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Neuroprotection by platelet-activating factor acetylhydrolase in a mouse model of transient cerebral ischemia

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HIGHLIGHTS

- It seems that human recombinant PAF-AH (rPAF-AH) may have neuroprotection in middle cerebral artery occlusion mice.
- In these mice models we found that rPAF-AH might provide neuroprotection against ischemic injury.
- Neuroprotection might be induced not only by decrease in MMP-2 and MMP-9 expression, but also by increased VEGF expression.

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ABSTRACT

Neuronal damage after transient cerebral ischemia is exacerbated by signaling pathways involving activated platelet-activating factor (PAF) and ameliorated by PAF-acetylhydrolase (PAF-AH); but whether cerebral neurons can be rescued by human recombinant PAF-AH (rPAF-AH) remains unknown. Adult male mice underwent a 60 min middle cerebral artery occlusion (MCAO) and reperfusion for 24 h. Then, the mice received intravenous tail injections with different drugs. Neurological behavioral function was evaluated by Bederson's test, and cerebral infarction volume was assessed with tetrazolium chloride (TTC) staining. mRNA and protein expression levels of matrix metalloproteinase-2 (MMP-2, collagenase-1), MMP-9 (gelatinase-B), and vascular endothelial growth factor (VEGF) were determined by quantitative real-time PCR (RT-PCR) and western blot analysis, respectively. Compared with the vehicle group, rPAF-AH significantly improved sensorimotor function (42%, $P = 0.0001$). The volume of non-infarcted brain tissue was increased by the rPAF-AH treatment ($16.3 \pm 4.6\%$ vs. $46.0 \pm 10.3\%$, respectively). rPAF-AH significantly reduced mRNA and protein levels of MMP-2 and MMP-9, but increased the mRNA ($P < 0.001$) and protein levels ($P < 0.01$) of VEGF. These results demonstrate that rPAF-AH provides neuroprotection against ischemic injury. Neuroprotection might be induced not only by decrease in MMP-2 and MMP-9 expression, but also by increased VEGF expression.

1. Introduction

The platelet-activating factor (PAF) (1-O-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine) is a potent lipid mediator[1] that was initially extracted from immunoglobin E-stimulated basophils. It is reported to be involved in a large number of pathological processes, including shock, trauma, allergy, ischemia and inflammation [2,3]. Recent studies have shown that PAF is associated with ischemic injury in the central nervous system (CNS) and plays an important role in the aggravation of neuronal damage in postischemic and posttraumatic brains [4]. It was also reported that selective PAF receptor antagonists could prevent ischemia-induced CNS damage in animal models [5]. Collectively, the evidence indicates that the use of PAF receptor antagonists may be a potent treatment for stroke [6]. The acetyl group at the sn-2 position of the glycerol backbone in the PAF molecule is crucial to its biological activity. Deacetylation catalyzed by PAF-acetylhydrolase (PAF-AH) can directly deactivate PAF both in vivo and in vitro. Umemura et al. [7] reported that PAF-AH exerted strong neuroprotective effects against ischemic injuries, but the cellular mechanism underlying this effect is still poorly understood.

The concentration of the zinc-dependent endopeptidases, matrix metalloproteinases (MMPs), serves as an indicator of various cellular activities. MMP expression and activity are enhanced in various pathologic conditions, such as Alzheimer's disease, diabetes, cancer, atherosclerosis, or cerebral ischemia. In acute

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cerebral ischemia, the blood-brain barrier (BBB) disruption-mediated neuronal damage results in upregulated expression of MMP-2 and MMP-9[8]. Recent research has revealed that infarction volumes are reduced by inhibition of MMP-9 in mice [9]. These findings prompted us to hypothesize that modulating MMP expression and activity may be a potential therapeutic option for acute cerebral ischemia [10,11].

Vascular endothelial growth factor (VEGF) is the most significant regulatory element in the vascular system development and differentiation [12]. VEGF has pleiotropic functions in the brain, such as promoting neurogenesis and angiogenesis and mediating neuroprotection [13]. Existing studies on stroke have mainly concentrated on its neuroprotective effects, ignoring the potential ability of VEGF to reorganize ischemic vascular structure [14,15], even though angiogenesis and neurogenesis are known to occur simultaneously [16]. Increasing evidence suggests that vascular remodeling happens after stroke [15,17], and higher blood vessel counts are associated with longer survival in stroke patients [18]. These findings indicate that it is necessary to develop a new drug for stroke and ischemia that targets vascular remodeling in the brain.

The objective of the present study was to test the hypothesis that rPAF-AH is neuroprotective in a mouse model of transient cerebral ischemia. We also investigated the roles of MMP-2, MMP-9, and VEGF in cerebral vascular remodeling.

2. Materials and methods

2.1. Preparation and administration of rPAF-AH

Human recombinant PAF-AH (PeproTech EC Ltd., Rocky Hill, NJ, US) (4 mg/ml) was dissolved in 0.9% saline before it was intravenously injected into the tail vein within 15 min before the reperfusion in a middle cerebral artery occlusion (MCAO) mouse model. Reperfusion after 2 h embolism.

2.2. Ischemic animal model

All animals were obtained from the Experimental Animal Center of Sun Yat-sen University. Transient MCAO was performed as previously described [19]. Briefly, male Kunming mice (25–30 g) were temporarily anesthetized with 5% isoflurane in oxygen/nitrogen (N_2O/O_2 [30/70]) and a mixture of 2% isoflurane and N_2O/O_2 (30/70) during the course of surgery.

Rectal temperatures were maintained at 37 °C throughout the surgical procedures with a thermostat-controlled heating pad (Neuroscience, Tokyo, Japan). Changes in regional cerebral blood flow (rCBF) before and after MCAO were measured using laser-Doppler flowmetry (FLO-C1; Omegawave, Tokyo, Japan). The tip of the flexible probe was affixed with glue perpendicularly to the ipsilateral skull over the area between the secondary somatosensory cortex and the rostral part of the auditory cortex.

The right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were exposed through a ventral midline incision. An 8.0-monofilament nylon suture with a rounded tip was introduced into the CCA lumen and gently advanced into the ICA until it blocked the bifurcating origin of the middle cerebral artery (MCA). Sixty minutes after occlusion, animals were reperfused by withdrawing the suture from the CCA lumen. All experiments were conducted in compliance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 80–23; revised in 1996).

2.3. Experimental groups

Twenty mice were randomly divided into five groups according to different drug treatments after MCAO surgery. In the production of MCAO model preoperative 15 min pretreatment drug injected into the tail vein of mice, reperfusion 2 h after middle cerebral artery occlusion: (1) The sham-operation group in which the mice received equal volumes of saline solution ($n = 4$, Sham group), (2) the MCAO surgery group in which the mice received equal volumes of saline solution ($n = 4$, NS group), (3) the MCAO surgery group in which the mice received intravenous administration of 100 mg/kg ginaton ($n = 4$, Ginaton group), (4) the MCAO surgery group in which the mice received intravenous administration of 5 mg/kg ozagrel sodium ($n = 4$, OS group), and (5) the MCAO surgery group in which the mice received intravenous administration of 1 mg/kg rPAF-AH ($n = 4$, rPAF-AH group).

2.4. Behavior evaluation

A standardized battery of behavioral tests was used to quantify sensorimotor neurological function 2 h and 24 h after MCAO. The Bederson score was determined according to the following scoring system: 0, no deficit; 1, forelimb flexion; 2, forelimb flexion and decreased resistance to lateral push; 3, unidirectional circling; 4, longitudinal spinning or seizure activity; 5, no movement [20]. The test was conducted by an observer who was blinded to the treatment groups.

2.5. Evaluation of cerebral edema and infarction

Brains were dissected, and coronal slices (2 mm in thickness) were acquired from frozen forebrains using a mouse brain matrix (Braintree Scientific, Braintree, MA, USA). Brain slices were then stained with 2,3,5- triphenyltetrazolium chloride (TTC) (2%) (Sigma, St. Louis, MO, USA) at 37 °C for 15 min. The areas in the left hemisphere and those showing infarction were quantitated for each section with the ImageJ version 1.63 software (NIH, Bethesda, MD, USA). The relative infarction volume was expressed as the percentage of the infarct volume corrected to the ipsilateral hemispheric volume. The cerebral infarction volume was measured using an MPLAS-500 multimedia color pathological graphic analysis system. The investigators who performed the image analyses were blinded to the study groups.

2.6. Western blot analysis and enzyme assays

After reperfusion, mice were anesthetized and perfused with ice-cold phosphate-buffered saline. Hemispheric tissue was immediately frozen in liquid nitrogen and stored at −80 °C. Tissue was homogenized on ice in lysis buffer containing protease inhibitors, centrifuged at 10,000 rpm for 5 min, and the supernatant was collected. For each sample, 20 μg of protein was loaded on 4–15% sodium dodecyl sulfate polyacrylamide gels, separated by electrophoresis, and transferred to nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA). After blocking for 1 h at room temperature with 10% skim milk (Inner Mongolia Yili Industrial Group Co. Ltd., Inner Mongolia, China), blots were incubated overnight at 4 °C with any one of the following primary antibodies from Abcam (Cambridge, UK): rabbit anti-MMP-2 (2 μg/ml), rabbit anti-MMP-9 (1:400), and rabbit anti-VEGF (1 μg/ml). Horseradish peroxidase-conjugated anti-rabbit antibody was used as the secondary antibody, and signals were detected using the standard chemical luminescence method (ECL; Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.7. RT-PCR for MMP-2, MMP-9, and VEGF

Total RNA was isolated from tissues using TRIzol reagent (Invitrogen) and reverse-transcribed using oligo(dT) primers and Moloney murine leukemia virus reverse transcriptase