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MECHANISMS OF RESVERATROL-INDUCED NEUROPROTECTION IN RATS WITH INTRACEREBRAL HEMORRHAGE: INSIGHT ON THE ROLE OF ADENOSINE A1 RECEPTOR

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ABSTRACT

Stroke is a common cause of brain injury and contributes to significant mortality worldwide. Intracerebral hemorrhage (ICH) remains the least treatable stroke and is characterized by hematoma (HT) expansion and perihematomal (PHT) damage. Tumor necrosis factor-α (TNF-α) activation underlies PHT damage in ICH. TNF-α triggers apoptosis and necroptosis by increasing caspase-3 (CASP3) and receptor-interacting protein kinase 3 (RIPK3) respectively. None of the current therapy protects PHT. The only definite treatment is by evacuation of HT, which often results in incomplete recovery from HT-induced secondary injury. In view of the neuroprotective effects of *trans*-resveratrol (RV), we investigated the effects of RV on brain injury induced by collagenase (COL) in Sprague-Dawley rats. The objective of this study was to elucidate the neuroprotective effect and mechanisms of action of RV, with an insight on the role of adenosine A1 receptor (A1R), against COL-induced ICH in rats. In this study, rats were separated into 5 groups: (i) control, (ii) sham-operated rats, (iii) ICH rats pretreated with vehicle (0.1% DMSO saline, i.c.v.), (iv) ICH rats pretreated with RV (4 nmol, i.c.v.), and (v) ICH rats pretreated with RV (4 nmol) and the A1R antagonist, DPCPX (8 nmol, i.c.v.). Thirty minutes after pretreatment, ICH was induced by intrastriatal injection of COL (0.04 U). Forty-eight hours after ICH, rats were subjected to a variety of neurological and neurobehavioural tests and the brain tissues were processed for gross, histological and biochemical analyses. Sections of brain tissue were examined for HT volume through gross morphometry, while morphological features, midline shift, damaged area and neuronal density through histopathological examination using H&E and neuronal nuclei (NeuN) immunostaining. This study revealed that pretreatment with RV reduced HT volume, damaged area and neuronal depletion. DPCPX abolished effects by RV on damaged area and neuronal depletion. Overall, our data demonstrated that RV resolved HT and preserved PHT, while A1R is involved in the neuroprotective effects by RV in PHT. In the next part of our study, mechanisms of neuroprotection by RV against COL-induced PHT damage in rats was elucidated. Homogenate of PHT brain tissues were examined for expression of TNF-α, CASP3 and RIPK3 activity using ELISA, while A1R, mitogen-activated protein kinase P38 (MAPK P38), heat shock protein (Hsp90), brain-derived neurotrophic factor (BDNF) and tropomyosin-related kinase B (TrkB) expression using RT-qPCR. It was observed that pretreatment with RV restored the expression of TNF-α, CASP3, RIPK3, A1R and Hsp90, and increased transcriptional activity of BDNF/TrkB. DPCPX abolished effects by RV on CASP3, RIPK3, A1R, Hsp90 and BDNF. Overall, our data demonstrated that A1R is involved in the antiapoptotic and antinecroptotic effects by RV in PHT via upregulation of BDNF. The final study was done to evaluate the sensorimotor function of rats using neurological severity score, tail flick, open field, grip strength and rotarod tests. This part of study demonstrated that RV protected against signs of neurological deficit including general locomotor, forelimb activity and motor coordination, and partially preserved behavioural activities. DPCPX abolished the effects of RV. This data demonstrated that A1R is involved in the effects by RV against COL-induced neurological and neurobehavioural deficits in rats. In conclusion, this study demonstrates involvement of A1R in the mechanisms of neuroprotection by RV by inhibiting CASP3-dependent apoptosis and RIPK3-dependent necroptosis in PHT area, probably via the cross talk between A1R and BDNF/TrkB pathway.

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CHAPTER ONE
INTRODUCTION

1.1   Background of Study

Stroke is a common cause of brain injury and is the leading cause of long-term sensorimotor deficits. Stroke caused by cerebral hemorrhage is responsible for 15% of stroke incidence (Feigin et al., 2009). Despite its lower incidence in comparison with the ischemic subtype, it is generally a more severe type and is associated with highest mortality risk of all stroke subtypes (Jones et al., 2013).

Intracerebral hemorrhage (ICH) is the most common stroke caused by hemorrhage (Go et al., 2013). ICH is defined as a focal collection of blood within the brain parenchyma or ventricular system that is not caused by trauma (Sacco et al., 2013). The main underlying cause of ICH is believed to involve pathophysiological changes in deep perforating arteries of the basal ganglia, thalamus, cerebellum and cerebral cortex associated with chronic hypertension (Niu et al., 2017).

ICH is currently the least treatable subtype of stroke because of the lack of pharmacotherapeutic options. It is managed primarily by supportive and symptomatic treatment. The only definitive treatment is by surgical evacuation of hematoma (HT). The HT is believed to be the source of toxicity and inflammation (Aronowski and Zhao, 2011). Although surgical evacuation of HT can ensure patient survival, it often results in incomplete post-stroke recovery as it is associated with the occurrence of HT-induced secondary injury (Aronowski and Zhao, 2011). Therefore, a pharmacotherapeutic option which targets for reduction of HT volume and neuroprotection at perihematomal (PHT) area may be an ideal approach in the treatment of ICH.

Trans-resveratrol (RV) is one of the neuroprotective agents that was shown to improve HT resolution (Bonsack et al., 2017) as well as neurobehavioural functions in preclinical model of ICH (Cai et al., 2018; Bonsack et al., 2017; Singh et al., 2017). However, the mechanisms of action of RV-induced neuroprotection remain unknown.

In terms of the possible effects of RV on HT volume, in general, it has been reported that it has antiplatelet activity and was found to inhibit platelet aggregation (Dutra et al., 2017; Göçmen et al., 2013). In vitro and in vivo studies on the mechanisms