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# In Vivo Effects of Contrast Media on Coronary Thrombolysis

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***Objectives.*** **The aim of the present study was to evaluate the influence of radiographic contrast media (CM) on alteplase-induced coronary thrombolysis.**

***Background.*** **Contrast media inhibit fibrinolysis in vitro and interact with endothelial cells, platelets and the coagulation system. The in vivo effects of CM on thrombolysis are not known.**

***Methods.*** **Occlusive coronary artery thrombosis was induced in 4 groups of 10 dogs by the copper coil technique. After 70 min of occlusion the dogs were randomized to intracoronary injection of 2 ml $kg^{-1}$ of either saline, a low-osmolar ionic CM (ioxaglate), a low-osmolar nonionic CM (iohexol) or a high-osmolar ionic CM (amidotrizoate). Thrombolysis with alteplase and co-therapy with aspirin and heparin was initiated after 90 min of occlusion. The coronary artery flow was monitored with an electromagnetic flowmeter throughout the experiment.**

***Results.*** **Iohexol and amidotrizoate, but not ioxaglate, were associated with longer reperfusion delays (time to optimal reperfusion: 67 ± 48 min and 65 ± 49 min, respectively, vs. 21 ± 11 min after placebo; $p < 0.05$) and shorter periods of coronary perfusion (optimal perfusion time: 21 ± 26 min and 21 ± 28 min, respectively, vs. 58 ± 40 min after placebo; $p < 0.05$). No significant differences were observed between groups with regard to activated partial thromboplastin times, circulating thrombin-antithrombin III complex concentrations and fibrinogen.**

***Conclusions.*** **In this animal model administration of iohexol and amidotrizoate before thrombolysis significantly delayed reperfusion. This interaction should be considered in the design of clinical trials of thrombolytic therapy that evaluate coronary artery patency and in patients receiving local infusions of fibrinolytic agents.**

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The interaction between radiographic contrast media (CM) and hemostasis has been a subject of intense debate during the last decade. Since Robertson's first report (1) of clots being more likely formed in syringes containing nonionic than ionic CM, numerous studies have addressed the "clotting issue." Much has been learned about the interaction of CM with endothelial cells (2–4), platelets (5–7) and coagulation proteins (8–11). The effect of CM on endogenous fibrinolysis has also been described (12,13), but little is known about the interaction of CM with pharmacologic thrombolysis. In vitro studies have shown CM-induced inhibition of clot dissolution (14,15) and plasminogen activation (16). However, the mechanisms proposed are contradictory, one study suggesting that the inhibitory effect is due to the electrical charge of CM molecules (16), while another showed that iohexol (low-osmolar, nonionic CM) and diatrizoate (high-osmolar, ionic CM) but not ioxaglate (low-osmolar, ionic CM) delayed alteplase-induced thrombolysis (15).

Administration of thrombolytic agents for acute myocardial infarction has profound effects on hemostasis, activating both lytic and prothrombotic mechanisms. Since CM may interact with all factors involved in these reactions (i.e., platelets, endothelial cells and coagulation and fibrinolytic proteins), it is difficult to predict the result of CM administration at the time of thrombolytic therapy from in vitro experiments. The aim of our study was twofold: 1) to evaluate the interaction between different CM and the process of coronary artery thrombolysis and 2) to test the relative importance of biophysical properties (osmolality, electrical charge) for their effects in vivo.

## Methods

**Coronary artery thrombosis.** Mongrel dogs weighing 18 to 30 kg were sedated with 2.5 mg $kg^{-1}$ xylazin (Rompun, Bayer AG, Leverkusen, Germany), anesthetized with sodium pentobarbital (15 mg $kg^{-1}$ bolus and 0.1 mg $kg^{-1}$ $min^{-1}$ infusion; Nembutal, Sanofi, France), intubated and artificially ventilated (Mark 7A respirator, Bird Corporation, Palm Springs, CA, USA). A left thoracotomy was performed in the fifth intercostal space, and the heart was suspended in a pericardial cradle. The left anterior descending (LAD) coronary artery was dissected free distal to the first diagonal branch. An electromagnetic coronary flow probe (Skalar MDL1401, Skalar Med-

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**Abbreviations and Acronyms**

aPTT = activated partial thromboplastin time
CM = radiographic contrast media
LAD = left anterior descending coronary artery
TAT = thrombin-antithrombin III complex
TIMI = Thrombolysis In Myocardial Infarction

ical BV, Delft, The Netherlands) was positioned around the artery, distal to a silk snare. Coronary thrombosis was induced by placing a 3- to 5-mm long copper coil over an intracoronary wire into the LAD, as previously described (17). There were no visible diagonal branches between the copper coil and the flow probe. An occlusive thrombus was formed in 2 to 104 min (mean 26 ± 25 SD). The coronary thrombus was allowed to grow for 90 min before initiation of thrombolysis with alteplase (recombinant tissue plasminogen activator; Actilyse, Boehringer Ingelheim GmbH, Ingelheim, Germany; 0.1 mg $kg^{-1}$ intravenous bolus followed by a continuous infusion of 0.01 mg $kg^{-1}$ $min^{-1}$ for 30 min) and co-therapy with aspirin (5 mg $kg^{-1}$ of acetylsalicylic acid intravenous bolus; Aspegic, Synthélabo, France) and heparin (heparin naturium; Heparine Rorer, Rhône-Poulenc Rorer, Belgium; 100 IU $kg^{-1}$ bolus plus 25 IU $kg^{-1}$ $h^{-1}$ infusion). Ventricular arrhythmias were treated with lidocaine boluses (Xylocaine, Astra Pharmaceuticals, Brussels, Belgium). The ventilation was adjusted to maintain the pH and arterial blood gases within a physiologic range. Blood loss was compensated with a saline infusion adjusted according to the hematocrit. Body temperature was kept constant with a heating pad. All experiments conformed with the "Position of American Heart Association on Research Animal Use" and were conducted with the approval of the Ethics Committee of the University of Leuven.

**Contrast media.** After 70 min of coronary artery occlusion, the dogs were randomized to an intracoronary injection of one of the following: placebo (normal saline solution); the low-osmolar, ionic CM ioxaglate (Hexabrix 320, Laboratories Guerbet, France); the low-osmolar, nonionic CM iohexol (Omnipaque 350, Nycomed, Belgium); or the high-osmolar, ionic CM amidotrizoate (Urographin 76%, Schering AG, Germany). A 5F catheter was positioned in the proximal LAD under fluoroscopic control; fluoroscopy was not used during the intracoronary injections to maintain the blinding. A total volume of 2 ml $kg^{-1}$ was injected over 15 min, divided in multiple intracoronary boluses separated by periods of reflow. This dose was selected based on an estimated use of 150 ml CM (2 ml $kg^{-1}$ for a 75 kg patient) during catheterization. Five minutes after the end of CM injection, the intracoronary position of the catheter tip was verified by fluoroscopy, the catheter was withdrawn and thrombolytic therapy was initiated. CM or placebo were prepared in a separate laboratory room according to computer-generated randomization lists. All investigators remained blinded to the allocated treatment throughout the experiment.

**Venous blood samples.** Venous blood samples were collected on 4% citrate at baseline (sample 1), 70 min after coronary occlusion (before the administration of contrast media, sample 2), after 90 min of occlusion (sample 3), at the end of the alteplase infusion (sample 4) and at the end of experiment (sample 5). The samples were cooled on ice, centrifuged and stored at −20°C for analysis of activated partial thromboplastin time ([aPTT]; using Synthasil, ORTHO Diagnostics, Raritan, New Jersey, USA) and fibrinogen according to the Clauss method. Both assays were run on a KC10 coagulometer (Amelung, Germany) employing a mechanical end point. We have also measured $\alpha$2-antiplasmin with a chromogenic assay (Chromogenix, Sweden) and circulating thrombin-antithrombin III (TAT) complexes with enzyme-linked immunosorbent assay (Behringwerke, Germany).

**Data acquisition and analysis.** The aortic pressure, lead II electrocardiogram and coronary flow analog signals were digitized online at 1.000 Hz $channel^{-1}$ and recorded on a PC with a commercially available software package (Windaq, Dataq Instruments, Akron, Ohio, USA). The software stores the digital input of each channel together with the time reference in binary format (i.e., the signal intensity at each sampling point together with its time reference taken from the internal clock of the computer) and displays it on a screen resembling the millimetric paper. The advantage over classical paper recording is that at playback, the operator can choose the number of data-points that will be displayed between two consecutive grids on the screen. Typically, we have first used a low compression level (0.04 s of digital recording displayed on 1 mm of the screen, corresponding to the 25 mm $s^{-1}$ speed of paper recorders). This level of data compression allowed the best evaluation of the coronary flow pattern (phasic or not). The occurrence of cyclic flows was easier to identify at higher compression levels (1 to 5 min of digital recording displayed on 1 cm on the screen). Coronary zero flow was confirmed whenever needed by occluding the coronary artery for a few seconds with the proximal snare. We have performed qualitative and quantitative analysis of the flow curve. The qualitative analysis was focused on identification of events (reperfusion, cyclic flow and reocclusion; Fig. 1). The definitions of events and of the derived time indexes are given in Table 1. Another blinded investigator performed a second reading of the flow curves. The two readings agreed for event detection in 96% of the situations.

By storing data in digital format, we were able to perform computer-assisted quantitative analysis of the flow curve. We have used the built-in peak-detection software for labeling maximums and minimums of the coronary flow and aortic pressures curves for each heartbeat. The results of the peak detection were checked at the low compression level (0.04 s/mm) and manual corrections were performed when needed. The mean coronary flow and aortic pressure were then computed for every heart cycle. The peak coronary flow and the area under the flow curve (AUC) during therapy were measured. The individual variability in coronary flow and aortic pressure were minimized by baseline normalization.

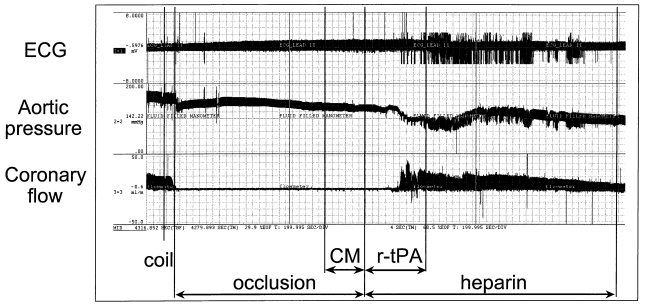


**Figure 1.** Strip chart (maximum compression). **Top curve** = ECG lead II; **middle curve** = aortic pressure; **bottom curve** = coronary flow. The experimental setup comprised a 90-min occlusion period followed by thrombolysis with recombinant tissue plasminogen activator, alteplase (bolus and 30-min infusion) and co-therapy with aspirin (bolus) and heparin (bolus and 2-h infusion). Contrast media (CM) or placebo were administered intracoronary, starting 20 min before thrombolysis. The events identified by strip chart analysis (occlusion, reperfusion, peak hyperemic flow and cyclic flow) were used for the calculation of time parameters.

**Statistical analysis.** Statistical analysis was performed with the SAS software, release 6.03 (18). The normal distribution was tested with Shapiro–Wilk statistic and transformations were performed when appropriate. The treatment effect on time parameters was tested with one-way analysis of variance (ANOVA) (overall effect) and Dunnett's *t* test (pairwise comparisons) for the normal distributions, and with the Kruskal–Wallis test (overall effect) and the Wilcoxon rank-sum test (pairwise comparisons) for nonnormal distributions. The main effects of time, group and the interaction time × group on hemostasis parameters were tested with ANOVA for repeated measurements. A p value of less than 0.05 was considered significant. The experiment had a power of 0.90 to detect a difference of 40 min in the reperfusion times, and of 0.65 to detect a 30-min difference. All calculations were performed with the SAS procedures UNIVARIATE, ANOVA, GLM and NPAR1WAY (18). Values are expressed as mean ± SD.

**Table 1.** Definitions of Events and Derived Time Indexes

| Events | Definitions |
|---|---|
| (Re)Occlusion | Disappearance of the phasic coronary flow pattern for at least 30 s; mean flow ≤1 ml $min^{-1}$ |
| Reperfusion | Appearance of the phasic coronary flow pattern for at least 30 s; mean flow >1 ml $min^{-1}$ |
| Optimal reperfusion | Period of stable coronary perfusion with evident hyperemia at the beginning and without cyclic flows |
| Cyclic flows | Cyclic variations of the mean coronary flow not explained by changes in the aortic pressure and/or arrhythmia |
| **Time indexes** | **Definitions** |
| Time to reperfusion | Time elapsed from initiation of thrombolytic therapy until the first episode of reperfusion, whether or not this was followed by sustained coronary artery perfusion |
| Time to optimal reperfusion | Time elapsed from initiation of thrombolytic therapy until the peak hyperemic flow |
| Total perfusion time | Total time of coronary artery perfusion during the 120 min of therapy |
| Optimal perfusion time | Time interval between onset of optimal perfusion and occurrence of the first episode of cyclic flow or reocclusion |

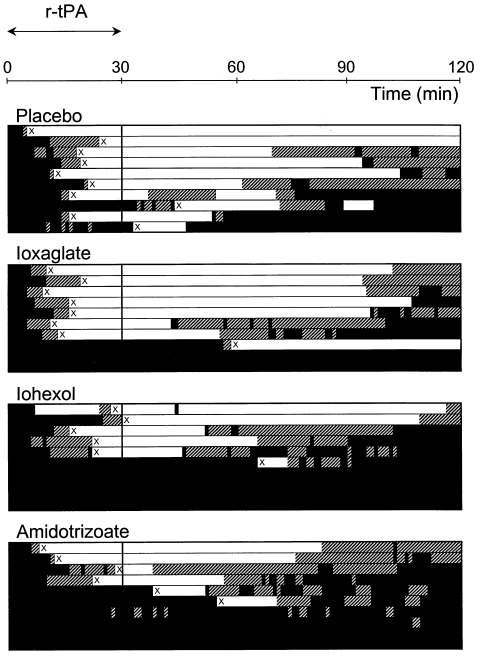


**Figure 2.** Schematic representation of the patency status of the LAD coronary artery. **Black squares** = zero flow; **striped squares** = periods with cyclic flows; **open (white) squares** = periods with optimal coronary perfusion. The time-point at which the peak hyperemic flow occurred (at the beginning of optimal reperfusion) is marked with an X. r-tPA = recombinant tissue plasminogen activator, alteplase.

## Results

A total of 59 experiments were performed. Nineteen dogs were excluded, eight before the randomization stage (death due to ventricular fibrillation early after occlusion: six dogs; problems with the coronary artery dissection: two dogs). The other 11 exclusions were due to recanalization of the LAD during the intracoronary injection of CM before initiation of thrombolytic therapy (six dogs; ioxaglate: one; iohexol: four; amidotrizoate: one), inadequate flow signal (four dogs; saline: two; ioxaglate: one; amidotrizoate: one) and death due to ventricular fibrillation during reperfusion (ioxaglate: one dog). The randomization process continued until 40 dogs completed the study protocol, giving a total of 10 dogs per group.

**Coronary flow.** The coronary artery patency is schematically represented in Figure 2. Thrombolytic therapy induced sustained reperfusion in the following proportions: 10 of 10

**Table 2.** Time Indexes, Aortic Pressure and LAD Flow

| | Placebo | Ioxaglate | Iohexol | Diatrizoate | p Value |
|---|---|---|---|---|---|
| Time indexes (min) | | | | | |
| Time to reperfusion | 15 ± 8 | 36 ± 47 | 61 ± 54 | 51 ± 47 | 0.06 |
| Time to optimal reperfusion | 21 ± 11 | 40 ± 44 | 67 ± 48* | 65 ± 49* | 0.02 |
| Total perfusion time | 80 ± 34 | 75 ± 43 | 45 ± 46 | 45 ± 43 | 0.08 |
| Optimal perfusion time | 58 ± 40 | 57 ± 37 | 21 ± 26*† | 21 ± 28*† | 0.01 |
| Aortic pressure (mm Hg) | | | | | |
| Baseline | 124 ± 18 | 129 ± 20 | 135 ± 20 | 123 ± 15 | 0.44 |
| Start medication | 103 ± 22 | 119 ± 16 | 107 ± 22 | 109 ± 23 | 0.33 |
| Pressure decrease during therapy | 29 ± 21 | 27 ± 21 | 36 ± 16 | 24 ± 19 | 0.52 |
| LAD flow | | | | | |
| Baseline (ml $min^{-1}$) | 8.6 ± 2.9 | 11.5 ± 7.9 | 8.6 ± 2.9 | 9.9 ± 3.7 | 0.43 |
| Peak flow during therapy (normalized values) | 1.7 ± 0.4 | 1.46 ± 0.98 | 0.96 ± 1.04* | 1.13 ± 0.15 | 0.11 |
| AUC (normalized values) | 69.6 ± 27.8 | 84.6 ± 63.7 | 41.6 ± 48.2* | 40.0 ± 40.2‡ | 0.09 |

*$p < 0.05$ vs. placebo; †$p < 0.05$ vs. ioxaglate; ‡$p = 0.053$ vs. placebo. Data are mean ± SD. AUC = area under the flow curve; LAD = left anterior descending coronary artery.

(placebo), 8 of 10 (ioxaglate), 6 of 10 (iohexol) and 6 of 10 (amidotrizoate). Late reperfusion (i.e., after the end of alteplase infusion) occurred in six dogs (placebo: two; ioxaglate: one; iohexol: one; amidotrizoate: two). At the end of the experiment the proportions of open LADs were 5 of 10 for the placebo, 5 of 10 for ioxaglate, 2 of 10 for iohexol and 2 of 10 for amidotrizoate. Optimal perfusion was maintained until the end of the experiment in only two dogs in the placebo group, and in none in the CM groups. Although more open LADs were observed in the placebo group than in the CM groups, these differences were not statistically significant. However, the effects of CM were evident on the time indexes (Table 2). Administration of iohexol and amidotrizoate prior to initiation of thrombolytic therapy resulted in a significant delay in the mean time to optimal reperfusion. With ioxaglate the reperfusion time was not significantly different from placebo ($p = 0.35$) or from the other two CM ($p = 0.16$ vs. iohexol, and $p = 0.19$ vs. amidotrizoate). A similar result was observed for the perfusion times, with dogs receiving iohexol and amidotrizoate showing shorter periods of optimal coronary artery perfusion (see Table 2). The optimal perfusion time after ioxaglate administration was similar to placebo and significantly longer than after both amidotrizoate and iohexol. All time indexes showed a large variability, mainly due to dogs in which reperfusion was not induced (ioxaglate: two; iohexol: four; amidotrizoate: two). When we excluded these experiments from the analysis, the deleterious effects of amidotrizoate on alteplase-induced thrombolysis were still evident (time to optimal reperfusion 52 ± 45 min vs. 21 ± 11 min for placebo, $p < 0.05$; optimal perfusion time, 26 ± 29 vs. 58 ± 37 for placebo, $p = 0.056$), although of a lower magnitude. For iohexol, the mean time to optimal reperfusion was significantly longer than for placebo (32 ± 18 min vs. 21 ± 11 min, $p < 0.05$); the optimal perfusion time, although shorter, was not significantly different from placebo ($p = 0.19$).

The results of the quantitative curve analysis are summarized in Table 2. In the absence of significant differences between groups with regard to pressure parameters, the area under the flow curve and the peak coronary flow during thrombolysis were significantly larger in the placebo group than in the iohexol group. A borderline significant difference was observed between placebo and amidotrizoate ($p = 0.053$ for AUC and $p = 0.09$ for the coronary peak flow); there were no significant differences between placebo and ioxaglate. Pressure decreased during thrombolysis to a similar extent in all groups.

**Hemostasis parameters.** The plasma TAT complex levels, the aPTTs and fibrinogen concentrations are given in Table 3. No significant changes were observed in these parameters during CM injection. However, a small but significant decrease in $\alpha$2-antiplasmin concentration was observed in dogs that received CM, but not in those receiving placebo (significant time × group interaction, $p < 0.01$). The sample taken at the end of alteplase infusion showed a marked decrease in fibrinogen and $\alpha$2-antiplasmin levels, probably due to continuous lysis in the vial, since blood samples were not collected on aprotinin (19). There was also a prolongation of the aPTT in all animals, no significant differences between groups being observed. The prothrombotic state induced by the copper-coil was neutralized by aspirin and heparin to a similar extent in all groups, as shown by the return of the TAT complex concentration to baseline values.

## Discussion

**Effects of CM administration on coronary thrombolysis.** This report represents the first study of the in vivo effects of CM on pharmacologic thrombolysis. In designing the experiment we tried to mimic the clinical situation of acute myocardial infarction: occlusive coronary thrombosis aged for 90 min, standard intravenous thrombolysis with alteplase, co-therapy with aspirin and heparin. Various parameters were defined for the evaluation of the interference between CM and thrombolysis. The reperfusion times and the normalized peak coronary

**Table 3.** Hemostasis Parameters

| | Placebo | Ioxaglate | Iohexol | Diatrizoate |
|---|---|---|---|---|
| Fibrinogen (g/liter) | | | | |
| Baseline | 1.85 ± 0.54 | 1.74 ± 0.28 | 1.96 ± 0.54 | 1.75 ± 0.35 |
| Start CM | 1.90 ± 0.59 | 1.77 ± 0.39 | 1.89 ± 0.62 | 1.66 ± 0.43 |
| Start r-tPA | 1.82 ± 0.60 | 1.48 ± 0.61 | 1.70 ± 0.58 | 1.55 ± 0.32 |
| End r-tPA | 0.07 ± 0.02* | 0.06 ± 0.01* | 0.07 ± 0.02* | 0.06 ± 0.02* |
| End of experiment | 1.61 ± 0.60 | 1.55 ± 0.46 | 1.63 ± 0.51 | 1.40 ± 0.52 |
| aPTT (s) | | | | |
| Baseline | 15 ± 1 | 15 ± 1 | 15 ± 1 | 16 ± 1 |
| Start CM | 16 ± 2 | 16 ± 2 | 16 ± 2 | 16 ± 1 |
| Start r-tPA | 15 ± 2 | 17 ± 3 | 15 ± 2 | 17 ± 2 |
| End r-tPA | 371 ± 19* | 384 ± 13* | 376 ± 12* | 349 ± 69* |
| End of experiment | 78 ± 103* | 118 ± 144* | 44 ± 18* | 66 ± 37* |
| TAT ($\mu$g/ml) | | | | |
| Baseline | 5.1 ± 3.8 | 6.9 ± 3.9 | 6.3 ± 6.9 | 5.9 ± 4.1 |
| Start CM | 9.8 ± 7.0* | 10.6 ± 8.8* | 11.9 ± 10.6* | 13.2 ± 11.9* |
| Start r-tPA | 15.2 ± 15.7* | 21.0 ± 21.7* | 14.0 ± 8.4* | 12.7 ± 10.2* |
| End r-tPA | 8.7 ± 7.6 | 12.1 ± 16.9 | 7.5 ± 2.6 | 7.0 ± 2.2 |
| End of experiment | 7.3 ± 10.8 | 7.9 ± 8.9 | 11.1 ± 11.3 | 4.8 ± 1.4 |
| $\alpha$2-Antiplasmin (%) | | | | |
| Baseline | 91.0 ± 4.6 | 93.0 ± 8.4 | 93.1 ± 5.0 | 92.3 ± 3.5 |
| Start CM | 90.5 ± 4.3 | 91.4 ± 9.5 | 87.2 ± 9.3 | 88.7 ± 5.4 |
| Start r-tPA | 88.5 ± 4.5 | 84.2 ± 11.0* | 80.5 ± 10.9* | 80.6 ± 6.0* |
| End r-tPA | 0.0 ± 0.0* | 0.0 ± 0.0* | 0.0 ± 0.0* | 0.0 ± 0.0* |
| End of experiment | 56.0 ± 22.2* | 47.3 ± 23.4* | 52.1 ± 19.4* | 48.7 ± 23.1* |

*$p < 0.05$ vs. baseline. Data are mean ± SD. aPTT = activated partial thromboplastin time; r-tPA = recombinant tissue-type plasminogen activator, alteplase; TAT = thrombin-antithrombin complex concentrations.

flow are a measure of the direct effects of CM on alteplase-induced clot lysis. Reperfusion therapy however not only aims at opening the occluded coronary arteries, but also at preventing reocclusion and, therefore, we have defined indexes for the global effects of CM on the reperfusion process. The total perfusion time and the optimal perfusion time are a measure of both the quality of initial reperfusion (a smaller residual thrombus will have less procoagulant activity) and the absence of late reocclusion due to the prothrombotic effects.

All parameters analyzed showed an inhibition of alteplase-induced thrombolysis by iohexol and amidotrizoate: optimal reperfusion occurred 40 min later with these agents than after placebo. This delay may be highly relevant in the context of acute myocardial infarction since the only way to limit necrosis and preserve cardiac function is to restore blood flow as early as possible (20). Furthermore, also cyclic flows and reocclusion occurred more often and earlier after these two CM than after placebo, in spite of standard anticoagulant and antithrombotic therapy. Indeed, the aPTT levels at the end of the experiment were well above the 1.5 to 2-fold increase currently recommended (21).

**Relative importance of biophysical properties.** In contrast to other studies (16), our results show that the electrical charge of the CM molecule alone cannot predict the magnitude of the effects on in vivo coronary thrombolysis: for a similar osmolality, the ionic agent ioxaglate was superior to the nonionic agent iohexol. On the other hand, for a similar ionic charge, the low-osmolar agent ioxaglate was superior to the high-osmolar agent diatrizoate. The combinations low-osmolar nonionic (iohexol) and high-osmolar ionic (amidotrizoate) were associated with similar inhibition of alteplase-induced thrombolysis. Therefore, we hypothesize that both the ionic charge and osmotic properties are equally important for the inhibition of pharmacologic thrombolysis. The combination associated with the smallest effects seems to be of low osmolality and electrically charged molecules. Our results are supported by the findings of Dehmer et al. (15), who have shown that clots formed at thrombin challenge in the presence of iohexol and amidotrizoate are more resistant to alteplase than those formed in the presence of ioxaglate.

**Effects on hemostasis.** Intracoronary administration of CM did not induce changes in the aPTT or fibrinogen concentration. We consider that our findings are not in contradiction with the large body of evidence for an anticoagulant effect of CM. Indeed, the studies in which such effects were found have used higher CM concentrations (5,10,11,22). Similarly to our results, Manotti et al. (23) also could not find significant prolongations of the aPTT in patients receiving intraarterial CM. There were no significant differences between groups with regard to the TAT concentrations. It is likely that the intense prothrombotic state induced by the copper coil masked the effects of CM on thrombin generation. After initiation of antithrombotic and anticoagulant therapy, the TAT concentrations returned to baseline in all groups.

A different effect between CM and placebo was observed with respect to $\alpha$2-antiplasmin concentrations. Dogs receiving CM showed a small but significant decrease of this coagulation parameter during the intracoronary administration, in contrast to the placebo group in which there was no significant change. These changes probably reflect the weak stimulation of endogenous fibrinolysis by CM, an effect that was also observed in other studies (12,13). The differences between groups disappeared once intense activation of fibrinolysis was induced by alteplase administration, in spite of different effects on the reperfusion delay. This dual effect of CM (i.e., stimulation of endogenous fibrinolysis and inhibition of pharmacologic thrombolysis) is difficult to explain. The stimulating effect is known to be related to CM-induced release of endogenous tissue plasminogen activator (12,13). As for the resistance to lysis, Carr et al. (14) have suggested that CM-induced changes in fibrin structure represent the responsible mechanism, the clots formed in the presence of CM being composed of fibrin strands with lower mass/length ratio and with lower binding affinities for tissue plasminogen activator. Our experiments show that thrombi formed and aged in the absence of CM also become more resistant to thrombolysis after a short contact with certain CM.

**Clinical implications.** There are several potential implications of the interference between CM and thrombolysis. The clinical importance of coronary artery patency at the end of reperfusion therapy has been well established (24,25). As a consequence, most of the trials of thrombolytic therapy have used coronary angiography at various time points, typically at 90 min after initiation of therapy (26–30), but sometimes also at 30 and 60 min (29,30); the landmark Thrombolysis In Myocardial Infarction (TIMI) Phase I trial (31) used angiography also at baseline. The choice for one CM or another is generally left to the local investigator. Our results show that different CM inhibit in vivo thrombolysis to different extents. Therefore, uniform selection of CM for clinical trials may induce less bias in the results.

The importance of CM on the results of thrombolytic trials is also highlighted in a recent report of the TIMI 4 trial. In analyzing the angiographic results, Gibson et al. (32) found that in the absence of intracoronary injections before the 90-min angiogram, TIMI grade 3 flow was achieved significantly more often. In a meta-analysis of 61 trials of thrombolytic therapy, Verheugt et al. (33) have shown that reocclusion occurred more often in those trials that included a baseline angiography. These clinical observations might be explained by our findings.

Although restricted to a small number of selected patients, intracoronary infusions of thrombolytic agents may be valuable in the treatment of acute myocardial infarction, of periprocedural thrombosis in percutaneous transluminal coronary angioplasty (PTCA) and of chronic total occlusions (34). Local infusions of thrombolytic agents are also used in the therapy of peripheral arterial thrombosis (35). These are other clinical situations in which CM can exert inhibitory effects on thrombolysis. The interference may be more important since CM are administered prior to initiation of therapy in these situations. Indeed, thrombolytic agents seem to be less efficient in dissolving clots embolized into the coronaries during PTCA procedures (36).

**Study limitations.** Our model of coronary thrombosis has certain limitations. First, the clots formed within the copper coil are relatively platelet-poor in comparison to arterial thrombi (37). However, this model has been successfully used for the evaluation of thrombolytic and/or adjunctive therapies on ripe coronary thrombi (17,38,39). Second, the chest was opened and the heart was exposed. We have chosen this experimental setup because we had to measure the effect of therapy on coronary artery patency without the use of coronary angiography. The snare occlusions for zeroing may have influenced our results, especially the quantitative flow analysis. Finally, blood samples were collected from a peripheral vein and not from the coronary sinus and, therefore, we may have overlooked local effects of CM on hemostasis. The possible effects of CM on platelet function were also not evaluated.

**Conclusions.** In this animal model, administration of the low-osmolar nonionic agent iohexol and of the high-osmolar ionic agent diatrizoate significantly impaired alteplase-induced thrombolysis. The low-osmolar ionic agent ioxaglate was associated with the smallest delay in reperfusion and was not significantly different from placebo. Ioxaglate was superior to both iohexol and diatrizoate with regard to the maintenance of optimal coronary perfusion. Contrast media-inhibition of pharmacologic thrombolysis seems to be related to both ionic and osmolar properties. These effects should be considered in the design of clinical trials that use angiography for the evaluation of coronary artery patency and in patients receiving local infusions of thrombolytic agents.

## References

1. Robertson HJF. Blood clot formation in angiographic syringes containing nonionic contrast media. Radiology 1987;162:621–2.
2. Riemann CD, Massey CV, McCarron DL, Borkowski P, Johnson PC, Ziskind A. Ionic contrast agent-mediated endothelial injury causes increased deposition to vascular surfaces. Am Heart J 1993;125:71–8.
3. Barstad RM, Buchmann MS, Hamers MJ, et al. Effects of ionic and nonionic contrast media on endothelium and on arterial thrombus formation. Acta Radiol 1996;37:954–61.
4. Abeyama K, Oh S, Kawano K, et al. Nonionic contrast agents produce thrombotic effect by inducing adhesion of leukocytes on human endothelium. Biochem Biophys Res Commun 1995;212:776–83.
5. Stormorken H, Skalpe IO, Testart MC. Effect of various contrast media on coagulation, fibrinolysis and platelet function: an in vitro and in vivo study. Invest Radiol 1986;21:348–54.
6. Koza MJ, Shankey V, Walenga JM, Moncada R, Fareed J, Pifarre R. Flow cytometric evaluation of platelet activation by ionic or nonionic contrast media and modulation by heparin and recombinant hirudin. Invest Radiol 1995;30:90–7.
7. Chronos NAF, Goodall AH, Wilson DJ, Sigwart U, Buller NP. Profound platelet degranulation is an important side effect of some types of contrast media used in interventional cardiology. Circulation 1993;88:2035–44.
8. Levi M, Biemond BJ, Sturk A, Ten Cate JW. The effects of radiological contrast media in animal models of experimental thrombosis. Semin Hematol 1991;28 Suppl 7:27–30.
9. Brass O, Belleville J, Sabattier V, Corot C. Effect of ioxaglate—an ionic low osmolar contrast medium—on fibrin polymerization in vitro. Blood Coagul Fibrinolysis 1993;4:689–97.

10. Kopko PM, Smith DS, Bull BS. Thrombin generation in nonclottable mixtures of blood and nonionic contrast agents. Radiology 1990;174:459–61.
11. Andes WA. Effects of contrast media on fibrinogen and factor VIII. Invest Radiol 1988;23 Suppl 2:S346–50.
12. Fareed J, Moncada M, Messmore HL Jr, Walenga JM, Hoppensteadt D, Wehrmacher WH. Molecular markers of contrast media-induced adverse reactions. Semin Thromb Hemost 1984;10:306–28.
13. Levi M, Pascucci C, Agnelli G, Sturk A, Hoek J, Ten Cate JW. Effect on thrombus growth and thrombolysis of two types of low osmolar contrast media in rabbits. Invest Radiol 1990;25:533–5.
14. Carr ME, Carr SL, Merten SR. Effects of ionic and nonionic contrast media on clot structure, platelet function and thrombolysis mediated by tissue plasminogen activator in plasma clots. Haemostasis 1995;25:172–81.
15. Dehmer GJ, Gresalfi N, Daly D, Oberhardt B, Tate DA. Impairment of fibrinolysis by streptokinase, urokinase and recombinant tissue-type plasminogen activator in the presence of radiographic contrast agents. J Am Coll Cardiol 1995;25:1069–75.
16. Schilvold A, Bjornsen S, Ing C, Brosstad F. The effects of various contrast media on activation of plasminogen by streptokinase or recombinant tissue plasminogen activator in vitro. Invest Radiol 1994;29:705–8.
17. Van de Werf F, Bergmann SR, Fox KA, et al. Coronary thrombolysis with intravenously administered human tissue-type plasminogen activator produced by recombinant DNA technology. Circulation 1984;69:605–10.
18. SAS Institute Inc. SAS/STAT™ User's Guide, Release 6.03 Edition. Cary, NC: SAS Institute Inc., 1988:1028 pp.
19. Garabedian HD, Gold HK, Leinbach RC, et al. Laboratory monitoring of hemostasis during thrombolytic therapy with recombinant human tissue-type plasminogen activator. Thromb Res 1988;50:121–33.
20. The GUSTO Angiographic Investigators. The effects of tissue plasminogen activator, streptokinase, or both on coronary-artery patency, ventricular function, and survival after acute myocardial infarction. N Engl J Med 1993;329:1615–22.
21. Antman EM, Braunwald E. Acute myocardial infarction. Pharmacotherapy for acute infarction. In: Fauci AS, Braunwald E, Isselbacher KJ, et al., editors. Harrison's Principles of Internal Medicine, 14th ed. New York: McGraw-Hill, 1998:1359.
22. Ing JJ, Smith DC, Bull BS. Differing mechanisms of clotting inhibition by ionic and nonionic contrast agents. Radiology 1989;172:345–8.
23. Manotti C, Quintavalla R, Ugolotti U, Del Favero C, Dettori AG. Variation in hemostatic parameters after intra-arterial and intravenous administration of iodinated contrast media. Invest Radiol 1992;27:1025–30.
24. Kleiman NS, White HD, Ohman ME, et al. Mortality within 24 hours of thrombolysis for myocardial infarction. The importance of early reperfusion. Circulation 1994;90:2658–65.
25. Lenderink T, Simoons ML, Van Es GA, Van de Werf F, Verstraete M, Arnold AER. Benefit of thrombolytic therapy is sustained throughout five years and is related to TIMI perfusion grade 3 but not grade 2 flow at discharge. Circulation 1995;92:1110–6.
26. The GUSTO Investigators. An international randomized trial comparing four thrombolytic strategies for acute myocardial infarction. N Engl J Med 1993;329:673–82.
27. Vanderschueren S, Barrios L, Kerdsinchai P, et al. A randomised trial of recombinant staphylokinase versus alteplase for coronary artery patency in acute myocardial infarction. Circulation 1995;92:2044–9.
28. Neuhaus K-L, von Essen R, Tebbe U, et al. Improved thrombolysis in acute myocardial infarction with front-loaded administration of alteplase: results of the rt-PA-APSAC patency study (TAPS). J Am Coll Cardiol 1992;19:885–91.
29. Zeymer U, von Essen R, Tebbe U, et al. Recombinant hirudin and front-loaded alteplase in acute myocardial infarction: final results of a pilot study. HIT-I (hirudin for the improvement of thrombolysis). Eur Heart J 1996;17 Suppl D:22–7.
30. Smalling RW, Bode C, Kalbfleisch J, et al. More rapid, complete, and stable coronary thrombolysis with bolus administration of reteplase compared with alteplase infusion in acute myocardial infarction. Circulation 1995;91:2725–32.
31. The TIMI Study Group. The thrombolysis in myocardial infarction (TIMI) trial. N Engl J Med 1985;312:932–6.
32. Gibson MC, Marble SJ, Rizzo MJ, et al. Relation between injections before 90-minute angiography and coronary patency: results of the Thrombolysis in Myocardial Infarction 4 trial. Am Heart J 1997;134:351–4.
33. Verheugt FWA, Meijer A, Lagrand WK, van Eenige MJ. Reocclusion: the flip side of coronary thrombolysis. J Am Coll Cardiol 1996;27:766–73.
34. Tiefenbrunn AJ. Intracoronary recombinant tissue-type plasminogen activator (rt-PA). Coron Artery Dis 1996;7:637–40.
35. Navarro F, Bacharach MJ. Treatment of peripheral arterial and venous diseases. Coron Artery Dis 1996;7:649–55.
36. Davidson CJ, Mark DB, Pieper KS, et al. Thrombotic and cardiovascular complications related to nonionic contrast media during cardiac catheterization: analysis of 8517 patients. Am J Cardiol 1990;65:1481–4.
37. Bush LR, Shebuski RJ. In vivo models of arterial thrombosis and thrombolysis. FASEB J 1990;4:3087–98.
38. Collen D, Lu HR, Lijnen HR, Nelles L, Stassen JM. Thrombolytic and pharmacokinetic properties of chimeric tissue-type and urokinase-type plasminogen activators. Circulation 1991;84:1216–34.
39. Jun L, Arnout J, Vanhove P, et al. Comparison of a low-molecular-weight heparin (nadroparin calcium) and unfractionated heparin as adjunct to coronary thrombolysis with alteplase and aspirin in dogs. Coron Artery Dis 1995;6:257–63.