Delayed treatment with the defibrinogenating agent, Acutobin, salvages brain tissue in a rat MCAO model

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Synopsis
The effect of Acutobin, a purified protein fraction of venom from Deinakistrodon acutus, on a rat right middle cerebral artery intraluminal hollow filament insertion model (MCAO/R) was evaluated. The proton MRI results showed that internal carotid artery infusion of Acutobin (10U/kg, 2 h after MCAO/R) reduced T₂WI measured lesion by 31% and the residual CBF in the pre-ischemic area recovered to baseline as compared to saline controls at 24 h after MCAO/R. The results suggest that Acutobin improved cerebral microcirculation which may offer a safe and effective therapy following an ischemic insult.

Introduction
Acutobin has been studied and used clinically in Mainland China for decades as reperfusion therapy for conditions such as peripheral vascular disease, deep venous thrombosis, and central retinal venous thrombosis. Acutobin (MW 25,000 ~ 27,000Da), similar to Ancrod¹ from the venom of the Malayan pit viper, is a serine protease that rapidly proteolyses fibrinogen to form a fibrin clot, but unlike thrombin, it only cleaves the Αα chain of fibrinogen and does not activate factor XIII and other blood coagulation factors. The resulting circulation of soluble, noncrosslinked “ancrod-fibrin” appears to stimulate the release of endogenous tissue plasminogen activator (t-PA) from the endothelium of vessel walls². Our previous study on hyperglycemic rat MCAO/R models show that intravenous injection of Acutobin (10U/kg body weight) 30 min after MCAO has a beneficial effect, including improved blood flow and reduced lesion volume³. The chief aim of this study was to evaluate whether Acutobin, injected directly into the MCA territory 2 h after occlusion, right before MCAO reperfusion, can reduce lesion size and/or improve cerebral blood circulation.

Materials and Methods
Male Sprague-Dawley rats (300-330g) were divided into two groups and anesthetized with 3% isoflurane in 30%O₂/70%N₂, then intubated and mechanically ventilated with isoflurane maintained at 1.0-1.5% during the surgery and MRI procedures. The femoral artery was cannulated for monitoring blood gasses and mean arterial blood pressure. A cannula was inserted in the tail vein to deliver contrast agents. A 2 h MCAO was performed with a 4.7-T imaging system (Varian). Rats were positioned supine on a Plexiglas cradle with a 5cm surface coil. MRI was acquired before and 30min after MCAO and repeated at 15 min, then 1, 2, 3, 4 and 24 h after MCAO/R. The protocol consisted of: 1) Diffusion weighted image (DWI); 2) spin-echo T₂-weighted image (T₂WI); 3) Single slice dynamic bolus tracking gradient echo image. 4) Multislice plasma volume imaging (PVI). Imaging parameters for DWI and T₂WI which covered a 20 mm length from the cerebellum to vessel walls³. Our previous study on hyperglycemic rat MCAO/R models show that intravenous injection of Acutobin (10U/kg body weight) 30 min after MCAO has a beneficial effect, including improved blood flow and reduced lesion volume³. The chief aim of this study was to evaluate whether Acutobin was injected after 6 h after MCAO/R by tail vein.

MRI was performed with a 4.7-T imaging system (Varian). Rats were positioned supine on a Plexiglas cradle with a 5cm surface coil. MRI was acquired before and 30min after MCAO and repeated at 15 min, then 1, 2, 3, 4 and 24 h after MCAO/R. The protocol consisted of: 1) Diffusion weighted image (DWI); 2) spin-echo T₂-weighted image (T₂WI); 3) Single slice dynamic bolus tracking gradient echo image. 4) Multislice plasma volume imaging (PVI). Imaging parameters for DWI and T₂WI which covered a 20 mm length from the cerebellum to the olfactory lobe with twelve contiguous coronal slices were: FOV=60×60mm, Matrix=256×256, TR=3.0s, TE=65ms, slice thickness=1.6mm and 128 phase encode steps. For the single slice dynamic MRI in the area of the caudate putamen, a T2* sensitive FLASH pulse sequence with FOV=6×6 was used. Each of the 35 frames was acquired with 64 phase encode steps, a TE of 3.0 ms, a TR of 8 ms and one acquisition per phase encode step. A bolus of 0.3mmol/kg gadopentate-dimeglumine (Magnevist®) or a superparamagnetic iron oxide (SPIO) tracer (2.0mg Fe/kg body weight) were injected into the tail vein after the sixth frame.

Results
MRI-measured lesion volume of cytotoxic edema from DWI, vasogenic edema from T₂WI and residual CBF of right hemisphere at 4 and 24 h after MCAO/R are summarized in the table. A * indicates p < 0.05 compared to saline treated rats. Data are presented as mean ± S.E.

Table Infarct volume and right hemisphere residual cerebral blood flow (CBF) measured by MRI

<table>
<thead>
<tr>
<th>Rats</th>
<th>4h after MCAO/R</th>
<th>24h after MCAO/R</th>
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<tbody>
<tr>
<td></td>
<td>DWI (mm³)</td>
<td>T₂WI (mm³)</td>
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<tr>
<td>Acutobin (n=5)</td>
<td>83.60 ± 12.71*</td>
<td>70.67 ± 17.90</td>
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<tr>
<td>Saline (n=5)</td>
<td>136.90 ± 25.57</td>
<td>96.62 ± 20.99</td>
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Discussion
The main finding of the present MRI study is a significant reduction in infarct volume by infusion of Acutobin via the internal carotid artery beginning 2 h post occlusion in a rat non-thrombotic MCAO/R model. The associated reduction in perfusion deficit suggests that cerebroprotection is afforded by the amelioration of local cerebral blood flow. The effects of Acutobin on this MCAO/R model are likely mediated by the same rheological and fibrinolytic mechanisms as described for Ancrod²,³. We conclude that Acutobin provides an effective therapy in acute cerebral ischemia, minimizing the extent of brain lesion and preventing further brain injury secondary to the cascading vascular insults.

References