Magnetic Resonance Imaging Studies on the Effect of the Antithrombotic Agent Acutobin on a Rat Model of Embolic Stroke

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Abstract

We conducted a study by using proton MRI to evaluate the efficacy of an antithrombotic agent, Acutobin, on a rat thrombotic MCAO model. Treatment was begun 1h after MCAO. Animals accepted intravenous infusion of Acutobin (2.5U/kg) or saline. The proton MRI measured vasogenic edema and perfusion deficit were significantly reduced at 24h after MCAO in the Acutobin-treated rats compared to the saline controls.

Introduction

Acutobin is a purified fraction of venom from the *Deinakistrodon acutus*, similar to Ancrod[1], mainly containing a serine protease, which cleaves fibrinopeptide A from fibrinogen. In contrast to thrombin, the enzyme does not cleave fibrinopeptide B and activate factor XIII. The therapeutic effect is ascribed to a lowering of plasma fibrinogen and stimulating the release of endogenous tissue plasminogen activator (t-PA) from the endothelium of vessel walls[2]. Our previous study showed that a significant reduction in infarct volume by infusion of Acutobin via the internal carotid artery in a rat non-thrombotic MCAO/R model. The present study was undertaken to investigate the effect of Acutobin treatment on a rat thrombotic MCAO model.

Materials and Methods

16 Male Sprague-Dawley rats (300-330g) were divided into two groups and anesthetized with 3% isoflurane in 30%O_2/70%N_2, 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 infuse Acutobin and deliver contrast agents. The MCA was occluded by placement of an embolus[3] at the origin of the MCA by a single intact, fibrin-rich, 24 h old, homologous clot via a 20mm length of PE-10 catheter. Acutobin (2.5U/kg, 600µl/animal) was infused through the tail vein at a rate of 10µl/min after 1h MCAO. The control group accepted saline only.

MRI was performed on 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 1,2,4 and 24h after MCAO. The protocol consisted of: 1) Diffusion weighted imaging (DWI); 2) spin-echo T_2-weighted imaging (T_2WI); 3) Single slice dynamic bolus tracking gradient echo imaging; 4) a multislice plasma volume imaging (PVI). Imaging parameters for DWI and T_2WI, which covered a 20 mm length from the cerebellum to the olfactory lobe with twelve contiguous coronal slices, were: FOV=6×6cm, 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=6×6cm 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 0.3 mmol/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 T_2WI and PVI 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 measured by MRI

Rats	4h After MCAO			24h After MCAO		
	DWI (mm3)	T_2WI (mm3)	PVI (mm3)	DWI (mm3)	T_2WI (mm3)	PVI (mm3)
Acutobin (n=9)	170.99 ± 23.72	58.95 ± 13.16*	119.06 ± 9.50	195.00± 31.45	158.64 ± 20.81*	148.04 ± 18.44*
Saline (n=7)	215.27 ± 42.81	154.71 ± 16.79	190.06 ± 41.69	328.03± 64.23	253.59 ± 35.76	213.03 ± 21.96

Discussion

The present MRI study shows a significant reduction in vasogenic edema and perfusion deficit by infusion of Acutobin via the tail vein beginning 1 h post occlusion in a rat thrombotic MCAO model. A significant increase of fibrinogen deposition in the ischemic core was observed in immunohistochemically stained brain tissue in this model. Intravenous injection of Acutobin reduces the concentration of plasma fibrinogen[4]. The effects of Acutobin are likely mediated by the same rheological and fibrinolytic mechanisms as described for Ancrod[2,5]. We conclude that Acutobin is an effective therapy in the treatment of thrombotic ischemia.

References

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