Inhibitory Effects of Heparin, Aspirin and Ketanserin on Coronary Artery Vasoconstriction After Arterial Balloon Injury in Hypercholesterolemic miniature Pigs

TAKESHI KUGA, MD, YUICHI OHARA, MD, HIROSHI HATA, MD, YOUJI HIRAKAWA, MD, HITONOBU TOMOIKE, MD, AKIRA TAKESHITA, MD
Fukuoka and Yamagata, Japan

Objectives. The present study aimed to clarify the effects of heparin, aspirin and ketanserin on coronary artery vasoconstriction after arterial balloon injury.

Background. The mechanisms of coronary artery vasoconstriction after coronary angioplasty are not well understood.

Methods. After being fed a cholesterol-rich diet for 1 month, 71 Göttingen miniature pigs were randomly allotted to five groups: 16 pigs with no pretreatment (group A); 21 pigs pretreated with heparin, 3,000 U (group B); 13 pigs pretreated with aspirin, 50 mg/day orally for 2 days (group C); 11 pigs pretreated with ketanserin, 1 mg/kg body weight (group D); 10 pigs pretreated with aspirin, 50 mg/day for 2 days, heparin, 6,000 U and ketanserin, 1 mg/kg (group E). After this pretreatment, the left anterior descending or the left circumflex coronary artery, or both, was denuded by a 2F balloon catheter.

Coronary artery vasoconstriction routinely occurs after percutaneous transluminal coronary angioplasty (1). Because abrupt vessel closure after coronary angioplasty is not infrequent and has an unfavorable prognosis (2–4), it is clinically important to elucidate the mechanisms related to coronary vasoconstriction after arterial injury. In experimental studies, vasoconstriction identical to that in humans has been reproduced in various vessels after arterial injury in vivo (5,6) and in vitro (7–9). These previous studies proposed two mechanisms of vasoconstriction after vessel injury: platelet-dependent and platelet-independent mechanisms. Platelet deposition is consistently demonstrated at the site of mechanical injury (5,6,10). A close positive exponential correlation was noted between the severity of vasoconstriction and the extent of platelet deposition (5). Intracoronary injection of aggregating platelets constricted denuded coronary arteries (11). These lines of evidence suggest a critical role of vasoactive substances released from aggregating platelets in coronary vasoconstriction after arterial injury; however, it is not known which substances play a major role in such platelet-dependent coronary vasoconstriction. Platelet-independent mechanisms may also play a role in coronary vasoconstriction after arterial injury (7–9); however, it also remains unknown to what extent platelet-dependent or platelet-independent mechanisms contribute to coronary vasoconstriction after arterial injury in vivo.

The present study was designed to elucidate pharmacologically 1) to what extent platelet-dependent mechanisms contribute to coronary artery vasoconstriction after arterial injury, and 2) which vasoactive substances released from aggregating platelets play a major role in such platelet-dependent coronary vasoconstriction.

Methods

Experimental protocol. Seventy-one male Göttingen miniature pigs were housed individually under conditions of...
controlled room temperature (12-14) and were fed a semi-
synthetic diet (14), including peanut oil (2.3%), cholesterol
(2%), sodium cholate (1.1%), salt mixture (1.4%), vitamin
mixture (3.5%) and cellulose (8.9%). After 1 month on this
diet, these pigs were lightly anesthetized with intramuscular
administration of ketamine hydrochloride, 12.5 mg/kg body
weight, followed by intravenous administration of sodium
dentobarbital, 20 mg/kg. The trachea was then intubated,
and the animals were ventilated with positive-pressure res-
piration via room air and supplemental oxygen (Shimano).
The carotid artery was aseptically exposed and a green Kifa
catheter was inserted. Just after catheter insertion, heparin,
500 U, was administered intravenously, and coronary arte-
tiography was performed before and 5 min after intravenous
administration of nitroglycerin, 20 μg/kg, as previously
described (12-14). The pigs were then randomly allotted to
five groups: group A, 16 pigs without any additional pretreat-
ment; group B, 21 pigs pretreated with intravenous heparin,
3,000 U 5 min before arterial injury; group C, 13 pigs
pretreated with aspirin, 50 mg orally 48 and 72 h before the
experiment; group D, 11 pigs pretreated with intravenous
ketanserin, 1 mg/kg 15 min before arterial injury, and group
E, 10 pigs pretreated with aspirin, 50 mg 48 and 72 h before,
ketanserin, 1 mg/kg, 15 min before and heparin, 6,000 U
5 min before arterial injury.
Sixty minutes after intravenous administration of nitro-
glycerin, 20 μg/kg, a 2F balloon catheter (Edwards Labora-
tory) was advanced under fluoroscopic monitoring into the
distal portion of the left anterior descending or left circum-
flex coronary artery. The balloon was inflated by the manual
injection of 0.06 ml of a 50% solution of contrast medium
(Urografin 76, Nihon Schering) and was withdrawn for 4 cm
toward the proximal portion of the artery. This procedure
was repeated three times in each pig within 1 min. Coronary
artery injury was performed in 14 left anterior descending
and 2 left circumflex coronary arteries in group A, 11 left
anterior descending and 10 left circumflex coronary arteries
in group B, 6 left anterior descending and 7 left circumflex
coronary arteries in group C, 5 left anterior descending and
6 left circumflex coronary arteries in group D and 5 left
anterior descending and 5 left circumflex coronary arteries in
group E. The region of balloon injury was recorded concon-
tantly on videotape and cine films for later analysis.
Selective coronary angiography was performed every 3 min for the initial 15 min, and then 20 and 30 min after arterial injury in 10 pigs from groups A, D and E. In other pigs, coronary angiography was performed 6 min after arterial injury. The electrocardiogram (ECG) was recorded with leads I, II, III, V₁ and V₆. Arterial pressure was measured with a Kifa catheter connected to a strain gauge
manometer and stored on a tape with a frequency modula-
tion data recorder (DFR 3915, Sony).
Quantitative analysis of coronary angiography. Cine film
was projected on a view screen (ELMO-35B, Nishimoto
Sangyo). The end-diastolic frame was selected by ECG
waves recorded on cine film, and photocopies (13 × 18 cm)
were made for measurements of the diameter of the coronary
artery. The diameter of the coronary artery was measured
with a caliper in blinded manner by at least two observers
(12-14). With this technique, we confirmed excellent corre-
lation between the repeated measurements (r = 0.99, p < 0.001) and between different observers (r = 0.96, p < 0.001).
Coronary artery diameter was measured at the site where
maximal diameter reduction was noted. Coronary artery
vasoconstriction was assessed as percent lumen diameter
reduction compared with that after intravenous nitroglycerin
(20 μg/kg) (12-14).
Statistics. Data are shown as the mean value ± SEM (n is the
number of experiments). Statistical analysis of temporal
changes in the coronary artery diameter evoked by interven-
tion was performed by one-way analysis of variance. A
p value < 0.05 was considered indicative of statistical
significance.
Results
There was no significant difference among the five groups
in body weight, baseline hemodynamic variables (arterial
pressure and heart rate) or serum cholesterol level (Table 1).
Intravenous administration of ketanserin, 1 mg/kg (groups D
and E), significantly decreased mean arterial pressure
(−12 ± 4 and −10 ± 3 mm Hg) and heart rate (−15 ± 6 and
−19 ± 5 beats/min), respectively (p < 0.05 vs. baseline), but
mean arterial pressure and heart rate after administration of
ketanserin in groups D and E were comparable to those in
group A.
Group without pretreatment. Figure 1 shows serial angio-
grams before and 6 min after arterial injury in a pig from
group A. The coronary artery vasoconstriction was not
homogeneous along the injured segment. Chronologic
changes in percent lumen reduction at the site of arterial
injury are summarized in Figure 2. In group A, coronary
vasoconstriction was maximal at 6 min after the injury and
then gradually reverted to the control level within 30 min,
whereas the lumen diameter of n uninjured coronary arteries
remained unchanged. Arterial pressure and heart rate were
stable during coronary vasoconstriction in all groups.
Groups with pretreatment. Figure 3 shows the inhibitory
effects of heparin, aspirin, ketanserin and a combination of
these three drugs on coronary vasoconstriction 6 min after
arterial injury. Coronary vasoconstriction was 28 ± 6% in
group B, 25 ± 5% in group C, 26 ± 7% in group D and 24 ±
5% in group E; these values were significantly lower than
that in group A (56 ± 3%, p < 0.01); however, vasoconstric-
tion in group E was comparable to that in groups B, C and D.
The time course of vasoconstriction after arterial injury was
also comparable in groups D and E (Fig. 2).
Discussion
To clarify the mechanisms of coronary artery vasocon-
striction after angioplasty, we used porcine coronary arteries
because the coronary circulation, platelet-coagulation system and nature of atherosclerosis in pigs closely resemble those in humans. Moreover, the pigs were fed a cholesterol-rich diet to mimic the cholesterol level of patients with ischemic heart disease. The present study demonstrates that coronary artery vasoconstriction after arterial injury was almost halved by heparin, 3,000 U, aspirin, 50 mg/day for 2 days or ketanserin, 1 mg/kg, and 2) a combination of heparin, 6,000 U, aspirin, 50 mg/day for 2 days and ketanserin, 1 mg/kg, showed no additive inhibitory effects on coronary artery vasoconstriction after arterial injury.

Platelet-dependent constriction. Although heparin is used clinically as an anticoagulant drug, it has an inhibitory effect on platelet aggregation at injured porcine carotid arteries after angioplasty (15). In porcine carotid arteries, aspirin, 1 mg/kg per day, also reduced constriction after angioplasty from 37% to 21%, which was accompanied by the inhibition

---

Table 1. Baseline Data on 71 Pigs in the Five Study Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>(n = 16)</th>
<th>(n = 21)</th>
<th>(n = 13)</th>
<th>(n = 11)</th>
<th>(n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>19.1 ± 0.4</td>
<td>23.3 ± 0.5</td>
<td>19.1 ± 0.9</td>
<td>19.0 ± 0.6</td>
<td>20.1 ± 1.0</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>103 ± 3</td>
<td>103 ± 3</td>
<td>108 ± 4</td>
<td>101 ± 4</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>80 ± 3</td>
<td>79 ± 4</td>
<td>81 ± 2</td>
<td>74 ± 5</td>
<td>77 ± 6</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>138 ± 7</td>
<td>131 ± 9</td>
<td>130 ± 15</td>
<td>137 ± 9</td>
<td>140 ± 14</td>
</tr>
<tr>
<td>Change in MBP by ketanserin (mm Hg)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Change in HR by ketanserin (beats/min)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>526 ± 38</td>
<td>501 ± 31</td>
<td>517 ± 29</td>
<td>501 ± 20</td>
<td>512 ± 59</td>
</tr>
</tbody>
</table>

Values are expressed as mean value ± SEM. DRP = diastolic blood pressure; HR = heart rate; MBP = mean blood pressure; SBP = systolic blood pressure. Pigs in groups C and E were given aspirin orally for 2 days before the study; however, this treatment did not alter baseline data. Intravenous administration of heparin did not change mean blood pressure or heart rate significantly in groups B and E. Changes in mean blood pressure and heart rate by intravenous ketanserin were evaluated 15 min after administration of ketanserin.

---

Coronary artery vasoconstriction in a pig from group A after arterial injury. Selective coronary angiograms before (A) and after (B) coronary artery injury. A, The injured segment is indicated by arrows. B, Coronary angiogram performed 6 min after balloon injury shows wall irregularity and >60% narrowing at the peripheral edge of balloon injury. There is no significant narrowing of the left circumflex coronary artery or the site distal to the balloon injury.

---

Figure 1. Coronary artery vasoconstriction in a pig from group A after arterial injury. Selective coronary angiograms before (A) and after (B) coronary artery injury. A, The injured segment is indicated by arrows. B, Coronary angiogram performed 6 min after balloon injury shows wall irregularity and >60% narrowing at the peripheral edge of balloon injury. There is no significant narrowing of the left circumflex coronary artery or the site distal to the balloon injury.

Figure 2. Time course of coronary artery vasoconstriction after arterial injury. The coronary diameter during treatment with nitroglycerin before injury was normalized at 1.0. Time indicates the minutes elapsed after the end of arterial injury. C = control condition before the injury. The extent of maximal coronary vasoconstriction in group D (△) or in group E (□) is significantly (p < 0.05) smaller than that in group A (○). The time course of coronary vasoconstriction after arterial injury was comparable in groups D and E. The diameter of noninjured coronary arteries in groups A (○), D (△) and E (□) did not show any significant change over 30 min. n = 10 in each group. Values are mean value + SEM.
of platelet aggregation (5). Therefore, the inhibitory effect of heparin or aspirin on coronary artery vasoconstriction after arterial injury in hypercholesterolemic pigs may suggest an involvement of a platelet-dependent mechanism, although platelet aggregation was not measured in the present study.

The inhibitory effect of ketanserin on coronary vasoconstriction suggests that serotonin may be one of the mediators in such vasoconstriction after arterial injury. The inhibitory effect of ketanserin did not differ significantly from that of a combination of ketanserin, heparin and aspirin. These results suggest that serotonin may be a major mediator in platelet-dependent coronary vasoconstriction after arterial injury. In canine coronary arteries, platelet-dependent coronary vasoconstriction is mediated by thromboxane A2 and serotonin (16), whereas in porcine coronary arteries, platelet-dependent vasoconstriction is mediated largely by serotonin but not by thromboxane A2 (17), a finding consistent with our present results.

Platelet-independent constriction. Balloon angioplasty has been reported to cause stretch-dependent contraction in isolated, perfused whole vessel segments of rabbit aortas, pig carotid arteries (7) and human coronary arteries (8) independent of platelet aggregation and neurogenic input. The inhibition of platelet-dependent constriction by heparin, ketanserin and aspirin in our study did not show additive effects and did not abolish coronary vasoconstriction after arterial injury. The in vitro study (17) indicated that platelet-induced vasoconstriction of porcine coronary arteries was totally abolished by ketanserin. In porcine carotid arteries (7), the cross-sectional areas of the angioplasty segments decreased by platelet-independent mechanisms an average of 41%, which is nearly equivalent to the degree of coronary vasoconstriction in the pigs in our group E (the decrease in cross-sectional area was ~42%). These results suggest that platelet-independent mechanisms may also contribute to coronary vasoconstriction after arterial injury.

In the present study, we did not examine the mechanisms of platelet-independent coronary vasoconstriction after arterial injury. This platelet-independent vasoconstriction appears to be mediated by endothelium-derived cyclooxygenase products because it is abolished by pretreatment with indomethacin or ibuprofen (7). In the present experiment, pigs in groups C and E received aspirin (50 mg) 48 and 72 h before arterial injury, and were aspirin free for 48 h before injury. Because the cyclooxygenase activity of endothelial cells may be recovered at the time of arterial injury (10), we cannot exclude the contribution of endothelium-derived cyclooxygenase products to platelet-independent constriction after arterial injury in this experiment.

We are grateful to Kensuke Egashira, MD and Hiroaki Simokawa, MD, Kyushu University, for comments on the manuscript.

References


15. Hasas M, Gnesett JH, Penny WI, et al. Importance of adequate heparin...
dosage in arterial angioplasty in a porcine model. Circulation 1986;78:
654–60.
vasoconstriction of large epicardial canine coronary arteries in vivo:
thromboxane A₂ and serotonin are possible mediators. Circulation 1989;
17. Shimokawa H, Aarhus LL, Vahanvaty PM. Porcine coronary arteries
with regenerated endothelium have a reduced endothelium-dependent
responsiveness to aggregating platelets and serotonin. Circ Res 1987;61:
256–70.
18. Jaffe EA, Weckler BB. Recovery of endothelial cell prostacyclin produc-