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Research Report

Astaxanthin reduces matrix metalloproteinase-9 expression and activity in the brain after experimental subarachnoid hemorrhage in rats

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ABSTRACT

We have previously shown that astaxanthin (ATX) reduces the blood–brain barrier (BBB) disruption and neurovascular dysfunction following subarachnoid hemorrhage (SAH) insults. However, the underlying mechanisms remain unclear. It is known that the matrix metalloproteinases (MMPs), especially matrix metalloproteinase-9 (MMP-9) plays a crucial role in the pathogenesis of secondary brain injury after SAH. And ATX has the ability to regulate MMP-9 in other models. Herein, we investigated whether ATX could ameliorate MMP-9 activation and expression in a rat model of SAH. A total of 144 rats were randomly divided into the following groups: control group ($n=36$), SAH group ($n=36$), SAH+vehicle group ($n=36$), and SAH+ATX group ($n=36$). The SAH model was induced by injection of 0.3 ml autologous blood into the prechiasmatic cistern. ATX (20 μl of 0.1 mmol) or vehicle was administered intracerebroventricularly 30 min after SAH induction. Mortality, neurological function, brain edema and blood–brain barrier (BBB) permeability were measured at 24 and 72 h after SAH. Biochemical and zymographic methods were used to analyze MMP-9 expression and activity in brain samples. Immunohistochemistry and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining were also evaluated at 24 h. Our data indicated that ATX could significantly reduce the expression and activity of MMP-9, leading to the amelioration of brain edema, BBB impairment, neurological deficits and TUNEL-positive cells at 24 h but not 72 h after SAH. The ATX-mediated down-regulation of MMP-9 was correlated with the decreased levels of IL-1β, TNF-α, oxidative stress, activated microglia and infiltrating neutrophils. These results suggest that the neurovascular protection of ATX in SAH is partly associated with the inhibition of MMP-9 expression and activity.

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1. Introduction

Early brain injury (EBI) is a major complication of aneurysmal subarachnoid hemorrhage (SAH) that contributes to the high incidence of morbidity and mortality from this disease (Sehba et al., 2012). And thus, prevention of EBI has been considered as a main goal in the management of patients with SAH. Different pathophysiological processes including cerebral ischemia, blood–brain barrier (BBB) disruption, brain edema, and cell death are involved in the pathogenesis of EBI (Cahill et al., 2006; Sehba et al., 2012). Among them brain edema, a consequence that occurs from disruption of the BBB disruption, has been considered as a crucial role in the development of EBI after SAH (Altay et al., 2012; Claassen et al., 2002).

Matrix metalloproteinase-9 (MMP-9), a member of the matrix metalloproteinase (MMP) family of zinc-containing proteinases, is considered to be a key mediator of BBB disruption in CNS diseases, including SAH (Feiler et al., 2011; Lee et al., 2004; Morancho et al., 2010; Sozen et al., 2009; Tsubokawa et al., 2006; Zhao et al., 2007). MMP-9 is released from glial cells, endothelial cells, neurons and infiltrating leukocytes et al., and MMP-9 can be activated by free radicals, serine proteases and other activated MMPs (Morancho et al., 2010). MMP-9 could degrade the extracellular matrix (ECM) of the BBB leading to BBB leakage, edema, leukocyte infiltration and progressive inflammatory reaction to brain injury over hours or days after the initial stroke (Asahi et al., 2001; Morancho et al., 2010; Zhao et al., 2007). Once neurovascular barriers are affected, there is a resultant activated of multiple neuro-inflammatory cascades, which could further increase the MMP-9 expression and activity, leading to much more damage to the BBB (Morancho et al., 2010). There is a considerable of evidence indicating that MMP-9 is up-regulated after the onset of SAH insults, associated with the disruption of BBB permeability and the formation of vasogenic edema (Feiler et al., 2011; Guo et al., 2010a; Guo et al., 2010b; Sozen et al., 2009; Suzuki et al., 2010). In addition, activation of MMP-9 could lead to cell death via degradation of the microvessel basal lamina protein laminin, a main component of ECM, after SAH (Gu et al., 2005; Guo et al., 2010b). Because early blockade of MMP-9 expression and activity stabilizes the BBB, reduces leukocyte infiltration, and confers both early and long-term neuroprotection, it suggests that early inhibition of MMP-9 may be a potential therapeutic strategy for SAH (Guo et al., 2010a; Guo et al., 2010b; Sozen et al., 2009; Suzuki et al., 2010).

Astaxanthin (ATX) has a variety of functions that may be beneficial in the treatment of stroke. In the central nervous system (CNS), it has been demonstrated that ATX could protect against cerebral ischemic injury, and neurotoxin-induced neurotoxicity both in vivo and vitro (Lin et al., 2010; Shen et al., 2009). At the same time, our earlier research has demonstrated that ATX has a powerful anti-oxidant and anti-inflammatory property in SAH (Zhang et al., 2014a; Zhang et al., 2014b). To date, few studies have been conducted to investigate the underlying molecular mechanisms of ATX in SAH. Previous studies reported that ATX could inhibit JNK activation and NF-kB pathways, and modulate MMP-9 activity and expression in different research fields (Kishimoto et al., 2010; Nagendraprabhu and Sudhandiran, 2011; Uekawa et al., 2014). It is known that JNK and NF-kB pathways could be involved in the modulation of MMP-9 in SAH (Kishimoto et al., 2010; Suzuki et al., 2010; Yatsushige et al., 2007). These seemed that ATX could also modulate MMP-9 in SAH. Thus, the aim of the current study was to extend our initial work and to verify whether ATX could rectify MMP-9 expression and activation following SAH, and, therefore, account for its neuroprotective function after SAH (Figs. 1 and 2).

2. Results

2.1. General observation and mortality

There was no significant difference in physiological parameters before, during, and after surgery. No statistical difference was observed among experimental groups regard to mean arterial blood pressure, arterial blood gases, and body temperature (data not shown). The mortality after surgery was 0% (0 of 36) in the control group, 18.2% (8 of 44) in the SAH group, 16.3% (7 of 43) in the SAH+vehicle group and 14.3% (6 of 42) in the SAH+ATX group (Fig. 3A).

2.2. Effects of ATX on neurological deficits, brain edema, and BBB disruption

Neurological scores were recorded prior to euthanasia at 24 h and 72 h after SAH induction. As shown in Fig. 3B, compared

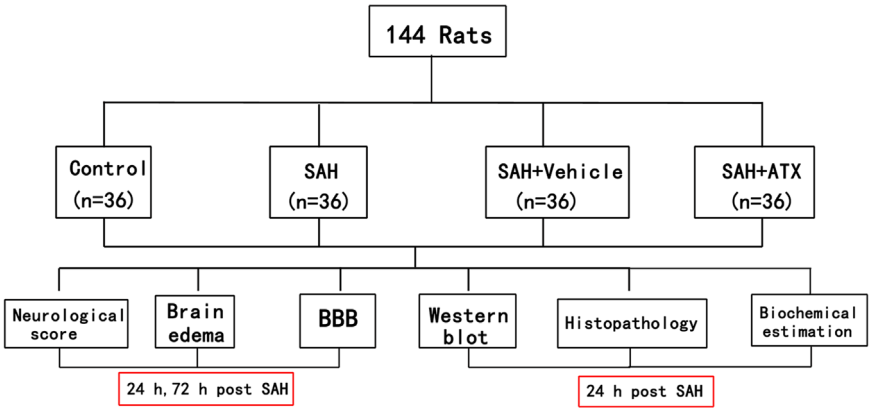

Fig. 1 – Schematic illustration of the experimental design. SAH, subarachnoid hemorrhage; ATX, astaxanthin.