

Effects of Epigallocatechin Gallate on Tissue Lipid Peroxide Levels in Traumatized Spinal Cord of Rat

*¹Ali Reza Khalatbary, ²Hasan Ahmadvand

Abstract

Objective(s)

Recent studies revealed the neuroprotective effects of epigallocatechin gallate (EGCG) on a variety of neural injury. The purpose of this study was to determine the effects of EGCG on the tissue lipid peroxidation after spinal cord injury (SCI).

Materials and Methods

Rats were randomly divided into four groups of 7 rats each as follows: sham-operated group, trauma group, and EGCG-treatment groups (50 mg/kg, i.p., immediately and 1 hr after SCI). The rats were euthanized 24 hr after injury and then, spinal cord samples were taken for determination of malodialdehyde levels, as an indicator of lipid peroxidation.

Results

The results showed that MDA levels were significantly decreased in EGCG-treatment groups.

Conclusion

On the basis of these findings, we propose that EGCG may be effective in protection of spinal cord tissue from injury.

Keywords: Antioxidants, Epigallocatechin gallate, Lipid Peroxides, Spinal Cord Injuries

1- Razi Herbal Medicine Research Center, Lorestan University of Medical Sciences, Khoramabad, Iran

*Corresponding author: Tel: +98-661-3218652; Fax: +98-661- 6200133; email: khalat90@yahoo.com

2- Department of Biochemistry, Lorestan University of Medical Sciences, Khoramabad, Iran

Introduction

Neurological damages after traumatic spinal cord injury result from both primary mechanical injury and secondary degeneration process (1). Outcome of spinal cord injury depends on the extent of secondary damage mediated by a series of cellular, molecular, and biochemical cascades, such as oxygen free radical-induced lipid peroxidation (2), inflammatory reaction (3), autoimmune response (4), and vascular events (5). In recent years, much attention has been focused on secondary injury because it appears to be susceptible to therapeutic interventions.

The chemical composition of green tea contains many polyphenolic compounds, generally known as catechins. Catechins have many actions such as free radical scavenging/antioxidant actions, preventing lipid peroxidation due to oxidative stress, modulating apoptotic pathways, prooxidant properties, and anti-inflammatory effects (6). Epigallocatechin gallate (EGCG) is the most abundant composition of the tea catechins and is thought to be responsible for the majority of biological activity of green tea extracts (7). EGCG has been shown to be of some protective effects against neuronal damage after transient ischemia (8), acute hypoxia (9), iron-induced oxidative stress (10), and aging (11). Recently, green tea extract has been demonstrated to attenuate secondary inflammatory response following spinal cord injury in mice (12).

In spite of some experimental evidence for the neuroprotective effects of EGCG in cerebral ischemia and neurodegenerative diseases, evidence regarding its effects on SCI is still limited. Accordingly, in the present study, we investigated the beneficial effects of EGCG administration on tissue lipid peroxidation after SCI in rat.

Materials and Methods

Animals

Male adult Spargue-Dawley rats were used (250-300 g) (Pasteur's Institute, Tehran, Iran) in this study. They were kept under standard conditions according to the guidelines of the university animal care codes to minimize the animals suffering.

Spinal cord injury

Contusive SCI was carried out using the weight dropping technique. The animals were anesthetized with ketamine (75 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). Laminectomy was performed at T9 level vertebra; the dorsal surface of the cord was then subjected to weight drop impact using a 10 g weight dropped from a height of 2.5 cm in order to produce contusive SCI. Following the surgery, the recovery of the animals was assisted by administering lactated Ringer's solution (12-25 ml) subcutaneously immediately after surgery and cefazolin (Jaber Ibn Hayan, Tehran, Iran) (50 µg/kg) which was administered twice daily for 3 days. The urinary bladders were pressed three times a day until the function was retained.

Experimental groups

The rats were randomly allocated into four groups, each containing 7 rats: (i) sham-operated group, which underwent laminectomy alone; (ii) trauma group, which underwent laminectomy followed by SCI and received saline (vehicle); (iii and iv) EGCG-treatment groups, which underwent laminectomy followed by SCI and received a 50 mg/kg single dose of EGCG (Sigma) intraperitoneally immediately (EGCGI) and 1 hr (EGCGII) after trauma, respectively.

Biochemical analysis

All rats were euthanized, and 1.5 cm traumatized spinal cord sample was removed for biochemical analysis 24 hr after SCI. The obtained samples were thoroughly cleaned of blood and the meninges were carefully removed. Then, the tissue samples were immediately frozen and stored in a -70 °C freezer for assays of tissue malondialdehyde (MDA) levels (13) as a product of lipid peroxidation which reacts with thiobarbituric acid (TBA). The assay procedure for lipid peroxide was as follows. Tissues were homogenized in 10 volumes (w/v) of cold 1.5% KCl. One-half millimeter (0.5 ml) of homogenate was mixed with 3 ml of 1% H₃PO₄ and 1 ml 0.6% thiobarbituric acid. The mixture was then heated in boiling water for 1 hr. After cooling, the color was extracted into 4 ml n-butanol. The absorbance of the supernatant

was measured by spectrophotometry at 535 nm. TBA reactant concentration was expressed as nanomoles per gram of wet tissue.

Statistical analysis

Statistical analysis was carried out using the SPSS package. Results were presented as mean values (\pm SD). The K-S test was used in order to evaluate the normality of the data. Also, the Tukey's multiple comparison test and the analysis of the variance were used in order to compare each two groups and compare the data among the groups, respectively. A value of $P < 0.05$ was considered significant.

Results

Lipid peroxidation levels

The histogram of the MDA levels for all groups at 24 hr post-injury is shown in Figure 1. The MDA levels were 4.34 ± 1.54 for the sham-operated group, 18.80 ± 2.54 for the trauma group, 11.59 ± 1.51 for the EGCGI-treatment group, and 9.88 ± 1.34 for the EGCGII-treatment group. Induction of SCI in the trauma ($P < 0.001$) and EGCG-treatment groups ($P < 0.01$) produced a significant elevation in lipid peroxidation level compared to the sham-operated group. The MDA levels in the EGCG-treatment groups were significantly lower than those in the trauma group ($P < 0.01$), while the differences between EGCG1 and EGCG2 were not significant ($P > 0.05$).

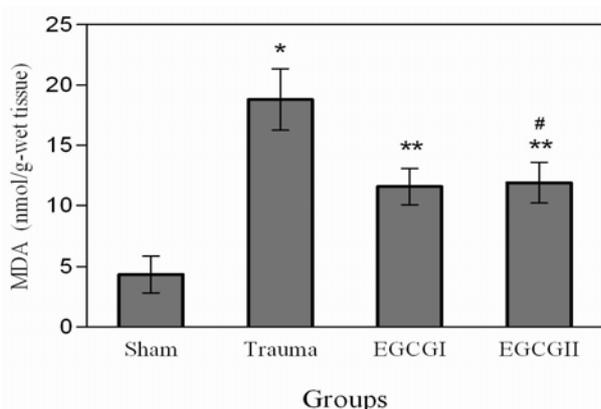


Figure 1. Effects of EGCG on MDA level. Histogram shows the levels of malondialdehyde (MDA) at 24 hr after SCI. Values are expressed as nanomoles per gram of wet tissue. * $P < 0.001$ versus sham group; ** $P < 0.01$ versus sham and trauma groups; # $P > 0.05$ versus EGCGI group.

Discussion

The main findings of the current study showed that treatment of SCI with EGCG attenuates the MDA levels, as an indicator of lipid peroxidation.

Secondary autodestructive processes of SCI have a highly debilitating pathology, considered to be a number of interrelated processes such as free radical generation (2), lipid peroxidation (1), and apoptosis (14). Lipid peroxidation is an important pathologic event in post traumatic neuronal degeneration, which has been demonstrated to reach peak values immediately after SCI (15). Thus, inhibition of lipid peroxidation is thought to be one of the principal mechanisms of action for therapeutic agents. In this study, malondialdehyde (MDA), which is formed from polyunsaturated fatty acids breakdown was measured as an index of lipid peroxidation. Our results showed that administration of EGCG immediately and 1 hr after SCI significantly attenuated the levels of MDA compared to those of trauma group. The neuroprotective properties of EGCG against neurodegenerative diseases and cerebral ischemia have been well documented (6). One of the neuroprotective mechanisms of EGCG is probably related with its effects on free radical-induced lipid peroxidation. Green tea polyphenols, mainly EGCG, due to the hydroxyl groups can bind to the free radicals and neutralize them (6). Previous studies reported that lipid peroxidation was reduced by EGCG administration after cerebral ischemia in rats (8). Recently, some investigators revealed that malondialdehyde (MDA) levels were decreased after EGCG treatment on aging mice model (11).

Conclusion

In summary, the present study demonstrates the therapeutic benefit of EGCG. EGCG protects the spinal cord from lipid peroxidation after SCI in rat.

Acknowledgment

This work was supported by a grant from the Razi Herbal Medicine Research Center (no. 100185384).

References

1. Amar AP, Levy ML. Pathogenesis and pharmacological strategies for mitigating secondary damage in acute spinal cord injury. *Neurosurgery* 1999; 44:1027-1039.
2. Hall ED. The role of oxygen radicals in traumatic injury: clinical implications. *J Emerg Med* 1993; 11:31-36.
3. Popovich PG, Wei P, Stokes BT. Cellular inflammatory response after spinal cord injury in Sprague-Dawley and Lewis rats. *J Comp Neurol* 1997; 377:443-64.
4. Popovich PG, Stokes BT, Whitacre CC. Concept of autoimmunity following spinal cord injury: possible roles for T lymphocytes in the traumatized central nervous system. *J Neurosci Res* 1996 ; 45:349-63.
5. Mauter AE, Weinzierl MR, Donovan F, Noble LJ. Vascular events after spinal cord injury: contribution to secondary pathogenesis. *Phys Ther* 2000; 80:678-687.
6. Sutherland BA, Rahman RM, Appleton I. Mechanisms of action of green tea catechins, with a focus on ischemia-induced neurodegeneration. *J Nutr Biochem* 2006; 17:291-306.
7. Kimura M, Umegaki K, Kasuya Y, Sugisawa A, Higuchi M. The relation between single/double or repeated tea catechin ingestions and plasma antioxidant activity in humans. *Eur J Clin Nutr* 2002; 56:1186-1193.
8. Choi YB, Kim YI, Lee KS, Kim BS, Kim DJ. Protective effect of epigallocatechin gallate on brain damage after transient middle cerebral artery occlusion in rats. *Brain Res* 2004; 1019:47-54.
9. Wei H, Wu YC, Wen CY, Shieh HY. Green tea polyphenol (-)-epigallocatechin gallate attenuates the neuronal NADPH-d/nNOS expression in the nodose ganglion of acute hypoxic rats. *Brain Res* 2004; 999:73-80.
10. Lin AM, Chyi BY, Wu LY, Hwang LS, Ho LT. The antioxidative property of green tea against iron-induced oxidative stress in rat brain. *Chin J Physiol* 1998; 41:189-194.
11. He M, Zhao L, Wet MJ, Yao WF, Zhao HS, Chen FJ. Neuroprotective effects of (-)-Epigallocatechin-3-gallate on aging mice induced by D- galactose. *Biol Pharm Bull* 2009; 32:55-60.
12. Paterniti I, Genovese T, Crisafulli C, Mazzon E, Dipaola R, Galuppo M, *et al*. Treatment with green tea extract attenuates secondary inflammatory response in an experimental model of spinal cord trauma. *Naunyn Schmiedebergs Arch Pharmacol* 2009; 380:179-192.
13. Mihara S, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal Biochem* 1978; 86:271-278.
14. Liu XZ, Xu XM, Hu R, Du C, Zhang SX, McDonald JW, *et al*. Neuronal and glial apoptosis after traumatic spinal cord injury. *J Neurosci* 1997; 17:5395-406.
15. Kwon BK, Tetzlaff W, Grauer JN, Beiner J, Vaccaro AR. Pathophysiology and pharmacologic treatment of acute spinal cord injury. *Spine J* 2004; 4:451-464.