Profound Platelet Degranulation Is an Important Side Effect of Some Types of Contrast Media Used in Interventional Cardiology

Nicolas A.F. Chronos, BSc, MBBS, MRCP; Alison H. Goodall, PhD; Darren J. Wilson, MSc; Ulrich Sigwart, MD; Nigel P. Buller, BSc, MBBS, MRCP

Background. Thrombotic complications occurring during coronary angiography and percutaneous transluminal coronary angioplasty (PTCA) are relatively frequent and can be influenced by the type of radiographic contrast media used. Low osmolar contrast media (LOCM), both ionic and nonionic, have been considered to be safer than the older high osmolar contrast media (HOCM), causing less haemodynamic and symptomatic side effects. Recently, however, nonionic LOCM have been associated with an increased incidence of thrombotic events, including coronary occlusion and stroke.

Methods and Results. The effects of commonly used contrast media on platelets in native blood were investigated using immunolabelling and flow cytometry to detect platelet activation in vitro. A nonionic LOCM (Omnipaque) caused profound platelet degranulation in nearly 80% of platelets compared with 2 to 5% of platelets in the control. Conversely, an ionic HOCM (Urografin) caused only 25% degranulation, whereas an ionic LOCM (Hexabrix) caused no platelet activation and, furthermore, it inhibited the effects of thrombin on platelets. Platelet degranulation, quantified by immunolabelling, was paralleled by release of β-thromboglobulin and platelet factor 4 from platelet α-granules. Blood from patients anticoagulated with heparin and pretreated with standard-dose aspirin in preparation for PTCA showed the same pattern of contrast media–induced platelet activation as normal subjects.

Conclusions. These results suggest that the type of contrast media used during invasive imaging of the vasculature could have a significant effect on platelets. Platelet degranulation within a PTCA-damaged vessel would be increased by a nonionic contrast medium, releasing procoagulant molecules and platelet-derived growth factors into the damaged vessel lumen, which might contribute to acute thrombosis and the initiation of the restenosis process. (Circulation. 1993;88[part 1]:2035-2044.)

KEY WORDS • contrast media • platelets • angiography

During coronary angiography and vascular intervention, eg, percutaneous transluminal coronary angioplasty (PTCA), many factors may cause platelet activation and degranulation. Exposure of highly thrombogenic subendothelial collagen and local thrombin generation occur on rupture of the atherosclerotic plaque during PTCA,1 and these will promote platelet recruitment and cause platelet degranulation. Any procedural factor that would increase the susceptibility of the circulating platelets to these agonists and cause increased platelet degranulation would promote the thrombotic tendency and the local release of growth factors (eg, platelet-derived growth factor, PDGF) within the damaged coronary artery lumen.

Contrast media used to opacify vessels during angiography can be divided into three groups by virtue of their osmolality and their ionic nature (Table 1). It has been suggested that the newer low osmolality contrast media (LOCM) are associated with fewer side effects and have the advantage of causing less nausea and vomiting than the high osmolality ionic contrast media.2,3 There have, however, been several reports of increased thrombotic complications with nonionic LOCM during diagnostic cardiac catheterization4,5 and coronary angiography.6,7

Paradoxically, contrast media are known to have an anticoagulant effect that has been widely investigated. Ionic contrast media have been found to be more anticoagulant than nonionic contrast media, and this has been suggested as a possible reason for the higher incidence of thrombotic events reported with nonionic contrast media.8 It has been proposed that ionic contrast media may delay coagulation by inhibiting thrombin formation and fibrin polymerization.9,10 Studies in vitro have shown decreased fibrinopeptide A and thrombin-antithrombin complex formation.11 The lesser anticoagulant effects of nonionic contrast media do not appear to operate via this mechanism12 and may depend on the osmolality and chemical structure of the iodinated molecules.

The effects of contrast media on platelets are unclear. Contrast media have been found to have an inhibitory effect on ex vivo platelet aggregation in patients within 20 minutes of angiography. In humans, there is a rapid
### Table 1. Physicochemical Characteristics of the Three Contrast Media Studied

<table>
<thead>
<tr>
<th>Example</th>
<th>Low Osmolar Nonionic</th>
<th>High Osmolar Ionic</th>
<th>Low Osmolar Ionic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical name</td>
<td>Omnipaque</td>
<td>Urografin</td>
<td>Hexabrix</td>
</tr>
<tr>
<td>Osmolality</td>
<td>852 mOsm/kg</td>
<td>2070 mOsm/kg</td>
<td>608 mOsm/kg</td>
</tr>
<tr>
<td>Iodine content</td>
<td>350 mgI/mL</td>
<td>370 mgI/mL</td>
<td>320 mgI/mL</td>
</tr>
<tr>
<td>Molecular structure</td>
<td>Monomer</td>
<td>Monomer</td>
<td>Dimer</td>
</tr>
</tbody>
</table>

![Molecular Structure Diagram]

This avoids the need for physical separation from other blood components, and hence for fixation, which would otherwise be required to prevent platelet activation during centrifugation or filtration procedures. In consequence, whole blood flow cytometry minimizes artifactual platelet activation and allows platelet response to exogenous stimuli or agonists to be analyzed in the presence of the other blood components.

This study investigated the effects of three routinely used contrast media on platelet activation and degranulation as assessed by immunolabeling and flow cytometric techniques in blood samples from both normal subjects and patients pretreated with heparin and aspirin before routine PTCA.

### Methods

**Reagents.** Platelets were identified by labeling with pan platelet MAbs to GPIb, (RFGP37) and GPIIb-IIIa (RFGP56) raised in our laboratory. These antibodies were purified from mouse ascitic fluid by ammonium sulphate precipitation followed by DEAE chromatography and coupled to fluorescein isothiocyanate (FITC) by standard techniques. The CD63 antigen was identified with an IgG, mouse MAb, RFAC4 raised in our laboratory, purified and conjugated to FITC as described above. P-selectin was identified with a FITC-conjugated IgG, mouse MAb obtained from Immuno-tech (The Binding Site, Birmingham, England). The fibrinogen binding site on GPIlb-IIIa was identified with the IgM MAb, PAC-1 

This is achieved by a whole blood flow cytometric method based on these described by Abrams et al and Warkentin et al. Whole blood flow cytometry provides a sensitive and rapid method for detecting platelet activation. Platelets are distinguished from other blood components by their forward and light scatter characteristics, reflecting their size and granularity, respectively.

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* decrease in platelet aggregation in response to standard agonists within 1.5 to 3 minutes of contrast injection. This inhibition is reversed within a few hours and is related to the amount of contrast medium injected. Examination of platelet release products, however, have given seemingly conflicting results. In vitro, contrast media have been reported to inhibit the release of platelet granule contents, yet in vivo rises in PF4, BτG, and thromboxane B₂ have been found. However, reported inhibition of the release of serotonin by contrast media in vivo. Adherence of platelets to the surface of the angiography catheters and guide wires is greater with nonionic than with ionic contrast media, especially when viewed by electron microscopy. This present study has sought to resolve these discrepancies by using flow cytometry to compare the degree of platelet activation and degranulation occurring when native blood is exposed to various contrast media in vitro.

Platelet activation is accompanied by a number of changes on the platelet surface. Weak agonists such as ADP or epinephrine cause conformational changes in the platelet membrane glycoprotein (GP) IIB-IIIa complex, exposing the receptor site for fibrinogen. This new epitope, which is present only after activation, can be recognized with a monoclonal antibody (MAb) PAC-1, whereas bound fibrinogen can be detected with a polyclonal antibody.

Strong agonists such as thrombin or vessel wall collagen, in addition to activating the GPIIb-IIIa complex, cause platelets to degranulate and release their granule contents. This process is accompanied by translocation of granule membranes to the platelet surface, bringing with them specific membrane glycoproteins that appear as neoantigens. Two such antigens are the P-selectin (GMP140, PADGEM, or CD62) of the α-granule membrane and the CD63 (or GP53) antigen of the lysosomal membrane.

These antigens can be identified in a whole blood flow cytometric method based on these described by Abrams et al and Warkentin et al. Whole blood flow cytometry provides a sensitive and rapid method for detecting platelet activation. Platelets are distinguished from other blood components by their forward and light scatter characteristics, reflecting their size and granularity, respectively.

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**Methods**

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**Agonists.** ADP was purchased from Sigma Chemical Co, Ltd (Poole, England) and used at final concentra-
tions of $10^{-7}$ to $10^{-4}$ M. Bovine thrombin was purchased from Diagnostic Reagents Ltd (Thame, Oxon) and used at final concentrations of 0.025 to 0.4 U/mL. Samples incubated with thrombin also contained glycyl-L-prolyl-L-arginyl-L-proline (GPRP) peptide, (Sigma), which inhibits fibrin cross-linking and hence clot formation. The 14 amino acid peptide (SFLLRNPNDKYEPPF) analogous to the cleaved N-terminal sequence of the human thrombin receptor was prepared by Glaxo Group Research, (Greenford, England) and was used at final concentrations of $10^{-6}$ to $10^{-4}$ M. Recombinant hirudin ( Hiruthin) was from Accurate Chemical and Scientific Corporation (Westbury, NY), and D-phenylalanine-L-prolyl-L-arginine chloromethyl ketone (PPACK) was from Calbiochem Corporation (La Jolla, Calif) and was added to blood at a concentration of $10^{-6}$ M.

**Contrast media.** The following contrast media were investigated: Omnipaque, 350 mg I/mL (iohexol), a low osmolar nonionic contrast medium obtained from Nycomed; Urografin, 370 mg I/mL (diatrizoate) high osmolar ionic contrast medium, obtained from Schering AG; and Hexabrix, 320 mg I/mL (ioxaglate), a low osmolar ionic contrast medium obtained from Mallincrodt Medical UK. Details of the properties of these contrast media are given in Table 1.

Solutions of equivalent osmolar and ionic strengths to the three contrast media were made from either sodium chloride (ionic equivalents of Urografin and Hexabrix) or mannitol (nonionic equivalent of Omnipaque) to elucidate whether the platelet activation seen was purely dependent on the osmolality of the media.

**Collection of Blood Samples and Incubation With Contrast Media.** Blood samples were obtained from 15 healthy, young, male volunteers (staff and students of the Royal Free Hospital School of Medicine) who denied taking any medication in the previous 2 weeks. Their ages ranged from 20 to 32 years, and all had full blood counts and platelet counts within the normal range. Samples were also taken from three patients at the Royal Brompton National Heart and Lung Hospital (RBNHLH) who were undergoing PTCA for symptomatic coronary heart disease before injection of any contrast media or placement of intraaortic guide catheters. All patients were on long-term aspirin therapy (300 mg per day) and were anticoagulated with 15 000 U of heparin to achieve an activated clotting time of >300 seconds or PTTK >400 seconds. A blood sample was also obtained from a patient with type 1 Glanzmann's thrombasthenia, a hereditary lack of the platelet membrane GPIIb-IIIa complex. Ethical committee approval was given by the RFHSM for use of normal volunteer blood and by the RBNHLH for use of patients' blood.

Blood was collected from the antecubital fossa, with minimal stasis, via a 21-gauge butterfly needle. The first 2 mL were taken into EDTA and used to determine the full blood count. Subsequent 5-mL samples were taken directly into 10-mL syringes (Plastipak, Becton Dickinson) containing volumes of contrast media ranging from 0.5 to 5 mL. An additional syringe containing 5 mL of 0.9% (wt/vol) saline was used as the standard control for each of the experiments. The syringes were inverted gently for 1 minute and then 4.5 mL of each mixture was transferred into Monovette tubes (Sarstedt Ltd, Leicester, England) containing 0.5 mL of 3.8% tri-sodium citrate. Within 2 minutes, these blood/contrast media mixtures were immunolabeled for flow cytometric analysis using a modification of the methods described by Warkentin et al. Five microliters of the blood/contrast media mixtures was added to LP3 tubes containing 50 μL of HEPES-buffered saline (0.145 M NaCl, 5 mM KCl, 1 mM MgSO4, 10 mM HEPES; pH 7.4) to which had been added 5-μL aliquots of appropriate concentrations of antibodies and agonists. After gentle mixing, the samples were incubated without stirring for a further 20 minutes, then diluted and fixed with 0.5 mL of 0.2% formyl saline (0.2% formaldehyde in 0.9% NaCl) to inhibit further activation. Samples analyzed for fibrinogen receptor expression were first incubated with 5 μL of PAC-1 for 15 minutes, then R-anti-MigFITC was added for a further 15 minutes. All incubations were carried out at room temperature (22° to 26°C), and all samples were assayed in duplicate.

**Flow Cytometric Analysis.** Samples were analyzed within 2 hours of collection in a Coulter EPICS Profile II flow cytometer (Coulter Electronics Ltd, Luton, England). The instrument was aligned daily with “Immunochek” and “Standard Brite” beads (Coulter Immunology) to calibrate the light scatter and fluorescence parameters, respectively. The platelet population was identified by its light scatter characteristics and identity confirmed using the anti-GPllb MA b, RFGP37-FITC. An electronic bit map was set around the platelet population, and >98% of the particles analyzed in all samples were positive for GPIb. The negative cut-off levels for each sample were set at 2% using appropriate isotype controls as described at Janes et al. Five thousand platelets were analyzed in each sample, and the data were expressed as the percentage of cells positive for fluorescent antibody binding.

**Detection of βTG and PF4 Release by Contrast Media.** Five-milliliter samples of blood were collected from normal volunteers into 10-mL syringes containing 5 mL of contrast medium or saline. After a 1-minute incubation, 5 mL of each blood/contrast media mixture was added to citrate and processed for flow cytometry as described above, and 5 mL was transferred into precooled Diatubes (Diagnostica Stago) containing a mixture of sodium citrate and citric acid with theophylline, adenosine, and dipyridamole. These were left on ice for 15 minutes and then centrifuged at 2500g for 30 minutes at 4°C. Samples of the plasma/contrast media mixtures were aspirated from the tubes, aliquotted, and frozen at −70°C for subsequent analysis of βTG and PF4 using the Asserachrom βTG and PF4 ELISA kits (Diagnostica Stago).

**Results.**

**Flow Cytometric Analysis of Platelets.** Identification of the platelet population in the flow cytometer is illustrated in Fig 1. The particles in the blood sample have been analyzed by their forward scatter (x-axis) and side scatter (y-axis) characteristics,
Indicative of size and granularity, respectively. The platelets have been enclosed in an electronic bit map (region 2) for analysis of binding of the fluorescent antibodies. Region 1 represents dust particles and machine noise, whereas region 3 is the red cells; Fig 1 illustrates the binding of the anti-GPIb MAAb, RFGP37-FITC to this population, confirming their identity as platelets.

**Influence of Contrast Media on Platelet Activation and Degranulation**

Incubation of blood from 12 control subjects, with equal volumes of contrast media for 1 minute, resulted in markedly different patterns of platelet activation for the three different media, measured by the expression of platelet granule membrane antigens, as illustrated in Fig 2. Results are expressed as mean±SD, and comparisons are made using the Student's unpaired t test. In control samples mixed 1:1 with saline, only 2.1±1.3% of platelets expressed P-selectin and 1.3±1.1% expressed CD63. This level of expression was not statistically different from that seen in blood samples taken directly into citrate anticoagulant without dilution with saline. Incubation of blood for 1 minute with an equal volume of the nonionic LOCUM (Omnipaque), however, induced marked platelet degranulation, with P-selectin and CD63 expression on 78.2±8.9% and 59.9±12.9% of platelets, respectively. This level of platelet degranulation approached that seen when blood was stimulated with maximal levels of thrombin (0.4 U/mL), in which P-selectin was expressed on 95.4%±1.0% and CD63 on 82.5±6.4% of platelets. The ionic LOCUM, Urografin, caused less degranulation, inducing 21.6±8.6% of platelets to express P-selectin and 29.7±12.5% to express CD63, a level of degranulation that was statistically significantly greater than the control (P<.01) and significantly less than with Omnipaque (P<.01). Conversely, the low osmolar contrast medium Hexabrix caused only 2.5±1.5% of platelets to degranulate and express P-selectin and 0.9±0.6% to express CD63. This was not significantly different from the control samples.

The presence of contrast media in the whole blood mixtures had no direct effect on the binding of monoclonal antibodies to antigens expressed on the platelet surface. No difference was observed in the level of binding of the two different pan platelet antibodies (against GPIb and GPIIb-IIIa) in any of the contrast media compared with the control samples.

**Effect of Contact Time and Concentration of Contrast Media**

The local concentration of contrast media in the coronary vessel during angiography will vary according to the time after contrast injection. Initially, the vessel is filled with contrast medium during injection and the contrast media concentration is at least 1:1 with blood. Subsequently, the ratio changes with resumption of blood flow. If a dissection is caused, however, contrast medium can become trapped in the wall of the coronary artery increasing the contact time with the whole blood.

The effect of prolonged incubation on the degree of degranulation seen with each contrast medium is shown in Fig 3. Equal volumes of contrast media and blood
were incubated together for 1 minute or 30 minutes, at room temperature, before transferring to citrate tubes and analysis of the platelets for the expression of P-selectin and CD63 antigens. The anticoagulant properties of the contrast media prevented the blood from clotting during the 30-minute incubation, but control samples in normal saline clotted within this time and were therefore only analyzed at 1 minute. Platelet degranulation induced by the nonionic LOCM Omnipaque was not significantly increased above the high level seen at 1 minute by prolonged incubation. There was, however, an increase in degranulation after 30-minute incubation with the ionic HOCM Urografin. P-selectin expression rose from 30.3±8.7% at 1 minute to 53.9±8.0% at 30 minutes, whereas the CD63 antigen rose from 37.7±17.5% to 54.3±9.8%. This increase was statistically significant (P<.01) with P-selectin but not with CD63 (P=.17). No degranulation was seen with the ionic LOCM Hexabrix even after the 30-minute incubation.

The ratio of contrast media mixed with blood was varied between 1:1 and 1:10 by taking 5 mL of blood into syringes containing volumes of contrast media between 0.5 and 5 mL (Fig 4). The nonionic LOCM Omnipaque still caused maximal platelet degranulation at a ratio of 1:3 (blood to contrast media). With blood at a ratio of 1:5, the effect decreased slightly to 76.2%, but even at a ratio of 1:10, 12.5% of platelets expressed P-selectin and 3% expressed the CD63 antigen. The effect of the ionic HOCM Urografin was virtually abolished, however, when diluted to 1:3 with blood. Hexabrix did not cause degranulation at any concentration tested.

**Role of the Fibrinogen Receptor on the Platelet Surface in Platelet Degranulation Induced by Contrast Media**

Platelet degranulation caused by the contrast media appeared to be independent of activation of the GPIIb-IIIa receptor and the binding of fibrinogen. This is illustrated in Fig 5, which compares P-selectin expression on platelets in blood incubated with an equal volume of contrast media with the activation of GPIIb-IIIa to form a functional fibrinogen receptor identified by the MAb PAC-1.

PAC-1 binding was only slightly increased by incubation with Urografin and was seen on only about 20% of platelets incubated with Omnipaque compared with P-selectin expression of 23% and 75%, respectively. Hexabrix caused no increase in PAC-1 binding com-
pared with control. These levels of PAC-1 binding were mirrored by fibrinogen binding in a single study (data not shown). This differs from the pattern of activation antigen expression seen with a weak agonist such as ADP, which at maximum concentration (10^-5 M) induced PAC-1 binding to 72.0±6.0% of the platelets, whereas P-selectin was seen on only 20.6±7.7%.

The role of the GPIIb-IIIa receptor in platelet degranulation induced by the contrast media was investigated further by measuring P-selectin expression on platelets from a patient with type I Glanzmann’s thrombasthenia who was shown to totally lack the GPIIb-IIIa receptor (determined by flow cytometry). As with normal platelets, Omnipaque caused marked degranulation of 60% of platelets expressing P-selectin; Urografin caused P-selectin expression on 7.0% of platelets and Hexabrix, 4.5%; 5.2% of platelets in the normal saline control expressed P-selectin. The magnitude of the responses in this patient with Glanzmann’s thrombasthenia were somewhat lower than in the normal subjects; however, this was also reflected by a lower response to 0.4 U/mL thrombin, which only caused 62.9% of the platelets to express P-selectin compared with >90% of control platelets.

Parallel Analysis of Platelet Granule Membrane Antigen Expression and Release of α-Granule Contents

Studies were performed on normal volunteer blood (n=3) to compare the degree of platelet degranulation, as assessed by immunolabeling, with the release of PF4 and BTG in samples incubated for 1 minute with an equal volume of contrast media. BTG and PF4 release paralleled the expression of P-selectin and CD63 antigens (Fig 6).

Platelet Degranulation in Blood From Anticoagulated Patients Undergoing PTCA

Contrast media are routinely used in PTCA in patients who are anticoagulated with heparin and aspirin. Blood was taken from aspirinized, heparinized patients (n=3) who were undergoing routine balloon angioplasty for symptomatic coronary disease. The same pattern of degranulation was seen as in normal subjects (Table 2).

Platelet Degranulation in Blood Treated With Specific Thrombin Inhibitors

Blood from normal volunteers was drawn into syringes containing Hirudin (10 U/mL) or PPACK (10^-6 M), immediately mixed with equal volumes of Omnipaque, Urografin, or normal saline (control), and platelets analyzed for P-selectin expression. Neither Hirudin nor PPACK caused any inhibition of the degranulation induced by either of the contrast media.
TABLE 2. Platelet Degranulation by Contrast Media Despite Patients Being Fully Anticoagulated With Heparin and on Chronic Aspirin Therapy

<table>
<thead>
<tr>
<th></th>
<th>CD63, %+ve (Lysosomal Antigen)</th>
<th>P-Selectin, %+ve (α-Granule Antigen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline control</td>
<td>5.5±5.4</td>
<td>7.5±3.1</td>
</tr>
<tr>
<td>Omnipaque</td>
<td>66.4±5.9*</td>
<td>76.2±8.6*</td>
</tr>
<tr>
<td>Urografin</td>
<td>29.9±9.7†</td>
<td>18.4±4.7†</td>
</tr>
<tr>
<td>Hexabrix</td>
<td>2.7±0.5</td>
<td>7.9±0.7</td>
</tr>
</tbody>
</table>

Values are mean±SD. *P<.001; **P<.01.

(Table 3). Addition of 0.4 U/mL of thrombin to the control (normal saline) samples, however, showed that the thrombin antagonists were effective in blocking thrombin-induced platelet degranulation.

Effect of Osmolarity on Platelet Degranulation

The three contrast media studied had significant differences in their osmolality (Table 1). To assess whether the different effects of contrast media on platelets could be explained by the osmolality of the contrast media alone, solutions of equivalent osmolality and ionic composition were prepared and incubated with equal volumes of whole blood in the standard method outlined above. Table 4 shows the degree of platelet degranulation occurring with each of the contrast media and the solutions of equivalent osmolality and ionic strength. The degree of degranulation caused by the three contrast media was not directly related to their osmolalities. Similarly, their osmolar/ionic equivalent solutions showed no direct relation between osmolality and degranulation. The HOCM (Urografin) osmolality equivalent (64.2 g/L NaCl, 2070 mOsm/kg) caused only 11% of platelets to express P-selectin and 16% to express CD63; however, the nonionic osmolar equivalent for Omnipaque (14.7% mannitol, 852 mOsm/kg) caused 80% of platelets to express P-selectin and 64% to express CD63, which was similar to the degree of degranulation seen with Omnipaque. The ionic LOCM equivalent of Hexabrix caused no activation.

Effect of Ionic Low Osmolar Contrast Medium on Platelet Response to Thrombin

Hexabrix (ionic LOCM), unlike the ionic HOCM or the nonionic LOCM, caused no activation of the platelets even after prolonged incubation with blood. To investigate whether Hexabrix had a specific, inhibitory effect on platelet activation, blood was incubated 1:1 with Hexabrix and then added to assay tubes containing thrombin at concentrations ranging from 0.01 to 0.2 U/mL. Fig 7 shows that Hexabrix markedly reduced thrombin-induced degranulation of the platelets at all agonist concentrations. In the control sample, (0.9% saline), thrombin caused platelets to express P-selectin and CD63 (Fig 7) in a concentration-dependent manner, rising to a maximum at 0.2 U/mL of thrombin. Hexabrix totally inhibited this effect at thrombin concentrations up to 0.05 U/mL and at maximum thrombin (0.2 U/mL). P-selectin was seen on only 45% of platelets compared with 93% in the control, whereas CD63 was seen on only 43% compared with 85% in the control. When platelets were stimulated with the 14 amino acid thrombin receptor activation peptide (TRAP), however, Hexabrix did not inhibit the expression of P-selectin or CD63 antigen. For example, at maximum TRAP concentration (1×10^{-4} M), 92.7% of platelets in the control samples expressed P-selectin compared with 89.7% in the blood incubated with Hexabrix.

Discussion

Although the anticoagulant properties of angiographic contrast media have been studied extensively, their effects on platelets have received less attention and the findings have been contradictory, reporting both increases in βTG and PF4 release in vivo and decreases in serotonin in vitro. Platelet aggregation has been reported to be inhibited ex vivo and in vitro. In addition, the differences between the types of contrast agents have not been fully investigated.

Flow cytometric examination of platelet activation marker expression allows such discrepancies to be resolved. The activation status of individual platelets, within the whole platelet population, can be examined directly. In addition, the use of whole blood flow cytometry avoids the need for fixatives that are required to prevent artificial platelet activation occurring during the separation and washing procedures, and this lack of fixatives allows the in vivo response of the platelets to agonists or other agents to be assessed.

The present in vitro study has demonstrated significant differences in effects on platelets in native blood of three commonly used types of contrast media. The nonionic LOCM (Omnipaque) caused profound platelet degranulation as evidenced by the expression of the α-granule and lysosomal membrane antigens on the platelet surface, which was paralleled by a marked release of βTG and PF4. The two ionic contrast media caused less platelet degranulation than the nonionic LOCM, but while the HOCM Urografin still produced

Table 3. Platelet Degranulation (P-Selectin Expression) In Blood Mixed With Hirudin or PPACK Before Exposure to Contrast Agents

<table>
<thead>
<tr>
<th></th>
<th>No Antithrombin</th>
<th>Hirudin</th>
<th>PPACK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline rest</td>
<td>3.67±0.78</td>
<td>3.2±2.83</td>
<td>2.8±0.53</td>
</tr>
<tr>
<td>Normal saline+0.4 U/mL thrombin</td>
<td>86.7±4.35</td>
<td>2.97±0.56</td>
<td>3.55±0.07</td>
</tr>
<tr>
<td>Omnipaque</td>
<td>58.3±8.72</td>
<td>63.9±1.56</td>
<td>59.15±9.81</td>
</tr>
<tr>
<td>Urografin</td>
<td>11.28±2.98</td>
<td>12.9±0.14</td>
<td>12.35±5.43</td>
</tr>
</tbody>
</table>

Values are mean±SD. n=3.
some degranulation, after 1 minute incubation, which increased in prolonged exposure, the LOCM Hexabrix caused no platelet degranulation even after 30 minutes incubation with blood and minimal release of βTG and PF4 similar to that seen with the controls.

In most studies on the effects of contrast media on platelets, the contact times have ranged from 20 minutes to several hours. The present study indicates that platelet degranulation occurs within the first minute of exposure of the blood to the contrast media.

The differences between the three contrast media could not be accounted for by possible inhibitory effects on antibody binding. Platelets incubated with equal volumes of contrast media showed no difference in the binding of the MAbs to the GPIb-IX and GPIIb-IIIa complexes, present on all platelets, compared with that seen in control samples; nor were their effects related solely to the osmolality of the media, as there was no direct correlation between osmolality and their platelet-degranulating effects.

The effects of the contrast media on platelets were linked more closely to the ionic nature of the media; solutions of similar osmolality and ionic strength produced very similar effects on platelets to their contrast media equivalents. However, when solutions of similar ionic strength were compared, increasing osmolality appeared to contribute to the degree of platelet degranulation seen. Optiray, another nonionic contrast medium, caused a similar degree of platelet degranulation to Omnipaque (data not shown), suggesting that the effects seen were linked to the nonionic chemical properties of the contrast media. This was further confirmed by the finding that the nonionic contrast mannitol caused a similar degree of platelet degranulation to that seen with the nonionic contrast medium Omnipaque. Together, these data indicate that it is the nonionic molecules per se that directly affect the platelets, implying that any contrast media based on nonionic molecules would be likely to cause platelet degranulation.

The platelet degranulation induced by the nonionic LOCM and by the ionic HOCM appeared to be independent of activation of the GPIIb-IIIa complex and of fibrinogen binding. Studies on a patient with Glanzmann's thrombasthenia showed that despite this absence of the GPIIb-IIIa receptor complex, both contrast media caused a similar degree of platelet degranulation to that seen with normal volunteer blood and to that seen when the blood from this patient was stimulated with thrombin.

The observation that contrast media cause minimal activation of the platelet GPIIb-IIIa receptor may help explain why the aggregation studies have been relatively

### Table 4. Relation of Osmolality of Contrast Media or Their Equivalent Solutions, Incubated 1:1 With Native Blood on the Degree of Platelet Degranulation

<table>
<thead>
<tr>
<th>Contrast Media</th>
<th>Osmolality, mOsm/kg</th>
<th>P-Selectin, %+ve</th>
<th>CD63, %+ve</th>
<th>Contrast Media</th>
<th>Osmolality, mOsm/kg</th>
<th>P-Selectin, %+ve</th>
<th>CD63, %+ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline, 9 g/L (control)</td>
<td>308</td>
<td>1.7</td>
<td>0.3</td>
<td>Saline, 9 g/L</td>
<td>308</td>
<td>1.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Hexabrix</td>
<td>608</td>
<td>1.4</td>
<td>0.3</td>
<td>Hexabrix equivalent, NaCl, 19.3 g/L</td>
<td>608</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Urografin</td>
<td>2070</td>
<td>13</td>
<td>20</td>
<td>Urografin equivalent, NaCl, 64.2 g/L</td>
<td>2070</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>Omnipaque</td>
<td>852</td>
<td>66</td>
<td>47</td>
<td>Omnipaque equivalent, mannitol, 14.7% (nonionic)</td>
<td>852</td>
<td>80</td>
<td>64</td>
</tr>
</tbody>
</table>

![Fig 7](https://circ.ahajournals.org/)

**Fig 7.** Plots show effects of ionic low osmolar contrast medium (Hexabrix) on platelet degranulation caused by thrombin (a and b) and the 14 amino acid thrombin receptor activation peptide (c and d). Panels a and c depict P-selectin expression; b and d, CD63 antigen expression.
uninformative. Platelets require fibrinogen binding to the activated GPIIb-IIIa receptor for platelet aggregation to occur. Even at the highest concentrations of contrast media, only a small increase in the fibrinogen receptor activation was seen, and this was to a level below that required for platelet aggregation to occur. The somewhat contradictory observation that the contrast media can inhibit platelet aggregation may be related to their viscosity, acting to slow down platelet-platelet contact and hence the rate of aggregation.

The concentrations at which the nonionic LOCM and the ionic HOCM caused degradation (>1:10 and >1:3, respectively), although markedly different, are concentrations that can be present, albeit transiently, during angiography and opacification of the coronary artery. During coronary intervention, the process of balloon dilatation causes local stretching of the narrowed section of atherosclerotic vessel. This barotrauma can cause significant micro- and macrodissections within the wall of the coronary artery. This is followed by injection of contrast media into the lumen of the balloon damaged vessel to allow angiographic assessment of the result of the angioplasty. Within the PTCA-induced dissections, high concentrations of contrast media can occur, and the contrast media remain relatively static because of decreased flow. A nonionic contrast medium and to a lesser extent an ionic HOCM would potentially cause significant platelet degradation within such a dissection flap in the vessel wall.

Patients undergoing routine PTCA are normally fully anticoagulated with heparin and receive standard-dose aspirin therapy. We have previously shown that aspirin itself does not affect the platelet degradation process in response to ADP and thrombin per se. Incubation of contrast media with blood from fully anticoagulated patients caused a similar pattern of platelet degradation to that seen with the normal volunteers. The nonionic LOCM caused marked platelet degradation, suggesting that the pretreatment of patients undergoing PTCA with aspirin and heparin will not modify this marked platelet degradation and that the nonionic LOCM does not stimulate the platelets through a thrombin mediated mechanism.

To confirm this and to test whether the contrast media had a heparin-neutralizing effect that would thus allow thrombin generation to occur in the samples, blood from normal volunteers was pretreated with thrombin antagonists before addition to contrast media. Neither hirudin nor PPACK abolished the degradation induced by Omnipaque or Urografin, suggesting that the degradation is not mediated simply by the production of thrombin in blood exposed to the contrast medium, in accord with observation of the effects of the agents on the coagulation cascade.

Although the activation caused by the nonionic LOCM appears to be independent of thrombin, high levels of local intracoronary thrombin generation in response to vessel wall injury and clot bound thrombin could independently cause platelets to degranulate. In this study we have shown that Hexabrix has a marked inhibitory effect on thrombin induced platelet degranulation, confirming an earlier report by Matsuda et al. Thrombin acts on the platelet thrombin receptor as a protease, cleaving the N-terminus of the extracellular domain of the receptor. The newly exposed N-terminal can then act as an autologous tethered ligand. This effect can be mimicked by a synthetic peptide analog of this ligand (thrombin receptor activating peptide, TRAP). Activation of platelets by TRAP was unaffected by the presence of Hexabrix. This suggests that the action of thrombin on its receptor is in some way inhibited by the large dimeric molecule of the ionic LOCM, yet activation of the receptor by the tethered ligand is unaffected. Thrombin is considered to be a cellular mitogen for smooth muscle cells, causing them to migrate and proliferate in the wall of the PTCA-damaged vessel. A contrast medium that attenuates the mitogenic effect might influence the post-PTCA healing process and thus reduce or alter the subsequent restenosis. The release of platelet-derived factors within the lumen of the balloon-damaged coronary artery during PTCA may influence the intracoronary prothrombotic environment and the stimulus to smooth muscle cell recruitment and proliferation, hypothesized to be part of the underlying mechanism in the restenosis process that occurs in 35% to 50% of patients after PTCA. Local dissections caused by the PTCA procedure would be likely to increase the contact time of the blood and contrast media and therefore enhance local release of these prothrombotic and vasoactive substances.

Conclusions

These studies have shown that routinely used contrast media cause significantly different degrees of platelet degradation, as seen by flow cytometric analysis of individual platelets in whole blood. The nonionic LOCM Omnipaque caused profound platelet degradation by a mechanism that appears to be related to the nonionic structure of compound rather than the osmolality of the medium. It is likely that this finding will be seen with all contrast media based on a nonionic molecular structure. The degradation caused was independent of thrombin generation and was not blocked by pretreatment with heparin or aspirin. The degradation was paralleled by release of prothrombotic factors from the platelet α-granule. These findings are consistent with previous reports of an increased incidence of thrombotic complications during coronary angiography and the development of clots in the angiography catheters and syringes reported with nonionic LOCM. The potential beneficial effect of ionic LOCM that not only fails to cause platelet degradation but also may modify the platelet response to intracoronary and clot bound thrombin is yet to be demonstrated in patients. These findings suggest that the choice of contrast media for angiography and PTCA in unstable coronary syndromes may influence the clinical outcome in these patients and suggest the need for studies to investigate whether these profound differences in platelet activation caused by contrast media in vitro are important in vivo.

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