ANTITHROMBOTIC EFFECT AND REPERFUSION BY LOW MOLECULAR WEIGHT HEPARIN IN A CANINE MODEL OF CORONARY ARTERY THROMBOSIS

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The lability of an intracoronary thrombus, which is common in the case of unstable angina, indicates the presence of endogenous fibrinolysis. To prove our hypothesis that suppression of coagulation facilitates the thrombolytic process, low molecular weight heparin (LMWH) was given intravenously to dogs with intracoronary thrombi induced by copper coils. LMWH caused reperfusion in this model and suppressed the formation of intracoronary thrombi, thus supporting our hypothesis. (Jpn Circ J 1993; 57: 553–557)

CORONARY arterial thrombus causes acute ischemic events such as unstable angina and acute myocardial infarction. An intraluminal thrombus facing blood has a potential to enlarge further by stimulating coagulation and platelet aggregation. However, a thrombus is not always stable. In the presence of unstable angina pectoris it is labile and intermittent. In acute myocardial infarction, the initially occluded infarcted vessel reopens spontaneously. The frequency of this reopening increases as time after onset increases. These findings indicate the presence of local endogenous fibrinolytic activity that might be triggered by a thrombus. The balance between the vector of thrombus expansion and that of fibrinolysis may determine the fate of the thrombus.

We therefore hypothesize that suppression of coagulation may facilitate the thrombolytic process. To prove our hypothesis, low molecular weight heparin (LMWH) was administered in a canine coronary thrombosis model. LMWH was employed because its half-life is longer than that of unfractionated heparin and it has a larger ratio of anti-Xa to anti-IIa activity than the unfractionated heparin which is considered beneficial in lowering the risk of complications due to bleeding.

MATERIALS AND METHODS

Coronary Thrombosis Model
Coronary thrombus was induced in adult mongrel dogs (10.0 to 23.5 kg), as previously described. The dogs were anesthetized with intravenous sodium pentobarbital (15 mg/kg)

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and received additional doses as required. Ventilation was maintained by an R-60 pump (Aika, Tokyo, Japan) delivering room air through an endotracheal tube.

An 8F Sones catheter was placed using fluoroscopy in the ascending aorta via the left carotid artery. A guide wire (0.014 in.) for coronary angioplasty was inserted through the catheter into the left anterior descending artery. A copper coil (2 mm in length x 1 mm in outside diameter) was advanced along the guide wire into the mid portion of the left anterior descending artery. The wire was then withdrawn. Coronary thrombus formed thereafter, and was confirmed by coronary angiography using a contrast medium (Urographin 76%, Nihon Schering Corp., Osaka, Japan). Cineangiograms were taken on a cineangiographic system (WHA-10, Shimadzu Company Ltd., Kyoto, Japan) equipped with a videographic apparatus (NV-9240X, Matsushita Electric Industrial Company Ltd., Osaka, Japan).

**Assessment of Coronary Angiograms**

Coronary angiograms were assessed based on TIMI gradings as follows: grade 0: complete occlusion; grade 1: visualization delayed and periphery not visualized; grade 2: visualization delayed and affected down to periphery, but contrast medium slowed; grade 3: visualization affected down to periphery without any delay in contrast medium.

**Monitoring and Blood Sampling**

The femoral artery and vein were cannulated to allow blood pressure recording, blood sampling, and drug administration. The electrocardiogram (I, aVL, and chest leads) was monitored and hemodynamic and electrocardiographic parameters were recorded on a 4-channel polygraph system (Nihon Kohden, Japan). Blood samples were collected in citrate, and were then immediately cooled on ice, centrifuged, and frozen at −80°C until they were assayed.

**Measurement of Coagulant and Anti-coagulant Activities**

Anti-Xa activities, anti-IIa activities, APPT3 prothrombin time; and alpha2-antiplasmin activity10 were measured, as previously described.

**Experimental Protocols**

**Study I:** Study I was performed to evaluate the inhibitory effects of LMWH on the generation of the coronary arterial thrombus. LMWH was administered intravenously at a dose of 0.5, 1, or 2 mg/kg, 10 min before insertion of a copper coil (n=5 at each dose). An equal volume of physiological saline was administered in the control group (n=5). After insertion of a coil, angiography was performed at 5 min intervals for 60 min. Blood samples were obtained before and 15, 20, 40, and 70 min after drug administration.

**Study II:** In study II, intravenous LMWH (1, or 2 mg/kg, n=6 at each dose) or saline (n=5) was given after the left anterior descending artery had been occluded (TIMI grade 0 or 1) following coil insertion. In order to determine whether
Reperfusion by Low Molecular Weight Heparin

Fig. 2. LMWH activates anti-Xa
Anti-Xa activity was determined before and after the intravenous administration of physiological saline (open triangles) or LMWH (0.5, 1.0, and 2.0 mg/kg represented by closed triangles, open circles, and closed circles, respectively). Data are mean ± SD (n = 5). *p < 0.05: placebo, 0.5 or 1.0 mg/kg vs 2 mg/kg at 20 and 40 min (by Tukey's test).

Fig. 3. Effects of LMWH on the anti-IIa activity
Anti-IIa activity was measured and expressed as in Fig. 2.

Fig. 4. Effects of LMWH on APTT
APTT was determined and expressed as in Fig. 2.

Fig. 5. Effects of LMWH on the prothrombin time
The prothrombin time was assessed and expressed as in Fig. 2.

Reperfusion developed, angiography was repeated at 5, 10, 15, 20, 25, 30, 40, 50, and 60 min after the administration of LMWH or saline. Blood samples were drawn before, 30 min after, and 60 min after drug administration.

Statistics
All data were expressed as mean ± standard deviation. Data were compared using either a chi-square test, a Dunn multiple comparison test with one-way analysis of variance, or Tukey's test. A p value of less than 0.05 was considered significant.

RESULTS

Study I
When LMWH was administered before coil insertion, LMWH significantly prolonged the time interval between coil insertion and thrombus formation (represented by TIMI 0) at a dosage of 2 mg/kg (Fig. 1). The time interval also tended to be longer at a dosage of 1 mg/kg. The activities of anti-
Fig. 6. LMWH elicits reperfusion
LMWH (1.0 and 2.0 mg/kg expressed by open and closed circles, respectively, n=6) or saline (open triangles, n=5) was administered after the left anterior descending artery had been occluded by the copper coil-induced thrombus. Coronary arteriography was then repeated periodically, and the degree of the distal flow was expressed by TIMI scores. Data are mean values. *p<0.05.

Xa and anti-Ⅱa increased dose-dependently with a peak at approximately 15 min, as shown in Figs. 2 and 3. Similarly, APTT prolonged in a dose-dependent fashion (Fig. 4). PT did not change significantly (Fig. 5).

**Study II**

The flow of the segments distal to the coil was progressively restored with time, as shown in Fig. 6. Sixty minutes after the administration of LMWH, reperfusion was observed in 2 of 6 cases and in 5 of 6 cases at doses of 1 and 2 mg/kg of LMWH, respectively. No reperfusion occurred in the control group. There was no difference in the blood pressure or heart rate between the control and LMWH group and Alpha2-antiplasmin activity did not change in any group (data not shown).

**DISCUSSION**

In the present study, preadministered LMWH significantly prolonged the time interval between coil insertion and occlusion of the artery, elevating anti-Xa and anti-Ⅱa activities (Study I). This finding indicates that LMWH may have suppressed the formation of coronary arterial thrombus.

LMWH caused reperfusion when it was administered after the vessel had been occluded following coil insertion. This is the first report showing that LMWH can potentially induce reperfusion without concomitant use of any fibrinolytic agent. LMWH did not change the activity of plasma alpha2-antiplasmin, which indicates that fibrinolysis was not systemically activated. There may be 2 local vectors around the thrombus: thrombosis expansion and endogenous fibrinolysis. Actually, in acute myocardial infarction, the initially occluded infarct-related vessel sometimes reopens spontaneously as time from onset increases indicating lysis of the occlusive intracoronary thrombus. In unstable angina pectoris, the coronary arterial thrombus is labile and intermittent. These observations of spontaneous thrombolysis may be caused by endogenous fibrinolysis. In the present study, reperfusion was considered to be induced by the relative enhancement of local endogenous fibrinolysis secondary to suppression of coexisting thrombosis by LMWH.

The effects of LMWH mentioned above may be beneficial in the prevention or treatment of coronary arterial thrombosis that is the principal underlying lesion leading to unstable angina or myocardial infarction. The combined use of LMWH with fibrinolytic agents (such as urokinase, streptokinase, or tissue-type plasminogen activator) may potentiate the action of fibrinolytic agents and enable thrombolysis to be achieved at a lower dosage of these agents. However, the potential benefit of LMWH may be less in unstable angina than in acute myocardial infarction, because thrombi in unstable angina are thought to be rich in platelets and to be resistant to fibrinolytic therapy.

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