AN ANIMAL MODEL OF CORONARY THROMBOSIS AND THROMBOLYSIS
— Comparisons of Vascular Damage and Thrombus Formation in the Coronary and Femoral Arteries after Balloon Angioplasty —

MASAYUKI KATSURAGAWA, M.D., HISAYOSHI FUJIWARA, M.D., ATSUSHI KAWAMURA, M.D., THAN HTAY, M.D., YOSHIKI YOSHIKUNI, Ph.D.*, KAZUYA MORI, M.S.* and SHIGETAKE SASYAYAMA, M.D.

The purpose of this study was to compare vascular damage and thrombus formation in the coronary and femoral arteries after balloon angioplasty, and to develop a physiological animal model of intracoronary occlusive thrombus using the balloon angioplasty technique. Angioplasty of the left anterior descending coronary arteries of 14 dogs was performed with an oversized balloon catheter at a high inflation pressure (150 PSI). This was followed angiographically (PTCA protocol). Dogs that showed arterial occlusion were divided into 2 groups. The dogs in 1 group were killed with an overdose of sodium pentobarbital, and those in the other group were infused with a tissue-type plasminogen activator (t-PA; 300,000 unit/kg). Angioplasty of the femoral and profunda femoris arteries (n=5) was performed in 5 other dogs (PTA protocol). All of the animals were eventually sacrificed and tissue preparations were made from all 3 types of arteries. In the PTCA protocol, acute arterial occlusion was seen angiographically within 2 h in 10 of the 14 dogs. A histological study of the acutely occluded arteries (n=5) showed thrombotic occlusion and severe arterial damage with medial tearing. T-PA was infused to 5 of the dogs with acute occlusion, and all showed reperfusion. A histological study of these animals showed severe arterial damage, but no macroscopic thrombus. In 4 dogs without acute occlusion, none of the 10 arteries examined were acutely occluded. In the PTA protocol, none of the 10 arteries were acutely occluded. A histological study showed fewer thrombi and less severe arterial damage. The media and adventitia of normal coronary arteries had less elastic fiber than the normal femoral and profunda femoris arteries. Differences in arterial structure can account for the differences in arterial damage and thrombus formation. Thus, oversize balloon angioplasty of canine coronary arteries frequently resulted in acute thrombotic occlusion. This method may be useful as an animal model of thrombosis. (Jpn Circ J 1993; 57: 1000—1006)

Intra-coronary thrombolysis, an interventional therapy used for acute coronary thrombosis, is as important as percutaneous transluminal coronary angioplasty (PTCA). Several plasminogen activators and their adjunctive agents have been developed and investigated with regard to their use in intra-

Key words: Coronary thrombosis Balloon angioplasty Animal model

(Received December 1993; accepted April 28 1993)
The Third Division, Department of Internal Medicine, Faculty of Medicine, Kyoto University, Kyoto, Japan and *Research Laboratories, Nippon Shinyaku Co. Ltd., Japan
Mailing address: Hisayoshi Fujiiwara, M.D., Third Division, Department of Internal Medicine, Kyoto University Hospital, 54 Kawaracho, Shogoin, Sakyo-ku, Kyoto 606, Japan

1000 Japanese Circulation Journal Vol.57, October 1993
after angioplasty in rabbit iliac and pig carotid arteries. In these studies, acute thrombotic occlusion of the artery was not frequent. On the other hand in our previous study, acute thrombotic occlusion was frequently observed in canine coronary arteries after balloon angioplasty. We hypothesized that occlusive thrombi were more easily formed in coronary arteries than in systemic arteries after balloon angioplasty, due to differences in their arterial structure.

The purpose of this study was to compare vascular damage and thrombus formation in the coronary, femoral and profunda femoris arteries after balloon angioplasty, and to develop a novel animal model of occlusive thrombus that is based on angioplasty of the canine coronary artery. The thrombotic mechanism in this situation more closely resembles physiological conditions than the mechanisms of the methods described above.

METHODS

Nineteen male beagles weighing 10 to 13 kg were used. All of the dogs were fed a normal chow diet. The dogs were divided into 2 study groups. In 1 group (n=14), balloon angioplasty was performed on the coronary artery (PTCA protocol). In the other group (n=5), balloon angioplasty was performed on the femoral and profunda femoris arteries (PTA protocol).

Experimental procedure

The dogs were anesthetized with an intravenous injection of 30 mg/kg of sodium pentobarbital, after which they were intubated and mechanically ventilated with room air (Harvard respirator). Continuous electrocardiographic and arterial pressure monitoring was accomplished with a multichannel recorder. An incision was made under aseptic conditions that exposed the right carotid artery and external jugular vein. A drip infusion (0.09% NaCl, 0.149% KCl, 0.224% L-lactate Na and 4.3% Glucose) made via the external jugular vein was maintained at 10 ml/kg/h during the procedure. Systemic heparinization was not used in any of the protocols.

PTCA protocol

A sheath introducer (6-french) was in-
serted into the right carotid artery. An angiographic catheter (5-french; Kyoto special, Ueda L, Cook, Bloomington) was inserted into the orifice of the left coronary artery under fluoroscopy. Selective coronary angiography (right anterior oblique projection) was performed by manual injection of 3 ml of contrast medium (Iopamidol, Fig. 1-A), and was recorded on a videocassette recorder. A copper grid was then placed in the estimated position of the artery and recorded by fluoroscopy to measure the arterial diameter. A guide wire (Flexible-steerable, J-tip, .016", USCI, Billerica) was inserted through the angiographic catheter into the left anterior descending coronary artery. After exchanging the angiographic catheter, a balloon catheter (Mini-profile, 3.5×20 mm, USCI) was advanced into the distal half of the left anterior descending coronary artery over the guide wire. During this procedure, it was necessary to flush the lumen of the balloon catheter with saline containing 100 units/ml of heparin. Angioplasty was then performed by inflating the balloon catheter to 150 PSI for 5 min (Fig. 1-B). Angiography was performed at 30 min intervals until thrombotic occlusion was observed (up to 2 h). When thrombotic occlusion of the left anterior descending coronary artery was observed, 0.5 mg of isosorbide dinitrate was infused into the artery, and angiography was repeated to exclude the possibility of occlusion caused by vasospasm. Coronary angiography was repeated 1 h later to confirm the continued presence of occlusion.

Dogs that showed acute occlusion on the angiograms were divided into 2 groups. Dog in group 1 were killed with a lethal dose of sodium pentobarbital 1 h after thrombotic occlusion. Dogs in group 2 were given 30,000 unit/kg of tissue-type plasminogen activator (t-PA; Activase, Genentech, South San Francisco) as a bolus infusion 1 h after thrombotic occlusion. This was followed 1 h later by an intravenous drip infusion of 27,000 unit/kg of t-PA. Coronary angiography was performed at 30 min intervals. If reperfusion was observed during the infusion of t-PA, the infusion was immediately discontinued, and the dogs were killed with a lethal dose of sodium pentobarbital. Hearts of dogs that died due to reperfusion arrhythmia were removed immediately. TIMI trial criteria were used to define the occlusion or reperfusion of an artery8; TIMI grades of 0 and 1 were considered occlusion, and grades of 2 and 3 were considered reperfusion. If ventricular arrhythmia occurred during the thrombotic or
thrombolytic procedure, 20 mg of lidocaine was infused intravenously.

Dogs that did not show acute occlusion 2 h after angioplasty were killed immediately with a lethal dose of sodium pentobarbital.

**PTA protocol**

An 8-french sheath introducer was inserted into the right carotid artery. Selective arteriography of the bilateral femoral arteries was similarly accomplished with a 6-french Sones catheter (Fig. 2-A and C). PTA was performed in the femoral arteries and contralateral sides of the profunda femoris arteries of 5 dogs. Angioplasty of the femoral artery (n=5) was done with a balloon catheter (PE Plus II, 7×20 mm, USC1), and angioplasty of the profunda femoris artery (n=5) was accomplished with another balloon catheter (Mini Profile, 3.5×20 mm, USC1) at inflation pressures of 150 PSI for 5 min (Fig. 2-B and D). At 30 min intervals for up to 2 h after angioplasty, angiography was used to check for thrombotic occlusion, at which time all of the living dogs were killed with lethal doses of sodium pentobarbital.

**Tissue preparation and histological examination**

The heart and femoral arteries were removed from each dog after death. A 6-french polyethylene tube was inserted into the left main coronary artery or the proximal part of the femoral artery. Distal parts of the femoral and profunda femoris arteries, as well as the side branches, were ligated with silk braid. Each heart or femoral artery was perfused and fixed for 3 days with buffered 10% formalin at 100 mmHg. The site of angioplasty in the left anterior descending coronary artery, femoral or profunda femoris artery was cut serially and transversely into slices 2–3 mm thick. Each slice was inspected, and then embedded in paraffin. Slices 4 μm thick were then cut and stained with hematoxylin-eosin, elastic-van Gieson and phosphotungstic acid-hematoxylin (PTAH). Each specimen was examined under a light microscope, and the degree of thrombus formation and vascular injury were noted. Normal left anterior descending coronary arteries (n=5) were obtained from dogs in the PTA group, and normal femoral and profunda femoris arteries (n=5 each) were obtained from dogs in the PTCA group. In the histological study, these served as the normal coronary, femoral and profunda femoris arteries.

**Statistics**

The Fisher exact test was used to analyze the difference between the probability of arterial occlusion after angioplasty in the coronary and femoral or profunda femoris arteries. A p value of less than 0.05 was considered significant.

**RESULTS**

**Angiographic findings in the PTCA protocol group** (Fig. 3)

The diameters of the distal halves of the anterior descending coronary arteries were 1–2 mm, as measured on the angiogram. Acute occlusion of the left anterior descending coronary artery was seen in 8 of the 14 PTCA dogs within 1 h after angioplasty (Fig. 1-C), and in 10 dogs after 2 h. None of the arteries showed occlusion 30 min after angioplasty. In each of the 10 dogs, acute occlusion of the artery continued for 1 h. Five of
the 10 dogs with acute occlusion were randomly selected and killed 1 h after occlusion for the histological study (group 1). T-PA was infused into the other 5 beagles 1 h after occlusion (group 2). In all of the dogs in group 2, reperfusion (TIMI grade 3) took place within 1 h after t-PA infusion (Fig. 1-D). After reperfusion, ventricular arrhythmia, including ventricular tachycardia, occurred in all 5 dogs, 2 of which died of ventricular fibrillation. Coronary angiography showed a haziness or a filling defect in the dilated segment in the 4 dogs without acute coronary occlusion. There was no significant difference in the diameter of the left anterior descending artery before angioplasty between the dogs with and without acute occlusion. During the procedure, extravasation of the contrast media was not seen in any of the angiograms.

**PTA group (Fig. 3)**

The average diameter of the middle part of the femoral artery, as measured on the angiogram, was approximately 3–4 mm and that of the profunda femoris artery was 1–2 mm. At angioplasty, neither the balloon nor the femoral or profunda femoris arteries were fully expanded in most cases, even though the oversized balloon was inflated at a pressure of 150 PSI (Fig. 2-B and D). Two h after angioplasty, none of the arteries showed occlusion.

**Macroscopic and histological findings**

Macroscopically, the coronary arteries of...
the 5 dogs in group 1 were completely occluded with thrombi, which were composed of platelets, red blood cells and fibrin (Fig. 4-A). Serial slices of the coronary arteries showed that a mural thrombus had formed in the dilated segment, which became occlusive in the distal part of the segment. The coronary arteries of the dogs in group 2, however, showed few or no macroscopic thrombi (Fig. 4-B). The coronary arteries of the 4 dogs without acute coronary occlusion after angioplasty showed mild to moderate stenosis with thrombus. The coronary arteries of the dogs in groups 1 and 2 (Fig. 4-B and C) showed severe injury to the arterial wall with endothelial denudation, tearing of the media and elastica interna, and adventitial bleeding. The degrees of damage to the coronary arterial wall were similar for both groups. However, this damage was less severe in dogs that did not show acute occlusion.

In the PTA group, macroscopic mural thrombi formed in 4 arteries, but occlusive thrombi were not present 2 h after angioplasty. Arterial injury included endothelial denudation and stretched media in all arteries (Fig 4-D). Tearing of the media was seen in 5 arteries, but was less severe than that seen in the coronary artery (Fig. 4-E). A histological study of normal beagle arteries showed that the media and adventitia of the coronary artery have fewer elastic fibers than the femoral and profunda femoris arteries (Fig. 4-C and F).

**DISCUSSION**

We performed angioplasty of canine coronary arteries with an oversized balloon catheter. Acute coronary thrombotic occlusion was present in 10 of the 14 dogs 2 h after angioplasty. Reperfusion of the occluded artery was induced with a tissue-type plasminogen activator. All of these processes were clearly visible on the coronary angiograms. Therefore, this animal model can be used to evaluate other thrombolytic agents. In addition, since this is a closed-chest procedure, long-term follow-up is possible. The thrombotic mechanism after angioplasty is believed to be as follows: Dilatation of the coronary artery by the oversized balloon at high inflation pressure causes severe arterial injury with medial tearing. The connective tissues of the media and adventitia are exposed to blood flow, which causes platelet aggregation and thrombus formation. This is similar to the clinical process seen in intra-coronary thrombosis that follows rupture of atheromatous plaque and angioplasty. Our results showed that vascular damage was more severe in coronary arteries with occlusive thrombi than in coronary or femoral arteries without occlusive thrombi. This supports the idea that the extents of platelet aggregation and thrombus formation depend on the degree of vascular damage.

Angioplasty of the systemic arteries (aorta, carotid, femoral artery, etc.) is commonly used to study thrombosis and intimal proliferation after angioplasty. In the models currently in use, acute thrombotic occlusion is rare.

Our observation that occlusive thrombi were not formed in the femoral and profunda femoris arteries confirm previous reports. In our study, however, acute occlusive thrombi frequently formed after angioplasty of the coronary artery, and the degree of arterial damage was more severe in that artery than in the femoral and profunda femoris arteries. Although the balloons and arteries were fully expanded during inflation for coronary artery angioplasty, they were not fully expanded for angioplasty of the femoral and profunda femoris arteries. The diameter of the coronary artery before angioplasty was smaller than that of the femoral artery (1-2 vs 3-4 mm), but similar to that of the profunda femoris artery (1-2 vs 1-2 mm). The diameters of the balloons used were 3.5 mm for the coronary artery, 7 mm for the femoral artery and 3.5 mm for the profunda femoris artery. The pressure applied at angioplasty was the same for all of the groups (150 PSI). This indicates that the difference between the responses of the coronary artery and the 2 systemic arteries is not related to the angioplasty procedure.

A histological study of normal beagle arteries showed that the media and adventitia of the coronary artery had fewer elastic fibers than the femoral and profunda femoris arteries. This weaker basic structure of the coronary artery (in comparison to the structures of the systemic arteries) would account for the greater dilatation and more severe
medial damage to the coronary artery during angioplasty. Because occlusive thrombi frequently form in the coronary artery, angioplasty of that canine artery provides a novel animal model of thrombosis and thrombolysis. Moreover, our results indicate that it is better to use the coronary artery itself when studying intra-coronary thrombosis or intimal proliferation after coronary angioplasty because there is a similar difference in vascular structure between the coronary artery and middle to large systemic arteries in humans.

CONCLUSION

A high incidence of acute occlusive thrombus was found in canine coronary arteries within 2 h of experimental angioplasty with an oversized balloon catheter. This angioplasty method is considered to be a new and useful animal model of thrombosis.

Acknowledgments

We are grateful to Miss Yumiko Yamamoto and Mika Yoshida for their technical assistance.

REFERENCES

12. WALLER BF, GORFINKEL HJ, ROGERS FJ, KENT KM, ROBERTS WC: Early and late morphologic changes in major epicardial coronary arteries after percutaneous transluminal coronary angioplasty. Am J Cardiol 1984; 53; 42C–47C