Importance of Adequate Heparin Dosage in Arterial Angioplasty in a Porcine Model

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Acute occlusion after a successful angioplasty is a severe complication that has been reported in 2–12% of patients. Therefore, to determine whether there was a relation between the dosage of heparin and the presence of mural thrombosis, we studied in a pig model the relation between the dosage of heparin and acute platelet-thrombus deposition on the site of arterial injury. We retrospectively analyzed the effect of three heparin regimens on platelet deposition in 32 normal pigs (mean weight, 32.7 kg) that underwent bilateral carotid angioplasty and were sacrificed 90 ± 26 minutes later. Pigs in protocol 1 (n = 7) received an intravenous bolus injection of 4,000 units heparin 10 minutes before angioplasty. Pigs in protocol 2 (n = 11) received two bolus injections of 4,000 units heparin 40 minutes apart; the angioplasty was performed immediately after the second bolus. Pigs in protocol 3 (n = 14) had an initial 4,000-unit bolus injection of heparin followed immediately by an infusion of 4,000 units/hr; angioplasty was performed 20 minutes after starting the infusion. In-labeled platelet deposition on deeply injured (torn into the media) arterial segments were 86.3 ± 68, 56.2 ± 56.9, and 37.7 ± 37.7 × 10⁶/cm² for protocols 1, 2, and 3, respectively. A regression analysis showed an inverse relation between the log of platelet deposition and heparin units per kilogram per minute in arterial segments with deep injury (r = -0.70, p = 0.0002) for the whole group as well as for each protocol (protocol 1: r = -0.80, p = 0.11; protocol 2: r = -0.80, p = 0.008; and protocol 3: r = -0.90, p = 0.0008). Animals in protocol 2 had the lowest platelet deposition when heparin units per kilogram per minute was adjusted for each protocol. Platelet deposition in segments with subendothelial injury was very low (<10 × 10⁶/cm²) and independent of the dosage of heparin. Macroscopic thrombus was present in all arterial segments (100%) with deep injury in those animals that received less than 3.1 heparin units/kg/min, whereas it was present in only 25% of arteries from pigs that had 3.1 or more heparin units/kg/min (p < 0.001). No thrombus was observed in segments with subendothelial injury. This study indicates that acute therapy with adequate dosages of heparin can significantly reduce thrombosis after arterial angioplasty in the carotid artery in pigs. (Circulation 1988;78:654–660)

The primary success rate of percutaneous transluminal coronary angioplasty continues to improve as a result of increasing operator experience, better patient selection, and improvements in technology. Early reocclusion and restenosis cause the most significant morbidity and are the main limitations to further development of the technique. It is likely that platelet-thrombus deposition on the site of arterial angioplasty is not only an important factor in acute thrombotic occlusion but also a significant contributor to the pathogenesis of restenosis; therapies that limit this initial response to injury may also alleviate the problem of restenosis. Successful angioplasty in humans produces a deep arterial injury with a tear or fracture of the atherosclerotic plaque. The severity of platelet-thrombus deposition on the angioplasty site is related to the local shear forces (directly related to the...
velocity of flow and inversely related to the third power of the lumen diameter) and the pathological substrate, which is determined by plaque splitting or the depth of the arterial tear. A tear through the internal elastic lamina into the media or a splitting of a plaque is necessary for the development of mural thrombosis.2,6-8 Thus, most successful angioplasties are expected to have a mural thrombus at the site of injury. Deep arterial injury exposes collagen, smooth muscle cells, and elastic tissue, and it releases tissue thromboplastin; this combined with atherosclerotic gruel from atherosclerotic lesions activates platelets and the intrinsic and extrinsic pathways of coagulation.2,3 In contrast, superficial injury with only endothelial denudation causes deposition only of a monolayer of platelets and no thrombus formation.6-8

To limit the thrombotic complications of angioplasty, the use of antiplatelet and anticoagulant agents has evolved empirically.2 Coagulation proteins, especially thrombin, are generated at the platelet membrane, activate platelets, and require inhibition to effectively provide antithrombotic protection during angioplasty. Although in a previous study,9 long-term Coumadin treatment was no better than aspirin, neither therapy was started until after the procedure. Most patients are treated with heparin during angioplasty, but the optimal dose has not been established. Treatment with heparin for 24 hours after angioplasty may diminish the incidence of total thrombotic occlusion in patients at high risk.10 The antithrombotic effect in injured arteries supports the use of heparin during arterial angioplasty; in addition, heparin may have an antiproliferative effect on smooth muscle cell growth.11-13 Thus, in this study, we addressed the relationship between the dosage of heparin and acute quantitative platelet-thrombus deposition on the site of arterial injury in a porcine model of angioplasty to determine the dosage of heparin that prevents mural thrombosis on sites of arterial injury.

Materials and Methods

We studied thirty-two 4-month-old pigs, 14 females and 18 males, with a mean weight of 32.7±9.2 kg (range, 21-49 kg). These normal pigs were obtained from local farmers and were of the Babcock four-way cross stock (mixture of Landrace, Yorkshire, Hampshire, and Duroc breeds). They were housed at the Mayo Institute Hills Farm, Rochester, Minnesota, and were fed a normal chow diet. In all pigs, autologous platelets were labeled with 300-400 μCi 111In-labeled tropolon,14-16 24 hours before angioplasty.

Experimental Protocols

All pigs were sedated with 300 mg ketamine (Ketaset, Bristol Laboratories, Syracuse, New York) given intramuscularly. After inhalation of halothane (Fluothane, Wyeth-Ayerst Laboratories, Philadelphia, Pennsylvania), the pigs were intubated and mechanically ventilated with room air (Harvard respirator, South Natick, Massachusetts) mixed with 0.5% halothane to maintain anesthesia. The electrocardiogram and intra-arterial pressure were continuously monitored throughout the procedure. Regardless of weight, pigs were given one of three heparin dosages. In protocol 1 (n=7), angioplasty of both carotid arteries was performed within 10 minutes after an intravenous bolus injection of 4,000 units heparin. Pigs in protocol 2 (n=11) received two intravenous bolus injections of 4,000 units heparin within 40 minutes (mean); the angioplasty was performed immediately after the second bolus. Pigs in protocol 3 (n=14) received an initial intravenous bolus of 4,000 units heparin followed immediately by a heparin infusion of 4,000 units/hr; the carotid dilatation was performed 20 minutes after starting the infusion.

Carotid Angioplasty

Angioplasty of the common carotid arteries was performed with an 8F Medi-tech polyethylene balloon (8 mm×3 cm) dilatation catheter (Watertown, Massachusetts) inserted through a right femoral arteriotomy. After intravenous injection of the initial heparin bolus, the dilatation catheter was advanced under fluoroscopy into the common carotid arteries. The right and left common carotid arterial segments between the fourth and fifth vertebrae were dilated by five 30-second inflations to 6 atm (Medi-tech pressure manometer), with 60-second intervals between inflations. Spot films were taken before, during, and after the dilatation.

Histopathology

After the postdilatation angiogram, the pigs were given an overdose of pentobarbital and perfused antegrade with 2% glutaraldehyde and 1% paraformaldehyde in 0.1 M cacodylate (pH 7.25) at a pressure of 100 mm Hg for 15 minutes to allow fixation of the arteries in situ. The carotid arteries were then removed and cleaned of all adventitia. The dilated portion of the fixed artery was easily identified by the regions of vasoconstriction proximal and distal to it and from the spot films taken during and after the angioplasty. The dilated portion of the fixed carotid artery was divided into equal segments, and a similar-sized segment was taken from the adjacent proximal and distal ends. A twofold magnifying lens (Sunnex Laboratories, Needham, Massachusetts) was then used to examine for mural thrombus formation. We chose to look at macroscopic thrombus formation because easily visible thrombus has the potential of being physiologically and clinically relevant by embolizing, enlarging, obstructing blood flow, or contributing to more severe vasoconstriction. We have previously demonstrated that macroscopic mural thrombosis is associated with at least 20×10⁶/cm² platelets.8 From each arterial segment, two or three ring sections were stained with hematoxylin and eosin, and with
Heidenhain-Weigert-van Gieson's stain. Light microscopy was used to document the presence of a dissection or tear into the media at the site of dilatation. The consensus opinion of two observers was recorded. Deep arterial wall injury was defined as an intimal tear extending through the internal elastic lamina into the media; subendothelial injury was considered as endothelial denudation without a tear through the internal elastic lamina.

Quantification of Platelet Deposition
The extent of platelet deposition on the arterial segments was quantified by the method of Dewanjee et al16 with autologous 111In-labeled platelets.14 Three samples of blood were obtained at the time the animals were killed for determination of mean radioactivity in counts per minute per unit weight of blood (microbalance), which was then corrected for the amount of free 111In not bound to the platelets. The level of radioactivity (cpm) in each arterial segment was also measured by a gamma well counter (Gamma 8000, Beckman Instruments, Fullerton, California). The spectrometer of the counter was adjusted to include the photopeaks at 174,247 and 421 keV (sum peak) of 111In radionuclide. The 111In counts per minute per milligram of blood were transformed into counts per minute per milliliter of blood. The percentage of radioactivity bound to platelets was then determined, and the number of platelets per unit per minute was calculated in the known blood platelet count (Coulter counter, Coulter Electronics, Hialeah, Florida). The number of platelets deposited on an arterial segment per square centimeter was calculated by dividing the arterial segment counts per minute by both the number of platelets per counts per minute and the arterial endothelial surface area (area = \( \pi \times \text{diameter} \times \text{length of the arterial segment} \)).

Statistical Analysis
Because the variability of platelet deposition in the same pig or among pigs was directly related to the mean level of platelet deposition, a logarithmic transformation was first applied to all platelet deposition values. This removes the dependence of variability on the mean and allows variation to be expressed as a percentage of the mean rather than as an absolute term.

Two segments for each artery for each pig were analyzed for a total of four segments for each pig. Because of the large difference between deep and superficial injury in its effect on platelet deposition, all analyses were performed separately on deeply and superficially injured segments. Comparison of variation between pigs relative to variation between segments within pigs indicated that segments within pigs did not behave independently with respect to platelet deposition. Therefore, to maintain statistical validity, it was necessary to consider the pig as the independent observation. Means were taken for deeply injured and for superficially injured segments within each pig, and it was these means that were analyzed with respect to heparin effects.

One could consider weighting pigs in such analyses because, for example, some pigs had one and others had four deeply injured segments. However, because all segments within a pig were exposed to the same concentrations of heparin and because of the positive correlations among segments within pigs, there would be relatively little difference in the amount of information contributed by one-segment and four-segment pigs. For simplicity, unweighted analyses were performed. Such analyses are unbiased and have the property that the

<table>
<thead>
<tr>
<th>Protocol</th>
<th>1 (n = 7)</th>
<th>2 (n = 11)</th>
<th>3 (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>34.4 ± 9.8</td>
<td>33.5 ± 8.4</td>
<td>31.2 ± 10.0</td>
</tr>
<tr>
<td>Time to sacrifice (min)*</td>
<td>60.0 ± 15.0</td>
<td>114.5 ± 19.3</td>
<td>84.6 ± 12.6</td>
</tr>
<tr>
<td>Heparin (units/kg/min)</td>
<td>2.15 ± 0.78</td>
<td>2.31 ± 0.82</td>
<td>3.99 ± 1.06</td>
</tr>
</tbody>
</table>

All values are mean ± SD.
*Interval from the start of heparin treatment until sacrifice. Time from angioplasty to sacrifice was similar for each protocol.

FIGURE 1. Bar graph of platelet deposition in arterial segments with deep and subendothelial injury. Height of the bars represents mean platelet deposition for mean segments (number above the bars) with the same type of injury for each protocol. Vertical lines are standard error of the mean. Platelet deposition is significantly greater with deep injury compared with subendothelial injury (p<0.0001). There was no difference in platelet deposition between genders.
Regression analysis showed that the log of platelet deposition was inversely related to the heparin units per kilogram per minute for segments with deep arterial injury (Panel A) but not for segments with subendothelial injury (Panel B).

The method of relating platelet deposition to heparin dosage was to create an index of the amount of heparin given per unit of time per kilogram of weight of pig. This index should indicate the average concentration of heparin in the pig during the experiment. Regressions were performed with this normalized heparin value as the independent variable and with the log of platelet deposition as the dependent variable. The model allowed the three protocols to have different mean (adjusted) levels of platelet deposition, but the relation between units of heparin per kilogram per minute and platelet deposition was assumed not to depend on protocol (this assumption was tested).

**Results**

Table 1 lists the mean ± SD for weight, time to sacrifice, and units of heparin per kilogram per minute for the pigs in each protocol. Although we counted the platelets deposited in each vessel's segment, the analysis is based on the mean number of platelets deposited in all the segments with the same type of injury (subendothelial or deep) for each pig. In the presence of subendothelial injury, platelet deposition was always less than $10^9$/cm². On areas of deep injury, platelet deposition was much higher (Figure 1). Animals treated as in protocol 1 had an average activated partial thromboplastin at time of sacrifice of four times the basal level in controls; three pigs in protocol 2 and 11 pigs in protocol 3 had activated partial thromboplastin times that were three and four times higher than basal control levels, respectively.

**Relation Between Platelet Deposition and Heparin Dosages**

On areas of deep injury, the regression analysis, with log of platelet deposition (LPD) as a dependent variable, showed that log of platelet deposition was...
inversely related to the heparin units per kilogram per minute (HKM) (\(r = -0.70, p = 0.0002\), LPD = 5.1 – 0.57 HKM, Figure 2A). The proportion of variance accounted for was 49%, whereas the platelet deposition in subendothelial injured segments was low and independent of the heparin units per kilogram per minute (\(r = -0.38, p = \text{NS}\), LPD = 2.02 – 0.13 HKM, Figures 2A and 2B). The relation between log of platelet deposition and heparin units per kilogram per minute was also assessed for each protocol. In protocol 1, the regression analysis between these two variables showed an inverse relation that was not significant for mild injury (\(r = -0.76, p = \text{NS}\)) or for deep injury (\(r = -0.80, p = 0.11\), Figure 3A), but the number of observations was small. In protocol 2, there was a significant inverse relation between the two variables in deep injury (\(r = -0.80, p = 0.009\), LPD = 5.76 – 1.10 HKM, Figure 3B) but not with mild injury (\(r = -0.63, p = \text{NS}\)). In protocol 3, there was also only a significant inverse relation between the two variables in deep injury (\(r = -0.90, p = 0.0008\), LPD = 6.76 – 0.88 HKM, Figure 3C) but not in mild injury (\(r = -0.002, p = \text{NS}\)).

In deep injury, the differential effect of the three protocols on platelet deposition was tested by an analysis of covariance. When adjusted for heparin units per kilogram per minute, the platelet deposition was significantly less in protocol 2 than in protocol 1 (\(p = 0.04\)) or than in protocol 3 (\(p = 0.0007\)). Although pigs in protocol 3 received more heparin units per kilogram per minute, pigs in protocol 2 received a bolus injection of heparin (4,000 units) just before the dilatation; the lower platelet deposition for heparin units per kilogram per minute in protocol 2 compared with protocol 3 is reflected by a shift to the left and a steeper slope in the regression relation (Figure 3B compared with 3C). Thus, the relation of log of platelet deposition with heparin units per kilogram per minute was strengthened when the protocol was adjusted for heparin dosage because the difference between protocols in platelet deposition was much less than what would have been expected on the basis of differences between protocols in heparin units per kilogram per minute. The relation for that analysis is given in the Appendix, and it accounted for 73% of the variation in log of platelet deposition.

**Thrombus**

No thrombus was seen in segments with subendothelial injury. We looked at the heparin units per kilogram per minute that prevented thrombus formation in deeply injured segments. All macroscopic thrombus was formed by at least \(20 \times 10^6 / \text{cm}^2\) platelets as had been shown previously.\(^6\) Figure 4 shows pigs with deep injury by high dosage (\(>3.1 \text{ units/kg/min}\)) or low dosage (\(<3.1 \text{ units/kg/min}\)) heparin and by the presence or absence of thrombus. All pigs that received less than 3.1 heparin units/kg/min had thrombus, whereas only two of eight with high dosage of heparin developed mural thrombosis; the difference was statistically significant (\(p < 0.001\)).

**Discussion**

To assess the effect of heparin on platelet deposition after acute angioplasty, we retrospectively analyzed the effect of different heparin dosages in our pig model of arterial injury. All pigs were given the same dosage of heparin according to one of three protocols regardless of their weight. We have shown that platelet deposition in deeply injured vessels is inversely related to the amount of heparin per kilogram of body weight administered per minute of the procedure; that is, the more heparin given, the less the platelet deposition. Furthermore, mural thrombosis that occurred in all animals with deep arterial injury with low dosages of heparin (\(<3.1 \text{ units/kg/min}\)) was reduced to 25% of all animals given higher dosages of heparin (\(>3.1 \text{ units/kg/min}\)). In addition, administering a bolus injection of heparin just before angioplasty (protocol 2) appeared to maximize the effect of the total dosage of heparin. Pigs in protocol 2 received a second bolus injection of heparin just before arterial injury; this suggests that the heparin level at the time of the angioplasty is more important for platelet deposition than the mean concentration given throughout the experiment. This study showed an inverse relation between the acute heparin dosage and platelet deposition.

Compared with deep arterial injury, arteries with superficial injury (deendothelialization) were found to have significantly less platelet deposition with only a monolayer of platelets being deposited as previously reported.\(^6\) Heparin does not affect platelet deposition on these areas. To our knowledge, only Adelman et al.\(^\text{17}\) have reported a decrease in platelet adhesion to deendothelialized rabbit's aorta by infusing very large amounts of prostaglandin I\(_2\) (850 ng/kg/min). To date, no other agent has been reported that decreases platelet deposition on arteries with superficial injury. Ibuprofen and aspirin given in addition to heparin, which was given in a dose sufficient to prolong the activated partial thromboplastin time, had no additional benefit.
boplastin time by five times, are ineffective in reducing platelet deposition after subendothelial injury but do significantly reduce platelet deposition and thrombus formation on sites with deep arterial injury.18,19

Deep arterial injury exposes components of the arterial media, particularly collagen, elastic tissue, and smooth muscle cells, and it releases tissue thromboplastin. The generation of thrombin plays a pivotal role in thrombus formation because it activates platelets and the coagulation cascade. Thrombin is generated on the platelet surface by generation of the prothrombinase complex through both the extrinsic and intrinsic pathways of coagulation, but small amounts of thrombin exert a potent positive feedback, in part through activation of factor V; factors Va and Xa by binding with calcium to specific platelet membrane receptors can produce thrombin 280,000 times faster than factor Xa alone.20 Heparin is predominantly antithrombin, but it also acts earlier in the coagulation cascade to inactivate factor X (which is necessary for the prothrombinase complex to function) by increasing the rate at which antithrombin III combines with factor Xa.21 Thus, larger doses of heparin may reduce the generation of thrombin by the prothrombinase complex. As described here, the inhibitory effect of heparin is most apparent when platelet deposition exceeds a monolayer (as in deep arterial injury), and thus, heparin appears to inhibit platelet-to-platelet interaction. However, the mode of action of heparin on platelet-thrombus deposition during arterial injury remains to be fully elucidated.

In deep arterial injury, there is increased thrombogenicity of the substrate in the wall. Collagen types I and III, which are found in the arterial media and plaque, are more thrombogenic and stimulate greater platelet deposition than collagen types IV and V, which are found in the basement membrane and the subendothelial layer.22 Mural thrombosis forms in over 90% of deeply injured arteries despite therapeutic doses of heparin (100 units/kg) that prolong the activated partial thromboplastin time to between four and five times control.8 We have shown that this does not occur after heparin doses greater than or equal to 3.1 units/kg/min, and a bolus injection of heparin immediately before the injury is most effective. One previous study describes in a qualitative manner the prevention of thrombosis after arterial injury in dogs by heparin 200 units/kg.23 Heparin prolongs the bleeding time, and the mechanism of this effect is not known, but it may relate to a direct effect of heparin on platelet function.24,25 There have been several reports describing varying effects of heparin on in vitro platelet aggregability.26-28 The use of low molecular weight fractions of heparin that have a much higher anti Xa:anti IIa ratio than heparin itself24,29 has inhibited intracoronary thrombus formation after electrical injury.30 However, the antithrombotic potency of low molecular weight heparin was not related to its anti-Xa activity, which suggests that the anti-Xa activity of heparin is not the factor responsible for its antithrombotic effect.29 Further studies with more specific antithrombin agents are needed to elucidate the antithrombotic action of heparin and its low molecular weight derivatives.

Heparin has inhibited the proliferation of smooth muscle cells in vivo and in vitro,11-13,31-33 and this effect may also be of value after angioplasty by inhibiting an important step in the postulated mechanism of restenosis. However, our experimental design does not address this question; other studies addressing both this issue in vivo and the optimum duration of heparin therapy need to be performed.

Although the results of this study indicate that acute therapy with heparin is beneficial, further studies are necessary after balloon injury in patients to determine the optimal dosage, duration, and type of heparin therapy. These studies are also needed to resolve the controversy between proponents of using up to 15,000 units heparin as a bolus injection followed by an infusion of heparin immediately before the procedure and those who doubt the benefits of any heparin at all.

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Appendix
Relation obtained for each protocol when adjusted for heparin units per kilogram per unit of time.
Protocol 1. Log of platelet deposition = 6.22− 0.97 heparin units/kg/min
Protocol 2. Log of platelet deposition = 5.49− 0.97 heparin units/kg/min
Protocol 3. Log of platelet deposition = 7.14− 0.97 heparin units/kg/min

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