Similar inhibition of platelet adhesion, P-selectin expression and plasma coagulation by ioversol, iodixanol and ioxaglate

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ABSTRACT. Contrast media (CM) are reported to possess both prothrombotic and anticoagulant properties. The mechanisms are not clearly understood, and early reports are contradictory. To study the effects of CM on haemostasis, we analysed the *ex vivo* effects of ioversol and iodixanol on platelet adhesion and P-selectin expression, and the *in vitro* effects of ioversol, iodixanol and ioxaglate on platelet adhesion, P-selectin expression and plasma coagulation. A novel enzymatic assay was used to measure platelet adhesion to protein surfaces, and an enzyme-linked immunosorbent assay was used to measure platelet P-selectin surface expression. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were used to measure plasma coagulation. The *ex vivo* study consisted of blood from 27 outpatients administered ioversol and 9 patients administered iodixanol intravenously. Samples were collected before and 5 min after CM administration. Healthy donors were used for the *in vitro* studies on the effects of CM. The *ex vivo* study showed significantly ($p < 0.05$) decreased platelet adhesion and P-selectin expression after administration of ioversol and iodixanol. Adhesion was more affected than P-selectin expression. The *in vitro* study showed that ioversol, iodixanol and ioxaglate significantly ($p < 0.05$) and dose-dependently (beginning at 3 mg ml⁻¹) decreased platelet adhesion and P-selectin expression. APTT and PT were significantly ($p < 0.01$) prolonged at concentrations of 10 mg ml⁻¹ and 30 mg ml⁻¹, respectively. In conclusion, ioversol, iodixanol and ioxaglate inhibit platelet adhesion and P-selectin expression, as well as plasma coagulation. Platelets are more sensitive in relation to the inhibiting effect on plasma coagulation.

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Radiology

Iodinated contrast media (CM) for intravenous administration are routinely used worldwide in daily radiography practice, and approximately 60 million doses are applied per year [1]. All CM used today are in the form of either a monomer or a dimer and, unrelated to this, classified as ionic or non-ionic. The ionic and most of the non-ionic CM are hypertonic in relation to plasma, but the osmolalities are significantly lower than the former generation of CM, explaining the terms “low-osmolar” and even “iso-osmolar” CM.

CM are reported to possess both prothrombotic and anticoagulant properties, but it seems that ionic CM are more anticoagulant in behaviour and non-ionic CM more prothrombotic [2–4]. The mechanism for these two effects is not clearly understood, and reports on the effects of CM are partly contradictory and difficult to interpret. Different mechanisms are suggested. On the prothrombotic side, there is evidence of increased platelet degranulation with surface expression and release of inflammatory mediators and factors promoting haemostasis, *i.e.* P-selectin, and increased thrombin

production [4–7]. On the anticoagulant side, suggested mechanisms include inhibition of thrombin generation and interference with FV and FVIII activation [5, 8, 9] and resistance to thrombolysis [10].

One of the well-known adverse effects of CM is the induction of acute renal failure (ARF). CM is the third leading cause of hospital-acquired ARF, which in some cases leads to the need for dialysis [11]. Little is known about the underlying mechanism, but there are studies in which platelet P-selectin has been associated with neutrophil-mediated acute post-ischaemic renal failure [12] and glomerulonephritis [8]. It has also been shown that platelets are critical to enhanced glomerular arachidonate metabolism in acute nephrotoxic nephritis in rats [13]. Thus, platelets play a crucial role in haemostasis and the inflammatory response, and have been connected to renal impairment.

Normally, platelets circulate in a quiescent state, but, upon activation, the expression of P-selectin is induced, which acts as a cell adhesion molecule in activated platelets and endothelial cells; inflammatory mediators and factors promoting haemostasis are also released. P-selectin is stored in α -granules and is mobilised and expressed on the platelet surface after stimulation with agonists such as thrombin, adenosine diphosphate (ADP) and adrenaline [14]; this mobilisation is agonist

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and dose dependent [15]. In earlier studies of platelet function, P-selectin was the major parameter analysed, mainly with flow cytometry as the method of choice. Observations of the parameters prothrombin time (PT) and activated partial thromboplastin time (APTT) in response to CM have been made previously [2, 16, 17], but no studies on platelet adhesion to protein surfaces have previously taken both *ex vivo* and *in vitro* effects into account. In this study, the *ex vivo* effect of two non-ionic CM — ioversol (monomer) and iodixanol (dimer) — on platelet adhesion to protein surfaces was measured with a novel assay. In addition, the *in vitro* effects (including dose responses) of ioversol, iodixanol and ioxaglate (an ionic dimer) on platelet adhesion, platelet P-selectin surface expression, PT and APTT were analysed.

Methods and materials

Ethical consideration

The study protocol was approved by the Ethics Committee of the Faculty of Health Sciences, Linköping University, Sweden, and was conducted according to the Declaration of Helsinki.

Contrast media

Three commercially available CM were studied: (i) ioversol, a non-ionic monomer (Optiray[®], 300 mg of I ml⁻¹; tyco HealthcareDeutschland GmbH, Neustadt/Donau, Germany); (ii) iodixanol, a non-ionic dimer (Visipaque[®], 320 mg of I ml⁻¹; Amersham Health AB, Solna, Sweden); and (iii) ioxaglate, an ionic dimer (Hexabrix[®], 320 mg of I ml⁻¹; Laboratoire Guerbet, Roissy Charles-de-Gaulle, France). All three CM were tested *in vitro*, whereas only ioversol and iodixanol were used in the *ex vivo* experiments. Ioxaglate were not included in the *ex vivo* test owing to practical problems in acquiring standardised samples.

Patients and controls

Blood samples from 40 subjectively healthy individuals (21 females and 19 males; 45–69 years old; median age, 63 years) were used as controls for the *ex vivo* study. For the *in vitro* study, blood from six healthy individuals was used for each experiment.

Patient samples consisted of blood from a total of 41 consecutive outpatients: 32 with no history of renal impairment (14 females/18 males; range, 26–81 years; median age, 55 years) and 9 with renal failure (3 females/6 males; range, 43–80 years; median age, 75 years). In the group with no renal impairment, samples were taken from five patients undergoing intravenous urography for a time-study experiment (pilot study). The remaining samples ($n=27$) were from patients undergoing contrast-enhanced CT, in the vast majority of cases as a consequence of malignant disease ($n=21$). In the pilot study group, 50 ml of ioversol (240 mg ml⁻¹) was administered intravenously to each

patient; in the CT group, 90 ml (300 mg ml⁻¹) was administered. The patients in the group with renal failure ($n=9$) underwent CT angiography owing to suspected renal artery stenosis and were administered 50 ml of iodixanol (320 mg ml⁻¹) intravenously.

Collection of blood samples

Blood samples were collected within 1 h of preparation and analysis. In the *ex vivo* time-study experiments (pilot study), samples were collected at four different time-points in relation to the ioversol administration: immediately before (0') and 1 min (1'), 5 min (5') and 15 min (15') after the end of the injection. For all other *ex vivo* experiments, samples were collected immediately before and 5 min after the injection. To minimise the risk of contamination, waste was discarded in between sampling in all *ex vivo* experiments. In both *ex vivo* and *in vitro* experiments, blood was collected in sodium heparin tubes (Becton Dickinson, San Jose, CA) for adhesion and P-selectin assays. For coagulation assays, blood was collected in tubes containing 3.8% trisodium citrate (Becton Dickinson).

After collection, blood was centrifuged at $220 \times g$ for 20 min at room temperature to obtain platelet-rich plasma (PRP). The top two-thirds of the PRP was collected and gently suspended 1:4 in 0.9% NaCl. For adhesion and P-selectin assays, PRP was further supplemented with MgCl₂ to obtain a final assay concentration of 5 mmol l⁻¹ [18]. Platelet-poor plasma (PPP) was prepared by further centrifugation of PRP for 10 min at $1500 \times g$ at room temperature. The platelet suspension was kept under a gentle shaking motion at room temperature before use.

Platelet adhesion assay

A new assay, recently described by Eriksson and Whiss [19, 20], was used to measure platelet adhesion to different protein surfaces in microplates. Microplate coating was performed by adding 100 µl of one of the following solutions to 96-well microplates (Nunc Maxisorp, Roskilde, Denmark): (a) 2 mg ml⁻¹ albumin (Pharmacia & Upjohn AB, Stockholm, Sweden); (b) 2 mg µl⁻¹ fibrinogen (American Diagnostica Inc., Greenwich, CT); or (c) 100 µg ml⁻¹ collagen S from calf skin (Roche Diagnostics, Mannheim, Germany) combined with 2 µg ml⁻¹ horse tendon collagen (Biopool International, Ventura, CA). The coated microplates were incubated overnight at 4°C. The albumin-coated surface served as a negative control surface, as it causes a low number of platelets to adhere. After incubation, microplates containing coating solution were washed twice in 0.9% NaCl. Immediately after washing, the wells were supplemented with either (i) CM for the *in vitro* studies of the effects of CM or (ii) CM together with soluble activators for the studies of the *ex vivo* effects of CM. In the *in vitro* studies, CM were added to achieve final assay concentrations 0.3, 1.0, 3.0, 10.0 and 30.0 mg ml⁻¹. The contrast agents were diluted in 0.9% NaCl. In the studies of the effects of CM *ex vivo*, the platelet activators used were ADP and 1- α -lysophosphatidic acid (oleoyl-

Figure 1. Platelet adhesion to (a) albumin, (b) collagen and (c) fibrinogen before (grey) and after (black) intravenous administration of ioversol (90 ml, 300 mg ml⁻¹) and *in vitro* stimulation with adenosine diphosphate (ADP), ristocetin (Risto), adrenaline (Adr), L- α -lysophosphatidic acid (LPA) and a combination of LPA/adrenaline at the stated concentrations. Significant differences calculated with analysis of variance (ANOVA) are indicated by *, ** and ***, corresponding to $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively (mean $\pm$ standard error of the mean; $n = 27$).

sn-glycero-3-phosphate; LPA) (Sigma-Aldrich, St Louis, MO), ristocetin (Diagnostica Stago, Asnières-sur-Seine, France) and adrenaline (NM Pharma, Stockholm, Sweden). In the studies of the effects of CM *in vitro*, the protein surface was the only activating stimulus. After addition of CM or CM + activators, 50 μ l of PRP was added to each well. The microplates were incubated for 1 h at room temperature and then washed twice in 0.9% NaCl. Thereafter, 140 ml of a substrate solution containing 1 mg ml⁻¹ *p*-nitrophenyl phosphate (Sigma-Aldrich) dissolved in 0.1 mol l⁻¹ sodium citrate, 0.1 mol l⁻¹ citric acid and 0.1% Triton-X-100 (pH 5.4) was rapidly added to each well. For estimation of total platelet count, 50 μ l of PRP was mixed with 140 ml of substrate solution and added to an uncoated microplate; 50 μ l of 0.9% NaCl was used as blank. Background absorbance was measured at 405 nm using a Spectramax microplate reader

(Molecular Devices, Sunnyvale, CA) and the microplates were incubated for 40 min under gentle shaking motion. After incubation, the enzymatic reaction was stopped and colour was developed by the addition of 100 μ l of NaOH (2 mol l⁻¹) to each well. The coloured *p*-nitrophenol produced by the reaction was measured by absorbance readings at 405 nm, and the background absorbance was thereafter subtracted from these values. The percentage of adherent cells was calculated using the formula:

$$(\text{sample-blank})/(\text{total-blank}) \times 100 \quad (1)$$

Measurement of P-selectin on adhered platelets

To study the activation state of platelets adhered to the different protein surfaces, platelet exposure of CD61 and

Figure 2. Platelet adhesion to (a) albumin, (b) collagen and (c) fibrinogen before (grey) and after (black) intravenous administration of iodixanol (50 ml, 320 mg ml^{-1}) and *in vitro* stimulation with adenosine diphosphate (ADP), ristocetin (Risto), adrenaline (Adr), L- α -lysophosphatidic acid (LPA) and a combination of LPA/adrenaline at the stated concentrations. Significant differences calculated with analysis of variance (ANOVA) are indicated by * and ** corresponding to $p < 0.05$ and $p < 0.01$, respectively (mean $\pm$ standard error of the mean; $n = 9$).

P-selectin were measured by a previously described enzyme-linked immunosorbent assay [14, 18]. The procedure was identical to the adhesion assay described above until the washing procedure to remove non-attached platelets. Before this washing procedure, the platelets were fixed for 5 min in phosphate-buffered saline (PBS; 137 mmol l^{-1} NaCl, 2.7 mmol l^{-1} KCl, 8 mmol l^{-1} Na_2HPO_4 and 1.5 mmol l^{-1} KH_2PO_4 , pH 7.4) containing 0.04% formaldehyde. Following two washing procedures, residual protein binding sites were blocked with PBS containing 5% bovine serum albumin (BSA) for 30 min. The wells were washed twice with 0.9% NaCl containing 0.05% (vol/vol) Tween 20 (NaCl-T) and were further incubated with mouse monoclonal antibodies (mAbs) against CD61 (RUU-PL 7F12; Becton Dickinson Immunocytometry Systems, Erembodegem-Aalst, Belgium) or P-selectin (Clone 1E3; DakoCytomation, Glostrup,

Denmark) for 30 min. Expression of CD61 was used as a control in the P-selectin expression assay to exclude loss of platelets during the washing procedure before measurements [14]. After another two washing steps with NaCl-T, bound primary mAbs were detected with polyclonal rabbit anti-mouse antibodies coupled to alkaline phosphatase (D314; DakoCytomation, Glostrup, Denmark). All antibodies were diluted in PBS containing 1% BSA. The final concentration of mAbs was 0.3 $\mu\text{g ml}^{-1}$; secondary enzyme-conjugated antibodies were used at 1.0 $\mu\text{g ml}^{-1}$. Following a final wash with NaCl-T, 100 μl of 1 mg ml^{-1} *p*-nitrophenyl phosphate (Sigma-Aldrich) dissolved in 1 mol l^{-1} diethanolamine buffer (pH 9.8) was rapidly added to each well. Substrate hydrolysis by phosphatase was measured at 405 nm using a Spectramax microplate reader (Molecular Devices, Sunnyvale, CA) after a 10 min incubation. All incubations

Figure 3. Platelet P-selectin surface expression on platelets adhered to (a) albumin, (b) collagen and (c) fibrinogen before (grey) and after (black) intravenous administration of ioversol (90 ml, 300 mg ml⁻¹) and *in vitro* stimulation with adenosine diphosphate (ADP), ristocetin (Risto), adrenaline (Adr), L- α -lysophosphatidic acid (LPA) and a combination of LPA/adrenaline at the stated concentrations. Significant differences calculated with analysis of variance (ANOVA) are indicated by *, corresponding to $p < 0.05$ (mean $\pm$ standard error of the mean; $n = 14$).

were conducted at stated times with a gentle rocking motion at room temperature.

Plasma coagulation assays

PT and APTT were measured in PPP in the presence of the test compounds ioversol, iodixanol or ioxaglate at final concentrations of 0.3, 1.0, 3.0, 10.0 and 30.0 mg ml⁻¹, solvent (0.9% NaCl) and the reference compound heparin (0.1 U ml⁻¹; LEO Pharma, Malmö, Sweden). The plasma coagulation reagents APTT-synthetic phospholipids (liquid) and calcium chloride (0.025 mol l⁻¹) were obtained from the Instrumentation Laboratory (Lexington, MA), and Owren's PT GHI 131 was from Medirox AB (Studsvik, Sweden). A 270 μl aliquot of PPP plus 30 μl of test substance was pre-incubated for 5 min at room temperature before analysis using an ACL 10 000 (Instrumentation

Laboratory). The PT and APTT were determined according to standard procedures developed by the Instrumentation Laboratory. All tests were performed at 37°C.

Statistical analysis

PRP for the platelet assays and PPP for the coagulation assays were prepared from the stated number of patients or healthy controls for each experiment. The mean of duplicates was used for calculations. All data showed gaussian distribution, and the values were analysed with repeated measures of analysis of variance, followed by Dunnett's multiple comparison test for comparison with controls or solvent or Tukey's test for comparison of different treatments (GraphPad Prism®, version 4; GraphPad Software Inc, San Diego, CA). A p -value of < 0.05 was judged as statistically significant.

Figure 4. Platelet adhesion to albumin (bordered bars; black bars with CM) and platelet P-selectin expression (open bars; patterned bars with CM) after incubation with different concentrations *in vitro* (3 g l⁻¹, 10 g l⁻¹ and 30 g l⁻¹, respectively) of ioversol, iodixanol and ioxaglate. Significant differences compared with solvent calculated with analysis of variance (ANOVA) are indicated by * and **, corresponding to $p < 0.05$ and $p < 0.01$, respectively (mean $\pm$ standard error of the mean; $n = 6$).

Results

Time-study of platelet adhesion after intravenous ioversol administration

Initially, blood was collected from five individuals before and at 1 min, 5 min and 15 min after injection to study the effects of ioversol on platelet adhesion at different time-points after administration. With this small sample size, no significant effect of ioversol could be observed (data not shown). 5 min after injection was chosen as the sampling time for all subsequent experiments, as this time-point was most practical and easy to maintain.

Ex vivo effects of CM on platelet adhesion and P-selectin expression

Before administration of CM, platelet adhesion in the patient group was not significantly different from that in a group of 40 healthy controls (data not shown). Platelet adhesion to albumin, which normally is very low, was increased by activation with ADP, 1 $\mu\text{mol l}^{-1}$ adrenaline or the combination of adrenaline and LPA. In samples obtained after administration of ioversol, this increase

was significantly attenuated (Figure 1a). Excluding the adrenaline/LPA combination, this effect was also evident upon administration of iodixanol (Figure 2a). Upon platelet adhesion to collagen, all tested additional activators, besides LPA alone, increased adhesion. Adhesion to the collagen surface *per se*, as well as upon additional activation by all compounds besides LPA, was attenuated in samples with ioversol (Figure 1b). In samples with iodixanol, no significant effect was obtained (Figure 2b). Almost the same pattern was evident upon adhesion to the fibrinogen surface. Ioversol attenuated increased adhesion induced by additional activation by soluble compounds, ristocetin excluded (Figure 1c), but iodixanol did not cause any significant effect on platelet adhesion (Figure 2c).

In a subgroup of 14 patients with ioversol, surface expression of P-selectin on the adherent platelets was determined (Figure 3). A similar pattern to that observed in the adhesion assay was seen for the albumin surface, with the exception of the absence of inhibition by ioversol of ADP-induced activation. Thus, ioversol attenuated the increased P-selectin expression induced by 1 $\mu\text{mol l}^{-1}$ adrenaline and the adrenaline/LPA combination. However, on the collagen surface, ioversol attenuated only the increase induced by 0.1 $\mu\text{mol l}^{-1}$ adrenaline, and on the fibrinogen surface no significant

Figure 5. Platelet adhesion to collagen (bordered bars; black bars with CM) and platelet P-selectin expression (open bars; patterned bars with CM) after incubation with different concentrations *in vitro* (3 g l⁻¹, 10 g l⁻¹ and 30 g l⁻¹, respectively) of ioversol, iodixanol and ioxaglate. Significant differences compared with solvent calculated with analysis of variance (ANOVA) are indicated by * and **, corresponding to $p < 0.05$ and $p < 0.01$, respectively (mean $\pm$ standard error of the mean; $n = 6$).

effect was detected. Surface expression of P-selectin was also measured on platelets from three additional patients treated with iodixanol and, although the expression pattern was similar to the ioversol group, the sample size was too small to draw any conclusions (data not shown).

In vitro effects of CM on platelet adhesion and P-selectin surface expression

To examine whether the *ex vivo* effects of ioversol and iodixanol are measurable after *in vitro* challenge, platelet adhesion and P-selectin expression were measured in the presence of various concentrations of CM. In addition, ioxaglate was also tested for any *in vitro* effect, as this has been reported to elicit one of the most potent antiplatelet and anticoagulation properties [2, 4, 10, 21].

None of the CM affected platelet adhesion or P-selectin expression at 0.1 mg ml⁻¹, 0.3 mg ml⁻¹ or 1.0 mg ml⁻¹ (data not shown). Iodixanol alone inhibited the low amount of adhesion and P-selectin expression induced by the albumin surface (Figure 4). With the exception of ioxaglate and the collagen surface (Figure 5c), significant inhibition of platelet adhesion to collagen and fibrinogen was detected at 3 mg ml⁻¹ for all three CM and the inhibition was dose

dependent (Figures 5 and 6). Generally, a higher concentration (10 mg ml⁻¹) was required to inhibit surface expression of P-selectin on the adherent platelets. At 30 mg ml⁻¹, all of the CM were close to producing total block of adhesion and P-selectin expression. At 100 mg ml⁻¹, no additional inhibition was observed (data not shown).

All three tested CM contain the divalent cation chelator ethylenediaminetetraacetic acid (EDTA) and divalent cations are important for the platelet adhesion process [18]. Therefore, the possible effect of the EDTA content in the CM on platelet adhesion was studied. Corresponding concentrations of EDTA did not, however, affect platelet adhesion to any of the surfaces in the present study (data not shown). The highest concentration of EDTA tested in the platelet assay was 0.07 mg ml⁻¹, corresponding to the content in 100 mg ml⁻¹ Optiray®.

In vitro effects of CM on plasma coagulation

Table 1 lists the *in vitro* coagulation times after 5 min of pre-incubation with the different contrast agents. At 10.0 mg ml⁻¹ and 30.0 mg ml⁻¹, ioversol, iodixanol and ioxaglate exerted a concentration-dependent inhibition on the APTT and PT tests. At 10 mg ml⁻¹, the three

Figure 6. Platelet adhesion to fibrinogen (bordered bars; black bars with CM) and platelet P-selectin expression (open bars; patterned bars with CM) after incubation with different concentrations *in vitro* (3 g l⁻¹, 10 g l⁻¹ and 30 g l⁻¹, respectively) of ioversol, iodixanol and ioxaglate. Significant differences compared with solvent calculated with analysis of variance (ANOVA) are indicated by * and **, corresponding to $p < 0.05$ and $p < 0.01$, respectively (mean $\pm$ standard error of the mean; $n = 6$).

agents inhibited APTT slightly, but PT was unaffected. In both APTT and PT tests, the coagulation-inhibitory effect exerted by ioxaglate was significantly superior ($p < 0.001$ and < 0.01 , respectively) to those of ioversol and iodixanol at 30.0 mg ml⁻¹.

Discussion

In the *ex vivo* part of the present study, platelet adhesion was, in general, significantly lower 5 min after intravenous administration of ioversol and iodixanol than for prior administration. The same pattern appeared in the *in vitro* part of the study, in which all three tested CM yielded a significant dose-dependent decrease in platelet adhesion, P-selectin expression and coagulation. As the ionic state differs between the CM used in the present study, the platelet effect seems not to be related to this factor, a finding in line with earlier studies [6, 22]. The mechanism is unclear but appears to be unrelated to the different physiochemical characteristics, as all of the tested CM differ in that sense. We could not confirm a prothrombotic effect, as found previously [2, 5, 7, 23], although higher CM concentrations were used in those studies, in some cases up to a 50:50 mixture of CM/blood.

In an *in vitro* study by Chronos et al [5], there was profound release of platelet α -granule contents (P-selectin) in response to the non-ionic CM iohexol in a 50:50 mixture with blood. In our study using two other non-ionic CM, we instead saw dose-dependent inhibition of P-selectin expression and platelet adhesion with breakpoint to physiological concentrations used in daily practice. The ionic ioxaglate had slightly more of an inhibitory effect than the non-ionic CM (ioversol and iodixanol).

A CM-induced decrease in platelet activation could be caused by the presence of EDTA, as all iodinated CM contain EDTA. This probability was ruled out in the present study because corresponding concentrations of EDTA *per se* did not inhibit platelet adhesion. In the study of Heptinstall et al [7], there was evidence of inhibited platelet activation (relating to platelet aggregation) in the presence of citrate. This could not explain the inhibiting effect seen in this study, as no citrate was used. Concerning aspirin, exclusion was not considered necessary as only five of the 36 patients used the drug upon inclusion in the study, and no significant difference could be seen between groups when subdivided according to aspirin intake (data not shown). Our results are in accordance with the study of Chronos et al [5] in which patients pre-treated with large doses of aspirin showed

Table 1. *In vitro* effects of contrast media on coagulation tests on plasma from six healthy donors

Compound (concentration)	Concentration (g l ⁻¹)	APTT (s)	PT (s)
Solvent (0.9% NaCl)		29.3 ± 0.9	9.1 ± 0.0
Ioversol	3.0	29.6 ± 0.7	9.2 ± 0.0
	10.0	31.5 ± 0.9 ^a	9.3 ± 0.1
	30.0	38.2 ± 0.7 ^a	11.4 ± 0.4 ^a
Iodixanol	3.0	29.6 ± 0.7	9.2 ± 0.1
	10.0	31.6 ± 1.0 ^a	9.3 ± 0.1
	30.0	38.7 ± 0.8 ^a	11.0 ± 0.2 ^a
Ioxaglate	3.0	29.9 ± 0.7	9.2 ± 0.0
	10.0	32.6 ± 0.8 ^a	9.4 ± 0.1
	30.0	53.1 ± 1.6 ^a	14.7 ± 0.4 ^a

Data are the mean ± standard error of the mean. APTT, activated partial thromboplastin time; PT, prothrombin time.

^a*p* < 0.01 compared with solvent.

platelet activation to the same extent as untreated controls. However, the decrease in adhesion and platelet response was smaller in a subgroup of five patients who were taking aspirin in our study, but this result was not significant and was based on only a few individuals.

The non-ionic medium GdDTPA-bismethylamid (BMA) (Omniscan®) at 50:50 (CM/blood) has been reported to significantly increase platelet P-selectin expression [22]. Amongst patients undergoing CT angiography with iodixanol administration in the present study, the majority underwent an MRI scan with administration of GdDTPA-BMA prior to inclusion. Blurring of the results in this group owing to two concomitant CM cannot be excluded, but no indication of differences could be seen when the patients were subdivided.

The anticoagulant effect, measured by PT and APTT, of the ionic dimer ioxaglate in the present study was significantly greater at 30 mg ml⁻¹ than with the non-ionic monomer ioversol and the non-ionic dimer iodixanol. This is in accordance with several studies showing that ionic CM inhibit plasma coagulation more strongly than non-ionic CM [24, 25]. In agreement with a study in rat plasma by Valenti et al [26], iodixanol and ioxaglate significantly increased APTT at lower doses than PT in the present study. Iodixanol has also been reported to increase *in vitro* bleeding time in humans in the same manner as non-ionic CM, such as ioversol [17, 27]. The prolongation of PT and APTT in the present study was seen at a breakpoint at which concentrations of CM were approximately 10 times higher than in the adhesion and P-selectin tests (30 mg ml⁻¹); at lower concentrations, there was no significant effect. Hence, the inhibiting effect on humoral coagulation seems to be weaker than platelet inhibition. The inhibiting mechanism on humoral coagulation is unclear. Al Dieri et al [9] reported that ioxaglate had an inhibitory effect on the clotting of fibrinogen, as well as on activation of factor V and VIII, and of platelets by thrombin. It was suggested that ioxaglate interferes with the binding of macromolecular substrates to the anionic exosite I of thrombin, as stated previously by Li and Gabriel [8]. In the study by Li and Gabriel [8], the finding could be seen only in relation to the ionic ioxaglate, and not to the non-ionic CM iohexol and iodixanol. In the present study, ioxaglate increased APTT by more than 10 s more than did ioversol and iodixanol at 30 g l⁻¹. This difference in potency in increasing APTT might be dependent upon the mechanisms mentioned above.

Compared with other methods, the new assay used in this study reflects a new aspect of CM, *i.e.* inhibition of platelet adhesion to protein surfaces. In conclusion, the present study shows that all three tested CM at therapeutic doses elicit an inhibitory effect on platelet adhesion, as well as on platelet P-selectin expression and humoral coagulation. This inhibition of platelet adhesion is in line with one earlier *in vitro* study that used a 10% (vol/vol) solution of iodixanol, ioxaglate and iohexol to measure platelet-mediated membrane closure time [28]. The mechanisms are unclear, but the results indicate a general inhibitory effect, or multiple mechanisms, rather than a specific one, because platelets as well as humoral coagulation are affected. Several common physiochemical properties of CM, such as ion-binding capacity and protein interaction [29], have previously been suggested to influence cell volume regulation in human red blood cells [30]. These properties might also explain the inhibition of platelet adhesion and plasma coagulation in the present study, as both of these processes are highly dependent on the presence of divalent cations, as well as on protein interaction. Further studies are needed to show if this hypothesis can explain the inhibitory effects of CM on haemostasis.

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