intracoronary nitroglycerin. These animals were not included in the analysis.

Laboratory variables. Platelet counts, fibrinogen, hemoglobin and hematocrit were not significantly different in the two groups. Although the platelet count remained stable throughout the experimental period, fibrinogen values increased slightly in both groups. In hirudin-treated animals, a decrease in hemoglobin by 22 g/liter and in the hematocrit by 0.04 as a result of mild bleeding from the neck wound was observed 72 h after stent angioplasty (Table 1).

Activated partial thromboplastin time in heparin-treated animals (group 1) was still prolonged at 1 h after stent angioplasty and then returned to normal. In hirudin-treated animals, activated partial thromboplastin time was prolonged to >2 times the control value after 2 h and remained prolonged to >1.8 times for the experimental course of 72 h (Table 2).

After analysis for radioactivity, six slices/stent were analyzed histologically, and the injury score was determined. Segments were grouped as *deep arterial injury* if two or more of the six analyzed slices had an injury score ≥2 or as *superficial injury* if only one or none of the six slices had a score ≥2.

Platelet deposition. The mean values and ranges of values for platelets/segment are shown in Figure 1. In animals sacrificed after 12 h, platelet deposition was similar for segments injured only superficially (score <2) in both groups, but with deep injury (score >2), segments from heparin-treated animals showed four times more platelets deposited than segments from hirudin-treated animals. These results are in good agreement with our earlier observations (18) on the effect of hirudin

Table 2. Activated Partial Thromboplastin Time Values (multiples of baseline ± SD)

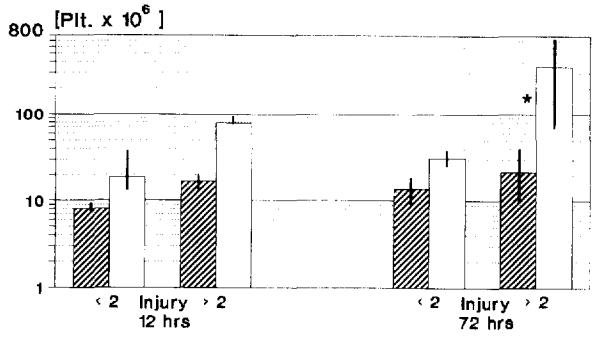
Group	Control Value	1 h After Angioplasty	3 h After Angioplasty	4 h After SC Hirudin	8 h After SC Hirudin
1 (heparin, n = 5)	1	2.6 ± 0.3*	1.2 ± 0.1		
2 (hirudin, n = 5)	1	2.1 ± 0.4*	2.0 ± 0.3*	2.7 ± 0.4* (n = 3)	2.2 ± 0.4* (n = 3)
3 (heparin, n = 5)	1	2.7 ± 0.4*	1.1 ± 0.1	1.0 ± 0.2	1.1 ± 0.3
4 (hirudin bolus, n = 5)	1	2.3 ± 0.5*	1.2 ± 0.2	1.2 ± 0.3	1.0 ± 0.2
5 (hirudin, n = 6)	1	2.1 ± 0.3*	2.0 ± 0.3*	2.5 ± 0.5*	2.1 ± 0.3*

*$p < 0.05$ versus control value. Data presented are mean value ± SD. SC = subcutaneous.

after this time period. We did not perform statistical analysis of these values because only two animals/group were analyzed after this 12-h period in the current study. After treatment with subcutaneous hirudin for 72 h, platelet deposition/segment remained in the range of the values after 12 h (range 9.7 to 39.7 × 10^6 platelets, mean 21) in the presence of deep injury. In contrast, it increased to 375 × 10^6 platelets (range 72 to 787) for segments in the heparin-treated minipigs showing an injury score >2 ($p < 0.05$). Segments with superficial injury only (score <2) showed no major increase compared with the 12-h values.

Fibrin deposition. Fibrin deposition closely resembled the pattern described for platelet deposition and is shown in Figure 2. The four segments in the heparin group (injury score >2) had an average of 157 μg (range 91 to 282) of fibrin deposited after 12 h. This deposition amounted to 1,856 μg (range 750 to 3,700) fibrin after 72 h. Animals in the hirudin group with an injury score >2 showed less fibrin deposition after 12 h (mean 16 μg fibrin/segment, range 6.8 to 28.8) as well as after 72 h (680 μg fibrin/segment, range 210 to 800). Similar to platelet deposition, fibrin deposition was greater in deeply injured segments than in vessels without deep injury.

Figure 1. Platelet (Plt.) deposition at 12 and 72 h after angioplasty (mean values **[columns]** and ranges **[vertical lines]**) in treatment groups 1 and 2 on a logarithmic scale. Segments were grouped for the presence or absence of deep arterial injury (score >2 or <2). **Columns** after 12 h represent two segments each from two animals in both treatment groups. After 72 h, three segments in the hirudin and four in the heparin group showed superficial injury (score <2), and six in the hirudin group and five in the heparin group showed deep injury (score >2). *$p < 0.05$. **Hatched bars** = hirudin; **open bars** = heparin.



Neointimal proliferation 4 weeks after coronary angioplasty. *Laboratory variables.* The values for hemoglobin, hematocrit, fibrinogen and the platelet counts did not differ among the three groups before angioplasty. Hemoglobin and hematocrit decreased significantly in the first 3 postoperative days in those animals with long-term hirudin as a result of bleeding from the neck wound. No renal or gastrointestinal bleeding nor any other hematoma formation was observed. At the end of the study period (4 weeks), these laboratory variables were not different among the three groups (Tables 3 and 4).

In all three groups activated partial thromboplastin time was <18 s at baseline and prolonged 1 h after start of anticoagulation (more than twice control value). In the heparin-treated minipigs, activated partial thromboplastin time was normal 3 h after implantation. In animals in group 4 (hirudin bolus), activated partial thromboplastin time prolongation after 1 h was more pronounced than in group 3 but had also normalized after 3 h. Group 5 minipigs had activated partial thromboplastin time values more than twice the control value after 3 h. During long-term treatment with subcutaneous hirudin, mean activated partial thromboplastin time values before the next injection were >1.8 times the control value. Hirudin doses were adopted to maintain minimal activated partial thromboplastin time >1.8 times the control value (Table 2).

Histologic analysis. In 16 animals, stent angioplasty was performed in 30 coronary arteries, and these were patent 4 weeks later. None of the animals died prematurely.

In six slices/angioplasty segment, the injury scores did not

Figure 2. Fibrin deposition at 12 and 72 h after angioplasty. *$p < 0.05$. Format and symbols as in Figure 1.

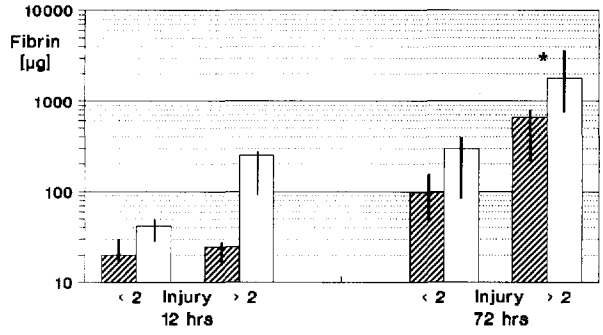


Table 3. Hemoglobin and Hematocrit Values Before and After Coronary Angioplasty

Group	Baseline		3 Days After Angioplasty		4 Weeks After Angioplasty	
	Hb (g/liter)	Hct	Hb (g/liter)	Hct	Hb (g/liter)	Hct
3 (heparin, n = 5)	117 ± 17	0.34 ± 0.04	118 ± 18	0.32 ± 0.02	106 ± 8	0.33 ± 0.04
4 (hirudin bolus, n = 5)	120 ± 12	0.37 ± 0.03	114 ± 8	0.33 ± 0.03	124 ± 11	0.35 ± 0.03
5 (hirudin, n = 6)	111 ± 9	0.32 ± 0.03	86 ± 3*†	0.25 ± 0.01*†	119 ± 6	0.34 ± 0.02

*$p < 0.05$ versus baseline values. †$p < 0.05$ versus baseline values and values at 4 weeks after angioplasty and values of groups 3 and 4 at the same time. Data presented are mean value ± SD. Hb = hemoglobin; Hct = hematocrit.

differ among the groups and averaged 1.84 ± 0.21 in group 3 (heparin); 1.84 ± 0.26 in group 4 (hirudin bolus); 1.83 ± 0.37 in group 5 (long-term hirudin treatment). The neointimal proliferative response was measured by microscopic examination and determination of mean neointimal thickness. Mean neointimal thickness (Fig. 3) was significantly lower in both hirudin groups (group 4, mean 460 μm, range 330 to 690; group 5, mean 480 μm, range 290 to 680) than in animals treated with heparin (mean 700 μm, range 660 to 820).

Vessel wall area accounted for 75.8% (range 74% to 96%) in the heparin-treated animals. In hirudin-treated minipigs, the vessel wall area amounted to 56% (range 39% to 71%) and 53% (range 42% to 68%) (Fig. 4). In all three groups the neointima was characterized histologically by a fibrocellular proliferation. Although the proliferative response in groups 4 and 5 was morphologically similar to that observed in group 3, the extent of this response was markedly lower in the hirudin-treated groups. Typical histologic sections through an angioplasty segment in groups 3 and 5 are shown in Figures 5 and 6.

Discussion

Restenosis after coronary angioplasty is the result of a complex response of the artery to a more or less severe trauma. Besides elastic recoil leading to an early loss of lumen gained, this trauma is followed by activation of the coagulation cascade resulting in the formation of a mural thrombus (8,24). The amount of thrombus formed can contribute to restenosis either by an organization and replacement by invading and proliferating medial smooth muscle cells or by the release of factors stimulating migration and proliferation of smooth muscle cells from the media (25) or, most likely, both.

The present study tested the hypothesis that the quantity of thrombus is related to the extent of late neointimal proliferation. Reduction of thrombus formation was achieved by using the direct thrombin inhibitor hirudin as an anticoagulant rather than the standard clinical practice of a heparin bolus. Hirudin reduces early thrombus formation after deep arterial injury in carotid arteries significantly more effectively than heparin, as originally shown by Heras et al. (17). This finding reflects several advantages of direct thrombin inhibitors over heparin. They can inhibit thrombin bound to fibrin clots or extracellular matrices, which is relatively resistant to heparin; in addition, they are not inhibited by release of platelet factor 4 from activated platelets and do not require antithrombin III (26,27).

Platelet and fibrin deposition. We previously showed (18) that hirudin also reduces thrombus formation after 12 h in a minipig coronary angioplasty model (18). The present study confirms the results of our earlier study of platelet and fibrin deposition 12 h after angioplasty. Although the number of angioplasty segments analyzed after 12 h in the present study is small (n = 6/group), platelet deposition in deeply injured segments is almost identical: The range of values in hirudin-treated animals was 14 to 33 × 10^6/segment in the earlier study and 12 to 20 × 10^6/segment in the present study. In heparin-treated animals, ranges were 53 to 164 × 10^6 and 78 to 98 × 10^6/segment, respectively. This effect is maintained for up to 72 h by subcutaneous administration of hirudin continuously prolonging the activated partial thromboplastin time. Assessment of platelet deposition after longer periods was not possible in our model because of the degradation of the short-lived indium. The present study shows not only that hirudin reduces platelet and fibrin deposition, but that this effect translates into a pronounced reduction in late neointimal proliferation compared with that for heparin.

Platelet deposition and neointimal proliferation. Hirudin was either given as a single bolus only or for 2 weeks after

Table 4. Fibrinogen and Platelet Values Before and After Coronary Angioplasty

Group	Baseline		3 Days After Angioplasty		4 Weeks After Angioplasty	
	Fibrinogen (μmol/liter)	Platelets (×10^9/liter)	Fibrinogen (μmol/liter)	Platelets (×10^9/liter)	Fibrinogen (μmol/liter)	Platelets (×10^9/liter)
3 (heparin, n = 5)	6.53 ± 2.75	505 ± 86	9.53 ± 2.09*	512 ± 28	8.88 ± 1.38	499 ± 83
4 (hirudin bolus, n = 5)	6.18 ± 1.35	510 ± 14.8	8.24 ± 0.50*	500 ± 83	8.32 ± 0.62*	580 ± 141
5 (hirudin, n = 6)	5.41 ± 1.77	529 ± 48	8.03 ± 1.44*	504 ± 14.7	6.94 ± 1.71*	539 ± 12.8

*$p < 0.05$ versus baseline values. Values in each treatment group were tested for significant differences using the Wilcoxon test. Measurements at each time point in different groups were compared using the Kruskal–Wallis test for significant differences. Data presented are mean value ± SD.

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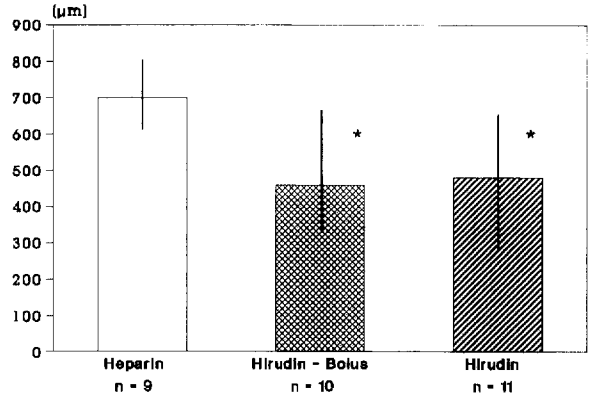
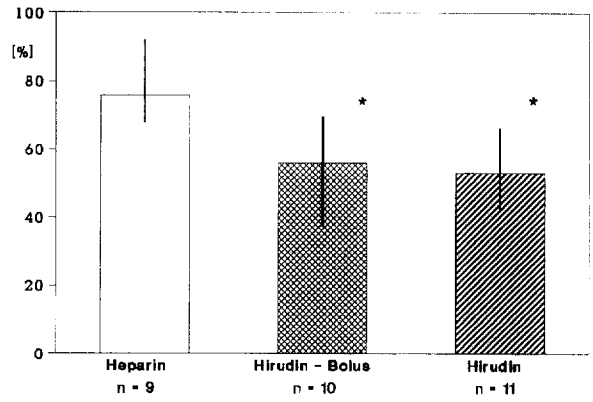


Figure 3. Mean values (**columns**) and ranges (**vertical lines**) for neointimal thickness in groups 3, 4 and 5. Numbers (n) correspond to the number of angioplasty segments in each group. *$p < 0.05$ versus group 3 at the 5% level.

angioplasty, a period after which reendotheliazation in this model is complete as assessed by electron microscopy in three animals (data not shown). The effect on neointimal proliferation was similar in both groups, that is, prolonging the activated partial thromboplastin time for <3 h (Table 2) resulted in the same reduction in neointimal formation as did long-term hirudin administration. These data suggest that it is mainly the platelet and fibrin deposition immediately after angioplasty that determines the degree of late neointimal proliferation. The continuation of anticoagulation until reendotheliazation is achieved seems to provide no additional effect. This interpretation is in accordance with several observations. We were unable to show an antiproliferative effect of hirudin on smooth muscle cells in culture (data not shown). The increase in platelet deposition between 12 and 72 h is minor compared with the number present at 12 h in the heparin-treated animals (i.e., the major effect of hirudin on platelet deposition was achieved in the first few hours after angioplasty), and subsequent anticoagulation has only a minor effect on thrombus formation. Moreover, thrombus formed after vascular injury

Figure 4. Mean values (**columns**) and ranges (**vertical lines**) for vessel wall area in groups 3, 4 and 5. Numbers (n) correspond to the number of angioplasty segments in each group. *$p < 0.05$ versus group 3 at the 5% level.



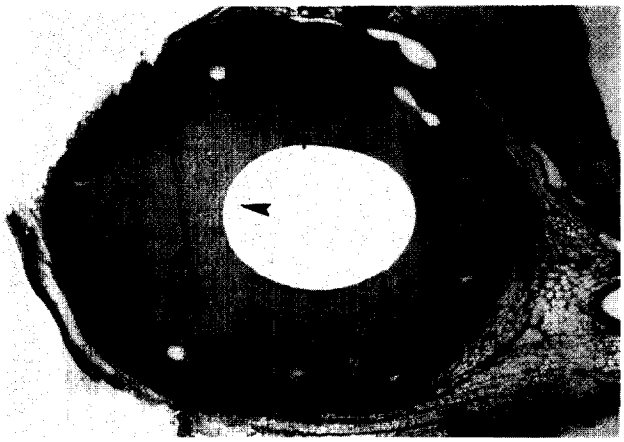
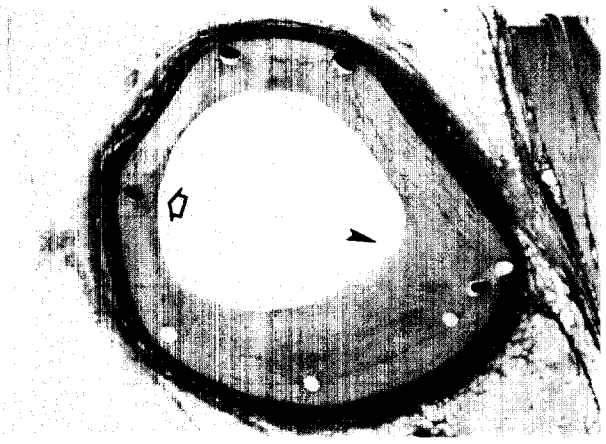


Figure 5. Section through an angioplasty segment after 4 weeks (heparin group 3). Neointimal proliferation is maximal at the site of severe vessel damage (**arrowhead**). Stent wires are **black** and partly "dropped out" from the thin sections. Elastica van Gieson stain ×40, reduced by 35%.

has been reported (28) to be largely resolved by 48 h. However, this interpretation may be valid only for segments with no residual lumen narrowing, as was observed in the present model.

The thrombogenicity of a largely irregular lumen in human coronary segments after angioplasty with a certain degree of residual stenosis might be substantially higher. This concept is supported in a study by Willerson et al. (29). In a dog model of combined coronary endothelium injury and external constriction, they reported cyclic flow variations as indirect evidence for the amount of platelet deposition. These flow variations were present in some animals for several days after injury, indicative of repetitive thrombus formation and resolution in this model with an external constrictor causing persistent

Figure 6. Section through an angioplasty segment after 4 weeks (hirudin group 5). Medial laceration is also associated with the most pronounced proliferation (**arrowhead**); however, the extent of this response is markedly reduced compared with that in the heparin treatment group (Fig. 5). There is only minimal poliferative reaction around a strut not penetrating the internal elastic lamina (**open arrow**). Elastica van Gieson stain ×40, reduced by 35%.



lumen narrowing after endothelial injury (29). Only if nearly complete elimination of cyclic flow variations by thromboxane and serotonin antagonists was achieved was subsequent neointimal proliferation decreased.

Nonetheless, the observation of a profound reduction in late proliferation obtained with a hirudin bolus alone has important implications for a major concern of this anticoagulation approach, namely, bleeding. Meyer et al. (30) recently showed that local hirudin administration with a very low dose compared with systemic application also results in a significant reduction in platelet deposition in a pig carotid angioplasty model. If subsequent proliferation is also reduced, this approach could further reduce bleeding complications. Evidence for a role of thrombin inhibition in late neointimal proliferation after angioplasty was previously provided by Walters et al. (31), who showed that the thrombin inhibitors hirudin and PPACK reduced neointimal proliferation in rabbit aortas. Similarly, Sarembock et al. (32) demonstrated a reduction of neointimal proliferation in rabbit iliac arteries by 50% after treatment with hirudin.

Coronary angioplasty model. In the present study we used intracoronary stent angioplasty to reproducibly create a deep arterial injury. Previous studies have shown that the degree of late neointimal proliferation in pigs depends on the degree of vessel wall injury. Leaving the internal elastic membrane intact results in a very modest neointimal proliferation, whereas deep tears into the media are associated with a much more pronounced proliferative response. This finding is in contrast to results obtained in smaller animal models such as the rat carotid artery. Here, apparently superficial injury limited to the endothelium already results in extensive neointimal formation (33). However, ballooning of porcine coronaries with significantly oversized (≥30%) balloons is associated with a significant incidence of vessel closure and animal death in the early hours after angioplasty (34). We lost every third animal to sudden death with histologic evidence of thrombotic vessel occlusion before switching to the stent angioplasty model. Apparently, stenting prevents vessel closure in the majority of cases because we lost only 2 of 28 animals prematurely in the present study. However, it might be argued that stent angioplasty cannot readily be compared with balloon angioplasty because of a higher thrombogenicity of and a foreign body reaction to the stent wire. Although we certainly cannot totally rule out such effects, we and others (35) found a histologic appearance of the neointima closely resembling results after balloon angioplasty and no evidence for foreign body reaction as a major contributor to the proliferative response. In contrast, the close correlation of the amount of proliferation and degree of vessel injury produced by stent struts suggests that the injury to the media mainly determines the amount of proliferation, and not any kind of foreign body reaction. If the latter were the case, struts over an intact internal elastic lamina should also be surrounded by a marked proliferative response, which we did not observe.

Nonetheless, because stent angioplasty in the clinical situation is associated with an increased incidence of thrombotic vessel closure compared with balloon angioplasty, this model certainly involves a higher thrombogenic potential. Extrapolation to human balloon angioplasty can be done only with appropriate caution. In a recent multicenter study (36), the effect of hirudin in patients with unstable angina undergoing coronary angioplasty was assessed. Restenosis was not different between hirudin and heparin treatment after 7 months. However, the incidence of early events was reduced in the hirudin group (36).

Summary. The present study shows that in a porcine coronary stent angioplasty model, reduced acute thrombus formation is associated with a significantly reduced neointimal proliferative response after 4 weeks. This finding could be of potential value for designing new anticoagulation strategies in human coronary angioplasty aiming at reduced acute platelet and fibrin deposition to reduce late restenosis.

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