Effects of Sulfaphenazole after Collagenase-Induced Experimental Intracerebral Hemorrhage in Rats

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ABSTRACT

Intracerebral hemorrhage (ICH) results from the rupture of a blood vessel in the brain, and is a major public health problem, because ICH leads to a high rate of death and disability in adults. The 30-d mortality rate is approximately 50%, and most survivors (more than 80%) are left with neurological disabilities. Primary ICH accounts for approximately 25% of all strokes in Asian populations including the Japanese, and spontaneous ICH represents one of the most devastating types of stroke.

The pathophysiology of ICH is caused by a complex chain of events starting with disruption of the blood–brain barrier (BBB) and infiltration of blood components into the brain parenchyma, resulting in a progressive edema, which starts in the first 24h and remains elevated over several days. The development of therapies relies on the use of animal models. The animal models are commonly produced by manipulation of events starting with disruption of the blood–brain barrier, especially the CYP2C subfamily, are expressed in not only brain regions such as the cortex, hippocampus and basal ganglia, but also in vascular endothelial cells, thus suggesting the possibility that ROS derived from CYP result in numerous cerebral vascular disorders.

In order to investigate the involvement of oxidative stress and CYPs in brain injury after ICH, this study examined the effects of sulfaphenazole (SPZ) on stroke, such as ICH in the rat brain. SPZ can directly scavenge ROS and suppress ROS production from the CYP2C subfamily, thus indicating that the SPZ-induced reduction of ischemia-reperfusion injury arises from both the ROS scavenging and CYP inhibition abilities of SPZ. The present results show that systemic SPZ treatment after ICH induction by COL helps to prevent neurological deficits, striatal dysfunction, brain edema and lipid peroxidation in rats.

MATERIALS AND METHODS

Animal Preparation of ICH. The experiments used...
for all 12 slices. T1WI images were obtained with 2D-SE-MS were TR (ms)/TE (ms) = 2500/69, FOV = 30 mm2, matrix = 128 × 256, voxel size = 0.246 × 0.246 × 0.246 mm, NEX = 8 a slice thickness of 1.5 mm for all 12 slices. T1WI images were obtained as the localizer images, and T2-weighted images (T2WI) was performed with the following parameters: T1WI parameters were TR (ms)/TE (ms) = 2500/69, NEX = 6, a slice thickness of 1.5 mm for all 12 slices. The rats were bolus injected via a femoral vein with 0.4 mmol/kg Gd-HP-DO3A (ProHance®, Bracco, U.S.A.). Gd-HP-DO3A does not cross the intact BBB and, therefore, does not accumulate in the normal brain.

**Figure 1.** Effects of SPZ on APO-Induced Rotation Behavior after ICH in Rats

(A) The number of ipsilateral rotations in the SPZ-treated (5, 25 mg/kg, i.v.) ICH groups was signiﬁcantly and dose-dependently decreased compared to the vehicle-treated ICH group (0 mg/kg) at 7d after ICH induction. (B) The number of ipsilateral rotations in the SPZ and Ac-SPZ-treated ICH groups (100 mg/kg, i.p.) signiﬁcantly decreased compared to the vehicle-treated ICH group (0 mg/kg) at 7d after ICH induction. The ipsilateral rotations were determined by counting the number of ipsilateral rotations every 5 min in a 60 min period after APO administration. Values each represent the mean ± S.E.M. (n=8) for each experiment.

**Behavioral Outcome** Apomorphine (APO, 1 mg/kg, i.p.)-induced ipsilateral rotational behavior was observed at day 1, 3, 5 and 7 after the ICH to determine the dysfunction of the striatum after ICH. The number of APO-induced ipsilateral rotations was counted for 20 min after APO injection.

**Magnetic Resonance Imaging (MRI) Acquisitions** The rats were anesthetized with 1.5–1.8% isoflurane (Escaïn®, 160 mL/min: Merck HOEI)-oxygen mixture. The body temperature was measured using a rectal thermocouple during the MRI measurements, and it was kept constant at 37±0.2°C with a feedback-controlled warm-water blanket (Yamashita Tech System, Japan) connected to a rectal probe (Photon Control Inc., Canada). The MRI data were acquired using a 1.5-Tesla MRmini-SA (DS Pharma Biomedical, Osaka, Japan) and a solenoid RF coil with a 30-mm inner diameter. Coronal MR images were obtained using a 2D spin-echo (SE) multi-slice (MS) T1-weighted imaging (T1WI) sequence. The typical T1WI parameters for a 2D-SE-MS were TR (ms)/TE (ms) = 500/9, FOV = 60 × 30 mm²; matrix = 128 × 256, voxel size = 0.246 × 0.246 × 0.246 mm, NEX = 8 a slice thickness of 1.5 mm for all 12 slices. T1WI images were obtained as the localizer
number of APO-induced ipsilateral rotation was gradually increased in ICH rats and reached a peak at day 7. These findings indicated that the ICH damage gradually caused striatum damage. The APO-induced ipsilateral rotation behaviors were significantly and dose-dependently inhibited by SPZ treatment day 7 after ICH (Fig. 1A). The evidence showed that SPZ had a pharmacologically protective effect against the progress of ICH dysfunction in the striatum. The APO-induced ipsilateral rotation was inhibited by a 3-day delayed treatment with SPZ at day 7 as well as by 7-day treatment (Fig. 1A). This result suggests that 3-day ICH is a critical period for causing striatum damage by ICH. In addition, although the contralateral (left) forelimb grasping behavior disappeared at 3 h and 1 day after ICH, the behavior later recovered after SPZ treatment. This result shows that the treatment of SPZ protects against such neurological deficits as hemiplegia, which appeared immediately after ICH.

SPZ may act via a different mechanism, such as ROS scavenging, in addition to the inhibition of superoxide production by CYP. Ac-SPZ lacked the ability to inhibit CYP after ICH. The APO-induced ipsilateral rotation behavior was inhibited in ICH rats following 7-day consecutive treatment with Ac-SPZ (100 mg/kg, i.p.) as well as SPZ (Fig. 1B), thus suggesting that the protective effect against striatum dysfunction by ICH might not be involved in the CYP inhibition by SPZ.

Effects of SPZ on Brain Edema after ICH in Rats

T2WI hyperintense hematoma core developed at 3 days, and the hematoma was gradually resolving at 7 days after ICH (Fig. 2A, upper row). Rats were treated with SPZ (25 mg/kg) for 7 days starting at 1 h after ICH to determine potentially beneficial SPZ effects for hematoma. SPZ treatment did not significantly reduce the hematoma volume at 3 or 7 days after ICH (Fig. 2A, bottom row). SPZ treatment for 7 days failed to significantly reduce the ipsilateral striatum atrophy in comparison to vehicle treatment, although there was a tendency for less brain tissue loss in the SPZ-treated rats (Fig. 2A).

The brain edema was observed by T2WI in the white matter for brain edema in posterior region from ICH site (Fig. 2B). The SI of T2WI was initially hyperintense in the ipsilateral white matter 1 h after ICH, and became uniformly hyperintense in the bilateral white matter 1 day after ICH (Fig. 2B, arrow heads). The brain edema in the bilateral white matter was caused by ICH at 3 days. The T2WI hyperintense persisted in the bilateral white matter at 7 days, although the T2WI gradually became more hypointense until 7 days after ICH. Therefore, these MRI findings indicated that the brain edema did continuously appear at the white matter during the 7 days after ICH. However, white matter edema in both cerebral
hemispheres, which was measured as hyperintensity in the T2WI, was observed to reach a maximum around 3 d after the induction of ICH (Fig. 2). Clear reductions in white matter edema, especially at the posterior region from the bleeding site, were observed in the SPZ treated rats. Although the effect of SPZ did not generate much edema around the hematoma after ICH induction, the values represented the mean±S.E.M. (n=5) for each experiment. **p<0.01 vs. 0mg/kg ipsilateral region of ICH rat. NS: not significant.

On the other hand, Gd-HP-DO3A accumulated in the hematoma, the treatment with SPZ improved the brain edema, especially the spreading to the contralateral white matter (Fig. 2B, bottom row). Brain edema formation was assessed by measuring the brain water content 3 d after the induction of ICH to confirm the effect of SPZ in the T2WI. ICH caused a significant increase in the water content of the right hemisphere (84.2±1.4% in ICH group vs. 78.5±0.9% in sham group). Treatment with SPZ (25 mg/kg, i.v.) for 3 d starting at 1 h after ICH reduced brain edema (80.2±2.4%).

Fig. 3. Effects of SPZ on the Attenuation of Oxidative Stress after ICH in Rats

TBARS levels were approximately 3 fold higher in ipsilateral striatum (i) of the ICH rat in comparison to the native rat. In the control ICH rats, the TBARS levels significantly increased, thus becoming approximately 150% higher in the ipsilateral striatum of the ICH rat in comparison to the contralateral region (c). The TBARS levels in both side of striatum were significantly inhibited by treatment with SPZ (5, 25 mg/kg, i.v., 100 mg/kg, i.p.) and Ac-SPZ (100 mg/kg, i.p.) at day 7 after ICH induction. The values represent the mean±S.E.M. (n=5) for each experiment. **p<0.01 vs. 0mg/kg ipsilateral region of ICH rat. NS: not significant.

Effects of SPZ on Lipid Peroxidation in ICH Rats

To examine the effects of SPZ on oxidative stress evoked by ICH, TBARS levels within the several brain regions at risk after 7 d of ICH was measured to indicate the level of lipid peroxidation as an oxidative stress marker (Fig. 3). The TBARS levels in the ICH region significantly increased by approximately 3 fold in all brain regions in comparison to the sham-operated rats. TBARS production of the ipsilateral striatum 7 d after ICH was significantly increased (150%) in comparison to the contralateral region. TBARS production in the ipsilateral striatum significantly decreased by approximately 40% after treatment with SPZ and Ac-SPZ for 7 d in comparison to the vehicle-treated rats (Fig. 3). These findings indicated that SPZ inhibited lipid peroxidation in the ipsilateral region, thus suggesting that brain damage could be suppressed by the reduction of oxidative stress by SPZ, but not due to CYP inhibition by SPZ.

Superoxide anion is converted to hydrogen peroxide via dismutation catalyzed by superoxide dismutase, followed by production of hydroxyl radicals in the presence of iron. Hydroxyl radical production could be facilitated in the localization of bleeding because there is excess iron in a hematoma or its distal region. SPZ might therefore suppress the chain-reaction of ROS causing tissue oxidation and injury via brain edema. Therefore, SPZ is considered to have a beneficial effect on the behavioral outcomes following ICH, thus suggesting that ROS scavenging, but not the CYP2C family, plays at least some type of role in the development of brain damage following ICH. In addition, the next step is to investigate the mechanism(s) of action of SPZ on brain damage after ICH.

These data indicate that SPZ could interrupt the cascade of oxidative processes that can contribute to tissue injury following the formation of ROS by ICH, and SPZ is therefore considered to be a potentially effective therapeutic approach for ICH.

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