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Evolution of early perihemorrhagic changes—Ischemia vs. edema: An MRI study in rats

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Abstract

Objectives: Cerebral ischemia has been proposed as a contributing mechanism to secondary neuronal injury after intracranial hemorrhage (ICH). The search for surrogate parameters that allow treatment stratification for spontaneous ICH continues. We aimed to examine perihemorrhagic ischemic changes with an animal experimental MRI study.

Methods: A high field MRI compatible setup for male Wistar rats was established using a double injection model. ICH was stereotactically placed into the right basal ganglia of 29 Wistar rats. Coronal T2-WI, T2*-WI and DWI were generated with a 2.35 T animal MRI scanner 15 min, 60 min and 210 min after ICH. Clot signal characteristics, clot volumes and normalized ADC values were analyzed in four hematoma regions (core, periphery, outer rim, healthy ipsilateral tissue) in all sequences.

Results: T2*-WI and DWI reliably demonstrated ICH in 100% with only small deviation from the applied volume (−20% to +26%) whereas T2-WI failed to conspicuously show ICH. There were no perihemorrhagic ADC decreases consistent with ischemic cytotoxic edema but a mild vasogenic edema surrounding the ICH could be observed.

Conclusion: T2*-WI and DWI are accurate for the diagnosis of hyperacute ICH. According to serial and crossectional ADC analysis, there is no hint towards the existence of a perihemorrhagic ischemic area that might be saved by early intervention. Future studies should focus on perfusion and metabolic/neurotoxic studies of this particular area and neurotoxic properties of the surrounding edema.

Keywords: Perihemorrhagic ischemia; Perihemorrhagic edema; Diffusion-weighted imaging; Intracerebral hemorrhage

Introduction

Although, primary intracranial hemorrhage (ICH) counts for up to 15% of all strokes, there is no consensus with regard to its treatment (Diringer, 1993; Qureshi et al., 1999). A recently presented, large, international, randomized multicenter trial for surgery versus best medical treatment failed to answer this question (Mendelow, 2004). Perihemorrhagic ischemia may be a potential surrogate parameter to identify patients, who may profit from surgical hematoma evacuation (Bullock et al., 1988). At present, there is only inconsistent information and contradictory findings from animal experiments as well as patient studies (Bullock et al., 1988; Deinsberger et al., 1999; Kidwell et al., 2001; Mun Bryce et al., 1993; Schellinger et al., 2003; Zazulia et al., 2001). In order to evaluate MRI signal characteristics, the diagnostic accuracy of MRI for ICH as well as eventual perihemorrhagic diffusion changes, we conducted a serial study using a double injection rat model (Deinsberger et al., 1996).

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Methods

Animal preparation

Animal protocols for these studies were approved by the institutional animal care and use committee. Studies were carried out on a total number of 29 male Wistar rats weighing 280 to 440 g (mean weight 334.7 g ± 32.7 g). Anesthesia was induced with an intraperitoneal injection of 10 mg/100 g ketamine hydrochloride (Wirtschaftsgenossenschaft deutscher Tierärzte), 0.01 mg/100 g succinylcholine (Rompun 2%, 0.5 mg, 0.05 ml/Tier), and, if necessary, maintained with a further 2.5 mg/100 g ketamine hydrochloride. PE-50 polyethylene tubing was inserted into the right femoral artery for the monitoring of mean arterial blood pressure and arterial blood gas analysis. Catheter placement in the right femoral vein was performed with PE-50 tubing for saline infusions. The temperature probe was inserted into the rectum and the animal was placed onto a warming pad that was connected to the temperature probe in order to maintain body temperature at 36.0 to 37.5°C. The animals were insufflated with oxygen but not intubated. Monitoring of physiological parameters (arterial blood gases, plasma glucose and body temperature) was done throughout animal preparation and directly before and after the MRI acquisitions. During MRI, the animals did not experience profound temperature fluctuations. Body temperature was measured continuously during anesthesia, and MRI scans were performed without additional surgery (Schäbitz et al., 2001).

Operative procedure

We adapted a double injection model for the use in a high field small animal MRI scanner (Deinsberger et al., 1996). Single injection models of ICH with unclotted autologous blood and all the modifications of this model share the problem that size and extension of the hematoma are not reproducible, because the injected blood either ruptures into the ventricular system or it extends to the subarachnoid or subdural space. In a double injection model of a total of 50 μl (15 μl and 35 μl), first, a small amount of fresh autologous blood is injected (preclotting) in order to block the way back along the needle track, and the actual hematoma is produced in a second step of the injection. In the series by Deinsberger et al., the final ICH volumes (stained serial sections) matched well with the injected volume with regard to size (41.1 ± 10.0 μl) and shape. Therefore, the model by Deinsberger et al. allows to generate reproducible hematomas in rats (Deinsberger et al., 1996).

Besides monitoring of blood gases and glucose, i.a.—lines served to gain autologous arterial blood for the intraparenchymal injections. The rat was placed into a stereotactic frame. A right-sided paramedian burrhole was placed behind the bregma and a plastic cannula (PE-50, outer/inner diameter 0.68 mm/0.38 mm) was introduced to 5 mm depth below the surface of the skull with an entry angle of 18° targeted at the basal ganglia. The plastic catheter ensured little artifacts and minor trauma to the parenchyma. A removable metal connector was put on the PE-50 catheter. A paraffin filled PE-50 catheter was used to draw the accurate arterial blood volume. This catheter was distally attached to the metal connector and after injection the connector and the distal PE-50 were removed. The hydrophobic paraffin within the PE-50 guaranteed a precise injection without air or saline. At two time points, arterial blood volumes of 15 μl and 35 μl each were injected (Deinsberger et al., 1996).

MRI protocol

The animals were examined in a 2.35 T animal scanner (Biospec 24/40, BRUKER Medizintechnik, Ettlingen, Germany). An actively shielded gradient coil with 120 mm inner diameter was used. This coil was driven by the standard 150 V/100 A gradient power supply. In this configuration, 180 mT/m was acquired in 180 ms. As RF-coil, we used a custom made birdcage resonator with 40 mm inner diameter. All animals were examined with an MRI protocol consisting of a T2-weighted turbo-spin-echo (TSE) sequence, a T2*-weighted fast low angle shot (FLASH) sequence and a diffusion-weighted (DWI) spin-echo echo-planar-imaging (EPI) sequence. Sequence parameters are listed in Table 1, planned imaging times were 15 min, 60 min and 210 min after intracerebral injection of the 35 μl arterial blood.

Measurements and signal characteristics

Signal characteristics, hematoma volume and apparent diffusion coefficient (ADC) values were evaluated serially in the course of the study. Signal intensity was arbitrarily categorized into hypointense, hypo/isointense, isointense, iso/hyperintense and hyperintense. Hematoma volume was calculated according to the A*B*C/2 method by Kwak et al.

Table 1
MR protocol parameters

	T2-W TSE	T2*-W GRE	DWI EPI
TR, ms	2000	160	9000
TE, ms	8–96 (12 values)	5.12	60
b, s/mm^2			200–700 (6 values)
Diffusion time, ms			15
Duration of diffusion gradient, ms			7
Flip angle, °	90	60	90
FOV, cm^2	4 × 4	4 × 4	4 × 4
Matrix	128 × 96	128 × 96	128 × 64
Thickness, mm	2	2	2
Slices, n	6	6	6

W, weighted; TSE, turbo spin echo; GRE, gradient echo; EPI, echo planar image; TR, repetition time; TE, echo time; FOV, field of view.