Cornel iridoid glycoside exerts a neuroprotective effect on neuroinflammation in rats with brain injury by inhibiting NF- κ B and STAT3

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Abstract

Traumatic brain injury leads to problems with movement and cognitive deficits and has a high mortality rate. Cornel iridoid glycoside, the active ingredient of *Cornus officinalis*, has been reported to ameliorate apoptosis and inflammation in rats. We investigated the therapeutic effect of cornel iridoid glycoside on neuroinflammation in rats with brain injury. Rats were treated with cornel iridoid glycoside, and then measurements of antioxidant, proinflammatory marker, and NF- κ B and STAT3 mRNA and protein levels, as well as a histopathologic analysis, were conducted. Cornel iridoid glycoside significantly reduced lipid peroxidation and TNF- α and IL-6 levels but increased the levels of antioxidants. In addition, cornel iridoid glycoside significantly decreased the expression of NF- κ B and STAT3. Histopathological analyses showed that cornel iridoid glycoside reduced the severity of neuroinflammation and apoptosis. Therefore, cornel iridoid glycoside significantly ameliorated neuroinflammation by reducing lipid peroxidation and proinflammatory marker levels and increasing antioxidant levels in rats with brain injury.

Keywords Neuroinflammation · Rats · Glycoside · mRNA · Protein

Introduction

Cornel iridoid glycoside is the active ingredient of *Cornus officinalis* (Ma et al. 2018), which is also known as Cornelian cherry, Japanese cornel, or Japanese cornelian cherry. *C. officinalis* is abundant in China, Korea and Japan (Wang et al. 2007). Supplementation with an ethanolic extract of *C. officinalis* ameliorated oxidative stress and liver injury (Ha et al. 2012). Yao et al. (2009) reported that supplementation of cornel iridoid glycoside from *C. officinalis* significantly promoted angiogenesis and neurogenesis in rats with ischemia (Yao et al. 2009). Supplementation with cornel iridoid glycoside also attenuated apoptosis and inflammation in rats with cerebral ischemia (Ya et al. 2010).

Traumatic brain injury leads to difficulty with movement and cognitive deficits and has a high mortality rate (Maas et al. 2008). Traumatic brain injuries are divided into penetrating head injuries and closed head injuries due to external brain damage (Collins and Dean 2002). Neuronal damage, increased oxidative stress, ischemia, inflammation, apoptosis, and excitotoxicity are the primary pathological features of traumatic brain injury (Werner and Engelhard 2007). Expression of nuclear factor kappa-B (NF- κ B) is

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abundant in the brain and constitutively activated in glutamatergic neurons of the central nervous system, such as those in the hippocampus and cerebral cortex (Shih et al. 2015). Signal transducer and activator of transcription (STAT) 3 is a transcription factor of the STAT family that mediates growth factor and cytokine-induced signaling leading to, for example, cell differentiation, proliferation, and apoptosis (Ihle 2001). STAT3-mediated signaling promotes astrocyte differentiation by inhibiting neuronal differentiation in the embryonic cortex (Wen et al. 2009).

Ya et al. (2010) reported that cornel iridoid glycoside supplementation attenuates apoptosis and inflammation in rats with cerebral ischemia. Supplementation with cornel iridoid glycoside protected against traumatic brain injury (Ma et al. 2018) and exerted a beneficial effect on Alzheimer's disease and other neurodegenerative diseases (Lin et al. 2009). In this study, we analyzed the therapeutic effect of cornel iridoid glycoside on neuroinflammation in rats with brain injury.

Materials and methods

Rats

Male albino Wistar rats were purchased from the School of Pharmaceutical Sciences, Health Science Center, Shenzhen University, Shenzhen, Guangdong, China, 518061. The rats weighed 180–210 g and were maintained in standard rat polypropylene cages at 25 ± 0.5 °C and $60 \pm 5\%$ relative humidity under standard atmospheric conditions and a 12/12 h light/dark cycle.

Cornel iridoid glycoside

Cornel iridoid glycoside extracted from the sarcocarp of *C. officinalis* as described previously (Yao et al. 2009) was purchased from Tong Ren Tang Company (Beijing, China). The purity of cornel iridoid glycoside was 79.5% as determined by reverse-phase high-performance liquid chromatography. The cornel iridoid glycoside contained 30.4% oganin and 69.6% morroniside.

Induction of traumatic brain injury

Traumatic brain injury was induced as reported previously (Katano et al. 1999).

Experimental groups and treatments

The rats were divided into the following groups: sham (group I), control (group II), 50 mg/kg cornel iridoid glycoside (group III), and 100 mg/kg cornel iridoid glycoside (group IV).

Assays of biochemical markers

Serum levels of interleukin (IL)-6 and tumor necrosis factor (TNF)- α were determined as described previously (Hend et al. 2014). The levels of lipid peroxidation, catalase, superoxide dismutase (SOD), glutathione peroxidase (Gpx), and reduced glutathione (GSH) were assayed (Kaddour et al. 2016).

Reverse-transcription polymerase chain reaction

RNA was extracted from brain tissue homogenates and converted into cDNA using oligo (dT) primers. NF- κ B and STAT3 mRNA levels were determined by reverse-transcription polymerase chain reaction (Table 1), and the relative expression levels were calculated according to the $2^{-\Delta\Delta CT}$ method (Masatoshi et al. 2001).

Immunofluorescence

Tissue homogenates were treated with anti-NF- κ B (ab16502, Abcam, Cambridge, UK) and anti-STAT3 (ab5073, Abcam) antibodies overnight. Next, the homogenates were treated with a fluorescein isothiocyanate-conjugated goat anti-rat antibody (Abcam, ab6840) for 1 h (Balic et al. 2011). Finally, NF- κ B and STAT3 expression was analyzed under a confocal microscope.

Histopathology

The rats were killed after being anesthetized with ketamine (70 mg/kg) and xylazine (10 mg/kg). The brain tissues

Table 1 List of real-time polymerase chain reaction primers used for the amplification of NF- κ B and STAT3

S. nos	Gene name	Forward primer	Reverse primer
1	NF- κ B	5'-GAAATTCCTGATCCAGACAAAAAC-3'	5'-ATCACTCAATGGCCTCTGTGTAG-3'
2	STAT3	5'-TGGAAGAGGCGGCAGCAGATAGC-3'	5'-CACGGC CCCATTCCCACAT-3'
3	GAPDH	5'-GGTCACCAGGGCTGCTTTT-3'	5'-ATCTCGCTCCTGGAAGATGGT-3'

were surgically removed and transferred to formaldehyde (4%). The hippocampal region was separated from the brain tissues, and sections (5 μ m thick) were cut using a rotary microtome. Finally, the sections were stained with hematoxylin and eosin and analyzed under a microscope (Muthuviveganandavel et al. 2008).

Statistical analyses

Data are presented as means with standard deviations. The data were compared by analysis of variance or Tukey's post hoc test. Differences were considered significant at $P < 0.05$.

Results

We investigated the therapeutic impact of cornel iridoid glycoside on neuroinflammation in rats with brain injury. The levels of TNF- α and IL-6 (7.5 and 8.7 U/mL) were substantially increased in the rats in group II compared to sham rats. However, cornel iridoid glycoside reduced the TNF- α and IL-6 levels to their normal ranges (Figs. 1, 2, $P < 0.05$). The altered levels of lipid peroxidation, SOD, GSH, Gpx, and catalase also returned to their normal ranges after treatment with cornel iridoid glycoside (Table 2). The MDA content was increased by 1.23 nmol/mL in control rats compared to sham rats, but was reduced $> 50\%$ by cornel iridoid glycoside treatment (Table 2, $P < 0.05$). In the control rats, the catalase, SOD, Gpx, and GSH levels were reduced by 83%, 75.5%, 72.7%, and 64.7%, respectively, compared to sham

rats ($P < 0.05$, Table 2). However, cornel iridoid glycoside returned these levels to within the normal ranges (Table 2, $P < 0.05$).

The NF- κ B and STAT3 mRNA levels were increased 1.5- and 1.3-fold in the control group compared to sham rats, respectively. However, cornel iridoid glycoside significantly reduced the NF- κ B mRNA level 0.24- and 0.46-fold (Fig. 4, $P < 0.05$) and the STAT3 mRNA level 0.16- and 0.45-fold (Fig. 3, $P < 0.05$) in groups III and IV, respectively. The NF- κ B and STAT3 protein levels were increased 1.2- and 1.1-fold in the control group, respectively. However, cornel iridoid glycoside significantly reduced the NF- κ B protein level 0.16- and 0.43-fold (Fig. 4, $P < 0.05$) and the STAT3 protein level 0.18- and 0.44-fold (Fig. 5, $P < 0.05$) in groups III and IV, respectively. Histopathological analysis revealed altered cellular morphology, inflammation, and apoptosis in the control rats, whereas cornel iridoid glycoside significantly reduced the apoptosis and inflammation (Fig. 6).

Discussion

We investigated the therapeutic effect of cornel iridoid glycoside on neuroinflammation in rats with brain injury. Cornel iridoid glycoside protected against traumatic brain injury (Ma et al. 2018) and exerted a beneficial effect on Alzheimer's disease and other neurodegenerative diseases (Lin et al. 2009). Supplementation with an ethanolic extract of *C. officinalis* ameliorated oxidative stress and liver injury (Ha et al. 2012). Yao et al. (2009) reported that cornel

Fig. 1 Effect of cornel iridoid glycoside treatment on the serum TNF- α level in rats with brain injury. * $P < 0.05$ versus group I and # $P < 0.05$ versus group II (brain injury)

Fig. 2 Protective effect of cornel iridoid glycoside treatment on the serum IL-6 level in rats with brain injury. * $P < 0.05$ versus group I and # $P < 0.05$ versus group II (brain injury)

Table 2 Effect of cornel iridoid glycoside supplementation on biochemical markers against neuroinflammation in brain injury induced rats

Parameters	Group I (sham)	Group II (control)	Group III (50 mg/kg of cornel iridoid glycoside)	Group IV (100 mg/kg of cornel iridoid glycoside)
MDA (nmol/ml)	0.31 ± 0.01	1.23 ± 0.1*	0.93 ± 0.05 [#]	0.48 ± 0.04 [#]
Catalase (U/ml)	15.3 ± 0.8	2.6 ± 0.11*	6.9 ± 0.33 [#]	13.6 ± 0.41 [#]
SOD (U/ml)	309.7 ± 18	75.8 ± 5.5*	149.5 ± 11.7 [#]	289.8 ± 14.5 [#]
Gpx (U/ml)	0.55 ± 0.02	0.15 ± 0.01*	0.28 ± 0.01 [#]	0.47 ± 0.03 [#]
GSH (nmol/ml)	0.51 ± 0.03	0.18 ± 0.01*	0.24 ± 0.03 [#]	0.44 ± 0.03 [#]

* $P < 0.05$

[#] $P < 0.05$

iridoid glycoside from *C. officinalis* significantly promoted angiogenesis and neurogenesis in rats with ischemia (Yao et al. 2009).

Cornel iridoid glycoside attenuated apoptosis and inflammation in rats with cerebral ischemia (Ya et al. 2010) and reduced the levels of proinflammatory markers in microglia (Zhao et al. 2019). Jiang et al. (2014) reported that cornel iridoid glycoside exerted a protective effect on D-galactosamine/TNF- α -injured L02 hepatocytes. Furthermore, retreatment with cornel iridoid glycoside reduced the IL-1 β and TNF- α levels in the brains of rats with focal cerebral ischemia (Ya et al. 2010). In our study, cornel iridoid glycoside significantly decreased the levels of TNF- α and IL-6 in rats with brain injury.

Cornel iridoid glycoside reduced lipid peroxidation and increased the GSH level in a mouse model of generalized tonic-clonic seizures (Taiwe et al. 2016). In this study, cornel iridoid glycoside significantly reduced lipid peroxidation

and increased the levels of catalase, SOD, Gpx, and GSH. Glial cells play a vital role in the pathophysiology of neuroinflammation in the brain. Normal activation of microglial cells is essential for scavenging exogenous substances and necrotic debris (Yu et al. 2013). However, over-activated microglial cells exhibit activation of proinflammatory mediators and proinflammatory transcription factors (Lakhan et al. 2009; Yi et al. 2007).

Therapeutic effects of cornel iridoid glycoside in rats with traumatic brain injury have been reported (Ma et al. 2018). Cornel iridoid glycoside promoted angiogenesis and neurogenesis and improved neurological function in rats with focal cerebral ischemia (Yao et al. 2009), and improved locomotor impairment, reduced tissue damage, and downregulated the myelin-associated inhibition signaling pathway in rats with spinal cord injury (Wen-jing et al. 2016). Yi et al. (2007) reported that NF- κ B and STAT3 activation plays a crucial role in neuroinflammatory events and cerebral injury by inhibiting

Fig. 3 Cornel iridoid glycoside treatment reduced the NF- κ B and STAT3 mRNA levels in rats with brain injury. * $P < 0.05$ versus group I and # $P < 0.05$ versus group II (brain injury)

Fig. 4 Cornel iridoid glycoside treatment reduced the NF- κ B protein level in rats with brain injury. * $P < 0.05$ versus group I and # $P < 0.05$ versus group II (brain injury). Scale bar = 100 μ m

Fig. 5 Cornel iridoid glycoside treatment reduced the STAT3 protein level in rats with brain injury. * $P < 0.05$ versus group I and # $P < 0.05$ versus group II (brain injury). Scale bar = 100 μ m

Fig. 6 Histopathological analysis of a brain section following cornel iridoid glycoside treatment in rats with brain injury. H&E hematoxylin and eosin staining

the production of proinflammatory factors. Cornel iridoid glycoside significantly inhibited phosphorylation of STAT3 during microglial activation (Zhao et al. 2019). STAT3 binds to DNA, inducing the expression of genes responsible for neuroinflammation (Satriotomo et al. 2006; Suzuki et al. 2001). Li et al. (2005) reported that an extract from *C. officinalis* significantly reduced the NF- κ B level in the cortex of rats with cerebral infarction. Yin et al. (2014) indicated that cornel iridoid glycoside inhibited JAK/STAT1/3 signaling in the brain and reduced the levels of proinflammatory cytokines in rats with autoimmune encephalomyelitis. Inconsistent with previous findings, we found that cornel iridoid glycoside significantly inhibited the expression of NF- κ B and STAT3. This is the first report of the effect of cornel iridoid glycoside on markers of oxidation and inflammation, as well as NF- κ B and STAT3, in rats with traumatic brain injury.

Conclusion

Cornel iridoid glycoside treatment significantly ameliorated neuroinflammation by reducing lipid peroxidation and the levels of proinflammatory mediators, and by increasing the

levels of antioxidants, in rats with brain injury. In addition, cornel iridoid glycoside significantly inhibited the expression of NF- κ B/STAT3. No mortalities occurred at any of the cornel iridoid glycoside doses evaluated (up to 100 mg/kg). Therefore, cornel iridoid glycoside at these doses may be considered safe.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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