Effects of Nonionic Contrast Media on Platelet Aggregation

Assessment by Particle Counting With Laser-Light Scattering

Takanori OGAWA, MD, Satoshi FUJII, MD, Kazushi URASAWA, MD, and Akira KITABATAKE, MD

SUMMARY
Intravascular radiographic contrast media used in angiography, particularly nonionic contrast media, may cause activation of platelets. This study was designed to determine which properties of nonionic contrast media were potentially responsible for this action. Platelet aggregation after adenosine diphosphate stimulation was studied in the platelet rich plasma obtained from 37 patients who underwent left ventriculography using the highly sensitive method of particle counting with laser-light scattering. Platelet activation by contrast media was studied in the platelet rich plasma from healthy volunteers using flow cytometric analysis to detect platelet degranulation as P-selectin expression. There was a significant decrease in platelet aggregation in patients injected with ioxilan or iomeprol compared with patients injected with iohexol. There was a significant increase in P-selectin expression with the three groups of contrast media compared to control. The platelet activation with ioxilan or iomeprol was significantly less compared to the activation with iohexol. The comparison showed that previous generalization regarding platelet activation by nonionic contrast media might not be valid. It is presumed that the higher osmolality of iohexol may contribute to the increase in platelet aggregation and activation.

Key words: Angiography, Blood, Coagulation, Complications, Flow cytometry, Osmolality, P-selectin, Thrombus

COMARED with high osmolar ionic contrast media nonionic low osmolar contrast media have been shown to have low toxicity and superior clinical tolerance, and to decrease the incidence of major complications associated with diagnostic cardiac catheterization. At present low osmolar contrast medium is considered to be safer than high-osmotic contrast medium. But an increase in thrombotic complications has been implicated with nonionic low osmolar contrast medium. When blood is mixed with nonionic medium in a syringe or cathe-
ter, thrombus formation might occur because its anti-aggregatory effect is weaker than ionic medium. Moreover, critical complications such as myocardial infarction by nonionic medium used for coronary angiography have been reported.

Although in vitro studies have shown that nonionic low osmolar agents possess less antithrombotic activity than do ionic contrast media, the effects on platelet aggregation among various nonionic contrast media have not been fully investigated. In this study blood samples from aorta through a catheter before and after left ventriculography were collected. Platelet aggregation was assessed by the highly sensitive particle counting method using laser-light scattering to investigate the effects of three types of routinely used nonionic contrast media on platelet aggregation. Platelet activation and degranulation were also assessed by immunolabeling and flow cytometric analysis of platelet P-selectin in samples from normal subjects.

**Subjects and Methods**

**Patients and contrast media used:** The subjects included 37 consecutive patients who underwent left ventriculography at Hokkaido University Hospital. In patients who were treated with medications which can potentially affect platelet function (such as non-steroidal anti-inflammatory agents, anti-platelets, steroid hormones, dipyridamole, heparin, fibrates), the agents were discontinued at least 3 days before the angiography procedure. Aspirin (81 mg orally) was discontinued 1 day before the angiography procedure. Before enrollment written informed consent was obtained from all patients in accordance with the local ethical guidelines established by Hokkaido University Hospital. Full clinical data were recorded in all patients. Routine laboratory estimation was performed for all patients including red blood cell count, hematocrit, white blood cell count, hepatic and renal function before and after the angiography. Contrast media used were ioxilan (Imagenil, Kyowa, Tokyo), iomeprol (Iomeron, Eisai, Tokyo) and iohexol (Omnipaque, Daiichi, Tokyo). Their physiochemical properties

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Ioxilan</th>
<th>Iomeprol</th>
<th>Iohexol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmolality (mOsm/kg)</td>
<td>698.6</td>
<td>620</td>
<td>880</td>
</tr>
<tr>
<td>Iodine content (mg/ml)</td>
<td>350</td>
<td>350</td>
<td>350</td>
</tr>
<tr>
<td>Viscosity (mPa•S 37°C)</td>
<td>8.1</td>
<td>7.5</td>
<td>10.7</td>
</tr>
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</table>
are summarized in Table I. There was no difference in the values of routine laboratory examination before the injection of contrast media among the three groups (results not shown).

**Blood sampling and platelet aggregation:** The blood samples were collected in a test tube containing 3.8% sodium citrate from the aorta through a catheter after sheath placement. The blood samples were collected immediately before and three minutes after left ventriculography (40 ml of contrast media for left anterior oblique view and 40 ml for right anterior oblique view), which was recorded after coronary angiography. Samples were centrifuged at 200 g for 15 min and platelet rich plasma (PRP) was obtained. The samples were then centrifuged at 3000 g for 10 minutes, and platelet poor plasma (PPP) was obtained. The PRP was diluted with PPP to adjust the number of platelets in PRP to 200,000 / mm³. Adenosine diphosphate (ADP) was added to PRP at final concentrations of 1, 3, and 5 µM. Platelet aggregation was measured by PA-200 (Mebanics, Tokyo) as previously described. In brief, a laser beam measuring 40 µm in diameter was generated using a 20 mW diode laser (675 nm, Toshiba, Japan) and was passed through PRP (300 µl) stirred at 22°C in a cylindrical glass cuvette with a 5 mm internal diameter. The light scattered from the observation volume (48 x 140 x 20 µm) was detected by a photocell array. Light intensity corresponds to particle size. Data were expressed as the change over time in the number of aggregates (counts / sec) of individual sizes (determined by light intensity expressed as volts). The total light intensities of small, medium and large aggregates were determined. Particles with an intensity of 25 to 400 mV represented small aggregates (9 - 25 µm, 50 - 1,100 platelets), those with an intensity of 400-1,000 mV represented medium aggregates (25 - 50 µm, 1,000 - 9,000 platelets), and those with an intensity of 1,000 - 2,048 mV represented large aggregates (50 - 70 µm, 9,000 - 25,000 platelets). Changes in signal intensity were recorded at 10 sec intervals for 10 min. Quantitative estimation was performed by determining the area under the curve representing the sum of 30 measurements of the light scattering intensity. Platelet aggregation was simultaneously conventionally measured by evaluating maximum percent decrease in optical density (%T).

**Flow cytometric analysis:** Blood was collected via a 19-gauge needle from non-smoking, healthy, young volunteers (n =5, male / female = 2:3, staff and students of Hokkaido University School of Medicine) taking no medications. Care was taken to ensure that there was no venous stasis or no excessive trauma on blood withdrawal. The first 5 ml were discarded and the next 5 ml used to prepare PRP. PRP (100 µl) was incubated in a water
bath maintained at 22°C with 16 mg/ml (final concentration) contrast media. This was likely to reproduce the concentration of contrast media 3 min after injection of contrast media. Our preliminary measurement showed that the concentration of contrast media 3 min after injection was 17.8 ± 2.5 mg/ml for ioxilan, 12.4 ± 1.6 for iomeprol, and 15.1 ± 2.5 for iohexol \( (n = 7) \). After 20 min PRP was fixed with 500 µl of paraformaldehyde (final concentration 1%) to inhibit spontaneous platelet activation. PRP was centrifuged for 5 min at 250 g and the platelets washed with 500 µl of phosphate buffered saline (Dulbecco's PBS without calcium, magnesium or phenol red, pH 7.2, Sigma) containing 0.1% sodium azide. Platelets were suspended in 500 µl of staining medium (PBS with 0.1% sodium azide, 2% fetal bovine serum, HyClone, Logan, UT) and incubated with phycoerythrin (PE) labeled CD62P antibody (0.2 ng/ml, Immunotech-Coulter, Tokyo), which recognizes P-selectin, for 30 min. All samples were washed with PBS twice before analysis by flow cytometry (Epics XL, Coulter Cytometry, Miami, FL) as previously described.\(^7\)

**Statistics:** Results are expressed as mean ± SD. Intergroup differences were compared using the Mann-Whitney test. Analysis was performed using SPSS for Windows (SPSS Inc) and statistical significance was defined as a two-sided \( p \) value < 0.05.

**RESULTS**

**Patients:** There were no differences in age, gender or total volume of contrast media used among the three groups (Table II). No significant differences were found in routine laboratory data including red blood cell count, hematocrit, white blood cell count, and hepatic and renal function measured before and after the angiography.

**Effects on platelet aggregation:** Detection of small, medium and large platelet aggregates after stimulation with ADP were compared and expressed as the ratio of platelet aggregation before and after the injection of contrast media. When PRP was stimulated with 1µM ADP, there was a significant

<table>
<thead>
<tr>
<th>Contrast media</th>
<th>( n )</th>
<th>age (years)</th>
<th>Male/female</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ioxilan</td>
<td>14</td>
<td>60.6±10.6</td>
<td>9/5</td>
<td>178.6±60.5</td>
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<tr>
<td>Iomeprol</td>
<td>10</td>
<td>56.2±15.9</td>
<td>5/5</td>
<td>151.0±22.8</td>
</tr>
<tr>
<td>Iohexol</td>
<td>13</td>
<td>59.5±13.8</td>
<td>7/6</td>
<td>187.7±54.6</td>
</tr>
</tbody>
</table>
ADP 1\(\mu\)M

Figure 1. Platelet aggregation induced by ADP (1 \(\mu\)M) was measured by a laser light scattering method. The small (S), medium (M) and large (L) aggregates were formed by 50-1,100, 1,000-9,000, and 9,000-25,000 platelets, respectively. Platelet aggregation was simultaneously conventionally measured by evaluating maximum percent decrease in optical density (%T). The results are expressed as the ratio of platelet aggregation after the injection of contrast media (post-injection) over the platelet aggregation before the injection of contrast media (pre-injection). Results are expressed as mean ± SD.

decrease in small aggregates in patients injected with ioxilan compared with patients injected with iohexol (Figure 1), whereas there was no significant difference in medium and large aggregates or the aggregation detected by the conventional optical density method. After stimulation with 3 \(\mu\)M ADP there was a significant decrease in medium aggregates in patients injected with ioxilan compared with patients injected with iohexol (Figure 2), whereas there was no significant difference in small and large aggregates. There was also a significant decrease in patients injected with ioxilan and iomeprol compared with patients injected with iohexol of the aggregation detected by the conventional optical density method. After stimulation with 5 \(\mu\)M ADP there was a significant decrease in medium aggregates in patients injected with iomeprol compared with patients injected with iohexol, and a significant decrease in large aggregates in patients injected with ioxilan and iomeprol compared with patients injected with iohexol (Figure 3), whereas there was no significant difference in small aggregates. There was also a significant decrease in patients injected with ioxilan or iomeprol compared with patients injected with
Figure 2. Platelet aggregation induced by ADP (3 μM) was measured by laser light scattering. The small (S), medium (M) and large (L) aggregates were formed by 50-1,100, by 1,000-9,000, and 9,000-25,000 platelets, respectively. Platelet aggregation was simultaneously conventionally measured by evaluating maximum percent decrease in optical density (%T). The results are expressed as the ratio of platelet aggregation after the injection of contrast media (post-injection) over the platelet aggregation before the injection of contrast media (pre-injection). Results are expressed as mean ± SD.

Figure 3. Platelet aggregation induced by ADP (5 μM) was measured by laser light scattering. The small (S), medium (M) and large (L) aggregates were formed by 50-1,100, by 1,000-9,000, and 9,000-25,000 platelets respectively. Platelet aggregation was simultaneously conventionally measured by evaluating maximum percent decrease in optical density (%T). The results are expressed as the ratio of platelet aggregation after the injection of contrast media (post-injection) over the platelet aggregation before the injection of contrast media (pre-injection). Results are expressed as mean ± SD.
iohexol of the aggregation detected by the conventional optical density method.

**Flow cytometric analysis of platelets:** Incubation of blood from healthy volunteers resulted in platelet activation for the three different contrast media measured by the expression of P-selectin (Figure 4). The platelet activation with ioxilan or iomeprol was significantly less compared to the activation with iohexol. Control samples mixed with saline expressed very low levels of P-selectin. Incubation with 5 µM ADP induced marked platelet activation compared to saline.

**DISCUSSION**

The introduction of nonionic low osmolar media has reduced side effects caused by the high-osmotic pressure of conventional ionic contrast media. Thus, angiography can be performed more safely than before.\(^1\),\(^5\),\(^9\)-\(^11\) However, thrombotic complications during angiography have been reported with the increased usage of nonionic low osmolar contrast media. One potential cause may be that nonionic media has a weaker anti-aggregation effect on platelets than conventional ionic high osmolar media or low osmolar media.\(^3\)-\(^5\),\(^12\)-\(^14\) Although there have previously been some com-
parisons between ionic and nonionic contrast media, no studies comparing various nonionic media to assess platelet aggregation using laser-light scattering have been reported.

Platelets play an important role in atherothrombosis such as acute myocardial infarction. Platelet aggregation has been conventionally measured by the optical density method and by the impedance method. These methods provide information on only large platelet aggregates and do not provide information regarding the number of platelet aggregates of different sizes after stimulation with an agonist. In the light scattering method the intensity of scattered light corresponds to particle size and provides information on small aggregates formed in the early phase of platelet aggregation. This is the first study to quantitate aggregates of different sizes after stimulation with different contrast media using this method.

The present study has demonstrated significant differences in the effects on platelet aggregation in native blood of three commonly used types of nonionic contrast media. Iohexol, which has the highest osmolality, caused significant platelet aggregation as evidenced by laser-light scattering, which was paralleled by an increase in optical density. The other two contrast media, ioxilan and iomeprol, which have lower osmolarity than iohexol, caused less platelet aggregation than iohexol, suggesting that there was some direct correlation between the increase in osmolality and the degree of platelet aggregating effects. Therefore, differences among the three nonionic contrast media could be accounted for, at least in part, by the effects related to the osmolality of the contrast media, suggesting that the degree of osmolality may be an important factor in its interaction with subsequent aggregation of platelets. These results are consistent with a previous study showing that increasing osmolality contributed to the degree of platelet degranulation when solutions of similar ionic strength were compared. This was further confirmed by the finding that iohexol caused significantly more platelet P-selectin expression as evidenced by flow cytometry. The other two contrast media, ioxilan and iomeprol, caused less P-selectin expression, suggesting that common mechanism(s) may be contributing to the differences in both platelet aggregation and degranulation. These results are consistent with the recent results of the COURT trial (contrast media utilization in high risk PTCA) which suggested that isomolar nonionic dimer iodixanol reduced in-hospital major clinical events. Taken together, these data indicate that nonionic molecules directly affect the platelets, implying that any contrast
media with nonionic molecules would likely cause some extent of platelet aggregation and activation.

In previous studies the blood samples were collected through a syringe needle, which may have affected the measurement of platelet aggregation. To reduce the effects of the blood sampling procedure samples were collected from the aorta using a 5 French diagnostic catheter before and after left ventriculography in this study. Each patient received the same amount of contrast media between the two blood sampling procedures during left ventriculography, and the total amount of contrast media used during coronary angiography and left ventriculography was similar among the three groups. Platelet aggregation was determined by particle counting with laser-light scattering to precisely investigate the effects of three types of nonionic media.

In conclusion, routinely used nonionic contrast media caused a significant degree of platelet aggregation. The nonionic contrast media iohexol caused profound platelet aggregation and platelet activation that may be related to the osmolality. This finding is consistent with the reports of increased thrombotic complications during angiography and the development of clots in catheters and syringes with nonionic contrast media. The interactions of various effects of contrast media with platelet function in vivo are unknown. Further investigations are necessary to determine whether these differences in the effects on platelet function caused by contrast media are important in vivo clinically.

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