Intra-Arterial Iodinated Radiographic Contrast Material Injection Administration in a Rat Middle Cerebral Artery Occlusion and Reperfusion Model
Possible Effects on Intracerebral Hemorrhage

Yuko Kurosawa, PhD; Aigang Lu, MD; Pooja Khatri, MD; Janice A. Carrozzella, RN, BA; Joseph F. Clark, PhD; Jane Khoury, PhD; Thomas A. Tomsick, MD

Background and Purpose—Observations in human interventional stroke treatment led us to hypothesize that iodinated radiographic contrast material use may contribute to intracerebral hemorrhage. Effects of intra-arterial iodinated radiographic contrast material on hemorrhagic transformation after middle cerebral artery occlusion and reperfusion were studied in a placebo-controlled, blinded preclinical study in rats.

Methods—Four groups of male Sprague-Dawley rats were studied: saline group (n=8), contrast group (n=12), heparin group (n=9), and contrast+heparin group (n=9). The middle cerebral artery was occluded for 5 hours using suture placement. Heparin was infused before suture removal and reperfusion. Saline and/or contrast were infused immediately during reperfusion. Incidence, location, and size of hemorrhage were determined by brain necropsy inspection at 24 hours.

Results—There was a significant increase in incidence of cortical hemorrhage from control (37.5%), contrast (75.0%), heparin (77.8%) to contrast+heparin (100%; Cochran-Mantel-Haenszel correlation, $P<0.01$). Both pooled contrast groups (85.7%) and pooled heparin groups (88.9%) had higher rates of cortical intracerebral hemorrhage compared with the control group ($P<0.05$). Similar trends for increased cortical intracerebral hemorrhage were seen in the contrast-only ($P=0.18$) and heparin-only ($P=0.18$) groups. There was a trend for decreased infarct edema in rats receiving contrast versus those without ($P=0.06$).

Conclusion—Intraarterial iodinated radiographic contrast material may increase cortical intracerebral hemorrhage, similar to heparin. Iodinated radiographic contrast material effect may be additive to heparin effect on the incidence of cortical intracerebral hemorrhage. (Stroke. 2010;41:1013-1017.)

Key Words: animal model ■ arterial occlusion ■ contrast media ■ heparin ■ intracerebral hemorrhage

Acute ischemic stroke injures the blood–brain barrier causing edema and swelling as well as intracerebral hemorrhage (ICH) in some instances.1 Acute stroke intraarterial intervention trials have encountered ICH rates higher than expected compared with untreated patients and with patients treated with intravenous recombinant tissue plasminogen activator. The Prolyse in Acute Cerebral Thromboembolism (PROACT) II Trial and Interventional Management of Stroke Trials I and II were associated with symptomatic ICH rates of 10%, 6.3%, and 9.9% as well as asymptomatic ICH rates of 35%, 42.5%, and 32.1%, respectively. Heparin-treated controls in PROACT I exhibited a symptomatic ICH rate of 7.1%, prompting a decrease in heparin for the PROACT II Trial.2 Heparin and thrombolytics are likely additive in their ICH effects.

Iodinated radiographic contrast material (IRCM) use is integral to intra-arterial thrombolytics as a diagnostic aid and to monitor therapy progress. In hyperacute stroke, iodinated CT contrast and gadolinium MR contrast accumulate under a variety of circumstances, and their passage across the blood–brain barrier by rapid CT or MR methods predicts blood–brain barrier disruption and subsequent ICH.3–5 Among patients treated with intravenous recombinant tissue plasminogen activator, those having CT angiography fared clinically than those who did not, raising the possibility of an unspecified harmful effect of intravenous IRCM.6

IRCM may accumulate in tissue and be visible on CT after intra-arterial thrombolysis.7–10 We hypothesize that IRCM itself may contribute to ICH and edema in acute stroke therapy. This report summarizes our preliminary observations on the effects of IRCM injection on ICH in a rat middle cerebral artery (MCA) reperfusion model.

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Materials and Methods

The animal protocol was approved by the University Animal Care Committee and conformed to the National Institute of Health Guide for Care and Use of Laboratory Animals. Male Sprague-Dawley rats (provided by Department of Neurology, University of Cincinnati, Cincinnati, Ohio) had unrestricted access to food and water and were housed with a 12-hour light–dark cycle. Throughout the study, the investigators and veterinarian staff closely monitored the rats’ health status. All chemicals and reagents were of reagent grade unless otherwise specified.

Stroke Model and Drug Injection

This was a single-blind, placebo-controlled study. The left MCA was occluded using the intraluminal filament technique. Rats were anesthetized with 1.5% of isoflurane and the left common carotid artery, external carotid artery, and left internal carotid artery isolated through a ventral midline neck incision. A 3.0 monofilament nylon suture was inserted into the external carotid artery and advanced approximately 20 mm beyond the carotid bifurcation until mild resistance was felt. The wound was closed temporarily and the suture kept in place for 5 hours followed by removal of the suture for reperfusion. The ventral midline neck incision was opened again, and a polyethylene-10 tube was placed into the external carotid artery with its tip at the internal carotid artery origin immediately after removal of the occluding suture (reperfusion), for infusion. The internal carotid artery was not directly catheterized. Four groups (n = 38) of male Sprague-Dawley rats (body weight = 309.7 ± 12.4 g) with different infusion regimens were studied: (1) saline−saline (control group, n = 8); (2) saline−contrast (contrast group, n = 12); (3) intravenous heparin−saline (heparin group, n = 9); and (4) intravenous heparin−saline−contrast (contrast+heparin group, n = 9). All animals received a 10-minute intra-arterial saline infusion using a KD Scientific Syringe (Model 210) of 1 mL/kg. Fifteen minutes later, contrast groups received an infusion of nonionic low-osmolar (672 mOs/mg/kg H2O) iohexol contrast (Omnipaque 300 mg/mL; Amersham Health, Inc, Princeton, NJ) at 30 μL/min (infusion time 622 ± 7.4 seconds). The external carotid artery was ligated and the incisions sutured. Five minutes before reperfusion, the heparin groups (heparin and contrast+heparin) received bolus intravenous heparin (APP Pharmaceuticals, LLC, Schaumburg, IL; 150 U/kg body weight) followed by 36 U/kg body weight (57.5 U average heparin group, 56.9 U average heparin−contrast group) through a hind-limb vein. During anesthesia, rectal temperature was monitored and body temperature maintained at 37 ± 0.5°C with a heating pad. Following a small craniotomy near the vertex, cerebral blood flow (CBF) was estimated from the left MCA using laser Doppler perfusion measurements (PF-5001; Perimed, Inc, Järfalla, Sweden) before and after MCA occlusion and after reperfusion with saline and/or contrast infusion. After the operation, rats were transferred to a temperature-controlled incubator at 37°C for 30 minutes before transfer to normal housing.

Neurological Deficits and Mortality

Neurological examinations at 45 minutes and 6.5, 8, 12, and 24 hours after induction of ischemia were scored according to a 7-point scale (0-6) from Zhang. Mortality was expressed as the number of rats dying within 24 hours, before euthanization, divided by the number in each group X 100.

Statistics

In the results, categorical variables were controlled for by use of a Mantel–Haenszel statistic to specifically examine the correlation over groups. Further analysis to examine overall contrast and heparin effects was done using logistic regression. Multiple comparisons, for categorical variables, were controlled for by use of a Bonferroni correction.

Results

CBF Measurements

CBF 5 minutes after MCA occlusion decreased to less than approximately 25.0% of preischemia baseline levels in all groups, indicating successful occlusion (Table 1). The contrast group exhibited numerically lower percent CBF after injection versus control and both heparin groups. The heparin and contrast+heparin groups exhibited higher percent CBF after injection versus the control group (nonsignificant).

Hemorrhage Incidence and Location

There was a significant increase in incidence of cortical hemorrhage from control (37.5%), contrast (75.0%), heparin (77.8%) to contrast+heparin (100%; Table 2; Cochran-Mantel-Haenszel correlation, \( P < 0.01 \)). The contrast+heparin group exhibited increased cortical petechial ICH compared with the control group (\( P = 0.03 \)). The contrast group (\( P = 0.18 \)) and the heparin group (\( P = 0.18 \)) exhibited similar trends toward increased cortical ICH compared with the control group (Figure). Using logistic regression to look at the effects of heparin and contrast, the odds of hemorrhage were higher in those given contrast compared with no contrast (OR, 6.76; 95% CI, 1.13 to 40.34; \( P = 0.04 \)) and the odds of hemorrhage was higher in those given heparin compared with those not given heparin (\( P = 0.03 \)). All animals exhibited deep, subcortical hemorrhage, including 1 parenchymal hematoma, the remainder of the hemorrhagic infarction (HI)-1 type.
Hemorrhage Area
The area of deep, undercortical hemorrhage for the heparin group was significantly larger than the control group ($P=0.04$; Table 3).

Cerebral Edema
No difference was seen in edema between the individual groups (Table 4). Both groups administered contrast (contrast and contrast + heparin) exhibited a strong trend toward less edema compared with the saline control group ($P=0.06$).

Neurological Deficit and Mortality
After MCA occlusion, all rats had forelimb flexion, circling to the right, indicating successful suture placement. Statistically significant differences were seen between the saline control group and all other groups ($P<0.05$) and between the contrast and contrast + heparin groups and between heparin and contrast + heparin groups ($P<0.05$ for both), but not between contrast and heparin groups ($P=0.16$).

Table 2. Effect of Contrast and/or Heparin Injection on Cortical Hemorrhage After 5 Hours of MCA Occlusion in Rats (Overall $P=0.03$)

<table>
<thead>
<tr>
<th></th>
<th>Rats With Hemorrhage (No.)</th>
<th>Rats With Hemorrhage (%)</th>
<th>$P$ (Versus Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=8)</td>
<td>3</td>
<td>37.5</td>
<td>N/A</td>
</tr>
<tr>
<td>Contrast (n=12)</td>
<td>9</td>
<td>75.0</td>
<td>0.18</td>
</tr>
<tr>
<td>Heparin (n=9)</td>
<td>7</td>
<td>77.7</td>
<td>0.18</td>
</tr>
<tr>
<td>Contrast + heparin (n=9)</td>
<td>9</td>
<td>100.0</td>
<td>0.03</td>
</tr>
<tr>
<td>Any contrast (contrast and contrast + heparin; n=21)</td>
<td>18</td>
<td>85.7</td>
<td>0.04</td>
</tr>
<tr>
<td>Any heparin (heparin and contrast + heparin; n=18)</td>
<td>16</td>
<td>88.9</td>
<td>0.03</td>
</tr>
</tbody>
</table>

N/A indicates not applicable.

Hemorrhage Area
The area of deep, undercortical hemorrhage for the heparin group was significantly larger than the control group ($P=0.04$; Table 3).

Cerebral Edema
No difference was seen in edema between the individual groups (Table 4). Both groups administered contrast (contrast and contrast + heparin) exhibited a strong trend toward less edema compared with the saline control group ($P=0.06$).

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Table 3. Effect of Contrast and/or Heparin Injection on Hemorrhage Area per Surface Number in the Cortex and Undercortex After 5 Hours of MCA Occlusion in Rats

<table>
<thead>
<tr>
<th></th>
<th>Cortex* (mm$^2$)</th>
<th>Undercortex† (mm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=8)</td>
<td>2.97±1.63</td>
<td>9.19±0.97</td>
</tr>
<tr>
<td>Contrast (n=12)</td>
<td>4.66±1.25</td>
<td>10.94±1.32</td>
</tr>
<tr>
<td>Heparin (n=9)</td>
<td>4.71±1.30</td>
<td>14.44±1.45</td>
</tr>
<tr>
<td>Contrast + heparin (n=9)</td>
<td>3.10±0.86</td>
<td>10.26±1.18</td>
</tr>
</tbody>
</table>

Data are expressed as the mean±SEM.
*P=0.61, †P=0.04 (control compared with heparin group; $P=0.04$).

There was no overall difference in mortality between groups ($P=0.12$). Highest mortality rate was in the heparin group (77.8%), followed by saline control (50.0%) and contrast group (41.7%) and the contrast + heparin group (22.2%).

Discussion
Rats with transient MCA occlusion, followed by reperfusion, demonstrate significantly higher cortical ICH compared with those with permanent occlusion (81.8% versus 18.2%, $P<0.05$). Hemorrhage scores were also higher with transient occlusion, supporting the postulate that reperfusion contributes to ICH.

If reperfusion leads to increased ICH, a trend for an agent to be associated with increased ICH after reperfusion implicates the agent in the etiologic mechanism. In our study, intra-arterial IRCM exhibited an effect on cortical ICH greater than saline control and similar to intravenous heparin. Pooled analysis of contrast groups exhibited a significant increase compared with saline, and when heparin and contrast were infused together, ICH effects appear additive, strongly suggesting IRCM contributes to ICH.

IRCMs differ in their ability to pass through a normal blood–brain barrier. Nonionic, low-osmolar iopromide and isos-
molar iodixanol do not cross the blood–brain barrier. Nonionic low-osmolar iohexol and iodixanol disrupt the blood–brain barrier with insignificant differences when injected intra-arterially in the rabbit. IRCMs may exhibit physiological effects mediated through leakage through an altered blood–brain barrier when administered intravenously for CT in the presence of intracranial tumors. Ionic iopromide sodium iothalamate administration has been associated with increased infarct volume and worse neurological status compared with iopamidol after permanent occlusion.

IRCM deposition in the brain after intra-arterial injection has been demonstrated after uncomplicated aneurysm coiling procedures and after carotid angioplasty and stenting after repetitive injections of IRCMs. Such deposition is usually transitory with local absorption decreasing local concentration with few clinical sequelae. IRCM may accumulate locally on CT after intra-arterial thrombolysis. Yoon defined deposition/accumulation as either contrast enhancement (any hyperdensity that disappeared within 24 hours) or contrast extravasation (a hyperdensity of Hounsfield units >90 that persisted on follow-up CT). Contrast enhancement appeared benign, whereas contrast extravasation was associated with increased symptomatic ICH as suggested by others.

Khatri linked IRCM identification, subsequent hemorrhage, and local microcatheter injections during revascularization, suggesting local deposition might have important implications.

In our model, IRCM may increase ICH, potentially associated with reduced CBF and infarct volume, effects that could be a result of diminished reperfusion. IRCM rats did exhibit lower CBF than control animals, and IRCM + heparin rats exhibited reduced CBF compared with heparin-only animals. IRCMs are known to have paradoxical prothrombotic, anticoagulant, and fibrinolytic effects in vitro.

Nonionic low-osmolar IRCMs and isosmolar ioxaglate permit greater thrombin generation and platelet and fibrin deposition in animal models than ioxaglate. Increased local thrombus formation during coronary intervention in humans occurs with nonionic compared with ioxaglate low-osmolar contrast material. A local intra-arterial thrombotic occlusive effect may lead to a prolonged locally administered IRCM deposition in cerebral vessels, which in combination with local intracerebral IRCM anticoagulant effects, may contribute to increased cortical ICH in a contradictory, paradoxical fashion.


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