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Ang-(1–7) exerts protective role in blood–brain barrier damage by the balance of TIMP-1/MMP-9

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ABSTRACT

Cerebrovascular disease (CVD) ranks as the top three health risks, specially cerebral ischemia characterized with the damage of blood–brain barrier (BBB). The angiotensin Ang-(1–7) was proven to have a protective effect on cerebrovascular diseases. However, its role on blood–brain barrier and the underlying molecular mechanism remains unclear. In this study, Ang-(1–7) significantly relieved damage of ischemia reperfusion injury on blood–brain barrier in cerebral ischemia reperfusion injury (IRI) rats. Furthermore, its treatment attenuated BBB permeability and brain edema. Similarly, Ang-(1–7) also decreased the barrier permeability of brain endothelial cell line RBE4. Further analysis showed that Ang-(1–7) could effectively restore tight junction protein (claudin-5 and zonula occludens ZO-1) expression levels both in IRI-rats and hypoxia-induced RBE4 cells. Furthermore, Ang-(1–7) stimulation down-regulated hypoxia-induced matrix metalloproteinase-9 (MMP-9) levels, whose silencing with (matrix metalloproteinase-9 hemopexin domain) MMP9-PEX inhibitor significantly increased the expression of claudin-5 and ZO-1. Further mechanism analysis demonstrated that Ang-(1–7) might junction protein levels by tissue inhibitor of metalloproteinase 1 (TIMP1)–MMP9 pathway, because Ang-(1–7) enhanced TIMP1 expression, whose silencing obviously attenuated the inhibitor effect of Ang-(1–7) on MMP-9 levels and decreased Ang-(1–7)-triggered increase in claudin-5 and ZO-1. Together, this study demonstrated a protective role of Ang-(1–7) in IRI-induced blood–brain barrier damage by TIMP1–MMP9-regulated tight junction protein expression. Accordingly, Ang-(1–7) may become a promising therapeutic agent against IRI and its complications.

1. Introduction

Cerebrovascular disease means brain dysfunction caused by temporary or permanent bleeding or lack of blood flow (Collins et al., 2004; Powers, 1991). Blood–brain barrier (BBB), playing a regulative and protective role in the microenvironment of brain, is a physical and metabolic barrier to separate peripheral circulation from central nervous system (Hawkins and Davis, 2005). The integrity of cerebral microvessel relies on the maintenance of endothelial permeability barrier (Thanabalasundaram et al., 2010). Loss of BBB integrity could cause intravascular proteins to penetrate into the cerebral parenchymal extracellular space, thereby incurring vasogenic edema formation and further brain damage (Yang and Rosenberg, 2011; Wang et al., 2011).

The main structure of BBB is composed of microvascular endothelium and intercellular tight junctions. Several identified proteins associated with tight junction in cerebral endothelial cell are transmembrane protein (mainly contains zonula occludens (ZO) and claudins protein family) and subsidiary cytoplasmic protein (composed of ZO protein family). It has been demonstrated that the redistribution and decreased expression of claudin-5 and ZO-1 in ischemia brain microvascular endothelial cells are closely associated with blood–brain barrier damage, and identified as the specific marks of BBB damage (Jiao et al., 2011).

Renin-angiotensin system (RAS) is an important circulative system in environmental homeostasis regulation. Classically, angiotensinogen (AGT) is hydrolyzed by renin to form the decapeptide Angiotensin I (Ang I), which is then converted into the biologically active peptide Angiotensin II (Ang II) by angiotensin converting enzyme (ACE). The Ang II receptor type (AT_1 receptor) is the primary receptor of Ang II, and the ACE-Ang-II-AT_1 receptor axis has long been thought to be the main path in blood pressure control and renal sodium–water reabsorption. Over-activation of RAS and the increase of Ang II are always closely associated with the development and maintenance of hypertension-related damages.

Angiotensin-(1–7) [Ang-(1–7)] is a newly established bioactive fragment of RAS and generated predominately from Ang II by angiotensin converting enzyme-related carboxypeptidase (ACE2) hydrolysis. Through acting with the receptor Mas, the

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ACE2-Ang-(1–7)-Mas axis is able to counteract most of the deleterious actions of ACE-Ang-II-AT_1 receptor axis, especially in pathological conditions (Ferrario et al., 2005; Nishimura et al., 2000; Fleegal-DeMotta et al., 2009). It has been confirmed that Ang-(1–7) has protective effect on cardiovascular dysfunction (Benter et al., 2007). Moreover, central administration of Ang-(1–7) is found to ameliorate oxidative stress, minimize the size of cerebral infarction and improve neurological functions in rat model of middle cerebral artery occlusion (MCAO). However, the mechanisms of Ang-(1–7) involved in the pathophysiology of cerebral ischemia are still unknown.

To explore the function of Ang (1–7) on stabilizing of BBB, we constructed a mouse model of ischemia reperfusion injury (IRI) for experiment, and revealed the relationship of Ang-(1–7), matrix metalloproteinase-9 (MMP-9)(Pan et al., 2008; Yang et al., 2007), tissue inhibitor of metalloproteinase 1 (TIMP-1)(Boz et al., 2006), brain edema and cerebral ischemia reperfusion injury in IRI rats and brain endothelial cell line RBE4. This may provide a promising therapeutic agent against IRI and its complications.

2. Materials and methods

2.1. Drugs and animals

Angiotensin-(1–7) was purchased from Bachem, Inc (Bachem, Switzerland). In this study, Ang-(1–7) was dissolved in an artificial cerebrospinal fluid (aCSF, pH7.4, composition in mM: NaCl 130, KCl 2.99, $CaCl_2$ 0.98, $MgCl_2$ $6H_2O$ 0.80, $NaHCO_3$ 25, Na_2HPO_4 $12H_2O$ 0.039, NaH_2PO_4 $2H_2O$ 0.46, equilibrated with 95%O_2/5%CO_2). Male Sprague-Dawley rats of 280–320 g were offered by National Rodent Laboratory Animal Resources Shanghai branch of China. They were housed in air-conditioned rooms under a photoperiod of 14 h of light (lights on, 0600–2000 h). Food and water were provided ad-libitum. The protocol was approved by the Experimental Animal Care and Use Committee of the First Affiliated Hospital of Henan University of Chinese Medicine. Every effort was made to minimize animal suffering and reduce the number of animals used.

2.2. MCAO model

Rats were subjected to middle cerebral artery occlusion (MCAO) for 90 min by an intraluminal filament. Transient intraluminal MCAO was conducted according to the methods before with some modification (Zhao et al., 1997). The rats were fasted overnight and initially anesthetized with 10% chloral hydrate (0.35 ml/100 g, i.p.). The right common carotid artery was exposed through a lateral incision, separated from the vagus nerve and ligated. A 4–0 nylon monofilament was introduced from the bifurcation of the internal carotid artery and advanced until resistance was felt (1.8–2.0 cm from the bifurcation). The occluder thread was removed post 90 min insertion. Body temperature was maintained at 37 ± 0.5 °C throughout the procedure. By using a 6-point grading scale, the neurological symptoms of each rat were evaluated. The range of the total score was from a minimum of 0 points (severe symptoms) to a maximum of 5 points (normal). Only rats scoring less than 5 were used for the subsequent experiments. Sham-operated rats underwent same surgical treatment without inserting the thread.

2.3. Drug application

About a quarter of an hour after MCAO, 48 rats were divided into 6 groups (8 rats for each group) and implanted catheters intracerebroventricularly in the lateral ventricles with an Alzet model 1007D minipump. Rats of control group were continuously delivered aCSF (0.5 μl/h), while 5 treatment groups were delivered angiotensin-(1–7) respectively (0.0005 pmol/0.5 μl/h, 0.005 pmol/0.5 μl/h, 0.05 pmol/0.5 μl/ h, 0.5 pmol/0.5 μl/h, 5 pmol/0.5 μl/h) through catheters, following placed in stereotactic frames (David Kopf, Tujunga, CA). Dental cement was used to affix the catheters to the skull, and wounds were closed with sutures.

2.4. Determination of blood–brain barrier permeability by using Evans blue

Samples were obtained from cerebral ischemia rats or hypoxia-treated cells. BBB integrity was evaluated by Evans blue (2% in saline, 4 ml/kg; Sigma) extravasation. Rats were injected intravenously in the right femoral vein with 100 μl of Evans blue at the onset of reperfusion. Three hours later, animals were perfused with NaCl (150 mmol/l). Upon removal of the brains, ischemic were dissected and centrifuged. Supernatants were diluted with 500 μl trichloroacetic acid overnight at 4 °C. After 30 min centrifugation at 21,000*g*, Evans blue in the samples was measured for absorbance at 620 nm by spectrophotometry using a standard Evans blue solution (from 0 to 6.25 μg/ml). The results were expressed as the percentage of Evans blue quantity in the contralateral cortex.

2.5. Brain water content

The hemispheres were weighed to obtain the wet and dry weights before and after drying at 100 °C for 48 h, and the percentage of water content was calculated as (wet weight/dry weight) 100/wet weight.

2.6. Cell culture and reagents

The rat brain endothelial RBE4 cells were cultivated in 50:50 α-MEM/Ham's F-10 medium mixture supplemented with 300 μg/ml Genetic in, 10% FBS. Basic fibroblast growth factor of 1 ng/ml was also added on rat tail collagen coated petri dishes and cover slips. $CoCl_2$ and DFO were added in RBE4 media directly.

2.7. Hypoxic exposures

Hypoxic experiments were carried out in a purpose-built hypoxic glove-box chamber (InVivo$_2$ 400, Ruskinn Technologies, Pencoed, UK). The hypoxic glove-box chamber was maintained at 37 °C with 5% CO_2. O_2 concentration was constantly monitored internally with an O_2 sensor. In all experiments, RBE4 cells were exposed to hypoxia (1% O_2).

2.8. Barrier function assessment using transendothelial electrical resistance (TEER)

Barrier function of RBE4 monolayers was assessed by real-time measurement of transendothelial electrical resistance (TEER). TEER experiments were performed using RBE4 monolayers grown on rat tail collagen-coated transwell filter as previously described (Patabendige et al., 2013). The resistance of transwell filter insert grown cells was corrected for resistance across an empty collagen-coated transwell insert, and multiplied by surface area. All measurements were normalized to the 0 h time point of the corresponding treatment.

2.9. Real-time reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was prepared using the Trizol reagent (Invitrogen) and the optical absorbance ratio at 260 nm/280 nm was measured to determine the content. Then, the RNA was reverse transcribed into cDNA with random hexamer primers. Quantitative RT-PCR analysis was performed with cDNA as the template to amplify ZO-1, claudin5, MMP-9, TIMP-1 mRNAs with specific primers. The reaction was as