Influence of Platelet–Vessel Wall Interactions on Leukocyte Rolling In Vivo

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The influence of platelet–vessel wall interactions on leukocyte rolling was investigated in rabbit mesenteric venules (diameter, 21–40 μm) using intravital videomicroscopy. Puncture of the wall with glass micropipettes (tip, 6–8 μm) evoked the formation of a thrombus in all venules. In most vessels, emboli were produced as well. The rolling of leukocytes (i.e., their movement along the vessel wall at a velocity clearly lower than that of the other blood cells) was quantitated simultaneously in vessel segments upstream and downstream from a thrombus up to 10 minutes after puncture. During embolization the number of rolling leukocytes decreased significantly from the upstream to the downstream vessel segment (median decrease, 45%; p<0.001). It was still decreased by ~50% after embolization had stopped, indicating that the decrease in leukocyte rolling was not caused by inclusion of leukocytes in the emboli. In venules without embolization, leukocyte rolling did not change systematically, indicating that fluid dynamic changes induced by the thrombus do not influence leukocyte rolling. Inhibition of prostaglandin formation with aspirin (100 mg/kg) almost completely abolished the influence of the thromboembolic reaction on leukocyte rolling, but blockade of thromboxane A2 receptors with sulotroban (30 mg/kg) had no effect. In conclusion, this is the first report on a functional interaction in vivo, at a site of vessel wall injury, between platelets, vascular cells, and leukocytes. The findings suggest that substances produced by activated platelets and/or damaged vascular cells diminish leukocyte rolling. The identity of these substances is not yet clear, but the present study indicates that prostaglandins other than thromboxane A2 are involved. (Circulation Research 1992;70:355–363)

A striking phenomenon in the microcirculation of the exposed mesentery is leukocyte margination. Leukocytes roll along the wall of venules at a velocity clearly lower than that of the flowing blood cells. This behavior, which is not observed in mesenteric arterioles even after vessel wall injury,1 may be the first step in the reaction of leukocytes to inflammatory stimuli and is followed by their adherence to the vessel wall, diapedesis, and migration into the tissue.2

During recent in vivo experiments in which the interaction of blood platelets with a microinjured vessel wall was studied,3 we noticed that this interaction influenced leukocyte rolling in venules. Although studies performed in vitro indicate that leukocyte behavior can be altered by substances derived from activated platelets4–6 or damaged endothelial cells,6,7 it is not clear whether such cell–cell interactions also occur in vivo.

The aim of the present study was to use intravital videomicroscopy to investigate in vivo, in rabbit mesenteric venules, whether interactions between platelets and a damaged vessel wall influence leukocyte rolling. Since this was found to be the case, the involvement of prostaglandins, if any, was investigated by repeating the study after pretreatment of rabbits with aspirin, in a dose sufficiently high to inhibit the enzyme cyclooxygenase in both platelets and the vessel wall.1 The possible role of thromboxane A2 (TXA2) was studied by administration of sulotroban, a selective TXA2-receptor blocker.8

Materials and Methods

Experimental Setup and Intravital Videomicroscopy

Experiments were performed on 34 rabbits of various breeds and either sex. Their weights ranged from 1.9 to 3.5 kg. Anesthesia was induced by intramuscular injection of 40 mg/kg body wt ketamine hydrochloride (Vetalar, Parke-Davis, Morris Plains, N.H.; or Nimatek, Ad Usem Veterinaria, The Netherlands) and 4 mg/kg body wt xylazine hydrochloride (Rompun, 2% solution, Bayer; or Sedamun, Ad
USem Veterinarum). It was maintained by continuous intravenous infusion of ketamine (40 mg/kg/hr) and xylazine (5 mg/kg/hr), dissolved in a lactetrol solution (15 ml/hr, Aesculap). The solution was infused through a catheter (PE-60) in the femoral vein.

After induction of anesthesia, blood was collected from a central ear artery in EDTA (0.1 vol, 0.027 M). In this blood sample, electronic platelet counts and hematocrit measurements were performed in duplicate with a Coulter counter (model ZF, Coulter Electronics Ltd., Luton, England) and an Autocrit II centrifuge (Clay Adams), respectively. For measuring arterial blood pressure (external pressure transducer, model CP-01, CTC) and heart rate, a second catheter (PE-60) was placed in the femoral artery. To keep this catheter patent, it was continuously perfused with physiological saline (5 ml/hr) via an Intralfo II-system (Sorenson Research). The rabbits were ventilated throughout the experiments. The trachea was canulated, and the cannula (3.5 or 4.5 mm i.d., Mallinckrodt) was connected to an infant ventilator (LOOSCO, model mk 2, Hoeckloos, Amsterdam, The Netherlands). Ventilation was performed with a mixture of oxygen (22%), nitrogen (76%), and carbon dioxide (2%). Respirations were 60/min, and the tidal volume varied from 17 to 20 ml, depending on the weight of the rabbit. Blood gas and pH values were assessed every hour in blood collected from a central ear artery, using an acid–base analyser (model ABL 3, Radiometer, Copenhagen). Blood gas values (mean±SD) during the experiments were 38.3±6.2 mm Hg for Pco2 and 98.5±12.7 mm Hg for Po2 at pH 7.42±0.03. These values are within the normal range for rabbits.3,9

Mesentery preparation and intravital videomicroscopy were performed, as described in detail before.3 In short, a segment of the distal ileum was brought outside the abdomen through a small midline incision. The mesentery was carefully spread over a siliconized glass plate mounted in an electrically heated microscope table (37°C). It was continuously superfused with a buffered Tyrode’s solution (37°C, pH 7.35–7.40). The ileum was kept moist with overlying wet gauze. All observations of the mesenteric microcirculation were made with a Leitz intravital microscope adapted to telescopmic imaging.10 Videomicroscopy was performed with a Leitz LL25 objective (long working distance; numerical aperture, 0.35) under transillumination with a tungsten lamp. Images were recorded on 1-in. videotape through a TV camera (Ultricon 4532, Bosch). The final magnification at the front plane of the camera was ×52.

The diameter of the selected venules was determined off-line with an image-catching device, as described by Intaglia and Tompkins11; red blood cell velocity was measured on-line using a prism grating system with the slit covering the whole vessel width.12 The velocity values, as measured in this way, were divided by a conversion factor of 1.1 to obtain actual mean red blood cell velocities.13,14 Reduced velocity (mean red blood cell velocity/vessel diameter), which is a measure of wall shear rate, was calculated from these parameters.

Vessel Wall Injury and Thromboembolic Reaction

Venules were selected, ranging in diameter from 20 to 40 μm. To induce an injury, the vessel wall was punctured with a glass micropipette (tip diameter, 6–8 μm), as previously described.3 In short, the tip of a micropipette was placed against the wall of a venule (see Figure 1) with the help of a micromanipulator. Puncture of the vessel was performed by tapping on the micromanipulator, which caused the micropipette to penetrate the wall, whereupon it spontaneously returned to its original position. Puncture was considered to be successful only if red blood cells left the vessel. The moment of puncture and the subsequent intravascular reactions were recorded on videotape for a period of 600 seconds.

Immediately after puncture, bleeding and the formation of a thrombus started in all vessels. Ongoing activation of circulating platelets with subsequent adhesion and aggregation occurred in most vessels, preferentially on the downstream side of the thrombus. After some time, such a downstream part broke loose and embolized. This process of thrombus growth and embolus formation could repeat itself several times. The size of the thrombus itself remained more or less the same after each embolization. This thromboembolic reaction was analyzed off-line. Bleeding time, thrombus height relative to the local vessel diameter, the number of emboli produced, and the duration of embolization were determined. Emboli were only counted if their short axis, perpendicular to the vessel wall, was greater than 25% of the local vessel diameter. Aggregates of smaller dimensions could not always be distinguished from the background with sufficient accuracy.

Leukocyte Rolling

The number of rolling leukocytes was determined off-line near the site of puncture by two independent observers. All leukocytes that were moving along the vessel wall and could be detected by eye were considered as rolling (Figure 1). Rolling leukocytes either moved at a rather constant velocity, which is approximately an order of magnitude lower than red blood cell velocity, or saltated (i.e., periods of contact with the wall were alternated with periods of traveling in the blood stream).15,16 To include also the saltating cells, counting was performed in a vessel segment with a length of 50–100 μm, adjacent to the thrombus-containing segment. Cells present in the segment at the beginning of the period of counting were not included. Rolling was expressed as the number of cells passing per minute through the cross section at the entrance of the vessel segment. The numbers found by the different observers were averaged.

In 36 venules of 20 control rabbits, the number of rolling leukocytes was determined before puncture and at several moments after puncture, both up-
FIGURE 1. Intravital videomicroscopy showing leukocyte rolling in a venule (diameter, 34 µm), immediately before puncture. The micropipette is already positioned against the vessel wall. In the time period between both video frames (1.4 seconds), the rolling leukocytes (arrows) have traveled along the wall at different velocities. The direction of the blood stream is from left to right.

stream and downstream from the thrombus. The periods of counting lasted 60 seconds. To evaluate the influence of puncture itself, in 15 of the venules the number of rolling leukocytes before puncture was determined proximal to the site of injury and compared with the number determined in the same vessel segment immediately after puncture. The first counting period after puncture started after bleeding had stopped but always within the first minute after induction of vessel wall injury. In six venules, a thrombus was formed, but no embolization occurred; in these vessels, counting was performed only once. In the other 30 venules, a thrombus as well as emboli were produced. In 15 of these vessels, the number of rolling leukocytes could be determined during the process of embolization and, in most instances (n=12), again after embolization had stopped. In the other 15 venules, the period of embolization was so short that counting started after embolization had already stopped.

In all experiments, the mid plane of a vessel, where the diameter was largest, was kept in focus. To assure that all rolling leukocytes in a selected vessel segment could be distinguished, including those not in the plane of focus, the objective was focused up and down through the lumen of some vessels (diameters, 30–40 µm). In this way it could be demonstrated that, indeed, leukocytes rolling along the upper or lower wall were seen when focus was in the mid plane of the vessel.

With increasing duration of an experiment, more substances produced by the exteriorized mesenteric tissue, if any, may preferentially enter the venules, because of their relatively high permeability as compared with that of arterioles. Such substances could influence leukocyte behavior and the thromboembolic reaction in the venules. Therefore, it was investigated whether the duration of an experiment (i.e., the time period between the moment of mesentery preparation and puncture) was related to leukocyte rolling and/or thromboembolic parameters.

Effects of Aspirin

To investigate the role of prostaglandins in the influence of the thromboembolic reaction on leuko-
cyte rolling, five rabbits received aspirin (acetylsalicylic acid, Sigma Chemical Co., St. Louis, Mo.) in a dose of 100 mg/kg. The aspirin was suspended in water at a concentration of 100 mg/ml. Subsequently, sodium carbonate crystals were slowly added until the aspirin crystals had dissolved. The pH of the solution remained just below 7.0, ensuring that hydrolysis of the aspirin was minimal. The aspirin solution was administered intravenously through a polyethylene catheter in the marginal ear vein in 1–2 minutes. Vessel wall puncture started 15 minutes after administration.

The influence of this dose of aspirin on platelet function and on leukocyte rolling was also studied separately. The effect on platelet function was determined by performing ex vivo aggregation tests. Before and 3 hours after administration of aspirin to the rabbits, 4.5 ml blood was collected from a central ear artery in 0.5 ml of 3.13% trisodium citrate (110 mM). The preparation of platelet-rich plasma (300,000 platelets/μl) and the aggregation tests were performed as described previously. Aggregation was induced by collagen (Horm, Hormon-Chemie, München, FRG); final concentrations were 5, 10, and 20 μg/ml. A final concentration of 5 μg/ml collagen normally induces a submaximal aggregation (90–95% of the maximal reaction). From the tracings the maximal degree of aggregation and the maximal rate of aggregation were determined. Drifts in gas tensions and pH in the platelet-rich plasma before and during the aggregation tests were prevented by keeping the platelet-rich plasma in closed volumes with 95% N2–5% CO2.

The influence of aspirin on leukocyte rolling was studied in three more rabbits. The number of rolling leukocytes was determined in duplicate immediately before as well as 20, 50, and 80 minutes after administration of aspirin. The results obtained were compared with data from three control experiments, in which the rabbits only received the vehicle.

**Effects of Sulotroban**

To determine whether TXA2 is involved in the cell–cell interactions investigated in the present study, three rabbits received 30 mg/kg sulotroban (BM 13.177, kindly provided by Boehringer Mannheim GmbH, Mannheim, FRG) at the beginning of an experiment. In rabbits, this substance acts as a TXA2 receptor blocker. Sulotroban was suspended in saline (154 mM NaCl) and dissolved by adding NaOH (1.0N) under stirring up to an equimolar concentration (final concentration of sulotroban, 30 mg/ml). The pH of the solution was adjusted to 7.4. The compound was administered like aspirin.

The effect of sulotroban on the sensitivity of blood platelets to TXA2 was tested ex vivo by means of platelet aggregation induced by the TXA2 mimetic agent U46619 (The Upjohn Co., Kalamazoo, Mich.). The final concentrations were 1 and 10 μM. In control situations, 1 μM U46619 induces a submaximal aggregation reaction (90–95% of the maximal reaction). Collection of the blood, aggregation tests, and analysis of the tracings were performed as described above.

**Statistics**

Because of their nonsymmetrical distribution, most data are presented as medians. Correlations were performed with Spearman's nonparametric rank correlation test (coefficient r). Paired data groups were compared using the Wilcoxon signed-rank test. The Mann-Whitney U test was used to compare independent data groups. In all tests the level of significance was set at 5%.

**Results**

In the control rabbits as well as in the rabbits treated with aspirin or sulotroban, electronic platelet counts (range, 170–632×106 platelets per liter), hematocrits (32–43%), mean arterial blood pressures (65±8 [mean±SD] mm Hg), and heart rates (118±21 beats per minute), as measured at the moments of puncture of the venules, were all within the normal ranges.

After puncture of the venular wall, bleeding times were short in the vessels of all groups, ranging from 0.1 to 15.0 seconds (median, 1.9 seconds). A thrombus was formed in all vessels within 1–2 seconds. The relative thrombus heights as well as the fluid dynamic conditions in the venules of control and aspirin- and sulotroban-treated rabbits are summarized in Table 1; no significant differences existed between the groups. No correlations were found between fluid dynamic parameters or the duration of the experiment (range, 15–182 minutes) and thromboembolic parameters or the degree of leukocyte rolling before puncture.

Rolling leukocytes were observed before vessel wall puncture in all venules (control: median, 46 rolling leukocytes per minute, range, 5–138 per minute; aspirin: median, 49 rolling leukocytes per

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**TABLE 1. Fluid Dynamic Parameters and the Thrombus Height, Relative to the Local Vessel Diameter, as Measured in Venules of Control Rabbits and Aspirin- and Sulotroban-Treated Rabbits**

<table>
<thead>
<tr>
<th>Group</th>
<th>Diameter (μm)</th>
<th>Mean RBC velocity (mm/sec)</th>
<th>Reduced velocity (sec⁻¹)</th>
<th>Thrombus height (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>Control (n=36)</td>
<td>30</td>
<td>21–40</td>
<td>1.5</td>
<td>0.7–4.7</td>
</tr>
<tr>
<td>Aspirin (n=10)</td>
<td>30</td>
<td>23–37</td>
<td>1.3</td>
<td>1.0–2.1</td>
</tr>
<tr>
<td>Sulotroban (n=8)</td>
<td>32</td>
<td>23–38</td>
<td>1.4</td>
<td>1.1–1.8</td>
</tr>
</tbody>
</table>

RBC, red blood cell; Reduced velocity, mean RBC velocity/vessel diameter.
minute, range, 17–92 per minute; sulotroban: median, 62 rolling leukocytes per minute, range, 5–284 per minute) but never in arterioles. Puncture itself did not influence leukocyte rolling, as determined upstream from the site of injury in 15 control venules (41 rolling leukocytes per minute before and 40 per minute immediately after puncture [median values]).

Influence of the Thromboembolic Reaction on Leukocyte Rolling

The thromboembolic reaction in the venules of the control group was similar to the reaction found in earlier studies. The number of emboli produced per vessel ranged from zero to 73, with a median number of only one.

In the venules in which only a thrombus was formed but no emboli, the number of rolling leukocytes was not systematically different in the upstream and downstream vessel segments, as is shown in Figure 2A. In contrast, in all venules in which emboli were produced and in which the number of rolling leukocytes could be counted during embolization, leukocyte rolling decreased from upstream to downstream from the thrombus (Figure 2B; median decrease, 45%; p < 0.001). The decrease in leukocyte rolling still existed after embolization had stopped (Figure 2C; counted on the average between the third and fourth minute after embolization; median decrease, 50%; p < 0.01). In nine venules, leukocyte rolling was counted again up to the 10th minute after embolization had stopped. Within this period, the decrease in leukocyte rolling hardly changed; it diminished by only 8% (median value) from the first period of 5 minutes to the second period of 5 minutes after embolization.

The change in the number of rolling leukocytes, expressed as the downstream/upstream ratio, was weakly correlated with the number of emboli produced per vessel (r = -0.45, p < 0.01, n = 36). The correlation with thrombus height (r = -0.41) was close to the level of significance (p < 0.10). Thrombus height did not change during and after the process of embolization; no adhesion of leukocytes to the thrombus was observed. No relation was found between the change in leukocyte rolling on the one hand and vessel diameter, mean red blood cell velocity, or reduced velocity on the other.

Effects of Aspirin

Ex vivo, aspirin treatment completely inhibited platelet aggregation as induced with 5 or 10 µg/ml collagen. With 20 µg/ml collagen, the inhibitory effect of aspirin was still strong: the maximal degree and rate of aggregation were reduced to 11% and 3%, respectively, of the reaction before aspirin administration.

The thromboembolic reaction, as induced by puncture in vivo, was not significantly influenced by aspirin treatment. Neither the relative thrombus height (Table 1) nor the number of emboli produced (median, 2.5; range, 0–7) was different from control.

Aspirin did not significantly influence the degree of leukocyte rolling in the course of an experiment. Twenty, 50, and 80 minutes after aspirin administration, the number of rolling leukocytes, as normalized to the number before administration, was 1.18, 0.80, and 0.63, respectively (median values, eight venules). These values were not different from the number of rolling leukocytes in the rabbits that only received the vehicle (0.82, 0.62, and 0.66, respectively; nine venules).

Figure 3 shows the effect of the thromboembolic reaction on leukocyte rolling in the aspirin-treated rabbits (10 venules). In the two venules in which only a thrombus was formed but no emboli, leukocyte rolling decreased from upstream to downstream from the thrombus in one vessel and increased in the other (Figure 3A). In the venules in which counting was performed during embolization (n=7), a small but nonsignificant decrease in leukocyte rolling was noted (Figure 3B; median decrease, 12%; p=0.10). The same holds for the eight venules in which
leukocyte rolling was counted after embolization had stopped (Figure 3C; median decrease, 14%; p>0.10).

Effects of Sulotroban

Ex vivo, sulotroban completely inhibited platelet aggregation as induced with 1 μM U46619. Aggregation induced with 10 μM U46619 was also inhibited; maximal degree and rate of aggregation were diminished to 77% and 65%, respectively, of the reaction before administration of sulotroban.

The thromboembolic reaction in vivo was not significantly influenced by sulotroban treatment. Both thrombus height (Table 1) and number of emboli produced per vessel (median, 3; range, 0–20) were not significantly different from control.

The influence of the thromboembolic reaction on leukocyte rolling in the venules (n=8) of the sulotroban-treated rabbits is shown in Figure 4. In two venules, a thrombus was formed, but no embolization occurred. In one of these vessels, leukocyte rolling decreased from upstream to downstream from the thrombus, whereas it increased in the other (Figure 4A). In the six venules in which emboli were produced, leukocyte rolling decreased significantly from the upstream to the downstream vessel segment during embolization (Figure 4B; median decrease, 66%; p≤0.05). In the four venules in which the degree of leukocyte rolling could be determined after embolization had stopped, the median decrease was also 66% (Figure 4C, sample too small to test statistically).

Comparison of Aspirin and Sulotroban Data With Control

In Figure 5, the median influence of the thromboembolic reaction on leukocyte rolling is shown for all three groups of rabbits. The influence was significantly different in the aspirin-treated and control rabbits. After embolization had stopped, the decrease in leukocyte rolling from upstream to downstream from the thrombus was significantly less in the aspirin-treated group (p≤0.05); during embolization the difference did not reach the level of significance (p>0.10). In the sulotroban-treated rabbits, the change in leukocyte rolling from upstream to downstream from the thrombus was significantly different from the control group (p≤0.05), and from the aspirin group (p>0.10).
bus was not significantly different from control, either during or after embolization.

**Discussion**

The thromboembolic reaction after vessel wall injury causes a significant decrease in the number of rolling leukocytes in rabbit mesenteric venules. This decrease is found only when one or more emboli are produced beside a thrombus. The effect lasts for at least 10 minutes after embolization has stopped. Inhibition of prostaglandin formation with aspirin significantly reduces the influence of the thromboembolic reaction on leukocyte rolling, whereas blockade of TXA$_2$ receptors has no effect.

It is unlikely that the decrease in the number of leukocytes rolling from upstream to downstream from the thrombus merely results from the presence of a partially occluding thrombus. The finding that no systematic decrease in rolling occurs in venules in which a thrombus is formed without subsequent embolization (Figures 2A, 3A, and 4A) implies that the relatively high velocity in the stenosis is not the cause of the detachment of rolling leukocytes from the wall into the central blood stream. In addition, the observation that thrombus heights and fluid dynamic conditions are similar to control in the venules of the aspirin- and sulotroban-treated rabbits (see Table 1) indicates that the effective cross-sectional area of the thrombi as well as the fluid dynamic conditions around the thrombi are similar in the three groups.$^{22}$ Hence, the differences in results among these three groups are not caused by fluid dynamic factors. These statements are supported by the findings that the degree of leukocyte rolling before puncture and the change in leukocyte rolling, as induced by the thromboembolic reaction, do not correlate with any of the fluid dynamic parameters measured. This is in accordance with the observation of Mayrovitz and coworkers$^{23,24}$ that the number of rolling leukocytes in venules is not influenced by changes in blood flow.

The decrease in rolling of the leukocytes downstream from the thrombus is not caused by attachment to and capture of leukocytes within the emboli: the number of rolling leukocytes is still decreased after embolization has stopped (compare Figures 2B and 2C). Because the size of the thrombus itself does not visibly change during and after embolization, it is unlikely that the decrease in leukocyte rolling results from adherence of leukocytes to the thrombus.

In the light of the above arguments, the diminished degree of leukocyte rolling distal to the thrombus during and after embolization is likely to be caused by substances released from activated platelets and/or from cells in the damaged vessel wall during the second phase of the thromboembolic reaction (i.e., the phase of embolization). This is supported by the significant correlation between embolus production and, hence, platelet activity on the one hand and the change in leukocyte rolling on the other, which indicates that such substances are produced in relatively large quantities when embolization activity is high. The finding that leukocyte rolling is still decreased up to 10 minutes after embolization has stopped, however, suggests that these substances are not involved in the activation of platelets themselves. Therefore, platelet-activating substances that have been shown to play a role in interactions between platelets, endothelial cells, and leukocytes in vitro, like 12-hydroxyeicosatetraenoic acid, serotonin, and platelet-activating factor,$^{4-6}$ are probably not directly involved in the in vivo cell-cell interactions observed in the present study. Moreover, these substances have been reported to augment rather than inhibit leukocyte adhesiveness in vitro.$^{4-6}$ An alternative possibility is that platelet antagonists are involved. Substances released by endothelial cells, like platelet-inhibiting prostaglandins or endothelium-derived relaxing factor, may simultaneously limit the duration of the thromboembolic reaction and the extent of leukocyte rolling. This notion is supported by our findings with aspirin and sulotroban.

The data obtained from the aspirin-treated rabbits indicate that the influence of the thromboembolic reaction on leukocyte rolling is at least in part mediated by prostaglandins. These prostaglandins probably do not play a major role in leukocyte rolling or thromboembolic reaction themselves, because aspirin influenced neither the degree of leukocyte rolling in the course of an experiment nor the number of emboli produced in venules. The experiments performed with sulotroban, which blocks TXA$_2$ receptors$^8$ but does not interfere with the production of other substances in the cyclooxygenase pathway, show that TXA$_2$ is probably not involved. This finding is in agreement with the above arguments that platelet-activating substances do not play a role in the decrease of leukocyte rolling from upstream to downstream from a thrombus. Moreover, this finding does not necessarily contradict reports on a mediating role...
of TXA₂ in leukocyte adhesiveness²⁵ and diapedesis,²⁶ because of the increasing evidence that different mechanisms are involved in the rolling of leukocytes and maintained leukocyte adherence.²⁷,²⁸

A substance that may be involved is prostacyclin (PGI₂). This prostaglandin, in concentrations present in vivo, inhibits adherence of polymorphonuclear leukocytes to cultured endothelial cells,⁷ whereas a synthetic PGI₂ analogue has been shown to reduce leukocyte accumulation in the ischemic myocardium in vivo.²⁹ The production of PGI₂, a substance that is synthetized by vascular cells and especially endothelial cells,³⁰ can be stimulated by platelet agonists³¹ that are produced at the site of vessel wall injury. It inhibits platelet activation³² and will limit the extent of the thromboembolic reaction. It is conceivable that the extra production of PGI₂ continues over a longer period of time than the thromboembolic reaction, as observed through the microscope. This would explain the finding that the decrease in leukocyte rolling is still present when embolization has stopped. Besides the possible involvement of PGI₂, a role for other prostaglandins produced by the vessel wall, like prostaglandin D₂ and prostaglandin E₂,³³,³⁴ cannot be excluded.

Inhibition of the cyclooxygenase pathway by 100 mg/kg aspirin in rabbits is associated by an overflow of arachidonic acid into the lipoxygenase pathway.¹ This may result in the formation of leukotrienes.³⁵ Leukotriene B₄ has been shown to induce in vivo an increase in leukocyte adherence to the venular wall, accompanied by a decrease in leukocyte rolling.³⁶ It is unlikely that in the aspirin-treated rabbits in the present study this overflow has led to a large enough production of leukotrienes to influence leukocyte behavior, because no increase in leukocyte adherence is observed, whereas the change in degree of leukocyte rolling over the thrombus was less than in the control group.

In conclusion, this is the first report on in vivo interactions between blood platelets, leukocytes, and vascular cells at a site of vessel wall injury. The rolling of leukocytes, a phenomenon found in venules and not in arterioles, is inhibited by platelet–vessel wall interactions after vessel wall damage. This phenomenon is not the result of fluid dynamic effects caused by the thrombus or capture of leukocytes in the emboli or the thrombus. Substances produced by activated platelets and/or damaged vascular cells that are able to inhibit leukocyte rolling are likely to be involved. The identity of these substances is not yet clear; the present study indicates that prostaglandins other than TXA₂ are involved.

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