Journal of Neuroimmunology 284 (2015) 67–73

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Total glucosides of peony attenuates experimental autoimmune encephalomyelitis in C57BL/6 mice

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ARTICLE INFO

Article history:
Received 5 February 2015
Received in revised form 9 May 2015
Accepted 11 May 2015

Keywords:
Total glucosides of peony
Experimental autoimmune encephalomyelitis
Th1/Th17
Th2/Treg
Brain-derived neurotrophic factor
2′,3′-cyclic nucleotide 3′-phosphodiesterase

ABSTRACT

Total glucosides of peony (TGP), an active compound extracted from the roots of *Paeonia lactiflora* Pall, has wide pharmacological effects on nervous system. Here we examined the effects of TGP on experimental autoimmune encephalomyelitis (EAE), an established model of multiple sclerosis (MS). The results showed that TGP can reduce the severity and progression of EAE in C57 BL/6 mice. In addition, TGP also down-regulated the Th1/Th17 inflammatory response and prevented the reduced expression of brain-derived neurotrophic factor and 2′,3′-cyclic nucleotide 3′-phosphodiesterase of EAE. These findings suggest that TGP could be a potential therapeutic agent for MS.

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease that leads to degeneration of the brain and spinal tissue which causes neurological disability in young adults (Minagar, 2013). There are many approved therapies for MS such as the beta-interferons, glatiramer acetate, fingolimod, teriflunomide and dimethyl fumarate (Cross and Naismith, 2014). However, the current treatments for MS have several shortages, such as expensive price, subcutaneous injection, limited efficacy, and occasionally irritation/infection injection site (Kieseier et al., 2007). Therefore, more effective immunomodulatory and neuroprotective agents, with good toleration and favorable safety profile, are required.

Total glucosides of peony (TGP) is an active compound extracted from the roots of *Paeonia lactiflora* Pall. It is a valuable traditional Chinese herbal medicine without evident toxic or side effects which has been used in the treatment of rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and hepatitis (Liu et al., 2006; Lin et al., 2012; Zhao et al., 2012). TGP can inhibit dendritic cell (DC) maturation, and reduce the production of interleukin-12 (IL-12) and IL-6 thus led to the impairment of the Th1 and Th17 differentiation in collagen induced arthritis mice (Lin et al., 2012). TGP also can activate the transcription factor Foxp3 and increase both the proportion and the number of CD4+CD25 + Treg cells in lupus CD4 + T cells (Zhao et al., 2012). And paeoniflorin (PF), the main active component of TGP, can ameliorate immunological liver injury through modulating inflammatory cytokines (tumor necrosis factor-alpha (TNF-α) and IL-6) and lipopolysaccharide receptor (lipopolysaccharide binding protein and CD14) expression (Liu et al., 2006).

Recent studies demonstrated that TGP/PF also has wide pharmacological effects in central nervous system (CNS) and the potential therapeutic applications in CNS diseases. PF can protect against ischemia-induced brain damages in rats via inhibiting mitogen-activated protein kinases/nuclear factor-κB-mediated inflammatory responses (Guo et al., 2012) and attenuate ischemic neuronal damage in vitro via adenosine A_1 receptor-mediated transactivation of epidermal growth factor receptor (Zhong et al., 2015). PF also ameliorates the spatial learning and memory deficits by attenuating oxidative stress and regulating

Abbreviations: TGP, total glucosides of peony; MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; CNS, central nervous system; MOG_{35-55}, myelin oligodendrocyte glycoprotein 35–55 amino acid peptide; IFN-γ, interferon gamma; IL-4, interleukin-4; IL-10, interleukin-10; IL-17, interleukin-17; BDNF, brain-derived neurotrophic factor; CNPase, 2′,3′-cyclic nucleotide 3′-phosphodiesterase; T-bet, T-box expressed in T cells; RORγt, retinoid-related orphan nuclear receptor γt; GATA3, GATA-binding protein 3; Foxp3, forkhead box protein 3; PTX, Pertussis toxin; CFA, Complete Freund's adjuvant; PBS, phosphate buffered saline; ELISA, enzyme-linked immunosorbent assay; qRT-PCR, quantificational reverse transcription and polymerase chain reaction; ANOVA, analysis of variance; p.i, post immunization.

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http://dx.doi.org/10.1016/j.jneuroim.2015.05.009
0165-5728/

the nerve growth factor-mediated signaling to reinforce cholinergic functions in the hippocampus of the $A\beta_{1-42}$-treated rats (Lan et al., 2013) and protects PC12 cells against $A\beta_{25-35}$-induced neurotoxicity by preventing mitochondrial dysfunction (Li et al., 2014). Moreover, TGP has its antidepressant-like activity by attenuation of oxidative stress, modulation of the hypothalamic–pituitary–adrenal axis and up-regulation of serotonergic and noradrenergic systems (Mao et al., 2009; Qiu et al., 2013). However, there are no reports about the effects of TGP against CNS immune inflammatory diseases, such as MS.

Here, the effects and mechanisms of TGP on C57 BL/6 experimental autoimmune encephalomyelitis (EAE) mice, an animal model for MS, were examined in this study.

2. Materials and methods

2.1. Animals and reagents

Six- to eight-week-old female C57 BL/6 mice weighing 16–18 g were obtained from the Experimental Animal Center of Sun Yat-Sen University (Guangzhou, China). Experiments were carried out according to the National Institutes of Health Guide for the care and use of laboratory animals and were approved by the Bioethics Committee of Sun Yat-Sen University. TGP (purity of paeoniflorin beyond 90%) was obtained from Nanjing Zelang Medical Technology (Nanjing, China). Myelin oligodendrocyte glycoprotein 35–55 amino acid peptide (MOG_{35-55}) (MEVGWYRSPFSRVVHLYRNGK) was synthesized by CL BioScientific (Xi'an, China). Amino acid sequences were confirmed by amino acid analysis and mass spectroscopy. The purity of the peptide was greater than 95%. Mycobacterium tuberculosis H37RA was purchased from Difco (Detroit, MI). Pertussis toxin (PTX) was purchased from Alexis (San Diego, CA). Complete Freund's adjuvant (CFA) was purchased from Sigma-Aldrich (St. Louis, MO). Polyclonal anti-brain-derived neurotrophic factor (BDNF) was purchased from Abcam (Cambridge, MA). The cytokine assay by enzyme-linked immunosorbent assay (ELISA) kits for interferon-gamma (IFN-γ), IL-17, IL-4 and IL-10 was purchased from RayBiotech (Norcross, GA). The polyclonal anti-2′, 3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) was purchased from Cell Signaling Technology (Danvers, MA).

2.2. Induction and assessment of EAE

The procedure used for the induction of EAE has been described previously (Chen et al., 2009). Briefly, mice received a subcutaneous injection in the flanks with 200 μg MOG_{35-55} peptide emulsified in CFA containing 500 μg mycobacterium tuberculosis H37RA. Immediately thereafter and again 48 h later, the mice received an intraperitoneal injection of 300 ng PTX in 100 μl phosphate-buffered saline (PBS). An additional injection of MOG_{35-55} peptide in CFA was delivered 7 days later. The animals were examined daily for disability. Clinical scores were defined as follows: 0, no signs; 1, loss of tail tonicity; 2, flaccid tail; 3, ataxia and/or paresis of hindlimbs; 4, complete paralysis of hindlimbs; 5, moribund or death.

2.3. Dose-finding experiments and treatment of mice

Mice were randomly assigned to three groups: control mice, vehicle-treated EAE mice, and TGP-treated EAE mice ($n = 7$). The dose of TGP was chosen on the basis of previous data *in vivo* (Zheng and Wei, 2005; Xu et al., 2007) and our preliminary dose-finding experiment. In our dose-finding experiment, TGP was suspended in 0.5% sodium carboxymethylcellulose. TGP was administered intragastrically at dosages of 50,100 and 200 mg/kg/day, respectively ($n = 7$). Treatment with TGP was initiated when the first mouse showed neurological signs (at the day 12 post immunization, p.i.), and medication was administered daily until mice were sacrificed at day 35 p.i.Vehicle-treated EAE mice were treated with intragastric administration of 0.5% sodium carboxymethylcellulose only.

2.4. Histological evaluation

To assess the degree of inflammation and demyelination in CNS, the vehicle- and TGP-treated groups were anesthetized and perfused with ice-cold PBS, followed by 4% paraformaldehyde from the left ventricle at day 21 p.i. ($n = 6$). Spinal cords were removed. Tissues were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin for revealing inflammatory infiltration. Solochrome cyanine technique was used for myelin staining. Histopathological examination was performed in a blinded fashion. The scale used to evaluate for inflammation was as follows (O Neill et al., 2006): 0, no inflammatory cells; 1, a few scattered inflammatory cells; 2, organization of inflammatory infiltrates around blood vessels; and 3, extensive perivascular cuffing with extension into adjacent parenchyma or parenchymal infiltration without obvious cuffing. Demyelination in the spinal cords was scored as previously described (Kuerten et al., 2007): 1, traces of subpial demyelination; 2, marked subpial and perivascular demyelination; 3, confluent perivascular or subpial demyelination; 4, massive perivascular and subpial demyelination involving one half of the spinal cord with presence of cellular infiltrates in the CNS parenchyma; 5, extensive perivascular and subpial demyelination involving the whole cord section with presence of cellular infiltrates in the CNS parenchyma.

2.5. ELISA assay

Spleens of different treated mice were aseptically harvested at day 21 p.i. Splenocytes (5×10^5 cells/well) from each group were incubated in 96-well flat-bottom plates in RPMI 1640 supplemented with 10% fetal calf serum, with MOG_{35-55}(12 μg/ml) used for the immunization. Culture supernatants were collected at 48 h and stored at −80 °C for assaying IFN-γ, IL-17, IL-4 and IL-10 according to the ELISA kit manual (Zhu et al., 2014).

2.6. RNA isolation and qRT-PCR

First-strand cDNA synthesis was performed with reverse transcription system (Promega).Transcript levels of the genes were detected by quantitative RT-PCR using the ABI7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with SYBR Green qPCR SuperMix (Invitrogen). The primer sequences synthesized by GENEWIZ (Suzhou, China) were as follows: T-box expressed in T cells (T-bet) sense, 5′-CGGTACCAGAGCGGCAAGT-3′, and antisense, 5′-CATGCTGCCTTCTG CCTTTC-3′; retinoic acid receptor-related orphan receptor (RORγt) sense, 5′-GCGGAGCAGACACACTTACA-3′ and antisense, 5′-TTGGCAAA CTCCACCACATA-3′; GATA-binding protein 3 (GATA-3) sense, 5′-TACT TGCGTTTTTCGCAGGA-3′, and antisense, 5′-GATCTGTCGCTTTCGGGC CT-3′; forkhead box protein (Foxp3) sense, 5′-CAGCTGCCTACAGTGC CCCTA-3′, and antisense 5′-CATTTGCCAGCAGTGGGTAG-3′; 18srRNA sense, 5′-CCTGGATACCGCAGCTAGGA-3′ and antisense, 5′-GCGGCGCA ATACGAATGCCCC-3′. The amplifications were performed in a final volume of 20 μl, containing 5 μl of the diluted cDNA template, and 10 μl of pcr-gradewater. The following program was employed for real-time PCR: 50 °C for 2 min, 95 °C for 2 min, 40 cycles of 95 °C for 15 s and 60 °C for 32 s. The mRNA expression levels of the target genes were normalized to the 18srRNA mRNA levels, and the results were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.7. Western blot analysis

To investigate the expressions of BDNF and CNPase in the lumbar spinal cord of different treated mice, we performed Western blot analysis as described previously (Jiang et al., 2013). Samples from different treated mice were loaded on 10% gradient sodium dodecyl sulfatepolyacrylamide