Neuropharmacology 126 (2017) 213–223

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

A green tea polyphenol epigallocatechin-3-gallate enhances neuroregeneration after spinal cord injury by altering levels of inflammatory cytokines

Lucia Machova Urdzikova [a, *, 1], Jiri Ruzicka [a, b, 1], Kristyna Karova [a, b], Anna Kloudova [a], Barbora Svobodova [a], Anubhav Amin [c], Jana Dubisova [a, b], Meic Schmidt [c], Sarka Kubinova [a], Meena Jhanwar-Uniyal [c], Pavla Jendelova [a, b]

[a] Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Vídeňská 1083, Prague, Czech Republic
[b] Department of Neuroscience, Charles University, Second Faculty of Medicine, Prague, Czech Republic
[c] New York Medical College, New York, USA

ARTICLE INFO

Article history:
Received 7 March 2017
Received in revised form
30 August 2017
Accepted 4 September 2017
Available online 9 September 2017

Keywords:
EGCG
Green tea
Spinal cord injury
Neuroregeneration
Cytokines
Neuroprotection
p65NFkB

ABSTRACT

Spinal cord injury (SCI) is a debilitating condition which is characterized by an extended secondary injury due to the presence of inflammatory local milieu. Epigallocatechin gallate (EGCG) appears to possess strong neuroprotective properties. Here, we evaluated the beneficial effect of EGCG on recovery from SCI. Male Wistar rats were given either EGCG or saline directly to the injured spinal cord and thereafter a daily IP injection. Behavior recovery was monitored by BBB, plantar, rotarod and flat-beam tests. The levels of inflammatory cytokines were determined on days 1, 3, 7, 10 and 14 after SCI. Additionally, NF-κB pathway activity was evaluated. The results demonstrated that EGCG-treated rats displayed a superior behavioral performance in a flat beam test, higher axonal sprouting and positive remodelation of glial scar. Cytokine analysis revealed a reduction in IL-6, IL2, MIP1α and RANTES levels on days 1 and 3, and an upregulation of IL-4, IL-12p70 and TNFα 1 day following SCI in EGCG-treated rats. Treatment with EGCG was effective in decreasing the nuclear translocation of subunit p65 (RelA) of the NF-κB dimer, and therefore canonical NF-κB pathway attenuation. A significant increase in the gene expression of growth factors (FGF2 and VEGF), was noted in the spinal cord of EGCG-treated rats. Further, EGCG influenced expression of M1 and M2 macrophage markers. Our results have demonstrated a therapeutic value of EGCG in SCI, as observed by better behavioral performance measured by flat beam test, modulation of inflammatory cytokines and induction of higher axonal sprouting.

© 2017 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Traumatic spinal cord injury (SCI) is irreversible, with an estimated annual incidence of about 15–40 cases per million (Sekhon and Fehlings, 2001). Much of the morbidity associated with SCI occurs due to the limited intrinsic ability of the spinal cord to recover following compression or contusion. The pathophysiology of SCI has 2 phases, namely primary injury and secondary injury. Primary injury is the consequence of mechanical insult that includes axons, blood vessels, and cell membrane disruption. Secondary injury that follows after primary injury includes secondary development of edema, ischemia, inflammation, local cytokine production, free radical damage, glial scar formation, apoptosis and necrosis. Recovery from SCI is often hindered by the degree of secondary injury. Perilesional astrocytes exhibit a strong reaction, and changes in morphology resulting in the formation of glial scar composed of a dense extracellular matrix, which occurs over the weeks following SCI (Renault-Mihara et al., 2008). Moreover, secreted inflammatory cytokines and growth factors enhance the activation of pro-survival pathways such as the NF-κB pathway

* Corresponding author.
E-mail addresses: urdzikl@saske.sk, urdzikl@gmail.com (L. Machova Urdzikova), j.ruzicka@biomed.cas.cz (J. Ruzicka), karova@biomed.cas.cz (K. Karova), anna.kloudova@gmail.com (A. Kloudova), barbora.svobodova@biomed.cas.cz (B. Svobodova), anubhav.amin@wmchealth.org (A. Amin), jana.dubisova@biomed.cas.cz (J. Dubisova), meic.schmidt@wmchealth.org (M. Schmidt), sarka.k@biomed.cas.cz (S. Kubinova), meena_jhanwar@NYMC.edu (M. Jhanwar-Uniyal), jendel@biomed.cas.cz (P. Jendelova).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.neuropharm.2017.09.006
0028-3908/© 2017 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

associated with c-Rel (Ghosh and Hayden, 2008; Ho et al., 2012). Major obstacles in complete recovery from SCI are, besides others, due to alterations of inflammatory molecules after SCI, therefore understanding therapeutic modalities that stimulate recovery could elucidate the pathophysiology of SCI. Compounds that modulate cytokine levels can be helpful for the recovery from SCI.

Epigallocatechin gallate (EGCG) is a chemical composition of green tea from the family of catechins, which have anti-oxidative/scavenging, anti-inflammatory and anti-apoptotic effects on neural tissue. It has been shown that EGCG attenuates lipid peroxidation, neural apoptosis, spinal tissue loss, motor dysfunction (Khalatbary et al., 2010), and inhibits induction of iNOS (Khalatbary and Ahmadvand, 2011). The neuroprotective effect has been successfully tested on Alzheimer's disease (Walker et al., 2015), Parkinson's disease (Reznichenko et al., 2010), amyotrophic lateral sclerosis (Xu et al., 2006), transient brain ischemia (Park et al., 2010) and in spinal cord injury (Ge et al., 2013).

The objective of this study was to elucidate the effect of EGCG on the levels of inflammatory cytokines, on activation of the NF-κB pathway, and other cellular and molecular mechanisms underlining the neuroprotective effect of EGCG that cause tissue preservation and better behavioral performance after SCI.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (n = 73) weighing 270–300 g were used in this study. Following spinal cord injury rats were randomly divided into EGCG or saline-treated groups. EGCG (Sigma, 50 mg/kg, volume 10 μl diluted in saline) was applied directly to the spinal cord surface immediately after spinal cord surgery, and then weekly for up to 28 days after SCI. The wound was reopened 7, 14 and 28 days in very limited range in comparison with the initial SCI surgery. The volume of 10 μl was applied directly on the spinal surface through laminectomy that was performed within SCI surgery. In the meantime, rats were injected with EGCG intraperitoneally (EGCG, Sigma, 50 mg/kg, volume 30 μl diluted in saline) daily. The control group received saline in the same regimen as the EGCG group. For morphometry, behavioral and immunohistochemical analysis, 12 animals from both groups (n = 24) were sacrificed nine weeks after SCI. For cytokine analysis, 5 animals per group and per timepoint (1, 3, 7,10, and 14 days) were utilized (n = 25). To determine the density of p65 nuclear translocation, 12 animals were used in control groups and 12 animals were used in groups treated with EGCG.

All experiments were performed in accordance with the European Communities Council Directive of 22nd of September 2010 (2010/63/EU) regarding the use of animals in research, and were approved by the Ethics Committee of the Institute of Experimental Medicine ASCR, Prague, Czech Republic. The number of animals was statistically optimized for each particular experiment to achieve their reduction.

2.2. Spinal cord injury

The balloon compression model was used to create SCI according to Vanicky et al. (2001). Briefly, after induction of anesthesia (isoflurane 3.5 vol%, Forane, San Juan, Puerto Rico), a 2 French Fogharty catheter (Edwards Lifesciences, Irvine, CA, USA) was inserted through a small opening in the T10 vertebra cranially, so that the center of the balloon rested at the T8 spinal level. The balloon was inflated with 15 μl volume of saline for 5 min. After removing the balloon, the wound was sutured in anatomical layers. To avoid hypo-hyperthermia (Urdzikova and Vanicky, 2006), the rat's body temperature was kept at 37 °C with a heating pad. The animals developed complete paraplegia for 2–3 days following SCI, with gradual recovery within 9 weeks. Manual bladder expression due to urine retention, and assisted feeding was provided during the recovery phase. The animal received gentamicin sulfate (5 mg/kg, Sandoz, Czech Republic) to prevent post-surgery infection. Animals were kept at a 12 h light/dark cycle with access to water and standard rat chow *ad libitum,* and examined daily for the occurrence of SCI side complications.

2.3. Functional analysis

The flat beam test was used to analyze the complex motor performance of the rats after SCI. The apparatus consists of a 3.4-cm-wide and 140-cm-long wooden rectangular beam with a goal box placed at the end. Only the central 1 meter long part of the beam was selected for the motor performance evaluation. A video-tracking system (TSE-Systems Inc., Bad Homburg, Germany) recorded and evaluated the latency and trajectory of the rat to traverse the beam for a maximum of 60 s. Animals were pre-trained before spinal cord surgery, and then again before each testing. Rats were scored using the modified Goldstein score (Goldstein et al., 1997) on a scale from 0 to 7, starting with no ability to balance and progressing to crossing the whole length of the beam properly using both hindlimbs.

The Basso Beattie and Bresnahan (BBB) test was used to determine hindlimb locomotor performance (Basso et al., 1995). The rats were placed into the circular arena and scored using 0–21 point scale. The BBB score reflects their joint movement, paw placement, weight support, forelimb-hindlimb coordination and trunk stability, 0 reflects no movement and 21 reflects normal performance.

Hindpaw withdrawal latency to a thermal stimulus was evaluated utilizing the specialized Plantar test apparatus (Ugo Basile, Comerio, Italy). Rats were placed into the closed chamber and received thermal stimulus to the hindpaws through a glass plate. The withdrawal latency was measured 5 times for each hindpaw. The average of five values was used for statistical evaluation.

BBB and plantar tests were performed prior to spinal cord surgery and then weekly after SCI.

A Rotarod unit (Ugo Basile, Comerio, Italy) was used to test the motor function and forelimb-hindlimb coordination of the rats. Rats were pre-trained before SCI. For evaluation fix speed (5 rpm) was used, and the latency to fall from the rotating rod was recorded.

The rotarod test and flat beam test require more complex motor abilities of the rats, so the testing procedures started the 3rd week after SCI and then 5, 7 and 9 weeks after surgery.

2.4. Histological and immunohistochemical analysis

For histological and immunohistochemical analyzes, 12 rats from each group surviving 9 weeks, and all rats destined for NF-κB activity analysis were transcardially perfused with 4% paraformaldehyde at the end of a designated end point. Briefly, animals were deeply anesthetized with an overdose of penthobarbital, administrated intraperitoneally; their chests were opened and transcardiac perfusion with phosphate buffer was followed by perfusion with 4% paraformaldehyde in phosphate buffer. Their spines were dissected and left in 4% paraformaldehyde for post-fixation overnight. The next day, 2 cm long spinal cords containing spinal cord lesions were removed from the spinal column for further processing.

For morphometric and immunohistochemical analysis, spinal cords were mounted in paraffin and serially sectioned (5 μm cross-sections, 1 mm interval). Luxol Fast Blue and Cresyl Violet staining were selected to distinguish white and gray matter. To measure the

Download English Version:

https://daneshyari.com/en/article/5548719

Download Persian Version:

https://daneshyari.com/article/5548719

Daneshyari.com