Efficacy of platelet depletion in counteracting the detrimental effect of acute hypercholesterolemia on infarct size and the no-reflow phenomenon in rabbits undergoing coronary artery occlusion-reperfusion

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ABSTRACT It has recently been demonstrated that acute hypercholesterolemia per se, independently of its atherogenic effect, increases the extent of myocardial injury in rabbits undergoing coronary artery occlusion-reperfusion. Estimation of myocardial blood flow after reperfusion indicated that this deleterious effect was due to a vascular obstruction that limited the efficacy of reperfusion. The goal of this study was to evaluate the role played by platelets in contributing to the occurrence of this deleterious effect. Accordingly, New Zealand White rabbits were fed a standard laboratory chow diet (plasma cholesterol 67 ± 12 mg/dl) or a 2% cholesterol-enriched diet for 3 days (plasma cholesterol 329 ± 70 mg/dl). In a first series of experiments autologous platelets were labeled with 111In-oxine. After labeling, platelets were reinfected in the same animal and 30 min later coronary artery occlusion (CAO) was induced. CAO was maintained for 30 min followed by 5.5 hr of reperfusion. The animals were then killed, their hearts were excised, and each left ventricle was divided into ischemic and normally perfused samples. Myocardial samples were then counted in a gamma counter. Platelet accumulation ratio, i.e., 111In activity in the ischemic myocardium per gram of tissue divided by 111In activity in the normal myocardium per gram of tissue, was calculated. The ratio was 2.4 ± 0.2 (mean ± SEM) in controls (n = 7) and 10.3 ± 1.0 in the cholesterol-fed group (n = 6, p < .001), indicating that a marked accumulation of platelets occurs in the ischemic myocardium of hypercholesterolemic rabbits. To evaluate the importance of this phenomenon, another series of experiments was performed. Twenty rabbits were fed a standard diet (n = 10) or a cholesterol-enriched diet (n = 10). One hour before CAO five controls and five hypercholesterolemic animals received 2 ml of goat anti-rabbit platelet serum (APS). One hour after administration of APS platelet count decreased by 97.8% and 97.6% in normocholesterolemic and hypercholesterolemic rabbits, respectively. CAO was maintained for 30 min followed by 5.5 hr of reperfusion. Infarct size (% of HZ) was 38.1 ± 1.4% and 83.5 ± 5.1% in normocholesterolemic and hypercholesterolemic animals that did not receive APS (p < .001). The nonreperfused myocardium (NRZ) was also strikingly larger in the cholesterol-fed group than in normocholesterolemic rabbits, being 86.1 ± 3.8% and 19.1 ± 3.7% of HZ, respectively (p < .001). Administration of APS had no effect on infarct size in normocholesterolemic rabbits (42.2 ± 2.5% of HZ), but caused a dramatic decrease to 47.1 ± 3.7% of HZ in cholesterol-fed animals, which was not statistically different from that observed in APS-treated or untreated controls. Similarly, APS reduced NRZ in hypercholesterolemic rabbits (to 26.5 ± 5.8% of HZ), but did not affect NRZ in normocholesteroleric rabbits (21.5 ± 2.7% of HZ). We conclude that (1) a marked intramyocardial accumulation of platelets occurs in hypercholesterolemic rabbits undergoing CAO-reperfusion, and (2) platelet depletion completely reverses the detrimental effect of acute hypercholesterolemia on myocardial infarct size and the no-reflow phenomenon.

tem independently of its atherogenic effect. Free or lipoprotein-bound cholesterol has been reported to sensitize isolated dog coronary arteries to various vasoconstricting agents. Vasoconstriction in response to ergonovine is augmented in coronary arteries from spontaneously hypercholesterolemic rabbits with no visible atherosclerosis, and is apparently mediated through a serotoninergic mechanism. In addition, elevated plasma cholesterol has been reported to potentiate the coronary artery response to norepinephrine in dogs. Furthermore, hypercholesterolemia may adversely influence the course of myocardial ischemia. We have recently demonstrated that acute hypercholesterolemia causes larger myocardial infarctions in hypercholesterolemic, nonatherosclerotic rabbits undergoing coronary artery occlusion-reperfusion than in normocholesterolemic animals. This increase in myocardial damage is accompanied by an increase in the extent of the area of no-reflow after reperfusion, thus suggesting that aggregation of some blood cellular components within the myocardial vasculature is responsible for this detrimental effect.

Platelet accumulation in areas of myocardial infarction has been demonstrated histologically, with 9Cr-labeled platelets, and with 111In-labeled platelets. Other studies also suggest the crucial role that platelet aggregate microemboli may play in infarct extension and fatal arrhythmias. Platelet function is known to be greatly influenced by the lipid composition of the extracellular fluid, as demonstrated by the observation that platelets from patients with hyperlipoproteinemia have higher responsiveness to various aggregating agents. The same finding was achieved by incubating normal platelets in a medium rich in cholesterol liposomes. Moreover, Burrell and Butler found that feeding rabbits a cholesterol-enriched diet significantly reduced the platelet half-life.

The present study was undertaken to determine whether platelets contribute to the increased myocardial damage during coronary artery occlusion-reperfusion when acute hypercholesterolemia is induced in rabbits. Accordingly, an initial series of experiments was performed to establish whether hypercholesterolemic rabbits undergoing coronary artery occlusion-reperfusion exhibit higher platelet intramyocardial accumulation than normocholesterolemic animals. It was found that hypercholesterolemic rabbits showed a 429% increase in myocardial platelet accumulation. In a second series of experiments platelet depletion, induced by injection of an antiserum to rabbit platelets, was found to completely abolish the increased myocardial injury due to hypercholesterolemia, so that rabbits treated with antiplatelet serum had infarcts similar in size to those observed in normocholesterolemic rabbits.

Methods

Ischemic myocardial injury was produced in New Zealand White rabbits by occlusion of the major marginal branch of the circumflex coronary artery for 30 min followed by 5.5 hr of reperfusion. In rabbits this artery supplies the anterolateral left ventricular wall with a distribution similar to that of the left anterior descending artery in man.

Three days before the experiment the animals were randomized into two groups: a control group fed a standard laboratory chow diet, and a cholesterol group fed a 2% cholesterol-enriched diet. On the day of the experiment the animals were anesthetized with a mixture of intramuscular ketamine (35 mg/kg) and xylazine (5 mg/kg) intubated through a tracheotomy, and ventilated with room air by a Harvard respirator. A catheter was placed into the aorta through the left common carotid artery to measure arterial pressure. A blood sample was obtained to measure plasma cholesterol level by the cholesterol oxidase method (Sigma Chemical, St. Louis). A left thoracotomy was performed at the fifth intercostal space of each rabbit, the heart was suspended in a pericardial cradle, and a surgical suture snare was placed around the coronary artery.

Protocol I — intramyocardial platelet accumulation

Preparation of radioiodinated platelets. Autologous platelets in each rabbit were isolated and labeled with 111In according to a modification of the method of Thakur et al. Briefly, 18 ml of blood was withdrawn in a plastic syringe that contained 2 ml of acid citrate dextrose. The blood was transferred into two 12 ml plastic test tubes and centrifuged at 180 g at room temperature for 15 min. The platelet-rich plasma (PRP) was removed from the packed cells and combined in a single 12 ml plastic tube. The PRP was centrifuged at 1000 g for 5 min and the platelet-poor plasma (PPP) was removed, leaving 1.5 ml of the PPP above the platelet pellet. Platelets were then gently resuspended. The desired quantity of 111In-oxine (Medi-Physics, California) was added to the platelets and allowed to incubate at 37°C for 30 min. After incubation, the platelet suspension was centrifuged at 1000 g for 5 min, the PPP was aspirated off the top of the platelet pellet, and the platelets were gently resuspended in 3 ml of autologous PPP. Labeling efficacy, defined as

\[
\frac{\text{111In bound to platelets}}{\text{111In bound to platelets} + \text{unbound 111In-activity}} \times 100
\]

was 82.2% (range 64% to 93%). The viability of the platelets after radiolabeling was assessed in vivo by calculating the percent of administered radioactivity bound to circulating platelets at different time intervals. Two, four, ten, thirty, and sixty minutes after the radiolabeled platelets were administered, a 1 ml blood sample was obtained from a peripheral vein. Platelets were isolated as described above and radioactivity was counted both in the platelets and in the PPP. Only a small amount of radioactivity was found in PPP (range 4% to 8%). The percent recovery of radiolabel was calculated by standard methods.

To assess whether platelet function was altered by labeling, platelet aggregation in vitro was performed in eight additional rabbits (four normocholesterolemic and four hypercholesterolemic) by the turbidimetric method of Born6 and with the use of a DP-247-E dual-sample aggregometer (Sieneco, Inc., Morrison, CO). After adjusting the platelet count to 300,000/mm³, aggregation was initiated by the addition of 10 μl of an ADP solution or 10 μl of a collagen suspension to 200 μl of PRP to yield final ADP concentration of 20 μM and collagen concentration of 600 μg/ml. All values are expressed as per-
percentage of light transmission standardized to the PRP and PPP samples yielding zero and 100% light transmission, respectively. These experiments were also conducted to test the hypothesis that 3 days of cholesterol feeding would enhance platelet aggregation.

**Experimental design.** Thirteen rabbits were included in this protocol: seven received the standard diet and six were fed a 2% cholesterol-enriched diet. After re-injection, platelets were allowed to circulate for 30 min before coronary artery occlusion (CAO) was induced. Systemic blood pressure and heart rate were recorded continuously before and after CAO throughout the experiment. CAO was maintained for 30 min and was followed by 5.5 hr of reperfusion. At the end of the experiment, the animals were killed by an intravenous injection of KCl and the heart was promptly excised.

To clearly identify the ischemic myocardium from the normally perfused one, a perfusion technique ex vivo was developed so that the mass of tissue that did not receive blood flow after coronary occlusion could be identified. The aorta was cannulated above the coronary ostia. The coronary vasculature was first perfused with 100 ml of warm (37°C) saline to ensure that radioactive blood was not nonspecifically trapped within the coronary vasculature. The coronary artery was then reoccluded at the same site and the heart was perfused again with 100 ml of a 1% solution of methylene blue. Both solutions were perfused through the same cannula at a constant pressure of 75 mm Hg. Thereafter, the left ventricle was dissected free from all other structures and was sliced into several sections parallel to the atroventricular groove. The slices were then cut according to the methylene blue staining into transmural ischemic and nonischemic samples to determine radioactivity by gamma spectrometry. Care was taken to be certain that the tissue samples from the ischemic zone did not contain tissue that was part of the nonischemic zone. These tissue sections were weighed and placed in a gamma spectrometer (Beckman Gamma 8000) to determine the amount of 111In radioactivity present in each piece of tissue.

The accumulation of 111In-labeled platelets is expressed as the increase in 111In radioactivity in the ischemic myocardium over 111In radioactivity in the normal myocardium, subsequently referred to as the platelet accumulation ratio (PAR). The raw radioactivity counts of the ischemic and nonischemic myocardium were divided by the tissue wet weight to yield counts per minutes per gram of tissue. By dividing the activity of the area at risk (cpm/g) by the activity of nonischemic tissue (cpm/g), the increase in 111In radioactivity in the ischemic zone was calculated, yielding the PAR.

**Protocol II—effect of antiplatelet serum (APS) on infarct size and the area of no reflow**

**Preparation of goat anti-rabbit platelet serum.** Rabbit platelets were isolated from a rabbit fed a standard diet. One hundred fifty milliliters of blood was withdrawn from the animal in three 60 ml syringes that contained 6 ml of acid citrate dextrose. The whole blood was centrifuged at room temperature at 180 g for 30 min; the PRP was transferred into other plastic test tubes and centrifuged at 1000 g for 10 min. The platelets were then gently resuspended in a total volume of 40 ml of autologous PPP. The erythrocytes and leukocytes contamination of this preparation was less than 0.001%. APS was obtained by immunizing a goat with this suspension of platelets. The goat was inoculated initially with 15 ml of platelet suspension given as an intradermal injection followed by four injections of 5 ml each at 10 day intervals. Two weeks after the last injection the goat was bled and the serum was stored at −70°C. The serum used had a titer of 1:3200.

**Experimental design.** Twenty rabbits were included in this protocol. The animals were randomly assigned to four groups: group A, normocholesterolemic rabbits not receiving APS (n = 5); group B, hypercholesterolemic rabbits not receiving APS (n = 5); group C, normocholesterolemic rabbits receiving APS (n = 5); and group D, hypercholesterolemic rabbits receiving APS (n = 5). To avoid bleeding, APS was administered after all the surgical procedures were completed. APS was given in a single 2 ml dose via a peripheral vein. Platelet count was performed immediately before and 1 hr after the administration of APS. One hour after the injection of APS, rabbits underwent CAO-reperfusion as above described. Hemodynamics were continuously recorded before and after CAO throughout the experiment.

For later assessment of the zone of hypoperfusion (HZ, the area at risk of infarction), 2 × 10^6 99mTc-labeled albumin microspheres (15 μm in diameter, activity 0.8 mCi) were injected into the left atrium 1 min after CAO. To estimate the distribution of myocardial blood flow after reperfusion the animals received an injection of a 6% solution of thioflavin S (1 ml/kg) via a peripheral vein 10 sec before excision of the heart according to a previously described technique. Thioflavin S is a fluorescent dye with a relatively high affinity for endothelium. Thus, the extent of the capillary bed receiving blood flow can be visualized in slices of myocardium observed under ultraviolet light. The rabbits were then killed by intravenous injection of KCl and the left ventricle was dissected free from all other structures and weighed. The left ventricle was cut into eight 10 slices parallel to the atroventricular groove. Slices were observed first under ultraviolet light; the areas that received blood flow were brightly fluorescent. Contours of the fluorescent and the nonfluorescent areas were traced onto transparent plastic sheets. The total cross-sectional area of the slices and of the nonreperfused zones (NRZs) were determined by planimetry.

The slices were then incubated in a 2% solution of triphenyl tetrazolium chloride (TTC, Sigma Chemical) for 10 min at 37°C. The normal myocardium stained dark red and the damaged tissue appeared gray or pale yellow. Again, a transparent plastic sheet was placed over the slices and the necrotic as well as the total areas were traced: the total cross-sectional areas of the slices and the areas of damaged myocardium were determined by planimetry.

Thereafter, the extent of the HZ was determined on the same slices. Slices were placed into an x-ray film cassette adjacent to a sheet of high-speed x-ray film (Cronex 4, E.I., DuPont) sandwiched between two medium-contrast enhancing screens. The film was exposed overnight and developed (X-Omat Automatic Processor). To clearly identify the overall contours of the slices, "soft" x-ray (25 kVp–100 mA) images were obtained with the same slices held in position by the cassette. The soft x-ray image and autoradiographs were superimposed to permit tracing of the perfused (dark) areas and hypoperfused (white) areas onto clear plastic overlays. The total slice areas and the areas of hypoperfusion were determined by planimetry.

The following variables were calculated: (1) infarct size as the percentage of the left ventricle that showed myocardial damage by TTC staining criterion, (2) the HZ as the percentage of the left ventricle that was initially hypoperfused and therefore that was at risk of infarction, as determined by autoradiography, (3) the percentage of the HZ that evolved to infarction, calculated by dividing infarct size by HZ and multiplying by 100 and representing the percentage of the area at risk that actually evolved to necrosis and, (4) the NRZ, which was that not receiving blood flow at the end of the 5.5 hr of reperfusion as determined by thioflavin S fluorescence.

Three additional hypercholesterolemic rabbits underwent the same procedure described above but received 2 ml of nonim-
mune goat serum to determine whether goat serum per se could nonspecifically modify the measured variables.

**Statistical analysis.** Results are expressed as the mean ± SEM. Comparisons between two means were made with Student's t test for group observations. In making multiple comparisons among groups, analysis of variance was performed. For comparison of heart rate and arterial pressure among groups, an analysis of variance for a design with repeated measures was used (program BMDP-2V, see Dixon and Brown).

**Results**

A total of 44 rabbits underwent the surgical procedure described above. On the day of the experiment plasma cholesterol levels were 67 ± 12 mg/dl in rabbits receiving the standard diet and 329 ± 70 mg/dl in animals fed a 2% cholesterol-enriched diet for 3 days (p < .001). Data from eight rabbits were excluded from analysis because the animals did not survive the 6 hr protocol. Of the 36 rabbits that successfully completed the experiments, 13 were included in protocol I and 20 in protocol II. Three hypercholesterolemic rabbits underwent protocol II but received nonimmune goat serum. Finally, eight additional rabbits (four normocholesterolemic and four hypercholesterolemic) were used to test platelet aggregation in vitro before and after labeling with $^{111}$In and to estimate platelet reactivity after 3 days of cholesterol feeding.

**Protocol I — intramyocardial platelet accumulation.** The fragility of platelets demands that particular care be taken in handling during labeling in vitro with $^{111}$In-oxine. To verify that platelet viability is not altered during labeling, two independent tests on platelet function were performed. The method in vivo assumes that $^{111}$In-labeled platelets that are injured during the labeling process are sequestered by the spleen and liver within 30 min of administration into the circulatory system. In these experiments, an average of 77.3% of total administered $^{111}$In activity was present in the circulation after 30 min (figure 1). Platelet aggregation in vitro, induced by ADP or collagen, was nearly identical for the unlabeled and $^{111}$In-labeled platelets, being 82 ± 7% and 80 ± 6% light transmission (max) for ADP and 68 ± 6% and 63 ± 8% light transmission (max) for collagen in normocholesterolemic rabbits (figure 2). Similarly, percent maximal aggregation induced in platelets harvested from hypercholesterolemic animals was not significantly different before and after radiolabeling. However, platelets from hypercholesterolemic animals were more responsive to ADP and collagen than platelets isolated from normocholesterolemic rabbits. In fact, they elicited the same aggregatory response to only 12 ± 4% of the concentration of ADP and to 18 ± 5% of the concentration of collagen as compared with platelets isolated from normocholesterolemic animals (p < .01 for both values vs the respective concentrations of ADP and collagen used in platelets isolated from normocholesterolemic rabbits) (figure 2). These data indicate that labeling in vivo does not substantially alter normal platelet viability and that platelets isolated from rabbits fed a 2% cholesterol diet for 3 days are more responsive to aggregating agents in vitro than platelets harvested from normocholesterolemic animals.

Quantitative assessment of platelet accumulation in ischemic myocardium was obtained by calculating the increase in radioactivity present in ischemic tissue.
mic rabbits not receiving APS; group C (n = 5), normocholesterolemic rabbits receiving APS; group D (n = 5), hypercholesterolemic rabbits receiving APS.

Injection of APS decreased platelet count in normocholesterolemic rabbits (group C) and in hypercholesterolemic rabbits (group D) to a similar extent (97.8% vs 97.6%, respectively). Concurrently, there was a decrease in the number of circulating leukocytes by 56.3% in group C and by 47.8% in group D (table 1). The number of circulating platelets and leukocytes did not change in rabbits that received nonimmune serum.

HZs created by CAO in the four groups of animals were similar in magnitude. HZ after CAO was 31.9 ± 1.0%, 31.4 ± 2.6%, 32.9 ± 2.9%, and 33.9 ± 1.7% of left ventricle in groups A, B, C, and D, respectively (p = NS), showing that coronary occlusion resulted in comparable areas at risk in all groups. The fraction of the HZ that actually evolved to necrosis, i.e., the infarct size, was strikingly different in the various groups (figure 4): 38.1 ± 1.4% in group A, 83.5 ± 5.1% in group B, 42.2 ± 2.5% in group C, and 47.1 ± 3.7% in group D. Thus, as previously reported, acute hypercholesterolemia increased infarct size after CAO-reperfusion by about 100% (group B vs group A). Analysis of variance of these data indicated that the untreated hypercholesterolemic rabbits (group B) were, in fact, the only group in which infarct size was statistically different from the other (p < .001); infarct sizes in all other groups were similar and did not statistically differ from one another. Thus, platelet depletion in hypercholesterolemic rabbits (group D) completely reversed the adverse effect of hypercholesterolemia on infarct size. It is important to note that platelet depletion in normocholesterolemic rabbits had no effect on infarct size (group C vs group A).

In a similar way the NRZ, or the portion of myocardium that did not receive blood flow after reperfusion, was affected by the administration of APS. In fact, NRZ (% of HZ) was strikingly larger in group B (86.2 ± 3.8%) than in the other three groups and included

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Platelet count/mm³</th>
<th>Leukocyte count/mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before APS</td>
<td>After APS</td>
</tr>
<tr>
<td>Normocholesterolemic rabbits (group C)</td>
<td>449,800 ± 16,178</td>
<td>9,600 ± 1,208</td>
</tr>
<tr>
<td>Hypercholesterolemic rabbits (group D)</td>
<td>457,200 ± 33,663</td>
<td>10,600 ± 2,420</td>
</tr>
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</table>

Data are mean ± SEM.
essentially all of the infarcted tissue. Values in the other groups were as follows: 19.1 ± 3.7% in group A, 21.5 ± 2.7% in group C, and 26.5 ± 5.8% in group D (p < .001, figure 5).

No differences between the groups with respect to mean arterial pressure or heart rate were observed at any time during or after CAO (table 2).

Finally, the administration of nonimmune goat serum to hypercholesterolemic rabbits had no effect on either infarct size or NRZ (81.4 ± 4.7% of HZ and 82.4 ± 3.5% of HZ, respectively; p = NS vs group B).

Discussion

We have previously reported that in hypercholesterolemic, nonatherosclerotic rabbits undergoing CAO-reperfusion, infarct size is about twice the size of that observed in normcholesterolenic animals. An estimation of myocardial blood flow distribution after reperfusion revealed that hypercholesterolemic rabbits had NRZs strikingly larger than those observed in controls and that these zones included essentially all the infarcted tissue. These findings strongly suggest that a vascular obstruction by aggregation of some blood cellular components in the vessels of ischemic myocardium could be responsible for this deleterious effect.

The aim of the present investigation was to test the hypothesis that platelets are involved in this phenomenon. By labeling autologous platelets with \(^{111}\)In we have been able to demonstrate that, in this reperfusion preparation of myocardial ischemia, platelet accumulation within the ischemic myocardium is greatly enhanced when moderate hypercholesterolemia is induced by cholesterol feeding: a 429% increase in PAR was observed in cholesterol-fed rabbits as compared with the PAR in control animals.

Proper consideration should be given to the fact that platelet deposition within regions of myocardial damage is strictly dependent on reperfusion and is directly related to its duration. Moschos, Ruf, and Romson and their colleagues have reported that in an animal preparation of permanent CAO, radiolabeled platelet accumulation in the ischemic myocardium was negligible. Romson et al. also demonstrated that, in a reperfusion preparation of myocardial ischemia, a significant intramyocardial platelet accumulation occurred. Moreover, Laws et al. demonstrated that platelet accumulation in the ischemic regions is inversely proportional to blood flow and maximal in the lowest flow regions. These investigators showed that duration of reperfusion is also important for platelet accumulation, since platelet uptake is negligible before reperfusion, increases at 3 hr after reperfusion, and maximizes at 24 hr after reperfusion. The mechanism responsible for the accumulation of platelets in the ischemic myocardium seems to be related to microvascular damage. It has been demonstrated that early after experimental CAO, morphologic ultrastructural abnormalities occur not only in the myocardial cells, but also in the microvasculature. In particular, endothelial cells appear to be very sensitive to the interruption of blood flow, with a consequent endothelial disruption and exposure of the subendothelium. The exposed subendothelial structures represent a strong aggregating stimulus for blood platelets that may activate and adhere to the subendothelium when blood reenters the vasculature of the ischemic myocardium.

![FIGURE 4. Infarct size (IS) as percent of the HZ in normcholesterolenic (con) and hypercholesterolenic rabbits (chol) undergoing CAO-reperfusion with (+ APS) or without (- APS) APS. Each bar represents five animals. Note the dramatic reduction in IS caused by APS in hypercholesterolenic rabbits (chol + APS). Note also that APS had no effect on IS in normcholesterolenic animals (con + APS), as determined by comparison with IS in normcholesterolenic rabbits that did not receive APS (con - APS). *p < .001 by analysis of variance test.](image)

![FIGURE 5. Extent of myocardium that failed to reperfuse (i.e., NRZ) as a percent of the initial HZ in normcholesterolenic (con) and hypercholesterolenic rabbits (chol) undergoing CAO-reperfusion with (+ APS) or without administration of APS (- APS). Each bar represents five animals. *p < .001 by analysis of variance test.](image)
TABLE 2

Hemodynamic variables during 30 min of CAO and 5.5 hr of reperfusion

<table>
<thead>
<tr>
<th>Time after CAO</th>
<th>Heart rate (min⁻¹)</th>
<th>Mean arterial pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>0</td>
<td>167 ± 5</td>
<td>161 ± 6</td>
</tr>
<tr>
<td>15 min</td>
<td>173 ± 4</td>
<td>169 ± 3</td>
</tr>
<tr>
<td>30 min</td>
<td>169 ± 3</td>
<td>170 ± 4</td>
</tr>
<tr>
<td>1 hr</td>
<td>174 ± 4</td>
<td>169 ± 6</td>
</tr>
<tr>
<td>2 hr</td>
<td>172 ± 4</td>
<td>172 ± 4</td>
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<tr>
<td>3 hr</td>
<td>167 ± 4</td>
<td>165 ± 6</td>
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<td>165 ± 5</td>
</tr>
<tr>
<td>5 hr</td>
<td>170 ± 3</td>
<td>167 ± 5</td>
</tr>
<tr>
<td>6 hr</td>
<td>167 ± 4</td>
<td>168 ± 6</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

Furthermore, Davies et al.11 and Falk10 have demonstrated a significant intramyocardial platelet accumulation in patients who die of sudden ischemic cardiac death.

The fact that hypercholesterolemia induced a marked increase in myocardial platelet accumulation is not totally unexpected. It has, in fact, been reported that platelets isolated from patients with familial hyperlipoproteinemia or normal platelets incubated in a cholesterol-rich medium were hypersensitive to different aggregating substances in vitro.12, 13 In the present study, rabbits fed a 2% cholesterol diet for 3 days had a fivefold increase in plasma cholesterol. This was associated with an enhanced responsiveness of platelets to ADP and collagen in vitro: platelets from hypercholesterolemic rabbits aggregated in response to one-eighth of the concentration of ADP and one-sixth of the concentration of collagen as compared with platelets isolated from normocholesterolemic animals. The mechanism of the cholesterol effect is uncertain; although changes in membrane fluidity have been postulated,25 enhanced synthesis of thromboxane B₂ has also been suggested.26

Whatever the mechanism by which hypercholesterolemia increases platelet reactivity and intramyocardial platelet accumulation, data from the second series of experiments of the present study demonstrate the biological importance of this phenomenon. In accordance with our previous study,5 a 3 day period of cholesterol feeding was associated with a striking increase in both infarct size and NRZ as compared with those in normocholesterolemic rabbits. Administration of APS to normocholesterolemic animals had no effect on either infarct size or NRZ as compared with those in normocholesterolemic animals that did not receive APS. By contrast, administration of APS to hypercholesterolemic animals caused a dramatic reduction in both infarct size and NRZ compared with control values. These data demonstrate that platelets do not significantly contribute to the development of myocardial injury in normocholesterolemic rabbits. This finding is in accordance with two recent reports that demonstrated that platelet depletion had no effect on infarct size in normocholesterolemic dogs undergoing CAO reperfusion.27, 28 Furthermore, the fact that platelet depletion in hypercholesterolemic rabbits completely reversed the detrimental effect of hypercholesterolemia indicates that the extension of the infarct size observed in cholesterol-fed animals is platelet dependent.

Since it has been demonstrated that platelet accumulation occurs primarily during the early stages of infarction6, 21 when myocardial cells are still undergoing biochemical transformation leading to cell death, preventing this accumulation by lowering circulating platelet number leads to a dramatic decrease in myocardial injury. Data from the present study do not allow us to identify a specific mechanism by which platelets increase infarct size in hypercholesterolemic animals. One possibility is that hypercholesterolemic platelets form bigger and more persistent aggregates within the microvasculature of the myocardium at risk, thus precluding the occurrence of effective reperfusion. This, in turn, may cause necrosis of that portion of myocardium that would have otherwise been salvaged by reperfusion. This hypothesis is suggested by the fact that the no-reflow phenomenon, i.e., the NRZ, included essentially all of the infarcted tissue. It is also possible, however, that platelets contributed in part to the induction of myocardial damage through a release of a number of vasoactive substances such as serotonin and thromboxane A₂,29, 30 which are known to cause marked vasoconstriction.
It was noted that the antiplatelet serum also caused a concomitant reduction in circulating leukocytes, averaging 56% and 48% of control values in normocholesterolemic and hypercholesterolemic rabbits, respectively. Since it has been demonstrated that leukocytes play an important role in the evolution of myocardial infarction and that their depletion is associated with a decrease in the extent of myocardial injury, one might speculate that the reduction in infarct size we observed could be attributed at least in part to this depletion of circulating leukocytes. Although this possibility cannot be absolutely ruled out, it seems unlikely. In fact, if it were so, we should have observed a decrease in infarct size in normocholesterolemic rabbits that received APS as compared with that in normocholesterolemic animals to which APS was not given. This observation indicates that the leukocyte depletion observed in the present study was probably not severe enough to affect the ultimate size of myocardial necrosis in control animals. However, we would like to point out that the data from the present study do not allow us to exclude unequivocally the possibility that the leukopenia we observed, even if moderate, may have contributed to the salvage of part of the ischemic myocardium in hypercholesterolemic rabbits. Nevertheless, if put into the proper perspective, the results of the present study provide useful insights into the involvement of platelets in increasing ischemic myocardial injury in the setting of acute hypercholesterolemia.

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References
30. Holmsen H: Prostaglandin endoperoxide-thromboxane synthesis and dense granule secretion as positive feedback loops in the propagation of platelet responses during the "basic platelet reaction." Thromb Haemost 38: 1030, 1977
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