Dynamic intracoronary thrombosis does not cause significant downstream platelet embolization

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Received 21 December 1999; accepted 18 April 2000

Abstract

Objective: A mural intracoronary thrombus is a potential source of platelet emboli that may obstruct downstream microvessels, but this phenomenon has not been characterized. The present study aimed to assess the magnitude of myocardial platelet accumulation downstream of a mural intracoronary thrombus and its modification by a concomitant transient coronary occlusion (OC) or by treatment with aspirin.

Methods: The myocardial content of Tc-labelled platelets was analyzed in 26 pigs submitted to intimal injury of the left anterior descending coronary artery (LAD) followed by no intervention (n=6), 25-min OC (n=6), or 48-min OC preceded (n=8) or not (n=6) by intravenous administration of 250 mg aspirin.

Results: After 2 h, 24 animals had had 12±6 cyclic flow reductions (CFRs) reflecting dynamic LAD thrombosis. Myocardial platelet content in the inferior region was similar among groups. Platelet content in the LAD region was not significantly different to that in the inferior region (129±19%, P=NS) in the no intervention group, but was increased following OC (172±20 and 312±71% after 25- and 48-min OC, respectively, P<0.05). Pre-treatment with aspirin lessened the number of CFRs but did not reduce platelet accumulation in LAD myocardium (483±148%). Myocardial platelet accumulation was not associated with the magnitude of platelet deposition in the LAD nor with the number of CFRs, but was correlated with myeloperoxidase activity (r=0.91, P<0.001) and with infarct size (r=0.52, P=0.05). Histological analysis frequently showed sparse platelets or small platelet or leukoplatelet aggregates in small vessels, but arteriolar emboli were rare. In none of seven additional experiments coronary angiography showed obstructions of arterial branches during CFRs.

Conclusion: The magnitude of platelet embolization from a mural intracoronary thrombus into downstream myocardium is small despite the presence of repetitive CFRs.

Keywords: Coronary circulation; Ischemia; Platelets; Reperfusion; Thrombosis/embolism

1. Introduction

Oclusive intracoronary thrombosis may cause myocardial ischemic injury and cell death. Several data suggest that non-occlusive thrombosis may also be harmful to downstream myocardium by mechanisms independent from flow limitation at the thrombus site. Intracoronary thrombosis has been associated with impaired endocardial flow [1] and increased resistance at the distal vascular bed [2,3], and a previous study in our laboratory showed that coronary intimal injury blunted the hyperemic response and increased infarct size after a prolonged coronary occlusion (OC), an influence that was attenuated by pretreatment with aspirin [4]. Moreover, experimental reperfusion through a stenotic artery, which could favor mural thrombosis, has also been associated with increased necrosis [5,6], an effect that may be prevented by platelet depletion [6].

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Time for primary review 22 days.
Whether these deleterious effects of mural intracoronary thrombosis are due to the release of vasoactive substances or to a mechanical limitation of flow by multiple platelet emboli remains unclear [3,7]. Intracoronary thrombosis induces an increased release of platelet-derived products that cause local and distal vasoconstriction [8,9] but, on the other hand, the characteristic cyclic flow reductions (CFRs) that are associated with the development of intracoronary thrombosis in experimental animals strongly suggest the occurrence of repetitive embolization of thrombotic material [10]. However, although periodic dislodgment of the thrombus has been demonstrated during CFRs [11], neither the magnitude of platelet embolization in myocardial microvessels nor its modification by antiplatelet drugs have been investigated.

Accordingly, we aimed to quantify the magnitude of myocardial platelet embolization downstream of a mural intracoronary thrombus and its modification by aspirin. We measured the myocardial content of radiolabelled platelets in pigs submitted to coronary intimal injury to induce thrombosis with CFRs. Since the adhesiveness of the microvascular endothelium is enhanced by ischemia [12–14], the modification of platelet accumulation by a concomitant transient OC of different duration was also analyzed. Finally, additional angiographic studies were performed to detect macroscopic embolization during CFRs.

2. Methods

2.1. Animal preparation

The experimental methods were approved by the Research Commission of the Hospital General Vall d’Hebron and conform with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Thirty-two farm pigs of either sex (35±6 kg) were premedicated with 10 mg/kg intramuscular azaperone, anesthetized with 10 mg/kg intravenous thiopental, intubated and mechanically ventilated with room air (Bennett MA-1B, Santa Monica, CA). A continuous infusion of thiopental was administered to maintain anesthesia. The right femoral artery and vein were catheterized, the right carotid artery was dissected, a sternotomy was performed and the pericardium opened. The left anterior descending coronary artery (LAD) was dissected free in its mid segment at two adjacent points. A 2-mm width Doppler flow probe (Transonic Systems, Ithaca, NY) was placed around the proximal point, and the distal point was surrounded by an elastic snare and served as the site for coronary injury and occlusion.

2.2. Study protocol

To produce coronary intimal injury, the LAD was catheterized with a Judkins 8F guiding catheter introduced by the carotid artery, and a 2.5F catheter (Cordis) was advanced into its distal segment and moved backwards and forwards several times while the elastic snare was gently tightened. After this intervention, which has been shown to cause endothelial denudation, disruption of the internal elastic lamina and mural thrombosis [4,15], the catheters were withdrawn and the carotid artery ligated. Animals were allocated (Fig. 1) to no further intervention or to a 25- or 48-min ligature of the LAD. After these two periods of OC, that in this model result, respectively, in no histochemically detectable myocardial necrosis and in detectable but incomplete necrosis [4,16], the effects of coronary injury on the hyperemic response and on infarct size were manifest, respectively, in our previous study [4]. Ninety minutes before OC, animals allocated to 48-min OC were randomized to receive, blindly to the investigators performing the experiment, an intravenous solution of 250 mg aspirin in 5 ml of saline or vehicle. This dose of aspirin has been shown to prolong bleeding time and significantly inhibit in vitro platelet aggregation in the same model, and attenuated the deleterious influence of coronary intimal injury on infarct size in our previous study [4]. Two hours after reperfusion, or after coronary intimal injury in animals without OC, the heart was excised.

Six animals were excluded due to technical reasons (two animals), coronary reoclusion (two animals), refractory ventricular fibrillation (VF) (one animal) and severe hypotension (one animal). Thus, 26 experiments remained valid, six without OC, six with 25-min OC, six with 48-min OC without aspirin, and eight with 48-min OC and aspirin.

2.3. Study monitoring

Frequent measurements of arterial blood gases were performed to adjust the ventilatory parameters. Blood platelet count was determined at the end of the experiment. Continuous monitoring of aortic blood pressure with a crystal quartz transducer (Coulbourn, Lehigh Valley, PA),

![Fig. 1. Study design. OC, coronary occlusion; ID, intimal damage; ASA, aspirin.](https://academic.oup.com/cardiovascres/article-abstract/47/2/265/364657/fig1)
and of LAD blood flow with a flow meter (T106, Transonic Systems) were obtained. In five animals (three without OC and two with 48-min OC and aspirin) in which coronary flow did not change spontaneously after intimal injury, the LAD was constricted with a ligature around it and a 20-Gauge needle to facilitate the development of CFRs. If blood flow decreased to zero, the artery was gently shaken to allow recovery of flow. The lead II of the electrocardiogram, aortic pressure, and the signals from the flow meter were amplified in a Coulbourn modular instrument system and continuously recorded by a thermocap recorder (MT-9500, Astro-Med, West Warwick, RI) at a sampling rate of 200 kHz and at a paper velocity of 10 mm/min, with several additional recordings at 10 mm/s. When VF occurred, it was converted to sinus rhythm by internal direct current shocks of 10–20 J.

2.4. Platelet deposition in the LAD and accumulation in myocardium

Platelet content was quantified in the injured LAD and in myocardial samples by using \(^{99m}\text{Tc}\)-labelled platelets as previously described [4]. Briefly, the previous day animals were anesthetized and ventilated as described, one femoral vein was catheterized percutaneously and 51 ml of blood was withdrawn through a 5F catheter. The blood was centrifuged and acidified with acid citrate dextrose, and the resultant pellet was resuspended in platelet-poor plasma and incubated with \(^{99m}\text{Tc}\)-labelled hexamethy(propyleneamineoxime (HMPAO) [17]. After centrifugation, the pellet of \(^{99m}\text{Tc}\)-labelled platelets was resuspended in plasma and injected intravenously into the animal. The labelling efficiency averaged 55.5±2.4% and a mean of 6.7±0.3 mCi of \(^{99m}\text{Tc}\)-labelled platelets was injected into each animal. The femoral catheter was withdrawn, anaesthesia was stopped and animals were allowed to recover and returned to the animal room until the next day.

After excising the heart, the aortic root was cannulated and a solution of 0.5% albumin in Ringer was perfused at 120 mmHg pressure for 5 min to eliminate any trapped blood from the coronary vasculature. One 10-mm segment of the LAD including the injured site and one segment from the right coronary artery were obtained, opened and measured. The heart was immersed in Ringer solution at 4°C and cut in 5–7-mm slices perpendicular to its long axis. The slices were weighed in a high precision scale and transmural myocardial fragments were obtained from the anteroseptal region in the third and fourth slices, downstream of the damaged LAD (2.24±0.19 g of tissue per animal). Two fragments from the inferior region (2.36±0.18 g) were obtained in the same slices. The radioactivity of these fragments, along with that of the arterial segments and of 1 ml blood withdrawn at the end of the experiment, was counted for 20 s in a gamma counter (1282 Compugamma, Wallac, Turku, Finland). The activity in the LAD was corrected by sample surface. Specific activity in LAD-dependent myocardium (number of counts/g) was calculated and expressed as percentage of the specific activity in control myocardium. Platelet content in arterial and myocardial samples was calculated from the activity and platelet count in total blood.

2.5. Myocardial PMN accumulation and infarct size

Myocardial PMN content was quantified in 16 experiments (five with 25-min OC, four with 48-min OC without aspirin and seven with 48-min OC and aspirin) by determining myeloperoxidase (MPO) activity in myocardial tissue. Transmural samples (0.81±0.06 g) from the third slice were obtained and stored at −80°C until analysis. The samples were homogenized as previously described [4] and solubilized in 50 mmol/l potassium phosphate buffer at pH 6.0 with 0.5% hexadecyltrimethyl ammonium bromide (Sigma), and MPO activity was determined by spectrophotometry [18] after addition of o-dianisidine dihydrochloride and H₂O₂. MPO activity was expressed in units defined as the quantity of enzyme degrading 1 µmol peroxide/1/min at 25°C.

In animals with 48-min OC, the remaining slices were incubated at 37°C in 1% triphenyltetrazolium chloride, buffered for pH 7.4, for 5–10 min, and imaged from the basal side by a Sony TR-705E video camera. Instead of the third slice, used for MPO analysis, the apical surface of the fourth slice was imaged. The images, along with a reference scale, were digitized on line with a digitization card (Matrox IP8) into 768×576 pixels images. The area of necrosis was measured semiautomatically (Image Pro-Plus software, Media Cybernetics) in the digital images, and infarct size was calculated from these measurements and the weight of the slices.

2.6. Histological analysis

The slices from the animals with 48-min OC were fixed in 10% formaldehyde. Two slices were dehydrated in graded alcohol and embedded in paraffin, and serial transverse 4-µm thick sections were obtained (Polycut microtome, Reichert Jung Cambridge, Heidelberg, Germany), stained with hematoxylin and eosin, Schiff’s periodic acid, and Masson’s trichrome, and examined with an Olympus IMT2 microscope. The distal LAD was examined for the presence of luminal thrombosis. In myocardial tissue, the presence and distribution of platelets in the microvasculature was analyzed as well as the presence of red blood cell extravasation. Arteriolar plugs >50 µm in diameter were considered as microemboli [19,20].

2.7. Angiographic studies

In a separate series of seven animals, anesthetized and instrumented as described, the occurrence of macroscopic embolization was investigated by performing a coronary angiography during CFRs. The left coronary artery was
### 2.8. Statistical analysis

Statistical analysis was performed using SPSS software. Values are expressed as mean±S.E.M. Paired *t*-tests were used to assess changes in physiologic parameters and to compare the results obtained in the LAD or in LAD-dependent myocardium with those from a control zone within the same animal. Comparisons between groups were performed by Student’s *t*-tests or by one-way analysis of variance (ANOVA) after having assessed the data for normal distribution. Individual comparisons were performed by the less significant difference method if homogeneity was rejected by ANOVA. *P* values <0.05 were considered significant.

### 3. Results

#### 3.1. Hemodynamic data and ventricular arrhythmias

Hemodynamic data are summarized in Table 1. Heart rate increased slightly after OC and remained stable during the reperfusion period. Aortic pressure did not change significantly during the experiment. At baseline, mean blood flow at the mid LAD was 13±1 ml/min, without differences between groups. After LAD injury, 24 animals had a mean number of 12±1 CFRs (Fig. 2). The overall number of CFRs in each group was 9±2 in animals without OC, 17±3 in those with 25-min OC, 14±2 in those with 48-min OC without aspirin, and 7±2 in those with 48-min OC and aspirin (*P*<0.05 for the comparison between this group and the other groups with OC). Six animals had ischemic VF, 30±4 min after OC. All animals had repetitive bursts of idioventricular rhythm immediately after reflow, and 11 had VF within the first minutes after reperfusion. The number of CFRs was greater in animals with VF than in those without (14±2 vs. 9±2, respectively, *P*=0.05) and was not correlated with the number of electrical shocks (*r* =0.25, *P*=NS).

#### 3.2. Platelet deposition in the LAD and accumulation in myocardium

Blood platelet count averaged 404±23×10⁹ platelets/l, without between group differences. All animals had an

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**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>15 min OC</th>
<th>48 min OC</th>
<th>15 min reperfusion</th>
<th>2 h reperfusion</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Heart rate (bp)</td>
<td>Mean AP (mmHg)</td>
<td>Heart rate (bp)</td>
<td>Mean AP (mmHg)</td>
<td>Heart rate (bp)</td>
</tr>
<tr>
<td>No OC (n=6)</td>
<td>75±2</td>
<td>101±3</td>
<td>77±17</td>
<td>75±10</td>
<td>80±5</td>
</tr>
<tr>
<td>25 min OC (n=6)</td>
<td>76±6</td>
<td>73±7*</td>
<td>75±8</td>
<td>87±7</td>
<td>68±16</td>
</tr>
<tr>
<td>48 min OC (n=6)</td>
<td>85±10</td>
<td>92±7</td>
<td>92±20</td>
<td>89±10</td>
<td>83±17</td>
</tr>
<tr>
<td>48 min OC+ASA (n=8)</td>
<td>88±7</td>
<td>77±6</td>
<td>96±7</td>
<td>86±4</td>
<td>91±6</td>
</tr>
<tr>
<td>Total (n=26)</td>
<td>82±3</td>
<td>85±4</td>
<td>95±8</td>
<td>88±4</td>
<td>83±4</td>
</tr>
</tbody>
</table>

*Data are mean±S.E.M. *P*<0.05 respect to the groups with 25 min OC and 48 min OC+ASA; *P*<0.05 respect to the group with 48 min OC. OC, coronary occlusion; AP, aortic pressure; ASA, aspirin. In the first group, the last two measurements are from 15 min and 2 h after coronary intimal injury, respectively.

catheterized under fluoroscopic control (ARCOsi image intensifier, ATS, Pedrengo, Italy) with a 7F Judkins catheter introduced percutaneously through a femoral or carotid artery. A baseline coronary angiogram was performed by injecting 10 ml of iopamidol after selecting a projection with the best visualization of the distal LAD and its branches. After promoting the development of CFRs by mechanical injury of the mid LAD, several additional angiograms were obtained, at the times of the nadir of flow and as soon as the typical, brisk recoveries of flow occurred. Angiograms were examined to detect amputation of arterial branches distal to the damaged segment after recovery of flow.

![Fig. 2](https://academic.oup.com/cardiovascres/article-abstract/47/2/265/364657/172265564657-by-guest-on-06-February-2019)
increased platelet deposition in the injured LAD with respect to the right coronary artery (1847±626 vs. 23±5×10^6 platelets/cm², respectively, P=0.009). Myocardial platelet content in the control region averaged 13.0±1.2×10^6 platelets/g, without between group differences (Fig. 3). In LAD-dependent myocardium, platelet content was similar to the control value (129±19%, P=NS) in animals without OC, but was significantly increased after OC and this increase was related to the duration of ischemia receiving aspirin, and 7.2±3 without OC, but was significantly increased with respect to that in the control region only in animals submitted to 48-min OC and not with aspirin (ASA). Data are mean±S.E.M., * P<0.05 with respect to the first two groups.

3.3. Myocardial accumulation of PMNs and infarct size

MPO activity averaged 14±3×10^-3 IU/g in the control region (7±2, 21±8, and 16±4×10^-3 IU/g, respectively, in animals submitted to 25-min OC, 48-min OC without aspirin, and 48-min OC and aspirin, P=NS). In reperfused myocardium, MPO activity averaged 54±2×10^-3 IU/g (P=0.03 with respect to the value in control myocardium), and was not significantly different among groups (25±15, 34±10, and 92±36×10^-3 IU/g, respectively, P=NS).

MPO activity in reperfused myocardium was not associated with platelet deposition in the LAD nor with the number of CFRs, but was strongly correlated (r=0.91, P<0.001) with specific 99mTc activity in reperfused myocardium (Fig. 4).

Infarct size averaged 3.1±1.7 g (2.3±1.1% of ventricular mass) in animals submitted to 48-min OC and not receiving aspirin, and 7.2±2.2 g (5.3±1.7%) in aspirin-treated animals (P=NS). In the third slice, infarct size showed a positive correlation with specific 99mTc activity (r=0.52, P=0.05) and with MPO activity (r=0.70, P=0.04).

3.4. Histological analysis

The distal LAD was patent in 12 of the 14 animals with 48-min OC, and in the remaining two it was severely narrowed by a thrombus containing platelets, fibrin, and erythrocytes. In control myocardium, histological analysis did not show platelet aggregates, PMNs or hemorrhage. In LAD-dependent myocardium (Fig. 5), occasional arteriolar emboli were observed in the two animals with extensive thrombosis involving the distal LAD, and one microembolus was observed in another animal. In ten animals, however, histological analysis showed small platelet or platelet–PMN aggregates in capillaries and venules. These cells were attached to the vessel wall and occasionally occluded the lumen, and were distributed predominantly in areas of myocardial necrosis. In only three animals hemorrhage was observed.

3.5. Angiographic studies

The angiographic appearance of the LAD at baseline was normal in all cases. At the nadir of CFRs, angiography

![Fig. 3. Platelet content in samples from left anterior descending artery (LAD)-dependent and control myocardium. Platelet content in the control region was similar among groups. In the LAD territory, platelet content was significantly increased with respect to that in the control region only in animals with sustained ischemia, and the magnitude of this increase was related to the duration of coronary occlusion (OC). Among animals with 48-min OC, platelet content was similar in those pre-treated or not with aspirin (ASA). Data are mean±S.E.M. * P<0.05 with respect to the first two groups.](https://academic.oup.com/cardiovascres/article-abstract/47/2/265/364657/fig3)

![Fig. 4. Correlation between myeloperoxidase (MPO) activity and specific 99mTc activity in myocardial samples, reflecting polymorphonuclear leukocyte and platelet accumulation, respectively.](https://academic.oup.com/cardiovascres/article-abstract/47/2/265/364657/fig4)
Fig. 5. Histological sections of left anterior descending artery-dependent myocardium after intimal injury and 48-min occlusion of this artery and 2 h of reflow. (a) Plug consisting mainly of platelets and fibrin, and polymorphonuclear leukocytes (PMNs, arrow) in less proportion, occluding an arteriole (hematoxylin and eosin, 400×), that was considered as a microembolus. (b) Postcapillary venule with subendocardial edema (open arrow), activated PMNs (white arrow) and occasional platelets (black arrow) attached to the wall (Schiff’s periodic acid, 1000×).
showed a severe stenosis at the site of LAD injury, with slowed filling of the distal vessel, or a total occlusion. Immediately after recovery of flow, angiography showed a residual stenosis of variable severity with or without images suggestive of intracoronary thrombus, and improved or normalized filling of the distal vessel. Remarkably, none of these injections demonstrated acute obstruction of distal branches of the LAD suggestive of embolization.

4. Discussion

In the present study, in the absence of sustained ischemia, coronary intimal injury with repetitive CFRs was not associated with a detectable downstream accumulation of 99mTc-labeled platelets. Myocardial platelet content was increased in hearts submitted to transient OC, but was not correlated with the magnitude of platelet deposition in the injured artery nor with the number of CFRs, and was not lessened by pre-treatment with aspirin, an intervention that reduced the number of CFRs. By contrast, myocardial platelet content was associated with the duration of OC and was correlated with MPO activity and with infarct size. Histological analysis disclosed extensive platelet and PMN deposition on the microvascular endothelium within reperfused myocardium, but large arteriolar plugs consistent with microemboli were rarely seen. Coronary angiography failed to detect acute obstructions of arterial branches distal to the site of the dynamic thrombosis.

In the absence of aspirin, all animals with transient OC evolved spontaneous CFRs, while in one half of those without OC the LAD had to be constricted to facilitate their development. Moreover, the number of CFRs was greater in the former animals than in animals without OC or in those receiving aspirin. The apparently increased susceptibility for the development of CFRs after OC might be explained in part because coronary ligature may distort arterial shape and result in some degree of residual stenosis after reperfusion, and because these animals could have a more severe intimal injury than the rest. In this respect, we have previously observed that in the absence of additional catheter-induced injury, coronary ligature as produced in the present study determines a significant intimal damage at the occlusion site [15]. On the other hand, the finding of more CFRs in animals with VF suggests an increased thrombotic activity at the culprit lesion in these animals and concurs with a previous observation in our laboratory of a greater incidence of coronary reocclusion after ischemic VF [15].

Previous studies in experimental animals have shown a deleterious influence of non-occlusive intracoronary thrombosis on downstream myocardium [1–4]. The possible relevance of coronary microembolization has been suggested by the finding of platelet plugs in myocardial microvessels from patients with coronary disease who died suddenly [19–22]. However, the ultimate mechanism of this platelet plugging is difficult to establish, and has been attributed either to local aggregation or to embolization. It cannot be excluded that the 29% increase in platelet content in LAD myocardium over the value in the control zone observed in the present study in animals without OC could have reached statistical significance with a larger number of animals. However, its small magnitude suggests that embolization is not a relevant cause of sustained microvascular obstruction downstream of a mural intracoronary thrombus. This is further supported by the lack of association of myocardial platelet content with the severity of coronary thrombosis, by the rarerness of arteriolar obstructions at histological analysis, and by the absence of amputations of distal branches at angiography, an observation that is in agreement with a previous report in the canine model [11]. The failure of aspirin to reduce myocardial platelet accumulation despite its effect against CFRs also suggests that both phenomena are basically independent. Since strong evidence indicates that CFRs are the consequence of dynamic intracoronary thrombosis [9–11], the present results suggest a rapid deaggregation of platelets after they are released from the mural thrombus, which allows them to pass through the capillary bed and reach the systemic circulation.

The present findings of myocardial platelet accumulation after ischemia, its correlation with MPO activity and infarct size and the predominant distribution of platelets in areas of infarct concur with previous observations [23–27]. However, the mechanisms of platelet deposition in reperfused myocardium remain unclear. Several interventions aimed to reduce myocardial platelet accumulation after reperfusion or to modulate its effects have obtained disparate results [23,25–30]. In the present study, aspirin was ineffective in reducing the magnitude of myocardial platelet deposition. Furthermore, aspirin-treated animals showed a trend to increased platelet content, which might be due to chance or might be related to the trend to increased infarct size in these animals. It is improbable that the lack of effect of aspirin was due to an insufficient dose since this dose reduced the number of CFRs, it had an antiaggregatory effect in vivo and in vitro in the same model [4], and lower doses have been effective in animal models of coronary thrombosis [10] and in patients with acute coronary syndromes. Previous studies in the canine model have shown that neither ibuprofen [25] nor aspirin, 2–12 mg/kg [27] decreased the magnitude of myocardial platelet accumulation after reperfusion, although in none of these studies the occurrence of CFRs was reported. This lack of effect of aspirin indicates that other mediators different from or in addition to thromboxane A2 are involved. In this respect, the correlation between platelet content and MPO activity would suggest a role for activated PMNs, which is in agreement with previous studies demonstrating an enhanced platelet–PMN co-operation in reperfused microvessels [28] or the possibility to reduce platelet accumulation by neutrophil depletion [29] or by interfering with platelet–PMN interaction [26].
Aspirin-treated animals showed a trend to increased infarct size, which is in conflict with our previous observation [4]. The longer period of reperfusion in that study (6 vs. 2 h in the present one) might help explain this apparent discrepancy since it might have provided more room for the potential benefits of aspirin to manifest. Moreover, the present results on infarct size must be interpreted cautiously since they have not been corrected for the size of the area at risk. In a recent study in dogs submitted to 90 min OC and 6 h reperfusion through a critical residual stenosis, several doses of intravenous aspirin given before reperfusion did not significantly modify infarct size, although animals given 12 mg/kg showed a trend to smaller infarcts [27].

Several methodological considerations are pertinent. The myocardial content of radio-labelled platelets was quantified in two central slices downstream of the damaged LAD. A more comprehensive sampling of myocardial tissue would have reduced the possibility of missing platelet microemboli. However, considering the average size of the area at risk in our model, approximately 20% of it was sampled, which may be considered representative to detect a significant embolization from the mural thrombus. This strategy allowed the histological analysis of two additional whole slices. The hearts were perfused after excision for several minutes to avoid that blood sequestration in myocardial samples could distort the results. An important concern is that, if the perfusion was inadequate, the impact of CFRs could have been masked, either by an increased background activity or because microemboli had been flushed out of the microvessels. It seems unlikely that this could have occurred since similar perfusion protocols have been used in studies demonstrating the presence of platelet aggregates in myocardial microvessels [19,20,22], platelet counts in the control zone were practically identical among groups and platelet emboli or hemorrhage were rarely seen at histological examination. On the other hand, it is possible that more microemboli would have been detected had the experiments been prolonged for more than 2 h. However, this duration was selected to prevent that any increase in platelet content due to embolization could be masked by the progressive platelet deposition that occurs within the following hours after reflow [24], while it allowed for a rather large number of CFRs. Finally, thiopental and other barbiturates may impair in vitro and ex vivo platelet function [31], but it is unlikely that this drug has had a potent in vivo antiaggregant effect in our study since most animals developed a rapid and sustained pattern of CFRs reflecting dynamic intracoronary thrombosis.

5. Clinical implications

The potential importance of embolization in atherosclerotic vascular disease, especially after percutaneous coronary intervention, has recently been stressed [32]. Since the model of intracoronary thrombosis with CFRs resembles the pathophysiology of unstable angina or non-ST elevation acute myocardial infarction [33,34], the present results are against a significant mechanical obstruction of the microvascular bed by multiple microemboli in patients with these acute coronary syndromes. Although aspirin exerts a clear beneficial effect on the culprit lesion, the finding that myocardial platelet accumulation is not reduced by standard antiplatelet therapy may also be of interest. Finally, the results are in agreement with a cooperation between platelets and PMNs in reperfused microvessels [28], and stress the advisability of further studies that assess the effects of interventions targeting this cooperation, by interfering the interaction of P-selectin with its glycoprotein ligand or by preventing β₁-integrin activation, on platelet microvascular accumulation and myocardial salvage after reperfusion.

Acknowledgements

This study was supported in part by a grant from the Fondo de Investigación Sanitaria de la Seguridad Social FIS 99/1059, by the 1997 Beca Maratón TV3, and by the 1998 Beca Pfizer from the Sociedad Española de Cardiología.

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