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BRAIN RESEARCH

Research Report

# Experimental intracerebral hematoma in the rat: Characterization by sequential magnetic resonance imaging, behavior, and histopathology. Effect of albumin therapy

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ABSTRACT

We characterized acute intracerebral hemorrhage (ICH) in the rat by sequential magnetic resonance imaging (MRI) and correlated MRI findings with neurobehavior and histopathology. In addition, we investigated whether albumin treatment would reduce ICH-induced brain injury. ICH was produced in rats by a double-injection method in which 45 μl of fresh arterial blood was injected into the right striatum. Susceptibility-weighted (SWI) and T2-weighted (T2WI) MRI was carried out on a 4.7T magnet at 0–1 h, 6 h, 24 h, 72 h, and 7 days after ICH. Animals were treated with either 25% human albumin, 1.25 g/kg, or saline vehicle i.v. at 90 min after ICH. Neurological status was evaluated before ICH and after treatment (at 4 h, 24 h, 48 h, 72 h, and 7 days). Brains were then perfusion-fixed, re-imaged on an 11.7T magnet, and studied by histopathology and immunochemistry. MRI revealed a consistent hematoma involving the striatum and overlying corpus callosum, with significant volume changes over time. Lesion volumes computed from T2WI images and by histopathology agreed closely with one another and were highly correlated ($p=0.002$). SWI lesion volumes were also highly correlated to histological volumes ($p<0.001$) but overestimated histological hematoma volume by ~5-fold. Albumin treatment significantly improved neurological scores compared to saline at 72 h (3.8±0.6 vs. 1.5±0.7) and 7 days (3.8±0.4 vs. 1.3±0.5, respectively, $p<0.05$), but did not affect histological or MRI lesion volumes. Taken together, sequential MRI plus histopathology provides a comprehensive characterization of experimental ICH. Albumin treatment improves neurological deficit after ICH but does not affect MRI or histological hematoma size.



## 1. Introduction

Intracerebral hemorrhage (ICH) – focal hematoma formation within the brain parenchyma – accounts for ~10–15% of all strokes and is associated with higher mortality and more severe neurological deficits than other stroke subtypes (Broderick et al., 1999). ICH is a common consequence of chronic hypertension but has many other causes, including cerebral amyloid angiopathy;

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**Table 1 – Physiological variables**

| | Saline | Albumin |
|---|---|---|
| | $n=6$ | $n=8$ |
| *Before ICH (15 min)* | | |
| Cranial temperature (°C) | 36.7±0.20 | 36.7±0.12 |
| Rectal temperature (°C) | 36.3±0.42 | 36.3±0.25 |
| pH | 7.37±0.01 | 7.41±0.01 |
| $pO_2$, mm Hg | 346±65 | 356±46 |
| $pCO_2$, mm Hg | 44.8±1.4 | 44.3±1.9 |
| Plasma glucose, mg/dl | 121±20 | 107±10 |
| Hemoglobin, g/dl | 15.6±0.4 | 14.9±0.3 |
| *After ICH (15 min)* | | |
| Cranial temperature (°C) | 36.8±0.07 | 36.7±0.12 |
| Rectal temperature (°C) | 36.7±0.21 | 36.6±0.26 |
| pH | 7.38±0.02 | 7.44±0.02 |
| $pO_2$, mm Hg | 419±61 | 460±26 |
| $pCO_2$, mm Hg | 44.3±2.8 | 40.0±2.4 |
| Plasma glucose, mg/dl | 122±27 | 103±7 |
| Hemoglobin, g/dl | 15.0±0.5 | 14.5±0.5 |
| *After treatment (15 min)* | | |
| Cranial temperature (°C) | 36.4±0.24 | 36.5±0.14 |
| Rectal temperature (°C) | 35.8±0.79 | 36.5±0.26 |
| pH | 7.39±0.03 | 7.41±0.01 |
| $pO_2$, mm Hg | 370±74 | 329±54 |
| $pCO_2$, mm Hg | 47.5±1.5 | 43.7±1.1 |
| Plasma glucose, mg/dl | 106±17 | 124±13 |
| Hemoglobin, g/dl | 14.0±1.0 | 11.4±0.3* |

Values are mean±S.E.M.
ICH, intracerebral hemorrhage.
*Different from saline group ($p<0.05$, Student's t-test).

ruptured vascular malformations; bleeding diatheses secondary to disease or anticoagulant/thrombolytic therapy; bleeding into intracranial neoplasms; and cranial trauma (Butcher and Laidlaw, 2003; Norrving, 1998). Although recombinant factor VIIa therapy has shown promise in reducing acute hematoma growth in a phase II clinical trial (Mayer, 2003) [see http://www.strokecenter.org/trials/TrialDetail.asp?ref=431&browse=hemo for summary], no other specific therapies have been shown to improve outcome after ICH. The efficacy of surgical treatment of ICH remains unproven and controversial (Broderick et al., 1999). The development of pharmacological therapies for brain injury following ICH depends upon the use of appropriate and reproducible models of ICH. Because the putamen is the most common site of ICH in humans, in the present study we have used a rat model of striatal ICH that yields a consistent hematoma and neurological deficit.

High-dose human albumin therapy has proven to be remarkably neuroprotective in experimental models of focal cerebral ischemia (Belayev et al., 1997, 1998, 2001) as well as in global ischemia (Belayev et al., 1999b) and acute brain trauma (Belayev et al., 1999a). ICH and ischemic stroke share a number of common injury-mechanisms, which include oxidative injury, perilesional cellular and humoral inflammatory processes, blood–brain barrier (BBB) disruption and edema, and excitotoxic injury (Qureshi et al., 2003; Wagner and Broderick, 2002; Wagner et al., 2003). Albumin therapy is known to antagonize many of these mechanisms by directly protecting both parenchymal and vascular elements of the brain, exerting antioxidant effects, maintaining microvascular integrity, diminishing brain edema, and inhibiting endothelial cell apoptosis (Belayev et al., 2002; Brown et al., 1995; Emerson, 1989). These considerations provide a strong rationale for suspecting that albumin therapy might also be beneficial in acute intracerebral hematoma. Recently, we have shown that prompt therapy with moderate-dose human albumin reduces neurological deficits and improves BBB integrity in the setting of acute intracortical hematoma in the rat (Belayev et al., 2005).

MRI is an ideal method, with high sensitivity, for characterizing the temporal and spatial evolution of parenchymal alterations following ICH (Allkemper et al., 2004; Atlas and Thulborn, 1998; Brown et al., 1995; Del Bigio et al., 1996; Hartmann et al., 2000; Kuker et al., 2000). MR imaging of iron within the brain, either as part of hemoglobin or other non-heme-containing regions, has been studied extensively and has recently been reviewed (Haacke et al., 2005). MR and biochemical studies have shown that hemosiderin (proteolytic degradation of ferritin) is likely the MR visible degradation product after hemorrhage (Atlas and Thulborn, 2002, 1998; Haque et al., 2003). The purpose of the present study was twofold: First, to provide a multimodal characterization of the striatal blood-injection of ICH in the rat by combining sequential MRI and neurobehavioral testing with histopathology and immunochemistry; and secondly, to assess the potential of albumin as a neuroprotectant in this setting.

## 2. Results

Physiological variables showed no significant differences between groups except for modest hemodilution after treatment in the albumin group (Table 1).

### 2.1. *Neurobehavioral assessment*

These data are presented in Fig. 1. A moderate neurological deficit was initially apparent in both albumin- and saline-treated

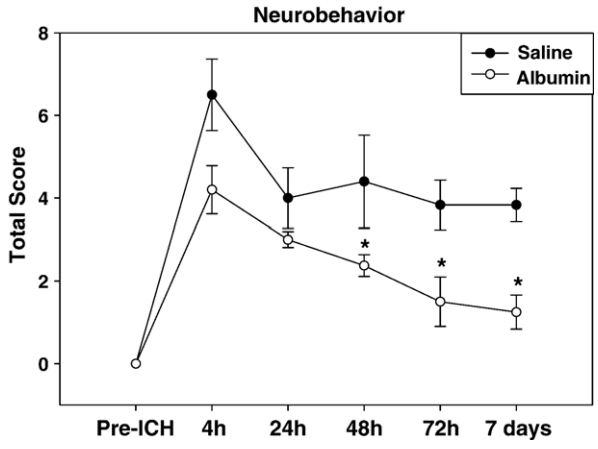


**Fig. 1 – Total neurobehavioral scores in albumin- and saline-treated rats with acute intracerebral hemorrhage (ICH) (mean±SEM). *, significant inter-group difference, $p<0.05$, repeated-measures ANOVA followed by Bonferroni comparisons.**