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Research Report

Pharmacologic blockade of vascular adhesion protein-1 lessens neurologic dysfunction in rats subjected to subarachnoid hemorrhage

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ABSTRACT

Aneurysmal subarachnoid hemorrhage (SAH) is a potentially devastating clinical problem. Despite advances in the diagnosis and treatment of SAH, outcome remains unfavorable. An increased inflammatory state, one that is characterized by enhanced leukocyte trafficking has been reported to contribute to neuronal injury in association with multiple brain insults, including hemorrhagic and ischemic stroke. This study was designed to investigate, in rats, the neuropathologic consequences of heightened leukocyte trafficking following SAH, induced via endovascular perforation of the anterior cerebral artery. Experiments focused on the initial 48 h post-SAH and sought to establish whether blockade of vascular adhesion protein-1 (VAP-1), with LJP-1586, was able to provide dose-dependent neuroprotection. Treatment with LJP-1586 was initiated at 6 h post-SAH. An intravital microscopy and closed cranial window system, that permitted examination of temporal patterns of rhodamine-6G-labeled leukocyte adhesion/extravasation, was used. Effects of LJP-1586 on neurologic outcomes and leukocyte trafficking at 24 h and 48 h post-SAH were examined. In VAP-1-inhibited vs control rats, results revealed a significant attenuation in leukocyte trafficking at both 24 h and 48 h after SAH, along with an improvement in neurologic outcome. In conclusion, our findings support the involvement of an amplified inflammatory state, characterized by enhanced leukocyte trafficking, during the first 48 h after SAH. VAP-1 blockade yielded neuroprotection that was associated with an attenuation of leukocyte trafficking and improved neurologic outcome.

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1. Introduction

Subarachnoid hemorrhage (SAH), frequently resulting from intracerebral aneurysm rupture, is associated with a high incidence of morbidity and mortality. Initially, results from clinical and pre-clinical investigations revealed the presence of vasospasms in segments of large cerebral conduit arteries at several days post-SAH (Kamii et al., 1999; McGirt et al., 2002). Since these vascular dysfunctions often coincide with clinical observation, it was postulated that treatments targeting vasospasm might limit SAH-linked ischemic brain damage. Yet, contrary to expectations, clinical treatments designed to prevent vasospasm in conduit vessels have not been consistently efficacious in improving patient outcomes (see, for example, Etminan et al., 2011; Munoz-Guillen et al., 2013; Sehba et al., 2011).

However, delayed *large-vessel* vasospasm does not necessarily represent the only vasculopathy that may contribute to cerebral ischemia after SAH. Evidence obtained in rodents (Britz et al., 2007; Jorks et al., 2011; Koide et al., 2012) suggested that vasodilatory impairments following SAH could arise in arterioles, and may be linked to the elevated presence of inflammatory factors (Kolias et al., 2009; Pradilla et al., 2010). Findings from our laboratory, in rats subjected to transient focal ischemia (Watcharotayangul et al., 2012) or transient forebrain ischemia (TFI), (Xu et al., 2006) suggested a direct correlation between post-ischemic pial venular leukocyte activity and brain damage. Also, a similar finding was obtained in mice subjected to collagenase-induced intracerebral hemorrhage (Ma et al., 2011). The common element in those 3 reports was that neuroprotection could be achieved through treatment with highly selective blockers of vascular adhesion protein-1 (VAP-1). That endothelial protein is thought to play a key role in promoting adhesion and transmigration of polymorphonuclear leukocytes, monocytes, and lymphocytes (e.g., Dunkel et al., 2011; Hernandez-Guillamon et al., 2010; Watcharotayangul et al., 2012).

As recently noted in a review article by Chen et al. (2014), no studies have yet examined whether VAP-1 plays any role in the neuropathogenesis of SAH. Thus, we addressed two hypotheses. The first hypothesis is that treatment with the selective VAP-1 blocker, LJP-1586 [Z-3-fluoro-2-(4-methoxybenzyl) allylamine hydrochloride], will attenuate the neurologic dysfunction observed following SAH. The second hypothesis is that SAH triggers a LJP-1586-treatable heightened cerebral inflammatory state—one that is linked to enhanced adherence of blood-borne leukocytes to pial venular endothelium and eventual leukocyte extravasation.

2. Results

In all rats studied, arterial PaO_2 values were maintained above 100 mmHg; and $PaCO_2$, pH, and MABP remained within normal limits over the course of the experiments. The only exception was the 2–3 min temporary 25–30 mmHg drop in MABP following ACA perforation. Furthermore, no significant differences were observed when comparing $PaCO_2$, pH, and MABP values at equivalent experimental time points in the sham surgical, vehicle and LJP-1586 treated SAH groups.

2.1. *Effect of LJP-1586 on SAH-associated neurologic deficits*

Neurologic deficits at 24 and 48 h after SAH were compared among the groups receiving either ip vehicle (normal saline) control solution or ip LJP-1586 treatment. Neurologic assessments were performed using the scoring system described in Section 5. The results are summarized in Fig. 1. The maximum achievable score was 21, which translates to no functional deficit. Median neurologic deficit scores in these 10 rats from the sham surgical groups were tightly distributed, ranging from 19 to 21. The actual values were 19 (1 instance), 20 (5 instances) and 21 (4 instances). Compared to one another, the neurologic scores measured in these two sham groups were not statistically different. Hence, they can be considered as a single group. All the SAH-exposed rats displayed median neurologic scores that were significantly lower than in the sham group. Within the SAH groups (Fig. 1), significant differences were revealed when comparing the neurologic function scores *only* in the groups receiving **10 mg/kg** treatments of LJP-1586 vs the appropriate vehicle groups in both 24 and 48 h time points. The median scores in the 24 h

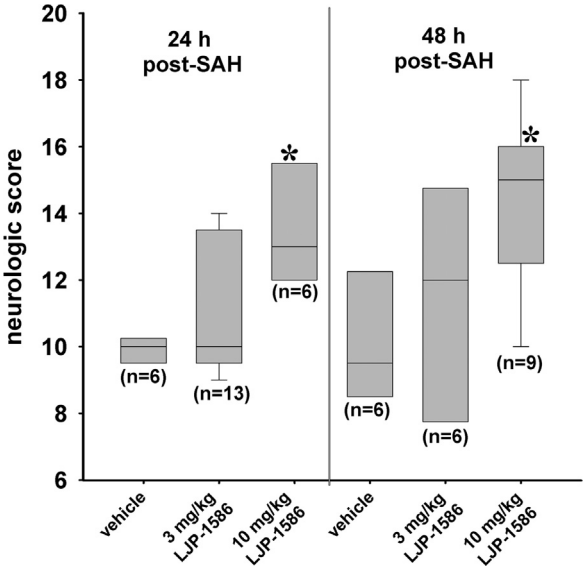

Fig. 1 – Neurologic outcome scores in LJP-1586-treated rats compared to their time-matched vehicle controls. Also included are findings from sham surgical control rats. The data, presented in boxplot form, represents scores obtained at 24 h to 48 h of reperfusion, where the upper and lower box edges represent the 25th and 75th percentiles, and the whiskers represent the 95% confidence intervals. The median values are depicted by the horizontal lines within the boxes, and outliers, are represented by closed circles. *$p < 0.05$ versus the time-matched vehicle-treated control (Mann–Whitney). Numbers of animals for each experimental group are displayed under the boxes. Also, please note that all scores are significantly different, when comparing the sham surgical control groups to the values found in the remaining groups (see text).