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Research Report

The protection by Octreotide against experimental ischemic stroke: Up-regulated transcription factor Nrf2, HO-1 and down-regulated NF-κB expression

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ABSTRACT

Background: Inflammatory and oxidative damage play a pivotal role in cerebral ischemic pathogenesis and may represent a therapeutic target. Octreotide (OCT) has been proved to elicit a variety of biological effects through its anti-inflammatory and anti-oxidant properties in the treatment of severe acute pancreatitis and ischemia-reperfusion injury in retina and intestine. However little is known regarding the effect of OCT in ischemic stroke. Here, we designed this study to investigate the protective effect of OCT in ischemic stroke and explore the potential underlying mechanisms. Methods: Male Sprague-Dawley rats were subjected to permanent middle cerebral artery occlusion (pMCAO) and randomly divided into four groups: Sham (sham-operated), MCAO (pMCAO+0.9% saline), OCT-L (pMCAO+OCT 50 μg/kg) and OCT-H (pMCAO+OCT 100 μg/kg) groups. OCT was administered intraperitoneally immediately after stroke. Neurological deficit scores, infarct volume and brain water content were measured at 24 h after stroke. Immunohistochemical staining and western blot were used to analyze the expressions of Nrf2, HO-1 and NF-κB. SOD and MDA were measured by spectrophotometer. Results: Compared with MCAO group, OCT significantly alleviated neurological deficit, lessened infarct volume and brain edema ($P<0.05$), upregulated the expression of Nrf2, HO-1 and SOD ($P<0.05$), and decreased the expression of NF-κB and MDA ($P<0.05$). Conclusions: OCT protected the brain against cerebral ischemic damage; this effect may be through upregulation of transcription factor Nrf2, HO-1 and downregulation of NF-κB expression.

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1. Introduction

Stroke is a major cause of disability and the second most common cause of death worldwide (Donnan et al., 2008). Previous studies have documented that during cerebral ischemia a burst of reactive oxygen species (ROS) is produced in brain tissue (Niizuma et al., 2009), which can activate diverse signaling pathways and result in oxidative stress and inflammatory response (Ji et al., 2012). Accumulating evidences have suggested that oxidative stress and inflammatory damage are important pathological mechanisms in cerebral ischemia (Chan, 2001; Huang et al., 2006). The strong correlation between oxidative stress, inflammatory damage and ischemic stroke has generated a lot of interest and excitement in seeking anti-oxidant and anti-inflammatory therapies to combat ischemia-induced damage (Simonyi et al., 2005; Du et al., 2012). Octreotide (OCT), a synthetic cyclic octapeptidewas, has been proved to elicit a variety of biological effects through its anti-inflammatory and anti-oxidant properties in the treatment of severe acute pancreatitis and ischemia-reperfusion injury in retina and intestine (Jingmin et al., 2012; Zhang et al., 2007, 2009; Takano et al., 2011; Celebi et al., 2002). Besides, in a rat chronic mild stress model, by virtue of its anti-oxidant and anti-inflammatory effects, OCT exerted potential antidepressant like activity (Schaalan and Nassar, 2011). Previous study has demonstrated that OCT could reduce brain edema induced by cerebral ischemia (Rauca et al., 1999). However the exact role and underlying mechanism of OCT in ischemic stroke is unclear.

Nuclear factor erythroid 2-related factor 2 (Nrf2), a master regulator of anti-oxidative defense responses, is an important cytoprotective transcription factor, which enhances the transcription of an expansive set of anti-oxidant enzymes and phase II detoxification enzymes, such as heme oxygenase-1 (HO-1), glutathione *S*-transferases (GSTs), NAD(P)H quinone oxidoreductase (NQO1) and so on. Recent studies indicate that Nrf2 is a critical protective survival factor for central nervous system (Liu et al., 2004; Shah et al., 2007). Our previous studies have proved that administration of curcumin or oxymatrine which increases the activity and expression of Nrf2 and HO-1 could attenuate oxidative injury induced by brain ischemia (Yang et al., 2009; Li et al., 2011).

Nuclear factor-kappa B (NF-κB), an important nuclear transcription factor, regulates the genes of a vast number of inflammatory mediators, such as IL-1, TNF-α, IL-6, iNOS and COX-2, all of which play a pivotal role in ischemic brain damage (Yi et al., 2007; Zheng and Yenari, 2004). Previous observation provides evidence that NF-κB was upregulated following ischemia (Duckworth et al., 2006). We recently found that downregulation of NF-κB showed significantly neuroprotective effects during cerebral ischemia (Fan et al., 2009; Liu et al., 2009; Ji et al., 2012; Qiao et al., 2012).

The aim of current study was to investigate the potential protective effect of OCT in the rat model of pMCAO and whether the therapeutic benefit of OCT was associated with the activities of Nrf2, HO-1 and NF-κB.

2. Results

2.1. OCT attenuated the neurological dysfunction

Neurological deficit was examined and scored on a 6-point scale at 24 h after MCAO (Fig. 1A). Compared with MCAO group, the neurological deficit scores were significantly reduced in OCT-H group (MCAO group vs. OCT-H group: 3.39 ± 0.78 vs. 1.94 ± 1.00, $P<0.05$), but no significant difference was found between MCAO group and OCT-L group (MCAO group vs. OCT-L group: 3.39 ± 0.78 vs. 3.00 ± 0.77, $P>0.05$).

2.2. OCT reduced brain edema

Brain water content at 24 h after ischemia was shown in Fig. 1B. In the Sham group, the brain water content was $79.49\pm0.4\%$. Compared with MCAO group, OCT-L and OCT-H group showed a significant decline in the brain water content in the ischemic hemisphere (MCAO group vs. OCT-L group: $85.31\pm0.93\%$ vs. $84.00\pm0.74\%$, $P<0.05$; MCAO group vs. OCT-H group: $85.31\pm0.93\%$ vs. $82.93\pm1.00\%$, $P<0.01$) ($n=6$ in each group).

2.3. OCT decreased brain infarct volume

No infarction was observed in Sham group. An extensive lesion was found in both striatum and lateral cortex in MCAO group (Fig. 1C). The infarct volume in high dose group was significantly decreased from $43.16\pm2.33\%$ to $30.56\pm4.01\%$ ($P<0.05$, Fig. 1D). But no significant difference was found between MCAO group and OCT-L group ($P>0.05$).

2.4. OCT increased the expression of Nrf2, HO-1, SOD and decreased MDA content

The immunohistochemical staining of Nrf2 and HO-1 at 24 h after operation is shown in Fig. 2A and B. Few Nrf2 and HO-1 positive cells were observed in the cortex of Sham group indicating a low baseline of Nrf2 and HO-1 in non-ischemic cortex. The number of cells labeled with Nrf2 and HO-1 in OCT-H group was significantly increased compared with MCAO group ($P<0.05$, Fig. 2D and E). But low dose of OCT did not reach a significant level. Compared with MCAO group, western blot showed the expression of Nrf2 was significantly increased in OCT-H group at protein levels ($P<0.05$, Fig. 3A and B), while HO-1 protein expression was significantly increased in OCT-L and OCT-H groups ($P<0.05$, Fig. 3D and E).

Meanwhile, the activities of superoxide dismutase (SOD) and malondialdehyde (MDA) content in ischemic cortical tissue were detected to examine the oxidative response at 24 h after ischemia. Compared with MCAO group, both dosages of OCT significantly increased the activities of SOD and decreased the production of MDA at 24 h after MCAO ($P<0.05$, Table 1).

2.5. OCT reduced the expression of NF-κB

The expression of NF-κB was upregulated after ischemia, and was significantly downregulated after systemic administration of