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Research Report

# Detection of BBB disruption and hemorrhage by Gd-DTPA enhanced MRI after embolic stroke in rat

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ABSTRACT

Thrombolytic therapy with rtPA increases the risk of hemorrhagic transformation (HT) after cerebral ischemia. We employed contrast enhancement MRI with Gd-DTPA to detect HT in a rat model of embolic stroke treated with rtPA and a glycoprotein IIb/IIIa receptor antagonist, 7E3 F(ab')$_2$, at 4 h after embolic stroke. Male Wistar rats were subjected to embolic stroke and treated with the combination of rtPA and 7E3 F(ab')$_2$ ($n$=12) or with saline ($n$=10) at 4 h after onset of stroke. MRI studies were performed immediately and at 24 h after embolization using a 7-T system. Histological measurements were obtained at 48 h. With Gd-DTPA, T1WI images and permeability related MRI parameters (the blood-to-brain transfer constant, $K_i$, and the distribution volume of mobile protons, $V_p$) of 15 out of 18 animals showed hyperintensity regions in gross or microscopic HT areas at 24 h, confirmed histologically at 48 h post stroke. Contrast enhancement MRI detected six of seven (86%) animals with gross HT and nine of eleven (82%) animals with microscopic HT at 24 h after ischemia. Two of eighteen animals with HT, had MRI indices of hemorrhage at 3 h post stroke. However, compared to HT data measured histologically at 48 h in embolic stroke rats, the enhanced areas by Gd-DTPA at 24 h were larger, and the patterns (time, intensity and region) did not directly correlate to the subtypes of HT, i.e., gross or microscopic hemorrhage. Contrast enhancement MRI using Gd-DTPA provides a method to detect gross and microscopic HT after stroke in rats.



## 1. Introduction

Hemorrhagic transformation (HT) of ischemic stroke has a natural incidence of 15% to 26% during the first 2 weeks (Hakim et al., 1983; Calandre et al., 1984; Horowitz et al., 1991) and increases up to 43% over the first months after cerebral infarction (Hornig et al., 1986). The risk of symptomatic HT during the first 36 h after the onset of stroke was significantly higher in patients receiving rtPA than in placebo treated patients (0.6% versus 6.4%), and 61% of the patients with symptomatic HT died within 3 months (NINDS, 1995). Increased acceptance of thrombolytic therapy will depend on the ability to decrease the incidence and to identify patients at risk of developing hemorrhagic complications. A method to assess the risk of HT in ischemic cerebral tissue after stroke would improve the safety of thrombolytic therapy.

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Computed tomography (CT) is the standard diagnostic test currently for identification of cerebral bleeding. Diagnosis of symptomatic hemorrhage using CT is 57% efficient (NINDS, 1997). However, while CT can diagnose hemorrhage once it has occurred, it cannot predict HT unless high-dose contrast-enhanced CT is used (Hayman et al., 1981; Schulte-Altedorneburg et al., 1996).

Magnetic resonance imaging (MRI) has shown promise in predicting and detecting HT after stroke (Knight et al., 1998; Neumann-Haefelin et al., 2002; Jiang et al., 2002, 2005). Contrast-enhanced $T_1$-weighted imaging (CE-T1WI) using Gd-DTPA detected the hyperintensity in the preoptic area after reperfusion at 3 h post intraluminal suture occlusion of the middle cerebral artery (MCA) of transient ischemic stroke in rat without any treatment (Knight et al., 1998), and enhancement was seen in 82% of the animals (all animals with HT by 24 h are enhanced) before any detectable hemorrhage. Using hand drawings of region-of-interest (ROI) in the areas related to HT, all the animals with HT exhibited increased signal intensity compared to the corresponding contralateral ROI (Knight et al., 1998). For embolic stroke rats with or without rtPA treatment at 4 h after MCA occlusion (MCAo), MRI detected the differences between ischemic regions with and without HT determined from histological staining (Jiang et al., 2002). Large increases in $k_{inv}$, the inverse of the apparent forward transfer rate ($k_f$) for magnetization transfer, were present at 3 h after embolism (Jiang et al., 2002). Contrast-enhanced MRI (CE-MRI) data revealed signal hyperintensity at 7 h after embolism in the region where gross hemorrhage was confirmed histologically at 48 h (Jiang et al., 2002). By drawing HT areas on histological section and then transposing the ROIs back onto the original MRI images, permeability related MRI parameters showed that at 5 h after embolic MCAo, significant differences of fibrin leakage were present between the ischemic tissue with and without BBB disruption (Jiang et al., 2005). CE-T1WI using Gd-DTPA predicted HT at 3 h post MCAo in spontaneously hypertensive rats of clot embolic model treated with rtPA via i.a. injection at 3 h after embolization (Neumann-Haefelin et al., 2002).

Although as noted, MRI has been employed to identify and to predict HT, MRI measurements and analysis methods that provide user independent identification and prediction of HT for clinical management of stroke patients need further development. Current MRI analysis methods to identify cerebral tissue areas destined for HT, using hand drawn ROIs (Knight et al., 1998), histological sections that identify HT (Jiang et al., 2002) and MRI methods post HT (Zhai et al., 2005), are not suitable for clinical practice.

In the present study, we employ an objective user independent method to detect HT. CE-MRI with Gd-DTPA, including CE-T1WI and permeability related MRI parameters, allows detection of microscopic (petechial) and gross hemorrhage in a model of embolic stroke.

## 2. Results

Control rats, treated with saline at 4 h after MCAo, were observed histologically at 48 h after MCAo with microscopic HT (6 of 10) or gross HT (4 of 10). None of them showed distinguishable Gd-DTPA enhancement in the HT related areas at 3 h after MCAo. Most rats (9 of 10), except for one with microscopic HT, showed distinguishable Gd-DTPA enhancement in the HT related areas at 24 h after MCAo. The situations of treated rats with combination of rtPA and 7E3 F $(ab')_2$ at 4 h after MCAo were more complicated: with 3 of 12 showing gross HT, 5 of 12 showing microscopic HT and 4 of 12 with no HT. Four rats (1 of 3 gross HT, 1 of 5 microscopic HT and 2 of 4 no HT) exhibited contrast enhancement at 3 h after embolic onset, and one of them did not show enhancement at 24 h. Among the other eight animals (2 of 3 gross HT, 4 of 5 microscopic HT and 2 of 4 no HT), five of them exhibited contrast enhancement at 24 h after stroke and three of them showed no Gd-DTPA enhancement at 24 h in the HT related areas. These data are listed in Table 1. A general pattern in this study is that large and intense Gd-DTPA enhancement at 24 h post stroke was associated with gross HT and small and weak Gd-DTPA enhancement with microscopic HT. HT was histologically confirmed at 48 h post stroke.

Figs. 1a–d present the $K_i$ and $V_p$ maps of a treated rat, that received combination treatment with rtPA and 7E3 F$(ab')_2$ at 4 h after onset of embolic stroke, with gross HT histologically evaluated at 48 h after stroke. Compared to the hemorrhagic areas in the histological section obtained at 48 h after stroke, as shown in Fig. 1g (Fig. 1h shows an enlarged hemorrhagic picture with a magnification of 20×), the acute (3 h) $K_i$ map (Fig. 1a) and $V_p$ map (Fig. 1c) do not clearly visually exhibit a hemorrhagic region. The 24 h $K_i$ (Fig. 1b) and $V_p$ (Fig. 1d) maps clearly identified the region of BBB disruption with Gd-DTPA leakage. With the 24 h $V_p$ map, three hyperintensity regions are separately identified which correspond to the histological hemorrhagic pattern. The 24 h $K_i$ map presents diffuse hyperintensity regions that encompass the distinct hemorrhagic spots. The CE-T1WI subtraction images show similar information of BBB disruption for this animal as the $V_p$ maps of 3 h and 24 h, shown in Figs. 1e and f. All T1WI subtraction images, $K_i$ and $V_p$ maps, visually, identify larger areas than the areas of gross hemorrhage.

For a treated rat with microscopic hemorrhage, i.e., no HT was visible without a microscope (Fig. 2g), parenchymal blood was measured with 20× magnification under the microscope (Fig. 2h). Increasing signal intensity at 3 h post stroke was detected by $K_i$ (Fig. 2a) and $V_p$ (Fig. 2c) maps, as well as the subtraction image of T1WI (Fig. 2e), respectively. $K_i$ and $V_p$ maps obtained at 24 h post stroke, accompanying the 24 h

**Table 1 – Gd-DTPA enhancement cases distributed with hemorrhagic situation of rats (C: control rat, T: treated rat)**

| Gd-DTPA enhancement | No HT (C/T) | Microscopic HT (C/T) | Gross HT (C/T) |
|---|---|---|---|
| Positive at 3 h | 2 (0/2) [a] | 1 (0/1) | 1 (0/1) |
| Negative at 3 h | 2 (0/2) [b] | 10 (6/4) | 6 (4/2) |
| Positive at 24 h | 2 (0/2) | 9 (5/4) | 6 (4/2) |
| Negative at 24 h | 2 (0/2) | 2 (1/1) | 1 (0/1) |

[a] One rat was positive to Gd-DTPA enhancement at 3 h after MCAo, who was negative at 24 h. Another was positive to Gd-DTPA enhancement at both 3 h and 24 h after embolization.
[b] One rat was negative to Gd-DTPA enhancement at 3 h after MCAo, who was positive at 24 h. Another kept negative to Gd-DTPA enhancement at both 3 h and 24 h after embolization.