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A NOVEL COMBINED MODEL OF INTRACEREBRAL AND INTRAVENTRICULAR HEMORRHAGE USING AUTOLOGOUS BLOOD-INJECTION IN RATS

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Abstract—Intracerebral hemorrhage (ICH) is the least treatable form of stroke and is associated with the worst prognosis. In up to 40% of cases, ICH is further complicated by intraventricular hemorrhage (IVH), which predisposes to hydrocephalus, and increases case-mortality to 80%. However, IVH is not present in widely used preclinical models of ICH. Here, we characterize a novel rat model of combined ICH and IVH. Rats were injected with different volumes of autologous whole blood into the right deep basal ganglia region (100 μL, 150 μL, 200 μL, and 250 μL, $n = 10$ per group). MRI was performed immediately, and at 24, 48, 72 h, and 1 week after blood injection, along with neurological evaluations. Injected blood volume reliably correlated with blood volumes measured from MRI obtained after blood injection. Brain edema was most prominent in the ⩾200 μL groups, peaking at 48 h in all groups, being statistically different between the ⩾200 μL and <200 μL groups at all-time points. Presence of hydrocephalus was detected in most of the animals, most clearly in the 200 μL and 250 μL groups, both being statistically different from the 100 μL group at all-time points, with tendency to worsen during the whole follow-up period. Most deteriorating neurological and behavioral outcomes as well as the highest mortality rates were detected in groups injected with 200 μL and 250 μL of autologous blood, 40% and 70%, respectively. These volumes were most similar to the clinical scenario of combined ICH and IVH, demonstrating that this novel rat model is a promising starting point for future ICH + IVH research.

Key words: intracerebral hemorrhage, intraventricular hemorrhage, rat, magnetic resonance imaging, hydrocephalus.

INTRODUCTION

Intracerebral hemorrhage (ICH) accounts for 10–15% of all strokes and is associated with the worst prognosis. Intraventricular hemorrhage (IVH) appears as a secondary complication in about 40% of cases (Meretoja et al., 2012), and leads to devastating outcomes (Tuhrim et al., 1988; Hemphill et al., 2001; Stein et al., 2010) The outcome of ICH patients with secondary IVH is further worsened by the presence of hydrocephalus, a common consequence of blood in the ventricular system (Shapiro et al., 1994; Ozdemir et al., 2008; Staykov et al., 2011a,b). At present, IVH is a disease with ineffective and limited treatment options (Engelhard et al., 2003). Even though recently proposed invasive treatment options for isolated IVH were proven potentially beneficial (Webb et al., 2012), combined IVH and ICH was less likely to resolve using invasive surgical procedures (Mendelow et al., 2010) although some promising results were reported using minimally invasive neurosurgery (Chen et al., 2011a).

Although the negative contribution of simultaneously existing IVH has been widely described in patients, up to date, no animal model of combined ICH and IVH have been described. Previously described isolated IVH animal models utilized both premature animals (Balasubramaniam and Del Bigio, 2006) mimicking neonatal IVH, and adult animals in various species (Pang et al., 1986a,b; Mayfrank et al., 1997) including rats (Lodhia et al., 2006). Although there are a number of representative ICH models in rodents (Strbian et al., 2008), none of them reproduce IVH or hydrocephalus.

In this study we developed a combined rat model of ICH and IVH using different blood volumes to (1) ensure the presence of blood both in brain parenchyma and ventricles, and verify its distribution by magnetic resonance imaging (MRI), (2) characterize changes in hematoma and hydrocephalus during follow-up, and (3) analyze animal outcome, behavioral changes, and survival rate up to one week after blood injection.

EXPERIMENTAL PROCEDURES

Animals

All experiments were approved by relevant authorities (PH 928A). Adult male Wistar rats (Harlan Nederland, The Netherlands) weighing 300–350 grams were anesthetized using injections of ketamine hydrochloride

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Abbreviations: CSF, cerebrospinal fluid; FLASH, fast low-angle shot; ICH, intracerebral hemorrhage; IVH, intraventricular hemorrhage; MRI, magnetic resonance imaging; RARE, relaxation enhancement.

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(i.p., 50 mg/kg, Ketalar, Parke-Davis, Sweden) and medetomidine hydrochloride (s.c., 0.5 mg/kg, Domitor, Orion, Finland). Animals were allocated into 4 groups (10 per group) with different volumes of injected blood (100 μL, 150 μL, 200 μL, and 250 μL). A PE-50 tube was inserted into the left femoral artery for continuous blood pressure monitoring (Olli BP Meter 533, Kone, Finland). Rectal temperature was maintained at 37 °C using a heating pad during the surgical procedures and a heating blanket during MRI. Body weight was observed daily and the general condition of the animals twice a day. The analgesic drug buprenorphine (0.05 mg/kg, Temgesic, Schering-Plough, USA) was administered every 8 h after the surgery for the first 48 h. The rats were housed under diurnal lighting conditions and given free access to food and water.

ICH + IVH model

To mimic the clinical situation of combined ICH and IVH, we modified the autologous whole-blood injection model previously used in our laboratory for the induction of isolated ICH (Strbian et al., 2007). The head of the anesthetized animal was fixed into a stereotaxic frame (Stoelting Co., Wood Dale, IL) and a midline scalp incision was made to disclose the calvaria of the skull. A burr hole, 1 mm in diameter, was drilled into the right side of the cranium, 0.26 mm anterior and 2.2 mm lateral to the bregma. A 27-gauge needle attached to a Hamilton syringe was inserted into the core of the right basal ganglia at 5 mm depth from the skull surface and subsequently lifted by 0.5 mm, thus producing a small pouch. Thereafter, depending on the group, different volumes of freshly collected autologous arterial blood (100–250 μL) was injected slowly into the brain (2 μL/12 s), after which the needle was kept in place for 5 min. The burr hole was then sealed with bone wax, and the scalp was sutured.

MR imaging

Presence, extent, and location of ICH and IVH were confirmed immediately after autologous blood injection and followed-up at days 1, 2, 3, and 7 using a 4.7-Tesla MRI scanner (PharmaScan, Bruker BioSpin, Germany) with a 90-mm shielded gradient capable of producing a maximum gradient amplitude of 300 mT/m with a rise time of 80 ms. A linear birdcage radiofrequency coil with an inner diameter of 38 mm was used. After shimming and scout images, coronal T2*-weighted images encompassing the whole brain were acquired with a fast low-angle shot (FLASH) sequence (repetition time 350 ms; echo time 10 ms; flip angle 40°; matrix size 256 × 128; field of view 40 × 40 mm; number of averages 4; 14 slices; and slice thickness 1 mm). Coronal T2-weighted images were acquired with rapid acquisition using a relaxation enhancement (RARE) sequence (repetition time 3000 ms; effective echo time 60 ms; rare factor 8; matrix size 256 × 192; field of view 40 × 40 mm; number of averages 6; 14 slices; and slice thickness, 1 mm).

Calculation of hematoma volume, hemispheric expansion, and ventricular volume

Hematoma volumes and hemispheric expansion were calculated at all-time points from T2*-weighted MR images. The borders of the hematoma, present both in parenchyma and ventricles, were outlined manually (Paravision, Bruker BioSpin) and resulting areas were multiplied by slice thickness and summed up to yield the total hematoma volume (Strbian et al., 2007). Thereafter, the volume of both hemispheres was calculated in an identical manner for measurements of hemispheric expansion. The percentage of hemispheric expansion was calculated as the volumetric increase of the ICH hemisphere compared to the intact hemisphere (% of hemispheric expansion = [(right hemisphere volume/left hemisphere volume) − 1] × 100) as previously described (Gerriets et al., 2004; Strbian et al., 2007; Marinkovic et al., 2009). Furthermore, the ventricle volumes (including blood and CSF) were calculated using T2-weighted sequences starting 4 mm caudally of rhinal sulcus. Analyses were performed blinded to quantity of injected blood and neurological outcome.

Neurological evaluation and mortality

We scored neurological performance at days 1, 2, 3, and 7 after ICH + IVH induction on an 8-point scale (**0**: no deficit; **1**: contralateral forepaw paresis; **2**: 1 + decreased resistance to lateral push, yet no circling; **3**: 2 + circling to the contralateral side; **4**: falling to the contralateral side; **5**: rolling; **6**: no spontaneous walking with a depressed level of consciousness; and **7**: death) (Marinkovic et al., 2009).

Behavioral evaluation

The assessment of behavioral status was performed based on the SHIRPA (Rogers et al., 1997) protocol from video records collected at days 1, 2, 3, and 7 after ICH + IVH.

Statistical analyses

Data are expressed as mean + S.D. for parametric data, medians for nonparametric data (also reported individually for each animal), or as proportions. As data sets were non-normally distributed, between-group comparisons of hematoma volume, hemispheric expansion, and ventricle size were performed at each time point using Kruskal–Wallis analysis of variance followed by the Dunn's post hoc test. This test was also used for comparing neurological outcomes between groups. Survival rates were compared with the log-rank test. A two-tailed $p < 0.05$ was considered significant. Analyses were performed using SPSS v.21.0 (IBM, Armonk, NY, USA).

RESULTS

Physiological parameters

The groups showed no significant differences in the mean arterial blood pressure or rectal temperature before or