Neuroprotective Effects of 6-Shogaol and Its Metabolite, 6-Paradol, in a Mouse Model of Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is an autoimmune disease characterized by progressive neuronal loss, neuroinflammation, axonal degeneration, and demyelination. Previous studies have reported that 6-shogaol, a major constituent of ginger (*Zingiber officinale* rhizome), and its biological metabolite, 6-paradol, have anti-inflammatory and anti-oxidative properties in the central nervous system (CNS). In the present study, we investigated whether 6-shogaol and 6-paradol could ameliorate against experimental autoimmune encephalomyelitis (EAE), a mouse model of MS elicited by myelin oligodendrocyte glycoprotein (MOG$_{35-55}$) peptide immunization with injection of pertussis toxin. Once-daily administration of 6-shogaol and 6-paradol (5 mg/kg/day, *p.o.*) to symptomatic EAE mice significantly alleviated clinical signs of the disease along with remyelination and reduced cell accumulation in the white matter of spinal cord. Administration of 6-shogaol and 6-paradol into EAE mice markedly reduced astrogliosis and microglial activation as key features of immune responses inside the CNS. Furthermore, administration of these two molecules significantly suppressed expression level of tumor necrosis factor-α, a major proinflammatory cytokine, in EAE spinal cord. Collectively, these results demonstrate therapeutic efficacy of 6-shogaol or 6-paradol for EAE by reducing neuroinflammatory responses, further indicating the therapeutic potential of these two active ingredients of ginger for MS.

Key Words: Multiple sclerosis, Experimental autoimmune encephalomyelitis, 6-Shogaol, 6-Paradol, Astrogliosis, Microglia

INTRODUCTION

Multiple sclerosis (MS) is a chronic neuroinflammatory disease of the brain and spinal cord in which focal lymphocytic infiltration leads to damage of myelin and axons (Compston and Coles, 2008). Experimental autoimmune encephalomyelitis (EAE) has been developed as a mouse model to investigate clinical, immunological, and histopathological features of MS (Lassmann and Bradl, 2017). Accumulating evidence suggests that both MS and EAE are processed by infiltrated immune cells (especially activated T cells) across the blood-brain barrier (BBB) to promote neuroinflammation, demyelination, gliosis, and axonal degeneration (Frischer *et al*., 2009; Goverman, 2009). The key morphological feature of MS and EAE is primary demyelination of nerve axons which results in a blockade of signal conduction or a decline of conduction at the site of demyelination (Fletcher *et al*., 2010). In addition, activated microglia and astrocytes can induce neuroinflamma-

tion, axonal damage, and demyelination through increasing the production of proinflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor-α (TNF-α) (Raivich and Banati, 2004; Wang *et al*., 2005; Lee *et al*., 2018). Therefore, all approved medications for MS exert anti-inflammatory properties, and they are more effective when applied at the early phases of disease development (Hauser *et al*., 2013; Torkildsen *et al*., 2016).

Ginger (*Zingiber officinale* rhizome) has been commonly used as a traditional medicine for food ingredient as well as a medicinal herb for common cold, nausea, asthma, cough, bleeding, and muscle pain (Mascolo *et al*., 1989). Previous studies have reported that ginger has anti-inflammatory, anti-oxidative, and neuroprotective activities in the central nervous system (CNS) (Choi *et al*., 2018). In particular, administration of ginger extract prior to EAE onset can reduce clinical symptoms of EAE via regulation of inflammatory cytokines and chemokines such as IL-17, IL-33, IFN-γ, CCL20, and CCL22

(Jafarzadeh *et al*., 2014, 2017). Interestingly, 6-shogaol [1-(4-hydroxy-methoxyphenyl)-4-decen-one] is a major active ingredient in dried ginger and 6-paradol [1-(4-hydroxy-methoxyphenyl)-3decen-one] is produced from 6-shogaol through biotransformation process (Dugasani *et al*., 2010; Ok and Jeong, 2012). Neuroprotective effects of ginger extract seem to be due to pharmacological actions of both 6-shogaol and 6-paradol because they exert anti-neuroinflammatory and anti-oxidative activities in the CNS. It has been shown that 6-shogaol can suppress the activation of glial cells, such as astrogliosis and microglial activation, and the expression of proinflammatory cytokines in both *in vitro* and *in vivo* models of transient global ischemia and Parkinson's disease (Ha *et al*., 2012; Park *et al*., 2013). It also protects against secondary damage and motor dysfunction in traumatic spinal cord injury in rats (Kyung *et al*., 2006) and lipopolysaccharide (LPS)-induced toxicity in astrocyte (Shim *et al*., 2012). Additionally, 6-paradol possesses anti-oxidant and anti-inflammatory activities, similar to 6-shogaol (Nagendra chari *et al*., 2013). We have previously demonstrated that 6-paradol can suppress the expression of proinflammatory cytokines in LPS-treated BV2 microglia and reduce brain damage in transient focal cerebral ischemia by inhibiting microglial activation (Gaire *et al*., 2015).

Accumulating findings on CNS diseases implicate that 6-shogaol and/or 6-paradol might be effective for other types of CNS diseases including MS based on their anti-inflammatory properties. In fact, it has been found that ginger extract can prevent disease onset in EAE mouse model (Jafarzadeh *et al*., 2014). However, it is currently unknown whether 6-shogaol and 6-paradol are effective for EAE. Furthermore, no study has reported whether these two active ingredients of ginger are effective for EAE as therapeutic regimen. In the current study, we determined whether 6-shogaol and 6-paradol were therapeutically effective in a mouse EAE model. We also assessed their effects on histopathological sequelae, including demyelination and increased cell density in injured spinal cord. In particular, we determined whether these ingredients reduced glial activation such as astrogliosis and microglial activation. In addition, we examined the link between their neuroprotective effects and suppression of TNF-α expression in EAE spinal cord because they could suppress its expression in other neuroinflammatory diseases such as cerebral ischemia (Gaire *et al*., 2015).

MATERIALS AND METHODS

Mice

Female C57BL/6 mice (7 weeks old) were purchased from Orient Co., Ltd. (Seongnam, Korea) and acclimated for a week under pathogen-free conditions with freely access to water and food on a 12-hour light/dark cycle. All experiments were performed under controlled conditions at temperature of 22°C ± 2°C and relative humidity of 60 ± 10% according to the Center of Animal Care and Use (CACU) guidelines of Lee Gil Ya Cancer and Diabetes Institute (LCDI) at Gachon University (approved animal protocol number: LCDI-2015-0028).

Materials

6-Shogaol and 6-paradol were provided by Dr. Dong Yoon Shin (Gachon University). A recombinant myelin oligoden-
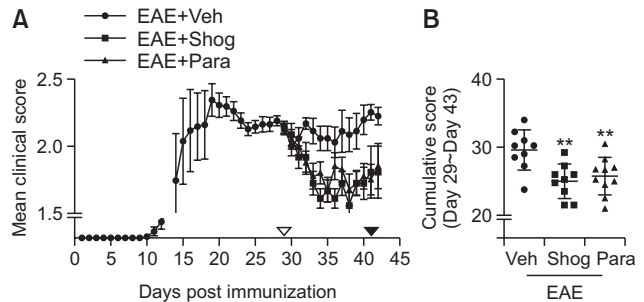

Fig. 1. Administration of 6-shogaol or 6-paradol improves clinical symptoms of EAE. 6-Shogaol (Shog, 5 mg/kg), 6-paradol (Para, 5 mg/kg), or vehicle (Veh) was orally given daily into symptomatic EAE mice, from days 29 to 42 after immunization. On day 43, the experiment was completed. (A) Mean clinical score of each group. ▽ or ▼ indicate the start or stop of a daily drug administration. Clinical scores of experimental groups were statistically analyzed by Mann-Whitney's test and actual *p* values were documented (Supplementary Table 1). (B) Cumulative score of each mouse was calculated as the sum of all scores from days 30 to 43 in each mouse. The mean value was then obtained for each group. n=9-10 per group. **$p<0.01$ vs. the vehicle-treated group (Newman-Keuls multiple range test).

drocyte glycoprotein (MOG$_{35-55}$, MEVGWYRSPFSRVVH-LYRNGK) was purchased from Peptron (Daejeon, Republic of Korea). Complete Freund's adjuvant (CFA) containing *Mycobacterium tuberculosis* H-37 RA (5 mg/mL) was purchased from Chondrex (Redmond, WA, USA). *Bordetella pertussis* toxin was purchased from List Biological Laboratories (Campbell, CA, USA). FluoroMyelin was purchased from Invitrogen (Waltham, MA, USA). Primary antibodies for GFAP and Iba1 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Primary antibody for TNF-α was purchased from Abcam (Cambridge, MA, USA). Alexa-Fluor® 488 secondary antibody was purchased from Jackson ImmunoResearch (West Grove, PA, USA). All other materials were of the highest grade available. They were obtained from normal commercial sources.

EAE induction and treatment

Mice were immunized with MOG$_{35-55}$ as described previously (Lee *et al*., 2018). Briefly, MOG$_{35-55}$ was emulsified in an equal amount of CFA. Mice were anesthetized with isoflurane and 200 μg of emulsion MOG$_{35-55}$ in CFA was injected subcutaneously at the start day of immunization (day 1). In addition, 400 ng of *Bordetella pertussis* toxin (PTX) per mouse was injected intraperitoneally on the start day of MOG immunization and 2 days later. Mice were weighed and monitored daily for clinical symptoms of EAE as follows: 0, no clinical signs of EAE; 0.5, some lack of tone, however, some strength at the base of tail; 1.0, total loss of tail tonicity and flaccid tail; 2.0, hind limb weakness; 2.5, incomplete paralysis of one or both hind limbs; 3.0, total paralysis of one or both hind limbs; 4.0, hind and fore limbs paralysis; 5.0, death from disease (Lee *et al*., 2018). For drug administration, symptomatic EAE mice were divided into three groups; (1) the vehicle-treated EAE group, (2) the 6-shogaol-treated EAE group, and (3) the 6-paradol-treated EAE group. Vehicle (10% Tween 80), 6-shogaol (5 mg/kg), or 6-paradol (5 mg/kg) was orally administered daily into symptomatic EAE mice from day 29 to day 42 (for 13 days) post immunization. Dosage of 6-shogaol and 6-paradol was
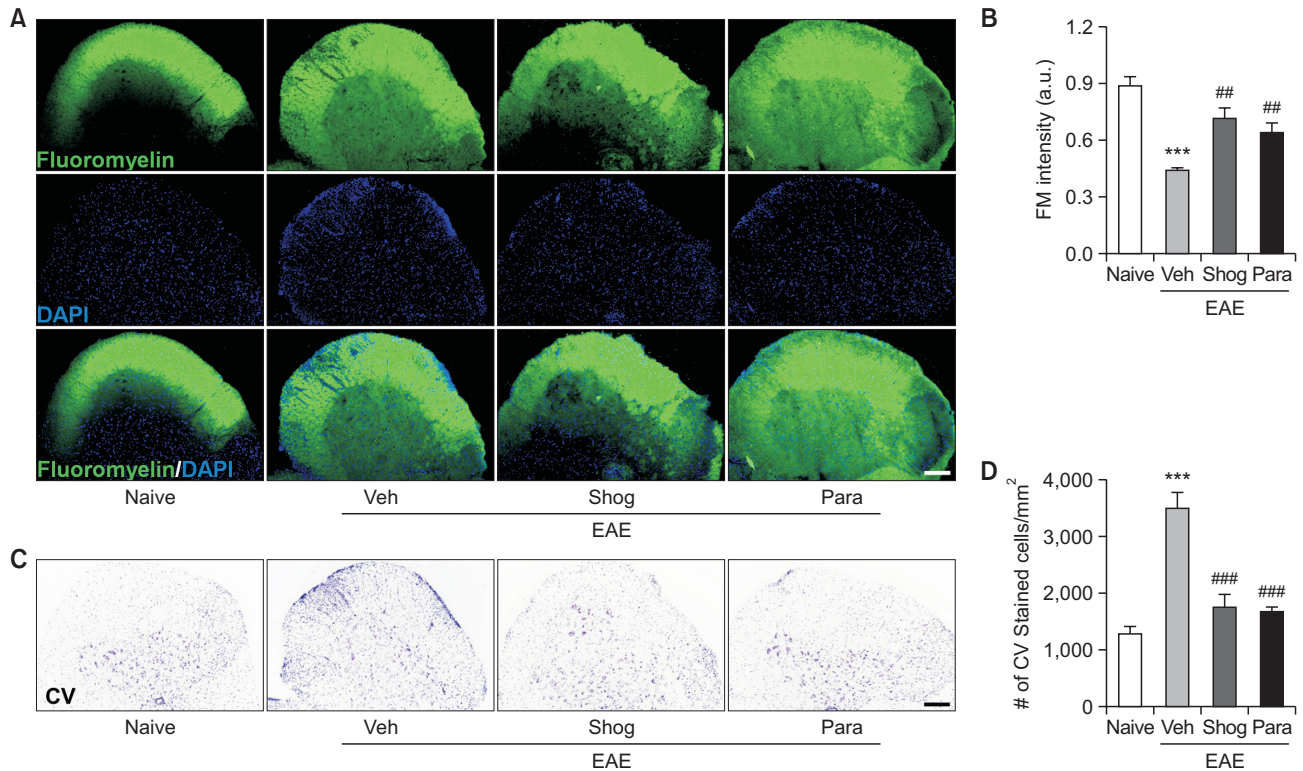
Fig. 2. Administration of 6-shogaol or 6-paradol reduces demyelination and cell accumulation in spinal cords of EAE mice. (A, B) Effects of 6-shogaol (Shog) and 6-paradol (Para) on demyelination were examined by FluoroMyelin staining (green). Sections were counterstained with DAPI (blue). Representative images of FluoroMyelin-stained lumbar spinal cord of each group (A) and quantification of FluoroMyelin intensity (B; a.u., artificial unit). (C, D) Effects of 6-shogaol and 6-paradol on increased cell density were examined by cresyl violet (CV) staining. Representative images of CV-stained lumbar spinal cord of each group (C) and quantification by counting the number of CV-stained cells (D). Scale bars, 100 μm. n=4 per group. ***$p<0.001$ vs. naïve. ##$p<0.01$ and ###$p<0.001$ vs. the vehicle-treated group. Newman-Keuls multiple range test.

set according to a previous report (Gaire *et al.*, 2015).

Tissue preparation

At the end of experiments, spinal cord was quickly removed from each mouse after CO_2 exposure. Removed spinal cord was cut into 5-6 pieces, embedded in Tissue-Tek Optimal Cutting Temperature (OCT) compound, and frozen using powdered dry ice. For histological analyses, frozen spinal cord samples were cut into 20 μm sections on a cryostat (J4800AMNZ, Thermo, Dreieich, Germany) and tissue sections were fixed in 4% paraformaldehyde (PFA) for 10 min.

Histological and immunohistochemical analysis

Cresyl violet staining was performed to determine increased cell density. Sections were blocked with chloroform/ethanol (1:1), rehydrated, stained with 0.5% cresyl violet acetate, dehydrated with increasing ethanol series and xylene, and mounted with Entellan medium.

FluoroMyelin staining was carried out to determine demyelination and remyelination. Sections were stained with FluoroMyelin solution (1:300) for 2 h, labeled with 4′,6-diamidino-2-phenylindole (DAPI) solution (1:10,000), and mounted with VECTASHIELD® mounting media.

For immunohistochemical study, sections were blocked with 1% fetal bovine serum containing 0.5% Triton X-100 and incubated with primary antibodies against glial fibrillary acidic protein (GFAP, 1:500, for astrogliosis), ionized calcium-binding adapter molecule 1 (Iba1, 1:500, for microglial activation), or TNF-α (1:100) overnight at 4°C. In case of signal development with 3, 3′-diaminobenzidinetetrahydrochloride hydrate (DAB) staining, sections were treated with H_2O_2 prior to blocking. To develop GFAP signals, sections were incubated with Alexa Fluor 488 donkey anti-rabbit (1:1000) as a secondary antibody, counterstained with DAPI, and mounted with VECTASHIELD® mounting media. To develop signals for Iba1, sections were labeled with secondary anti-rabbit antibody (1:200) and further labeled with avidin/biotin complex (1:100). Sections were developed using 0.02% DAB solution containing 0.01% H_2O_2, rinsed with PBS, dehydrated with ethanol, kept in xylene, and mounted with Entellan medium. To detect TNF-α in spinal cords, sections were exposed to Tris-EDTA solution at 90 to 100°C for 30 min before H_2O_2 exposure and labeled with a primary antibody for TNF-α (1:100) followed by labeling with secondary anti-rabbit antibody (1:200) and avidin/biotin complex. Signals were developed with DAB.

Colored images were collected using DP72 camera (Olympus Co., Tokyo, Japan). Fluorescent images were obtained with a laser scanning confocal microscope (Eclipse A1 Plus, Nikon, Tokyo, Japan). Quantification of FluoroMyelin and GFAP staining was carried out by measuring fluorescence intensity which was quantified using ImageJ program (National Institute of Mental Health, Bethesda, MD, USA). Quantifica-
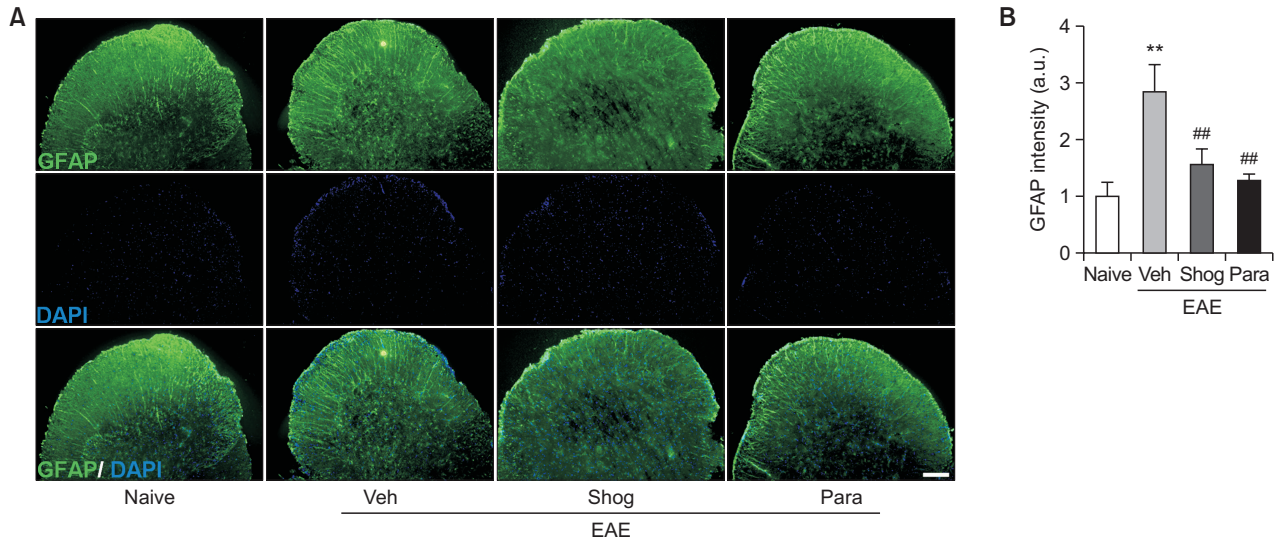
Fig. 3. Administration of 6-shogaol or 6-paradol reduces astrogliosis in spinal cords of EAE mice. Effects of 6-shogaol (Shog) and 6-paradol (Para) on astrogliosis were examined by GFAP immunolabeling (green). Sections were counterstained with DAPI (blue). (A) Representative images of GFAP immunoreactivity in the lumbar spinal cord of each group. (B) Quantification of fluorescence intensity (a.u., artificial unit). Scale bar, 100 μm. n=4 per group. **$p<0.01$ and ##$p<0.01$ vs. naïve and the vehicle-treated group, respectively, by Newman-Keuls multiple range test.

tion of CV staining, Iba1 immunohistochemistry, and TNF-α immunohistochemistry was done by counting the number of stained or immunopositive cells in the white matter region of the spinal cord. Data are expressed as total number of cells/ mm² of the white matter region.

Statistical analysis

All data analyses were done using GraphPad Prism Version 5.02 (GraphPad, La Jolla, CA, USA). Date are shown as mean ± SEM. Statistical significance among groups were analyzed using Student's *t*-test between two groups and one-way analysis of variance (ANOVA) followed by Newman-Keuls *post hoc* test for multiple comparisons. Statistical significance was set at $p<0.05$.

RESULTS

Effects of 6-shogaol and 6-paradol on clinical symptoms in EAE-challenged mice

We determined neuroprotective effects of 6-shogaol and 6-paradol in EAE mice under therapeutic regimen. EAE-symptomatic mice were treated with 6-shogaol or 6-paradaol (5 mg/ kg, *p.o.*) once daily for 14 days (between days 29 and 42 after immunization). Both 6-shogaol and 6-paradol significantly improved EAE-relevant symptoms from the fourth day after drug administration (between days 32 and 42) except days 36 and 37 in the 6-paradol-treated EAE group compared to the vehicle-treated EAE group (Fig. 1A, Supplementary Table 1). The effectiveness of 6-shogaol and 6-paradol was also clearly shown from cumulative clinical scores from days 30 to 43. Administration of 6-paradol or 6-shogaol significantly reduced the cumulative clinical score (6-shogaol, 25.25%; 6-paradol, 25.75%) compared to the vehicle-treated EAE group (Fig. 1B). These results demonstrate that 6-shogaol and its metabolite, 6-paradaol, exert neuroprotective effects against EAE. More-

over, these molecules are therapeutically effective for EAE.

Effects of 6-shogaol and 6-paradol on damage of spinal cords in EAE-challenged mice

Demyelination and cell accumulation are known to be key histopathological features of spinal cords in EAE mice (Lee *et al*., 2018). Since 6-shogaol and 6-paradol were therapeutically effective for EAE, we next investigated whether these therapeutic agents could reduce demyelination and cell accumulation in spinal cords of EAE mice. In the vehicle-treated EAE group, demyelination of myelin sheath (Fig. 2A, 2B) and cell accumulation (Fig. 2C, 2D) occurred in the white matter of the spinal cord, showing reduced FluoroMyelin staining (Fig. 2A, 2B) and increased number of cresyl violet-stained cells (Fig. 2C, 2D). These histological features were significantly reversed by 6-shogaol or 6-paradol administration (Fig. 2).

In addition to demyelination and cell accumulation, glial activation such as astrogliosis microglial activation is also a pathological feature of EAE. It occurs in the white matter of the spinal cord, leading to neuroinflammation and neuronal damage (Lee *et al*., 2018). We then examined whether the therapeutic efficacy of 6-shogaol and 6-paradol was associated with reduced astrogliosis and microglial activation in spinal cords of EAE mice by immunolabeling with GFAP and Iba1, respectively. In the vehicle-treated EAE mice, astrogliosis (Fig. 3) and microglial activation (Fig. 4) were observed in the white matter of the spinal cord. These were markedly reduced by administration of 6-shogaol or 6-paradol (Fig. 3, 4).

Proinflammatory cytokines such as TNF-α from activated microglia have been considered as major pathological mediators in EAE and MS (Sharief and Hentges, 1991; Renno *et al*., 1995). Previously, we have reported that 6-shogaol or 6-paradol can influence TNF-α expression in injured brain (Gaire *et al*., 2015). Thus, we determined whether the therapeutic efficacy of 6-shogaol and 6-paradol was also associated with downregulation of TNF-α expression in spinal cords of EAE
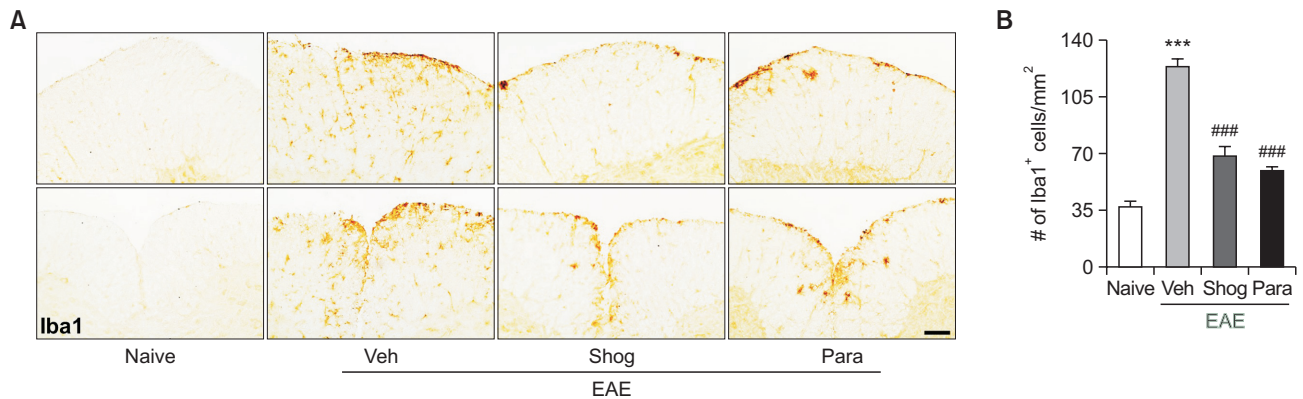
Fig. 4. Administration of 6-shogaol or 6-paradol suppresses microglial activation in spinal cords of EAE mice. Effects of 6-shogaol (Shog) and 6-paradol (Para) on microglial activation were examined by Iba1 immunolabeling. (A) Representative images of Iba1 immunoreactivity in the lumbar spinal cord of each group. (B) Quantification of the number of Iba1-immunopositive cells. Scale bar, 50 μm. n=4 per group. ***p<0.001 and ###p<0.001 vs. naïve and the vehicle-treated group, respectively, by Newman-Keuls multiple range test.

mice using immunohistochemical analysis. In the vehicle-treated EAE group, the number of TNF-α-immunopositive cells was increased in spinal cords of EAE mice, which was markedly attenuated by administration of 6-shogaol or 6-paradol (Fig. 5).

Taken together, these histological analyses on neuroinflammatory components demonstrate that 6-shogaol and 6-paradol exert therapeutic efficacy by alleviating neuroinflammatory responses, including reduced astrogliosis and microglial activation and downregulated TNF-α expression in injured spinal cords of EAE mice.

DISCUSSION

In the present study, we demonstrated that 6-shogaol and its biotransformed metabolite, 6-paradol, were therapeutically effective for EAE by reducing EAE clinical symptoms with histopathological sequelae including demyelination, cell accumulation, and astrogliosis in injured spinal cords of EAE mice. We also provided evidence that therapeutic effects of 6-shogaol and 6-paradol on EAE were associated with attenuating microglial activation and TNF-α upregulation in injured spinal cords of EAE mice. These aggregate findings implicate that 6-shogaol or 6-paradol might have therapeutic potential for alleviating MS symptoms by suppressing neuroinflammatory responses.

Ginger is known to possess anti-inflammatory and neuroprotective effects on diverse CNS diseases, including 3,4-methylenedioxymethamphetamine-induced neurotoxicity (Mehdizadeh et al., 2012), Alzheimer's disease (Zeng et al., 2013), focal cerebral ischemia (Wattanathorn et al., 2011), and EAE (Jafarzadeh et al., 2014). In addition, there have been many efforts to identify active ingredients in ginger extract in view of drug discovery for CNS diseases. Among constituents of ginger, pungent non-volatile compounds such as gingerols, shogaols, and zingerone and non-pungent biotransformed metabolites of shogaols, paradols, have been identified as active ingredients that exert pharmacological actions in the CNS (Choi et al., 2018). 6-Gingerol, a major pungent compound in fresh ginger, is converted to 6-shogaol through dehydration and hydrogenation of 6-shogaol can lead to the

formation of 6-paradol (Wohlmuth et al., 2005). Interestingly, anti-inflammatory and anti-oxidative properties of 6-shogaol and 6-paradol are higher than those of 6-gingerol (Dugasani et al., 2010), implicating that these two compounds might be active ingredients of ginger that exert neuroprotective effects on diverse CNS diseases. In case of EAE, one group has demonstrated neuroprotective effects of ginger extract in EAE under prophylactic regimen, in which ginger extract is given to immunized mice prior to disease onset (from day 3 post immunization) (Jafarzadeh et al., 2014). This administration has prevented disease onset of immunized mice, along with suppressed expression of immune response-relevant cytokines and chemokines (Jafarzadeh et al., 2014, 2017). Considering these previous findings along with our data, 6-shogaol and 6-paradol might be active ingredients responsible for the previously reported efficacy of ginger extract. It is notable in this study that these two ingredients are therapeutically effective because they could improve clinical symptoms of EAE mice even under therapeutic regimen.

Demyelination, increases in cell density, and astrogliosis are hallmarks of MS patients and rodent EAE models (Steinman, 1996; Tani et al., 1996; Malmestrom et al., 2003; Luo et al., 2007; Han et al., 2008). In this study, manifestation of these histopathological hallmarks of EAE was reduced by administration of 6-shogaol or 6-paradol. It has been reported that astrogliosis occurs prior to manifestation of clinical symptoms in EAE (Luo et al., 2007). In addition, astrogliosis and subsequent glial scarring can inhibit central repair processes in MS, including remyelination (Nair et al., 2008). Besides astrogliosis, microglial activation is also critically involved in the pathogenesis of MS and EAE (Benveniste, 1997; Minagar et al., 2002). Such glial activation is generally known to play a pivotal role in immune responses inside the CNS (Duffy et al., 2014). In MS and EAE, glial activation is also closely associated with disease severity. Suppressing gliosis has resulted in improved clinical symptoms of EAE (Reynolds et al., 2011). In a mechanistic view, astrogliosis and microglial activation consequently facilitate production of proinflammatory cytokines such IL-6 and TNF-α, both of which are involved in disease progression (Renno et al., 1995; Choi et al., 2011). These cytokines can also initiate myelin damage in the white matter of the spinal cord through death of oligodendrocytes (Luo et al.,
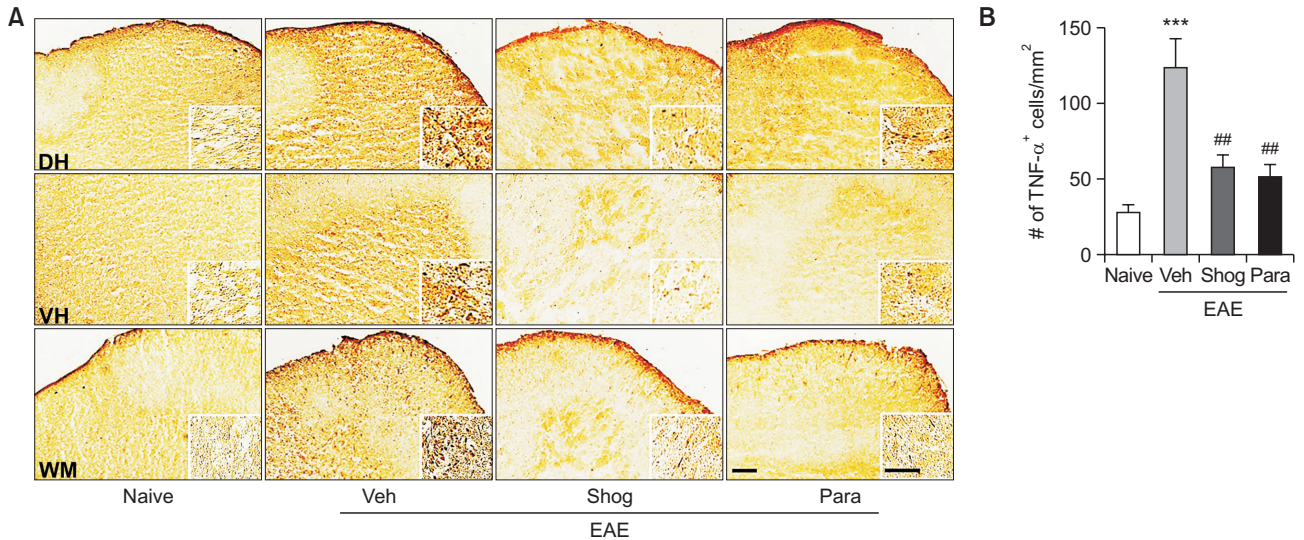
A

B

Fig. 5. Administration of 6-shogaol or 6-paradol suppresses TNF-α expression in spinal cords of EAE mice. Effects of 6-shogaol (Shog) and 6-paradol (Para) on TNF-α expression were examined by TNF-α immunolabeling. (A) Representative images of TNF-α immunoreactivity in the lumbar spinal cord of each group. (Insets) High magnification image of TNF-α-immunopositive cells from the dorsal horn (DH), the ventral horn (VH), and the white matter (WM) of the spinal cord. (B) Quantification of the number of TNF-α-immunopositive cells. Scale bars, 100 μm (inset, 50 μm). n=4 per group. ***$p<0.001$ and ##$p<0.01$ vs. naïve and the vehicle-treated group, respectively, by Newman-Keuls multiple range test.

2017). Our results showed that 6-shogaol and 6-paradol could reduce astrogliosis and the number of activated microglia in injured spinal cords of EAE mice. Furthermore, administration of these active ingredients reduced the number of TNF-α expressing cells in injured spinal cords of EAE mice. Therefore, neuroprotective effects of 6-shogaol and 6-paradol in EAE observed in this study might be associated with reduced inflammatory responses in the CNS such as astrogliosis, microglial activation, and TNF-α expression. This notion was supported by previous findings (Shim *et al*., 2011; Ha *et al*., 2012; Moon *et al*., 2014; Gaire *et al*., 2015). Treatment with 6-shogaol has suppressed secretion of proinflammatory cytokines in LPS-stimulated astrocyte (Shim *et al*., 2011). In animal studies, exposure to 6-shogaol or 6-paradol can attenuate ischemic injury through inhibiting microglial activation and suppressing expression of inflammatory cytokines (Ha *et al*., 2012; Gaire *et al*., 2015). In addition, 6-shogaol can attenuate β-amyloid-induced neuroinflammation and memory impairments through inhibiting glial activation in mice (Moon *et al*., 2014). Therefore, the neuroprotective efficacy of 6-shogaol and 6-paradol on CNS disorders might be through suppressing immune responses in the CNS.

In this study, 6-shogaol and its biotransformed metabolite, 6-paradol reduced clinical symptoms of EAE and associated histological sequelae to the similar extent, indicating that these two ingredients of ginger have similar pharmacological efficacy. 6-Paradol is a metabolized product which is derived from 6-shogaol by liver enzyme (Surh and Lee, 1992, 1994). These two ingredients exert similar pharmacological activities including anti-oxidative and anti-inflammatory actions (Nagendra chari *et al*., 2013; Jo *et al*., 2016). In addition, our previous *in vivo* study demonstrated that 6-shogaol and 6-paradol were neuroprotective against cerebral ischemia to the same extent (Gaire *et al*., 2015). Similarly, in the current study, we found that both 6-shogaol and 6-paradol exerted the similar neuro-

protective effects in EAE. These *in vitro* and *in vivo* studies showing the same efficacy of 6-shogaol and 6-paradol may further indicate that neuroprotective effects of 6-shogaol could be mediated by its metabolite, 6-paradol.

There has been growing evidence for the therapeutic potential of ginger itself as well as its active ingredients such as 6-shogaol and 6-paradol in diverse CNS diseases mainly through their anti-inflammatory properties. In case of MS, the extract can reduce tissue injury in spinal cords of EAE mice. Our *in vivo* results utilizing a mouse model of MS provided direct evidence that 6-shogaol and 6-paradol were active ingredients that could mediate neuroprotective effects of ginger extract. Furthermore, our current results clearly demonstrated that these active ingredients were therapeutically effective for EAE, further indicating their potential use to treat MS. In particular, 6-paradol could be more useful to avoid unwanted effects such as gastric irritation because it is a non-pungent metabolite unlike ginger or 6-shogaol.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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Table1. Statistical analysis for clinical symptoms by Mann–Whitney's test shown in Fig. 1a

Day	EAE vs Shogaol	EAE vs Paradol	Note
29	0.4816	0.4315	Drug start
30	0.1389	0.2622	
31	0.0868	0.2066	
32	0.0574	0.0054**	
33	0.0051 **	0.0127*	
34	0.0024**	0.0240*	
35	0.0057**	0.0092**	
36	0.0064**	0.1230	
37	0.0243*	0.0708	
38	0.0046**	0.0169*	
39	0.0164*	0.0178*	
40	0.0097**	0.0062**	
41	0.0129*	0.0023**	
42	0.0185*	0.0195*	Drug stop

P value of shogaol and paradol-treated groups compared with EAE from day 29.