Effects of a Novel Antiplatelet Agent in Mural Thrombogenesis on Collagen-Coated Glass

By Bernard J. Folie, Larry V. McIntire, and Andrew Lasslo

A parallel plate flow chamber and an epifluorescence video microscopy system were used to investigate the inhibitory effect of a novel antiplatelet agent (GT-12), a carbamoylpyrroperi dine congener, on surface platelet aggregation and on the kinetics of thrombus growth induced by collagen-coated glass under controlled flow. Both macroscopic and microscopic measurements revealed that increasing concentrations of the drug correspondingly decreased the reaction rate between platelets at the surface, thereby reducing thrombus rate of growth at the surface. Because of decreased platelet/platelet adhesion, there was some embolization of the larger thrombi near the inlet of the reactive surface. In the presence of GT-12, average thrombus size and number of platelets per thrombus were both strikingly lowered. In addition, the net rate of growth of individual thrombi decreased to zero after short exposure times (about 60 seconds), in sharp contrast to controls. In contrast to chlorpromazine, GT-12 was effective in inhibiting platelet aggregation and thrombus rate of growth at relatively low concentrations (<100 μmol/L) in whole blood. The drug's effectiveness relative to controls in impeding platelet/platelet interactions was found to increase with decreasing incubation time and increasing perfusion time.

© 1988 by Grune & Stratton, Inc.

MATERIALS AND METHODS

Blood sample preparation. Blood was collected from the antecubital vein of 12 consenting donors into 10 U/mL of heparin (anticoagulant; liquaemin sodium, Elkins-Sinn Inc. Cherry Hill, NJ) and 10 timol/L mepacrine (fluorescent probe; quinacrine for contrast payment. This article must therefore be hereby marked to indicate this fact.

From the Department of Chemical Engineering (Biomedical Engineering Laboratory), Rice University, Houston, and the Department of Medicinal Chemistry, Health Science Center, The University of Tennessee, Memphis.

Submitted November 13, 1987; accepted June 17, 1988.

Supported by Grants Nos. HL-17437, HL-18672, and HL-22236 from the National Institutes of Health, and Grant No. C-938 from the Robert A. Welch Foundation.

Address reprint requests to Larry V. McIntire, PhD, Department of Chemical Engineering, Rice University, PO Box 1892, Houston, TX 77251.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1988 by Grune & Stratton, Inc.
0006-4971/88/7204-0052$3.00/0
dihydrochloride; Sigma Chemical Co, St Louis). The donors were carefully chosen as being nonsmokers and aspirin-free for at least 2 weeks prior to the day of the experiment. Mepacrine was used to label platelets in whole blood and is reported to have no effect on normal platelet function at this low concentration.12 Blood was then immediately poured into polypropylene test tubes (Sarstedt Inc, Princeton, NJ) and kept at room temperature. Thirty minutes before perfusion, the blood sample was immersed in a water bath (Precision Scientific Group, Chicago) maintained at 37°C. Blood was always used within three hours after venipuncture.

**Drug sample preparation.** Drug samples were always prepared fresh daily. Chlorpromazine hydrochloride (35.53 mg) (F.W. C_{18}H_{20}N_{3}Cl,S, 355.52; Sigma Chemical Co) was weighed into a 1.0-mL volumetric flask filled with distilled water and covered with parafilm to make a 500-mmol/L solution; 2 mL of this solution for each milliliter of blood yielded a 100-μmol/L final concentration. Similarly, 31.63 mg of GT-12 (F.W. C_{19}H_{18}Br_{2}N_{2}O_{3}, 565.56) was weighed into a 1.0-mL volumetric flask filled with 95% ethyl alcohol and covered with parafilm to make a 50-μmol/L solution; 2 μL of this solution for each milliliter of blood yielded a 100-μmol/L final concentration. The flask was then placed in a water bath at 37°C for 15 to 30 minutes to facilitate dissolution of the drug and was then allowed to cool at room temperature. Concentrations of 95% ethyl alcohol up to 2.0 μL/mL of test medium were reported not to affect normal platelet function.13 For higher doses of the drug, distilled water was used as the dissolving medium.

**Collagen preparation.** One gram of type I acid-insoluble collagen (Sigma Chemical Co) from bovine achilles tendon was mixed with 12 mL of glacial acetic acid and 198 mL of distilled water for ten minutes at room temperature in a stirrer. This solution was placed in a homogenizer (The Virtis Co, Gardiner, NY) for two hours, and after addition of 200 mL of distilled water, it was again homogenized for one more hour. The final collagen solution was centrifuged (Model J-21B, Becthan, Palo Alto, CA) for ten minutes at 1,000 rpm. The supernatant was collected and stored at 4°C. The final collagen solution was assayed for its peptide bonds by a standard spectrophotometric technique4 (Protein Assay Kit No. P 5656, Sigma Chemical Co) and the final collagen concentration was calculated to be about 850 μg/mL in a 0.522-mol/L acetic acid solution at a final pH of 2.8.

**Flow chamber and collagen coating.** A parallel plate flow chamber, described in detail elsewhere,15 was used to establish a well-defined fluid-dynamic and rheologic environment, suitable for precise analysis of mural thrombogenesis. Before being assembled as part of the flow chamber, a glass cover slip (Corning Glass Works, Corning, NY; No. 1.24 × 50 mm), was coated with 200 μL of type I acid-insoluble collagen suspended in acetic acid. The fibrillar collagen solution was allowed to rest for 45 to 60 minutes on the glass cover slip before the supernatant was rinsed off with 10 mL of sterile isotonic saline. The amount of collagen in the supernatant solution was calculated to be on average 14 μg/mL by the same spectrophotometric method as previously mentioned.14 This leads to an average collagen density on the glass surface of 3.50 μg/cm². Finally, the flow chamber was assembled and filled with sterile isotonic saline.

**Perfusion and video microscopy equipment.** Whole blood was aspirated by a syringe pump (Model 935, Harvard Apparatus, South Natick, MA) at a controlled shear rate of 1,000/s (5.337 mL/min) from a test tube into the flow chamber. The test tube, the tubing, and the flow chamber were maintained at 37°C by a thermostatic air bath (Model 279, Laboratory Products, Boston). The flow chamber was mounted on an inverted-stage microscope (DIAPHOT-TMD, Nikon, Garden City, NY) equipped with an epifluorescent illumination attachment (TMD-EF, Nikon), a high-power 100 magnification oil immersion objective (CF Achromat, numerical aperture [NA] = 1.25 to 0.9, Nikon), and a silicon-intensified target (SIT) camera (Model C-1012, Hamamatsu, Waltham, MA) suitable for very low light levels. The microscopy was performed in episcopic (EP) or incident-light mode due to the opacity of whole blood. The experiments were recorded in real-time on a 0.5-inch color video cassette recorder (Model BR-3100U, JVC Industrial Audio/Video, Houston). Digitization of the video tape images and further image processing and analysis were then performed by a digital image processor (Model 327, Perceptive Systems, Houston). This technique involved the real-time, local visualization and analysis of mural thrombogenesis and was used to analyse the kinetics of thrombus growth, to model the three-dimensional structure of individual thrombi, and to obtain different morphologic information on each thrombus. A more detailed description of the equipment and the digital image processing techniques used in the microscopic data analysis is available elsewhere.14,15

Automatic scanning and direct recording of the locally averaged fluorescent intensity level in the entire field of view were performed by a microphotometric measurement system consisting of a photodiode (Model PIN-10DP/SB, United Detector Technology, Hawthorne, CA), and an amplifier with variable transimpedence gain (Model 101C, United Detector Technology). The amplifier acted as a current to voltage converter with the output signal directly recorded on a chart recorder. A 40× FLUOR objective (Nikon; CF Fluor, NA = 0.85) was used for that purpose. This technique was used to measure continuously the end-point accumulation of platelets on a collagen-coated surface.

In order to convert locally averaged fluorescent intensity to locally averaged platelet density at any given axial position along the coated surface, the analysis required the knowledge of the total number of platelets accumulated on the coated surface during the perfusion period, as previously reported.15 For that purpose, the glass cover slip was carefully removed from the flow chamber at the end of the perfusion period. The excess blood was flushed off the cover slip with 3.0 mL of isotonic saline; the cover slip was then dipped for 30 seconds in a stromatolysing agent (ZAP-OGLOBIN II; Coulter Electronics Inc, Hialeah, FL) solution consisting of 20.0 mL of ISOTON II and ten drops of the agent. This step was necessary to lyse all remaining RBCs sticking on the surface. The cover slip was then crushed in a 50.0 mL conical test tube containing 1.0 mL of 1% Triton X-100 cell lysing buffer. The sample was then sonicated for five seconds, centrifuged at 1,000 rpm for ten minutes to remove the glass fragments, and stored at 4°C before being assayed.

Two biochemical assays were performed on the experimental and/or the control samples with known concentrations of platelets and RBCs. All experimental and control samples were assayed for the platelet cytoplasmic enzyme lactate dehydrogenase (LDH) by a standard spectrophotometric technique.14 Because RBCs contain a large amount of LDH activity, the possible contamination of the control samples by a few RBCs might invalidate the results. Therefore, the samples were also assayed for the conjugated protein hemoglobin by another spectrophotometric technique.17 The amount of LDH activity per platelet was computed as

\[
\text{IU/platelet} = \frac{[\text{IU/L (PRP)}] - [\text{IU/L (FFP)}]}{\text{vol(EXP)} / [\text{platelets(PRP)}]}
\]

where IU stands for international unit and is defined as the amount of enzyme that catalyses the transformation of 1 μmol of substrate per minute. Finally, the total number of platelets accumulated on the collagen-coated surface was computed according to

\[
\text{No. platelets} = \frac{\text{IU/L (EXP)} \times [\text{platelets}]}{\text{IU/platelet}}
\]

EXP stands for values in experimental samples.
flow conditions were the same as those shown in Fig 1. The antiplatelet agent was tested at four different concentrations, namely, 20 \( \mu \text{mol/L} \) (---), 100 \( \mu \text{mol/L} \) GT-12-treated (— — —), and 200 \( \mu \text{mol/L} \) GT-12 for two minutes (— — —) at a wall shear rate of 1,000/s. The abscissa represents the axial position along the reactive surface.

RESULTS

Macroscopic measurements. The results of the macroscopic measurements are presented in Figs 1 through 4 and Table 1. Figures 1 through 4 show the platelet surface density, in number of platelets per 1,000 \( \mu \text{m}^2 \), along the collagen-coated surface. As can be seen from the control curves in Figs 1 and 4, after short-term perfusion (one or two minutes) of heparin anticoagulated whole blood, most platelet aggregation occurs at the entrance of the reactive surface. Figure 1 illustrates the difference in the inhibitory effects of chlorpromazine and GT-12 on platelet aggregation, both drugs tested at a same concentration of 100 \( \mu \text{mol/L} \). At that concentration, chlorpromazine has essentially no effect on platelet/platelet interactions on the collagen-coated surface, while the effect of GT-12 on platelet aggregation and thrombus formation is quite dramatic, as shown by a significant decrease in platelet density at the inlet of the coated surface. The effects of varying GT-12 concentrations on platelet surface aggregation are shown in Fig 2. As the drug concentration is increased from 20 to 50 \( \mu \text{mol/L} \), there is a significant decrease in platelet density, especially near the inlet of the reactive surface. However, as the drug concentration is further increased to 100 and 200 \( \mu \text{mol/L} \), surface platelet density increases again. The effect of incubation time on the aggregation-inhibitory potency of chlorpromazine and GT-12 is shown in Fig 3. Both drugs are more effective in inhibiting platelet aggregation when left only for a short period of time (five minutes) in contact with blood before perfusion. Finally, Fig 4 shows the effect of perfusion time on the aggregation-inhibitory potency of GT-12 at 50 \( \mu \text{mol/L} \) when incubated for five minutes. As shown by the two bottom curves in that figure, only a small increase in platelet density occurs between one and two minutes of blood flow, indicating that the net rate of platelet deposition on the reactive surface is very low.

Table 1 shows the average platelet density for different drug concentrations, incubation periods, and perfusion periods, expressed as percentage of the control value and calculated from the total number of platelets accumulated on the surface (see Materials and Methods) and the total area of the collagen-coated surface. The average platelet density for chlorpromazine-treated blood decreases with increasing concentration and decreasing incubation time. On the other
hand, the average platelet density for GT-12 decreases with increasing concentration up to 50 μmol/L but paradoxically increases again at higher concentrations, as was observed in Fig 2. The data for GT-12 at 50 μmol/L also indicate that the compound’s effectiveness in preventing platelet accumulation on a collagen-coated surface increases with decreasing incubation time and increasing perfusion time, which is consistent with the data presented in Figs 3 and 4.

**Microscopic measurements.** In order to analyse in detail the effect of an antiplatelet agent on platelet adhesion and aggregation on collagen, it is very informative to look at the morphology and the rate of growth of individual thrombi. The results of this microscopic study are presented quantitatively in Figs 5 and 6, and Table 2, and qualitatively in terms of two-dimensional contour images and three-dimensional models of thrombi, both pseudocolor enhanced, in Figs 7 to 9.

The typical morphologic information, length, width, average height, and basal area, are shown as a function of time in Figs 5 and 6 when the control or the 50 μmol/L GT-12–treated whole blood is exposed to collagen-coated glass for 120 seconds at a wall shear rate of 1,000/s. Also shown in Fig 6 is the total number of platelets constituting a given thrombus as a function of time. As can be seen from the shape of the control curves, the rate of growth of the thrombus, in terms of length, width, basal area, and number of platelets, is, in general, steadily increasing with time, as reported earlier.\textsuperscript{15,16} The average height of the thrombus, however, does not follow this pattern and levels off after about 75 seconds of flow. This can also be verified by looking at the three-dimensional structure of the thrombi in Fig 7, where there is no apparent difference in average thrombus height after 90 or 120 seconds of flow. The thrombus grows mostly from its basal area during that period of time. The rate of growth of the basal area is shown in 30-second intervals in the contour diagram (1) of Fig 9 for the control case. After 90 seconds of flow the thrombus grows mostly from its downstream edge, supporting the fact, noted by others, that platelets may become activated as they flow over the growing thrombus.\textsuperscript{19} It is likely, however, that the thrombus has to reach a certain size so that sufficient proaggregating substances are released in the medium and so that the flowing platelets have the time to become activated before this process occurs.

Very large differences in thrombus morphology and rate of growth, as it develops on collagen, can be observed in Figs 5 and 6 when heparinized whole blood is treated with 50 μmol/L GT-12. The shape of the curves in Fig 6 indicates that the thrombus rate of growth, in terms of number of platelets and basal area, practically vanishes after 45 to 60 seconds of flow. This is also shown in terms of the rate of change of length, width, and average thrombus height in Fig

![Fig 5. Length, width, and average height of a single growing thrombus as a function of time for the control (-----) and for 50 μmol/L GT-12–treated (---) blood. The flow conditions are identical to those shown in Fig 1.](image1)

![Fig 6. Basal area and platelet number per thrombus as a function of time for the control (-----) and for 50 μmol/L treated (---) whole blood. The flow conditions are identical to those shown in Fig 1.](image2)
Table 2. Effect of Drug Concentration on Thrombus Morphology

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (μmol/L)</th>
<th>50</th>
<th>100</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>GT-12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a)</td>
<td>40.1 ± 9.6</td>
<td>38.1 ± 4.5</td>
<td>33.4 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>(b)</td>
<td>34.5 ± 3.9</td>
<td>35.4 ± 5.1</td>
<td>31.1 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>(c)</td>
<td>13.3 ± 1.7</td>
<td>15.3 ± 2.8</td>
<td>10.3 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>(d)</td>
<td>38.1 ± 2.0</td>
<td>48.7 ± 8.4</td>
<td>20.9 ± 5.1</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a)</td>
<td>101.6 ± 9.9</td>
<td>39.9 ± 3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(b)</td>
<td>94.2 ± 10.1</td>
<td>65.6 ± 23.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(c)</td>
<td>93.6 ± 14.2</td>
<td>25.3 ± 0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(d)</td>
<td>101.4 ± 8.3</td>
<td>37.6 ± 4.4</td>
<td></td>
</tr>
</tbody>
</table>

Basal area (a), average thrombus height (b), total number of platelets per thrombus (c), and minimum number of aggregated platelets per thrombus (d) are expressed as percentages of the control values. Blood was perfused for two minutes at 1,000/s on collagen after a 30-minute incubation period with the drug.

Results are presented as mean ± SD.

5. The steady state maximum average height is about 4 μm. The dramatic effect of the compound on thrombus morphology and rate of growth can also be observed qualitatively by comparing the three-dimensional structures of thrombi at four different perfusion times in Figs 7 and 8, respectively, for the control case and the 50 μmol/L GT-12–treated case. Also, the contour diagrams (2) and (3) in Fig 9, when compared with diagram (1) for the control case, illustrate the effect of GT-12 at 200 μmol/L and 50 μmol/L, respectively, on the rate of growth of the thrombus basal area.

The data in Table 2 show the final inhibitory effects of both drugs on thrombus morphology in terms of basal area, average height, and total number of platelets, expressed as percentages of the control values, after two minutes of perfusion. Because the total number of platelets per thrombus includes both platelets involved in adhesion and aggregation, an effort to estimate the number of platelets involved in aggregation solely was undertaken to enable us to compare our results with previous data obtained in aggregometric studies with PRP. For that purpose, the surface coverage of a single platelet, adhered to a collagen-coated surface, was estimated to be approximately 4.35 μm$^2$ from measurements made from electron microphotographs and with the digital image processor in our laboratory. Using that number and the basal area of the thrombus, the maximum number of platelets adhered to the surface was computed and the minimum number of aggregated platelets, expressed as percentage of the control value, inferred as shown also in Table 2.

As previously mentioned and as illustrated in Table 2, chlorpromazine at 100 μmol/L has little effect on thrombus morphology or reaction rate between platelets. On the contrary, at that concentration, the compound might potentiate slightly the process of platelet aggregation as reported elsewhere. Chlorpromazine at 200 μmol/L and GT-12 at concentrations ranging from 50 to 200 μmol/L, on the other hand, greatly reduce both thrombus size and the aggregation process after two minutes of flow. The data also indicate that increasing the concentration of GT-12 from 50 to 200 μmol/L has little effect on thrombus morphology.

DISCUSSION

The results obtained in our experiments showed that the carbamoylpiperidine congener GT-12 profoundly interferes with the normal platelet function during blood and materials interaction in response to a strong stimulus such as a collagen-coated surface in vitro. Indeed, both our macroscopic and microscopic measurements revealed that the synthetic compound inhibits the chain reaction of platelet activation-secretion-aggregation and the subsequent thrombus formation and stabilization. In fact, the current findings corroborate previously reported observations. Specifically, GT-12 acts by interacting with and reducing the response sensitivity of anionic phospholipids, stabilizing membrane storage sites, and impeding mobilization of Ca$^{2+}$ into platelet cytosol. Obviously, through reinforcement of platelet membrane surfaces, it inhibits activation of phospholipase C and thereby precludes formation of inositol-1,4,5-triphosphate.

Fig 7. Kinetics of thrombus growth on collagen-coated glass in three dimensions for the control blood. Images were digitized at four different perfusion times as indicated on the figure, and the three-dimensional structures of the thrombi were generated digitally from local intensity measurements and pseudocolor enhanced. The length scale applies to both the length and the height.
Concurrently, as a result of reduced Ca\(^{2+}\) flux, it blocks stimulation of phospholipase A\(_2\) along with the pathway this generates.

When discussing the results of the macroscopic measurements, it is worthwhile pointing out, as mentioned previously,\(^2\) that the shape of the curves in Figs 1 to 4 represents the balance between platelet transport to the surface and platelet adhesion on, and aggregation at, the surface. Therefore, both mass transfer to the surface and rate of reaction at the surface must be considered when analyzing those results in this type of dynamic system. As illustrated by the control curves in Figs 1 and 4, most platelet aggregation occurs at the entrance of the coated surface, indicating that the process of mural thrombogenesis is limited downstream by platelet diffusion from the bulk fluid to the reactive surface. Indeed, after most platelets contained in the fluid layer adjacent to the reactive surface have reacted, this layer is depleted of platelets and is only slowly replenished with platelets due to the transport-limited process. The curve exhibited by chlorpromazine at a concentration of 100 \(\mu\)mol/L in Fig 1 is similar to the control curve; it is monotonically decreasing, showing that most platelet surface aggregation occurs at the inlet and indicating again that platelet transport to the surface further downstream is the rate-limiting step in the thrombosis process. It is clear that chlorpromazine, at that concentration, has virtually no effect on surface platelet aggregation. On the other hand, the nonmonotonic shape of the curve exhibited by GT-12, at a concentration of 100 \(\mu\)mol/L (see Fig 1), indicates that the drug weakens platelet to platelet bonds and that the larger thrombi at the inlet become more fragile and are dislodged from the surface by the fluid drag forces. In addition, by altering normal platelet function, the drug also decreases the rate of surface reaction between platelets, making the process somewhat less diffu-
sion-limited downstream. At the lowest concentration (20 mmol/L), the inhibitory effect of the drug is the least, as shown in Fig 2; platelet accumulation is decreased at the inlet but also spread out over a larger surface area; the process remains primarily diffusion-limited downstream. At 50 µmol/L, some embolization of the larger thrombi occurs at the inlet as indicated by the nonmonotonic shape of the curve. The process is still diffusion-limited, although the reaction rate between platelets is much lower. At 100 µmol/L, as mentioned above, the larger thrombi embolize from the reactive surface (but in a more restrained area than at 50 µmol/L), indicating that fewer large thrombi develop on the surface and that the drug reduces platelet to platelet interaction more effectively. As shown in Fig 2, the surface platelet density at that concentration is also becoming independent of axial position, meaning that the thrombogenesis process, under the effect of the antiplatelet agent, is slowly changing from a diffusion-limited to a reaction-limited process. The axial dependence is nearly completely removed when the drug is used at 200 µmol/L. Platelet reaction rate at the surface, mostly the rate of aggregation, becomes the rate-limiting step, preventing the formation of large thrombi and their subsequent embolization from the surface, as seen by the monotonic shape of the curve in Fig 2.

The average platelet density for GT-12 decreases with increasing concentration up to 50 µmol/L, but increases again at higher concentrations (see Table 1). This apparent anomaly can be explained by two facts: first, at 50 and 100 µmol/L, the larger thrombi embolize from the surface, decreasing significantly the number of platelets accumulated on the surface; second as the compound concentration is increased and platelet to platelet interactions are increasingly inhibited, it is likely, as shown by others,21 that platelet to collagen interactions (adhesion) are enhanced, leading to an increasing number of platelets accumulated on the reactive surface in the form of a monolayer. The results from our macroscopic measurements clearly show as GT-12 concentration is increased, the reaction rate between platelets at the surface (aggregation) also decreases, and that the platelet aggregates become so unstable that embolization of the larger thrombi can occur at the entrance of the flow chamber, indicating possibly that the compound weakens interplatelet fibrinogen bridges and/or affects indirectly the formation or conversion of thrombin to fibrin, thereby decreasing the overall thrombus stability. It should be stressed that the released emboli are miniature in size and are not prone to occlude vascular beds. While diminishing platelet aggregation, when used in increasing concentration, the antiplatelet agent indirectly potentiates platelet adhesion on the collagen fibrils. Indeed, since the fluid layer near the reactive surface is only slowly depleted of platelets when large concentrations of the drug are used, it is most likely that, downstream, the number of platelet collisions, contacts, and attachments to the surface, leading to platelet spreading and adhesion on the surface, might be enhanced.

When compared with the inhibitory effect of the well-known reference compound chlorpromazine, GT-12's action on platelet responses is much stronger, possibly indicating a molecular structure more specific for interactions with anionic phospholipids21 within the platelet plasma membrane's lipid bilayer, and hence more suitable for hindrance of platelet aggregation. Although both compounds alter normal platelet function predominantly by the same mechanism,4 chlorpromazine has been known also to have a stabilizing effect on RBC membranes, preventing hemolysis and release of the proaggregating ADP in situations of high shear rates.24-25 There is evidence, when comparing the results presented above with previous findings obtained in aggregometric studies with citrated PRP,23 that GT-12 interacts very little with red cell membranes or with most other plasma proteins.

The striking effect of GT-12 on the morphology of a single thrombus growing on a collagen-coated surface was demonstrated by the results of our microscopic measurements. As shown quantitatively in Figs 5 and 6, the thrombus rate of growth, when treated with 50 µmol/L GT-12, practically vanishes after approximately one minute of flow, possibly indicating that an equilibrium is reached at that time between the rate of embolization of individual platelets and/or platelet aggregates from the thrombus and the rate of platelet attachment to the thrombus. Qualitatively, the effects of the compound are shown in Figs 8 and 9, respectively, in terms of three-dimensional structures of thrombi and in terms of contour diagrams. Three important pieces of information regarding the mechanism of thrombogenesis under the influence of an antiplatelet agent are obtained from those contour diagrams. First, there is evidence, by comparing the contour diagram (3) and the curve of the thrombus basal area as a function of time for the GT-12 case in Fig 6, that the upstream part of the thrombus has embolized from the surface between 60 and 90 seconds, since the basal area was measured to be the same at those times; second, after 30 or 60 seconds of flow, the thrombus grows mostly from its downstream edges; third, the thrombus is clearly elongated in the direction of flow.

The first observation agrees with the results obtained from the macroscopic measurements that show that at 50 µmol/L, GT-12 induces the embolization of parts of the larger thrombi near the inlet of the reactive surface. The second and third observations indicate that, although the compound considerably affects platelet to platelet interactions, the way platelets are recruited at the downstream ends of the growing thrombus is still the same as reported earlier.13,19 Thrombin, generated enzymatically on the surface of the platelets in the presence of extracellular Ca²⁺, and shown to be of vital importance in inducing platelet activation in this type of flow system,19,25 might be responsible for the bulk of platelet adhesion and aggregation observed, since the antiplatelet agent does not directly inhibit its production. Although apparently peculiar, the fact that increasing concentrations of GT-12 do not decrease thrombus size, or the number of aggregated platelets accordingly, as illustrated by the data in Table 2 and the contour diagrams (2) and (3) in Fig 9, shows that the final inhibitory effect of the compound is due to both the embolization rate of parts of the thrombus from the surface and the reaction rate between platelets at the surface. At 50 µmol/L, platelet reactivity at the surface is the highest, but because the thrombus grows faster and bigger,
the embolization rate is also the largest due to the combined effects of the drug action on thrombus stability and interplatelet bonds and the fluid drag forces at the high shear rate of 1,000/s. At 200 μmol/L on the other hand, the reaction rate between platelets at the surface is so low that essentially no embolization occurs. At concentrations intermediate between 50 and 200 μmol/L, it is very probable that both the embolization rate and the reaction rate determine the inhibitory effect of the drug on thrombus growth at the end of the perfusion period.

REFERENCES

Effects of a novel antiplatelet agent in mural thrombogenesis on collagen-coated glass

BJ Folie, LV McIntire and A Lasslo