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Research Report

Anti-edema effect of epigallocatechin gallate on spinal cord injury in rats

Rui Ge[a], Yue Zhu[a,*], Yao Diao[b], Lin Tao[a], Wei Yuan[a], Xiao-chuan Xiong[a]

[a]Department of Orthopaedics, The First Affiliated Hospital of China Medical University, No.155 Nanjing North Street, Heping District, Shenyang 110001, Liaoning Province, PR China
[b]Center of Experiment and Technology, China Medical University, Shenyang 110001, Liaoning Province, PR China

ARTICLE INFO

Article history:
Accepted 7 June 2013
Available online 4 July 2013

Keywords:
Spinal cord injury
Edema
Epigallocatechin gallate
Aquaporin-4
Glial fibrillary acidic protein

ABSTRACT

Recent studies indicated that epigallocatechin gallate (EGCG) had neuroprotective effects on spinal cord injury (SCI).The current study was performed to determine the anti-edema effect of EGCG after SCI in rats. EGCG (100 mg/kg, i.p.) was administered to rats immediately following SCI. It was found that EGCG (100 mg/kg) could significantly reduce spinal cord water content. In addition, EGCG (100 mg/kg) significantly reduced the expression of aquaporin-4 (AQP4) and glial fibrillary acidic protein (GFAP) level at 24, 48 and 72 h after injury, but it did not have this effect at 12 h after injury. The changes of AQP4 and GFAP protein induced by EGCG (100 mg/kg) treatment were accompanied by a reduction of spinal cord edema. Our results indicated that EGCG (100 mg/kg) could reduce spinal cord edema after SCI, which could be correlated with the down-regulation the expression of AQP4 and GFAP protein level after SCI.

1. Introduction

Spinal cord injury (SCI) is a devastating pathology that may lead to neurological deficits. SCI is invariably associated with spinal cord edema that accounts for much of the morbidity and mortality of this condition (Fujiki et al., 2005; Leypold et al., 2008). Some researches show that spinal cord edema adversely affects the outcome after SCI (Verkman et al., 2011; Yang and Piao, 2011). Therefore, a strategy that reducing spinal cord edema in vivo would be ideal for the treatment of SCI.

The current recommended treatment for SCI involve administration of high-dose methylprednisolone, which has serious side effects. Epigallocatechin gallate (EGCG) is the main polyphenol present in green tea and thought to be responsible for the majority of biological activity of green tea extracts (Kimura et al., 2002). EGCG is known for its potent antioxidant (Frei and Higdon, 2003; Higdon and Frei, 2003) and anti-inflammatory properties (Thomas and Kim, 2005). EGCG also has been shown to have some protective effects against neuronal damage (Sutherland et al., 2006). A recent study shows that EGCG could attenuate neuronal apoptosis and improve locomotor function after SCI in rats (Khalatbary and Ahmadvand, 2011).

The aquaporins play an important role in water transport in many cell types. Aquaporin-4 (AQP4) protein is strongly expressed in the central nervous system(CNS) (Oshio et al., 2004; Saadoun and Papadopoulos, 2010). The previous studies demonstrate that the changes in AQP4 expression are associated with spinal cord edema and blood–spinal cord barrier permeability (Wang et al., 2009; Xu et al., 2008). The changes

*Corresponding author. Department of Orthopaedics, The First Affiliated Hospital of China Medical University, No.155 Nanjing North Street, Heping District, Shenyang 110001,Liaoning Province, PR China. Fax: +86 24 832 82772.
E-mail addresses: zhuyuedr@163.com, doctorzhuyue@126.com (Y. Zhu).

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http://dx.doi.org/10.1016/j.brainres.2013.06.009

in AQP4 expression in the injured spinal cords parallel changes in spinal cord water content (Nesic et al., 2006; Saadoun et al., 2008). Astrocytes swell also plays an important role in cellular edema after acute SCI. GFAP is a specific marker of astrocytes.

However, the effect of EGCG on spinal cord edema after SCI is poorly known. The aim of this study was to investigate the potential anti-edema effect of EGCG (100 mg/kg) on spinal cord edema after acute SCI and its underlying mechanism.

2. Results

2.1. Effect of EGCG on spinal cord water content

The result of spinal cord water content was shown in Fig. 1. The spinal cord water content after injury was significantly increased in SCI group compared with that of Sham group at 12, 24, 48 and 72 h after injury. The spinal cord water content after injury was significantly reduced in EGCG group compared with that of SCI group at 24, 48 and 72 h after injury, whereas it did not change significantly at 12 h after injury.

2.2. Effect of EGCG on AQP4 expression

The expression of AQP4 was detected using immunohistochemistry (IHC) and Western blot (WB) methods. The IHC result of AQP4 was shown in Fig. 2. AQP4 positive cells were strongly expressed in gray matter, around capillaries and in radial astrocytes with IHC. There was significantly up-regulation the number of AQP4 positive cells in SCI group compared with in Sham group at 24 and 72 h after injury ($p<0.05$). In EGCG group, the number of AQP4 positive cells was significantly down-regulation at 24 and 72 h after injury compared with that of SCI group ($p<0.05$).

The representative WB gels for AQP4 (34 kDa) and β-actin (42 kDa) were shown in Fig. 3. The level of AQP4 was quantified and normalized to β-actin. AQP4 was expressed at low level in Sham group and significantly increased at 12, 24, 48 and 72 h after injury($p<0.05$,Fig. 3A–D). The level of AQP4 in EGCG group showed a dramatic drop as compared with that in SCI group at 24, 48 and 72 h after injury ($p<0.05$, Fig. 3B–D). But at 12 h after injury, there did not have any significant difference between SCI group and EGCG group ($p>0.05$, Fig. 3A).

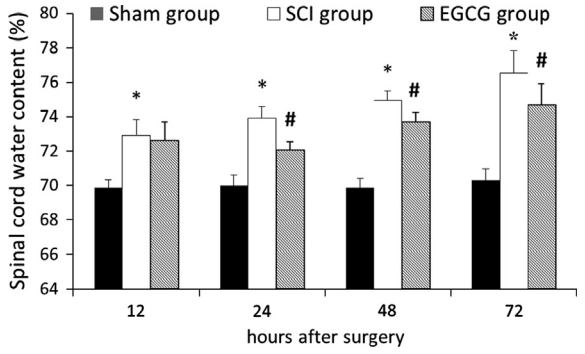

Fig. 1 – The spinal cord water content at 12, 24, 48 and 72 h after injury. The figures showed a significant increase in the spinal cord water content in SCI group when compared with that of Sham group at 12, 24, 48 and 72 h after injury, while a significant reduction was observed in EGCG group compared with that of SCI group at 24, 48 and 72 h after injury. Data present mean ± SD ($n=5$ every group, $^{*}p<0.05$ between Sham group and SCI group, $^{\#}p<0.05$ between EGCG group and SCI group).

2.3. Effect of EGCG on the GFAP expression

The IHC result of GFAP was shown in Fig. 4. The number of GFAP positive cells was significantly increased in SCI group compared with that of Sham group at 24 and 72 h after injury ($p<0.05$). In EGCG group, The number of GFAP positive cells was obviously decreased at 24 and 72 h after injury compared with that of SCI group ($p<0.05$).

With WB analysis, The IDVs of GFAP compared with β-actin were shown in Fig. 3. GFAP level was low in Sham group and significantly increased at 12, 24, 48 and 72 h after injury ($p<0.05$,Fig. 3A–D). Compared with in SCI group, the expression level of GFAP protein in EGCG group was showed a obvious drop at 24, 48 and 72 h after injury ($p<0.05$,Fig. 3B–D). While there did not have any significant difference between SCI group and EGCG group at 12 h after injury ($p>0.05$, Fig. 3A).

3. Discussion

In the previous studies about the treatment of EGCG on central nervous system injury, several researches used 50 mg/kg does of EGCG and had good efficacy (Khalatbary and Ahmadvand, 2011; Lee et al., 2003). Lee (Lee et al., 2004) reported the protective effect of EGCG against brain edema after unilateral cerebral ischemia in gerbils. In this research, it was shown that the treatment of EGCG at 50 mg/kg does at 30 min before and immediately after ischemia had better anti-edema effect, while gerbils received 25 mg/kg at 30 min before and immediately after ischemia did not have anti-edema effect. Another reaearch used EGCG at 25, 50 and 100 mg/kg does for the treatment of SCI, and the 100 mg/kg does group had better efficacy than 25 and 50 mg/kg does groups Deng et al. (2011). In this research, we chose 100 mg/kg does of EGCG for the treatment of SCI.

This study demonstrates that the EGCG (100 mg/kg) treatment has good therapeutic effects following SCI. The main results of the current study show that the treatment of SCI with EGCG (100 mg/kg) attenuates (1) spinal cord water content, (2) AQP4 level and (3) GFAP level.

The pathophysiology of SCI is complex and two mechanisms can be distinguished as the primary and secondary damages (Rowland et al., 2008). It is noteworthy that in most cases, the secondary damage rather than the primary damage, becomes the critical obstacle to successful treatment. Secondary damage after SCI include edema, altered blood flow, and changes in microvascular permeability (Rowland et al., 2008; Tator and Fehlings,1991). Traumatic injury to the spinal cord is often associated with edema. The edema is most prominent in the gray matter of the spinal cord (Oshio et al., 2004; Shepard and Bracken, 1999). In this study, we used the rat model of SCI