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FULL PAPER

Enhancement of IL-18 expression by *Paecilomyces tenuipes*

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Abstract *Paecilomyces tenuipes* is a popular medicinal mushroom, and has received extensive attention for medical application because of its various physiological activities. However, there is limited information about the anticancer and immunomodulatory activities of *Paecilomyces tenuipes* . This study attempted to evaluate the effect of an extract from the cultured fruiting bodies of *Paecilomyces tenuipes* (PTE) on the expression of the interleukin-18 (IL-18) gene in rat pheochromocytoma (PC) 12 cells and rat brain. Related mRNA levels were determined by reverse transcription-polymerase chain reaction (RT-PCR). Protein levels were measured by Western blot and immunohistochemistry. Our results demonstrated that PTE induced IL-18 gene expression both in vitro and in vivo. Treatment of PC12 cells and rat brain cells with 10 μg/ml and 20 mg/kg PTE, respectively, yielded significant increases of IL-18 levels. Significantly, IL-18-immunoreactive neurons were detected in the Purkinje cells of the cerebellum. IL18-immunohistochemical staining was markedly enhanced in animals treated with PTE compared to findings in the untreated controls. These results suggest that PTE could be a potential candidate as an immune activator or anticancer drug.

Keywords Brain · Cytokine · Entomopathogenic fungus · Immune system

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Introduction

Paecilomyces tenuipes (also referred to as *Isaria japonica*) is one of the famous Chinese medicinal entomopathogenic fungi, together with other fungi such as *Cordyceps sinensis* and *Cordyceps militaris*. It is a parasitic fungus on the larvae of Lepidoptera, and belongs to the subphylum Ascomycotina, class Pyrenomycetes, order Clavicipitales. Both Paecilomyces and Cordyceps are genera of the family Clavicipitaceae. *P. tenuipes* is traditionally used in Japan, Korea, and China as a time-honored tonic and to alleviate the effects of blood loss, fatigue, and anorexia (Zhu et al. 1998a, b). It has been reported that *P. tenuipes* and its glycoprotein constituent protect against anemia in mice treated with 5-fluorouracil (Takata et al. 2008). The known bioactive compounds obtained from *P. tenuipes* include cordycepin, tenuipesine, sterol, cyclopeptide, and polysaccharides (Kikuchi et al. 2004a; Yin et al. 2007). The fruiting bodies of *P. tenuipes* are highly valued as medicinal herbs, due to their various biological and pharmacological activities, including immuno-stimulating and

anti-tumor activities (Lee et al. 1996; Liu et al. 1996; Borchers et al. 1999; Song et al. 2007; Chen et al. 2008).

Cytokines are crucial intracellular regulators that have important physiological roles in a wide range of disease processes. Interleukins (ILs) are the largest group of cytokines that stimulate immune cell proliferation and differentiation. IL-18 is a multifunctional cytokine having roles in both innate and adaptive immune responses. IL-18 is a member of the IL-1 family of cytokines and was originally described as an interferon-γ-inducing factor (IGIF) (Okamura et al. 1995a). It has a variety of biological functions, including stimulation of the proliferation of activated T cells, enhancement of the activity of natural killer (NK) cells, induction of the production of interferon (IFN)-γ by helper T1 (Th_1) cells (Micallef et al. 1996; Okamura et al. 1995b), and the induction of granulocyte–macrophage colony-stimulating factor (GM-CSF) by activated T cells (Ushio et al. 1996). IL-18 induces gene expression and synthesis of tumor necrosis factor (TNF), IL-1, Fas ligand, and several chemokines (Dinarello 1999). Moreover, IL-18 has been shown to serve as an adjuvant in conjunction with certain DNA vaccines (Swencki-Underwood et al. 2006). However, little is known about the regulation of IL-18 gene expression, which would be modulated in the cytokine network, as seen in the regulation of other cytokines. Previously, it has been reported that *Cordyceps militaris* extract induced IL-18 in mouse brain (Kim et al. 2008). However, there is little information on the pharmacological actions of the cultured fruiting bodies of *P. tenuipes*. Moreover, despite the extensive research on several aspects of *P. tenuipes*, there is a lack of knowledge about the mechanism underlying the regulation of cytokines by *P. tenuipes.* Therefore, this study was designed to evaluate the effect of an extract from the cultured fruiting bodies of *P. tenuipus* on the expression of IL-18 in PC12 cells and in rat brain.

Materials and methods

Materials

RPMI 1640, fetal bovine serum (FBS), Dulbecco's phosphate-buffered saline (DPBS), Trizol^{TM} reagent, and trypsin-ethylene diamine tetraacetic acid (EDTA) solutions were purchased from Invitrogen (Carlsbad, CA, USA). Moloney murine leukemia virus (M-MLV) ribonuclease, oligo dT (deoxythymidine) primer, dNTPs (deoxynucleic acid triphosphate), Taq polymerase, specific primers (for IL-18 and β-actin), and 100 bp DNA ladder were purchased from BioNEER (Daejeon, Korea). A low-molecular-weight protein marker was purchased from BioRad (Richmond, CA, USA). A bicinchoninic acid (BCA) protein assay kit was purchased from Pierce (Rockford, IL, USA). Other reagents were special grade and purchased commercially.

Cultivation of *P. tenuipes*

For the study, *P. tenuipes* was purchased from the Korea Agricultural Culture Collection (KACC No-40503), Suwon, Korea. To cultivate a stock culture of *P. tenuipes*, potato dextrose agar (PDA) slants were inoculated with mycelia and incubated at 25°C for 7 days. This culture was then used for the seed culture inoculation. The mycelia were transferred to the seed culture medium by punching out approximately 5 mm^2 of the slants with a sterilized cutter. This seed was inoculated into a 500 ml flask containing 200 ml of synthetic medium (40 g/l glucose, 10 g/l yeast extract, 0.5 g/l KH_2PO_4, 0.5 g/l $K_2HPO_4 \cdot 3H_2O$, and 0.5 g/l $MgSO_4$) and incubated at 25°C in a rotary shaker (110 rpm) for 5 days. To cultivate *P. tenuipes* fruiting bodies, silkworms (*Bombyx mori*, China; 100 g) were packed into culture bottles and sealed using polypropylene. The bottles were sterilized at 121°C for 90 min, inoculated with the *P. tenuipes* seed culture, and incubated for 20 days at 25°C. Once the hyphae of *P. tenuipes* reached the bottom of the culture bottles the bottles were moved to a cold room at 16°C with 95% relative humidity, and placed under an incandescent light of 1,000 Lux to induce primordium formation. The incubator containing *P. tenuipes* was ventilated four times a day to provide fresh air.

Crude extract preparation

Two hundred grams of fruiting bodies of *P. tenuipes* were extracted with 70% ethanol at room temperature for 7 days. The extracts were then filtered and evaporated with a rotary evaporater. The residue was then stored at −80°C and freeze dried. The dried sample was weighed. The yield was calculated using the following formula:

$$\text{Yield (\%)} = (\text{mass of } P.\, tenuipes/\text{mass of dried extract}) \times 100$$

The yield was 5.7%. The dried extract was dissolved in phosphate-buffered saline (PBS) and filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA, USA) and then stored at 4°C before use.

Cell culture and treatment

Rat pheochromocytoma (PC) 12 cells, purchased from the Korean Cell Line Bank, were grown in RPMI-1640 supplemented with 10% FBS, L-glutamine (4 mM), penicillin (100 units/ml), and streptomycin (100 μg/ml) and incubated at 37°C in a humidified atmosphere of 5% CO_2 and

95% air. The cells were treated with saline or with 10 μg/ml of PTE for 2, 4, or 8 h. The treated cells were harvested by trypsinization.

Animals and treatment

Male Sprague–Dawley rats (280–320 g), approximately 3 months old, were used in all experiments. The animals were housed four per cage and maintained under controlled environmental conditions (22 ± 2°C, 12 h light/dark cycle). Food (Certified Rodent Diet 5002; Orient Bio, Seongnam, Korea) and tap water were available ad libitum. All efforts were made to minimize animal suffering and to reduce the number of animals used. All experimental procedures were performed in accordance with the NIH Guide for Care and Use of Laboratory Animals (NIH publication No.80-23 revised 1996) and related ethical regulations of our university.

Before treatment, the rats were weighed and PTE was prepared in PBS to achieve the desired dose, which was based on the average weight of the animals. For the dose–response study, 3 rats per group were treated intraperitoneally (i.p.) with 10, 20, 30, or 40 mg/kg body weight PTE and killed 4 h after dosing. Control groups received an equal volume of PBS. For the time-course study, animals were treated with 1 ml of PBS for a nominal dose of 0 (vehicle control) or 20 mg/kg body weight of PTE or lipopolysaccharide (LPS). Three animals were treated per dose group and time point, and groups for each dose and time point were housed in separate cages. Rats were killed 2, 4, and 8 h after dosing. An untreated group of rats was also included, which were killed at time zero when the other animals were dosed. Control and unstressed rats were killed immediately after the removal from their home cages. The brains were rapidly extirpated, cleaned of fat tissue, frozen in liquid nitrogen, and stored at −70°C for further analysis.

Total RNA isolation

Total RNA was extracted from the frozen tissues and cultures, using Trizol (Invitrogen) according to the manufacturer's instructions. Briefly, 1 ml of Trizol reagent was added to the cells harvested from each culture flask or 50 mg of rat brain tissue. A total of 200 μl of chloroform was then added to extract the RNA into the aqueous phase following centrifugation (12,000×g at 4°C for 15 min). The aqueous phase was removed and the RNA was collected by the addition of isopropanol and incubated at −20°C for 60 min, followed by centrifugation at 12,000×g at 4°C for 15 min. The RNA pellet was then washed in 70% ethanol and resuspended in 20 μl of diethyl pyrocarbonate-treated H_2O (DEPC H_2O). Total RNA was quantified by spectrophotometer and its integrity was established by formaldehyde agarose gel electrophoresis.

Reverse transcription-polymerase chain reaction (RT-PCR)

For the cDNA synthesis, 2 μg of isolated total RNA was used as the template for RT-PCR. A 40 μl aliquot for the RT reaction was set up as follows: 2 μg of total RNA was incubated with 4 μl of 10 pmol oligo dT primers and heated at 65°C for 10 min and then immediately stored on ice. To each tube was added: 0.6 μl of 200 unit reverse transcriptase (M-MLV), 5 μl of 5× reaction buffer, 4 μl of 100 mM dithiotreitol (DTT), 4 μl of 2.5 mM dNTPs, 10 μl of cDNA template, and 0.2 μl of 40 unit RNase. Synthesis occurred for 60 min at 42°C, followed by treatment at 70°C for 5 min to inactivate the RT enzyme. The quality of cDNA was verified by PCR amplification of β-actin.

Polymerase chain reaction (PCR) and the analysis of PCR products

For rat brain and PC 12 cells, IL-18 gene-specific primers; forward: 5′-ATGGCTGCCATGTCAGAAGA-3' and reverse: 5′-GTAGGTTATCATAAGGCTCG-3' (GeneBank Accession No. NM019165.1) for 653 base pair (bp) PCR product were used. For normalization, the β-actin gene was used (forward: 5′-CCTCTATGCCAACACAGT-3′ and reverse: 5′-AGCCACCAATCCACACAG-3′ (GeneBank Accession No. NM031144.2) for 155 bp PCR product). The cDNA in the RT product was amplified using Taq DNA polymerase. A PCR reaction was performed in 20 μl of the total volume using 10 pmol of the corresponding primers. The cDNA was amplified under the following reaction conditions: denaturation at 94°C for 45 s, annealing at 55°C for 45 s for IL-18, and at 56°C for β-actin, and polymerization at 72°C for 45 s. The cyclic process was performed 35 times for IL-18, and 30 times for β-actin. The PCR products were analyzed on 1.2% agarose gel and visualized with ethidium bromide. The stained intensity of individual bands was evaluated with Gel Quant software (DNR Bio-Imaging Systems, USA).

Immunoblot assay

Protein was isolated with Trizol (Invitrogen) according to the manufacturer's protocol. Subsequently, the protein concentration was determined using a BCA protein assay kit (Pierce). Twenty micrograms of the protein extract was separated by sodium dodecyl sulfate (SDS)-polyacryamide gel electrophoresis and then transferred to a polyvinylidene difluoride (PVDF) membrane. The blots were pretreated with 5% horse serum and then incubated with mouse monoclonal antibody (anti-IL-18, 0MA-04051, Affinity

BioReagents, 1:1,000) as the primary antibody at room temperature for 2 h. The blots were washed and incubated with biotinylated goat anti-mouse IgG as secondary antibody for 2 h. The enzyme productivity was visualized by the use of the Vectastain avidin–biotin conjugate (ABC) kit (Vector, Burlingame, CA, USA) according to the manufacturer's instructions. PBS containing 0.05% Tween 20 was used as washing buffer throughout the experiment. A digital image system was used to determine the density of the bands (Gel Quant; DNR Bio-Imaging Systems).

Immunohistochemical analysis

For immunohistochemical analysis, the experimental animals were anesthetized with ketamine and perfused with saline followed by 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4). The brain and stomach were removed, post-fixed in 4% paraformaldehyde for 24 h, and then transferred to 30% sucrose in 0.1 M PBS for at least 24 h for cryoprotection. Coronal 35 μm-thick sections were cut with a cryostat (Reichert-Jung). Endogenous peroxidase activity was blocked with 0.5% H_2O_2 in methanol. Non-specific binding sites were blocked by incubating the sections in PBS containing 1.5% normal goat serum (NGS), 0.5% bovine serum albumin (BSA), and 0.1% Triton X-100. These sections were then incubated for 24 h in primary antibody (anti-IL-18; Affinity BioReagents, 1:500). After removal of the primary antibody, sections were washed three times with PBS and incubated in peroxidase-linked secondary antibody (1:200) for 2 h at room temperature followed by three washes with PBS. Color for the peroxidase-linked antibody was developed with diaminobenzidene (DAB) as chromogen. Sections were transferred onto gelatinized glass slides, dehydrated, cleared, mounted in distyrene plasticizer xylene (DPX), and coverslipped. Finally, the sections were viewed under an inverted fluorescence microscope (TE2000; Nikon, Tokyo, Japan) and photographs were taken.

Statistical analysis

The data were expressed as means ± SD. Statistical significance was assessed with one-way analysis of variance followed by Tukey's post-hoc test for multiple group comparisons. Differences with p values less than 0.05 were considered statistically significant.

Results

Induction of IL-18 mRNA and protein

Cytokines are recognized as primary mediators of cachexia through their actions in the periphery and the central nervous system (CNS), and they have diverse actions in the brain, modulating and mediating both systemic host responses to disease and local changes caused by CNS inflammation, infection, and injury (Rothwell 1999). Because IL-18 is a pleiotropic cytokine, isolated as an important modulator of immune responses, and its expression in the brain is associated with the regulation of multiple functions, we examined the effects of PTE on the mRNA and protein expression levels of this cytokine in rat brain.

Figure 1 shows the dose–response effect of PTE on the IL-18 mRNA expression level. Significant changes were observed with all doses after 4 h treatments, which raised IL-18 mRNA levels in the rat brain by 168–268% above levels in the saline treated controls. As shown in Fig. 1, the most effective concentration was at 20 mg/kg, which yielded significant increases of IL-18 mRNA levels of about 268% above those in the untreated controls. However, two concentrations of PTE (10 and 30 mg/kg) elevated IL-18 mRNA levels by 200% above that of the control group. PTE administration elicited a significant dose-dependent effect on IL-18 mRNA levels at 4 h. PTE at a dose of 40 mg/kg was not as effective as other doses.

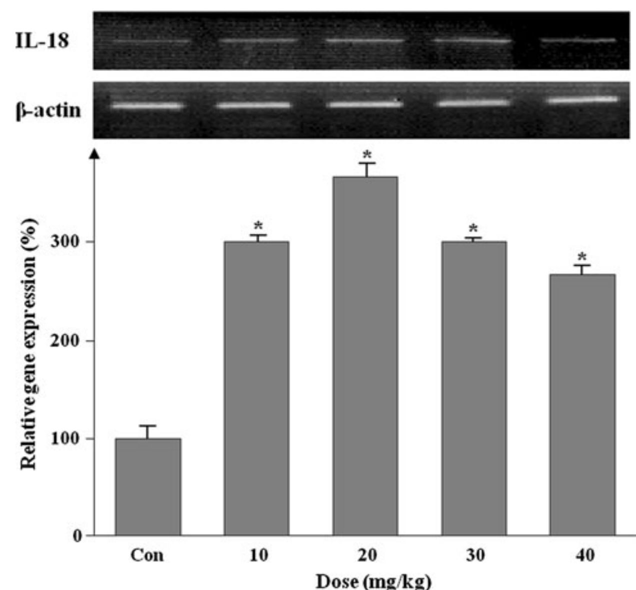

Fig. 1 Effect of different doses of *Paecilomyces tenuipes* extract (PTE) on interleukin-18 (IL-18) mRNA levels in rat brain. Rats were treated with 10, 20, 30, or 40 mg/kg PTE in saline as described in "Materials and methods". After 4 h treatment, RNAs were extracted from whole brain tissue isolated from individual animals and analyzed separately by reverse transcription-polymerase chain reaction (RT-PCR). Representative RT-PCR (*upper panel*) and quantification of relative intensity data (*lower panel*) are shown for IL-18 mRNA levels. The values were normalized to β-actin and are expressed as means ± SD ($n = 3$), with the level of IL-18 mRNA in the saline-treated control group taken as 100%. *Con* untreated control. $^{*}P < 0.05$ versus control group

Next, we examined the time course of the effect of PTE on IL-18 mRNA levels in rat brain. As shown in Fig. 2a, PTE significantly increased the IL-18 mRNA levels by 719% at 4 h, and by 619 and 514% at 2 and 8 h, respectively compared with the control, and this induction was much greater than that obtained with LPS treatment. This result suggests that IL-18 gene expression was significantly increased by the treatment with PTE.

To evaluate the in vitro induction of IL-18 mRNA by PTE, PC12 cells were treated with 10 µg/ml PTE for 2, 4, and 8 h. The related induction of IL-18 mRNA was determined by RT-PCR. As shown in Fig. 2b, maximum induction of IL-18 mRNA was observed at 4 h.

To determine whether the PTE-induced expression of IL-18 mRNA was accompanied by an increase in protein levels, Western blot analysis was performed with anti-IL18 antibody. As indicated in Fig. 3, treatment of rats with 20 mg/ml PTE yielded a remarkable increase in IL-18 protein levels above that of the saline-treated control.

Immunohistochemical analysis of IL-18 gene expression induced by PTE

We also evaluated the expression of IL-18 enzyme in rat brain by immunohistochemistry. Interestingly, in the cerebellum of the rat brain, IL-18 protein expression in Purkinje cells was activated by PTE treatment (Fig. 4). IL-18 immunohistochemical staining was significantly different in the PTE-treated groups as compared to that in the saline-treated animals.

Discussion

Paecilomyces tenuipes is one of the well-known medicinal entomogenous fungi, and has multiple therapeutic functions, including immunomodulatory and antitumor activities. However, much less is known about its effect on the regulation of cytokines. The present study has clearly demonstrated for the first time that PTE can induce the expression of the IL-18 gene at the mRNA and protein levels. These activities may be attributed to the presence of bioactive compounds. Besides cordycepin and polysaccharides (Yin et al. 2007), a number of bioactive constituents have been reported from *P. tenuipes*. These include: tenuipesine A (Kikuchi et al. 2004a), spirotenuipesine A and B (Kikuchi et al. 2004b), paecilomycine A, B, and C (Kikuchi et al. 2004c), 4β-acetoxyscirpene-3, 15-diol (Nam et al. 2001; Oh et al. 2001; Yoo et al. 2005), ergosterol (Nam et al. 2001), and beauvericin and its analogues (Nilanonta et al. 2000, 2002). Moreover, phthalic acid, stearic acid, di (2-ethylhexyl) phthalate, and cholesterol were also reported from this mushroom (Song et al. 2007). In our study, the active compounds in PTE were not analyzed; however, previous studies with a similar extraction process suggest that active ingredients may be present in the PTE. For example, a bioactive compound from *P. tenuipes* (BCTP), extracted with ethanol, contains cordycepin, tenuipesine, sterol, and cyclopeptide, and has been reported to exert an inhibitory effect on monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B) (Kan et al. 2006), and to have an antidepressant effect in

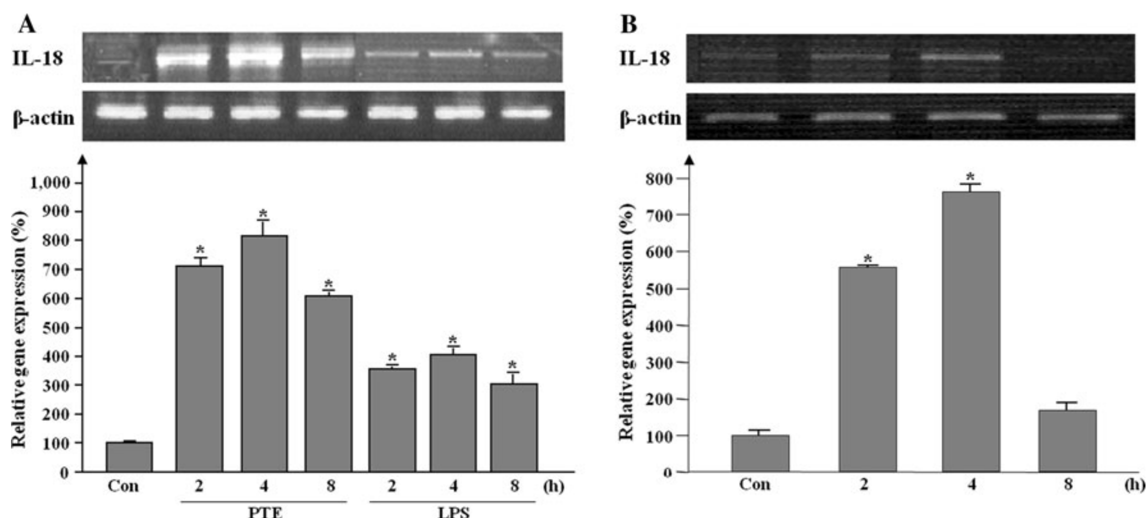

Fig. 2 Time-course effect of PTE and lipopolysaccharide (LPS) on IL-18 mRNA levels in rat brain (**a**), and effect of PTE on IL-18 mRNA levels in PC12 cells (**b**). Rats were treated with 20 mg/kg of PTE and 10 mg/kg of LPS and PC12 cells were treated with 10 µg/ml of PTE for 2, 4, or 8 h as described in "Materials and methods". Each treatment group was analyzed separately by RT-PCR. Representative RT-PCR and quantification of relative intensity data are shown for IL-18 mRNA levels. The values were normalized to β-actin and are expressed as means ± SD ($n = 3$), with the level of IL-18 mRNA in the saline-treated control group taken as 100%. *Con* control treated with saline. $^{*}P < 0.05$ versus control group. *Upper and lower panels* show representative RT-PCR products and relative intensity data, respectively

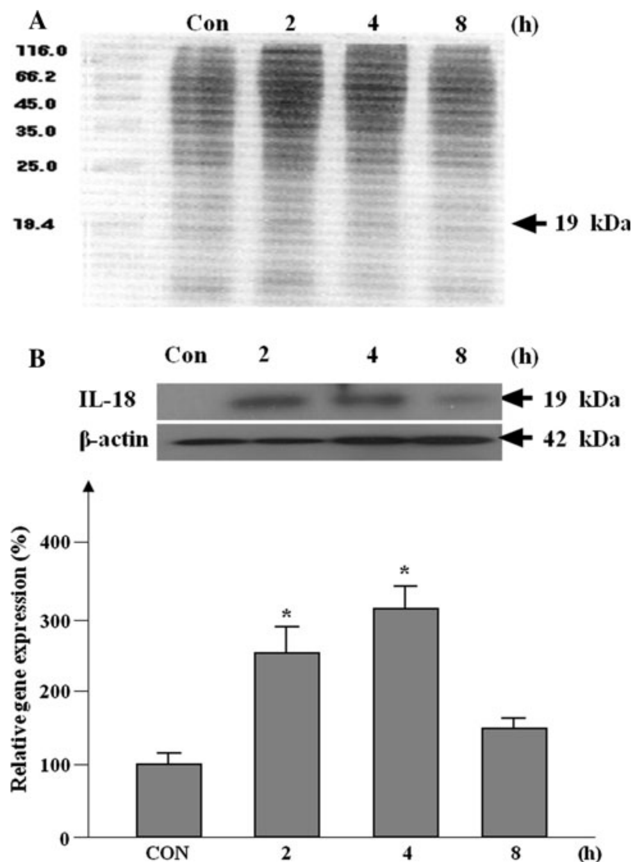

Fig. 3 The expression of IL-18 protein by Western blotting in rat brain. Rats were treated with 20 mg/kg of PTE for 2, 4, or 8 h as described in "Materials and methods". Equal amounts (20 µg) of protein were subjected to 12% sodium dodecyl sulfate polyacryamide gel electrophoresis (SDS-PAGE) (**a**) and Western blot analysis was carried out using anti-IL-18 (19 kDa) antibody (**b**); quantification of relative intensity data are shown for IL-18 protein levels (*lower panel*). The values were normalized to β-actin and are expressed as means ± SD ($n = 3$), with the level of IL-18 in the saline-treated control group taken as 100%. *Con* control treated with saline. $^{*}P < 0.05$ versus control group

rats (Yin et al. 2007). Similarly, some edible mushrooms, including *Cordyceps militaris,* extracted with ethanol have been shown to exhibit tyrosinase inhibitory activity (Chien et al. 2008). Ethanol extracts of the fruiting bodies of *Phellinus linteus* have demonstrated strong anti-angiogenic, antioxidant, and xanthine oxidase inhibitory activities (Song et al. 2003). As we know, ethanol is by far the most commonly used solvent for plant extracts. Recently, Koh et al. (2009) have reported that an ethanol extraction procedure (70–80% ethanol) allows the separation of small molecules including bioactive marker compounds (gallic acid, ellagic acid, rutin, rubusoside, and steviol monoside) from plants, without risking the loss of many unknown compounds. On the basis of these observations, it is reasonable to speculate that PTE may contain biologically effective ingredients.

The expression of IL-18 plays a crucial role in innate and adaptive immune responses. Its levels can be upregulated following LPS stimulation (Sugama et al. 2007) or treatment with IFN-γ (Suk et al. 2001). IL-18 was found to be associated with numerous inflammatory-associated disorders, including arthritis, infections, autoimmune diseases, and cancer (Boraschi and Dinarello 2006; Park et al. 2007; Dinarello and Fantuzzi 2003; Alboni et al. 2010). In the present study, we examined the transcriptional and translational changes of the IL-18 gene in response to treatment with PTE. The results indicate that there was an alteration in IL-18 gene expression in response to the PTE, and that the change occurred in a dose- and time-dependent manner. Although the mechanism for these changes is not completely understood, reports in the literature have suggested that IFN-γ increased IL-18 gene expression via IFN consensus sequence-binding protein (ICSBP) and activator protein-1 (AP-1) elements (Kim et al. 2000). One possible mechanism could be the activation of IFN-γ production via

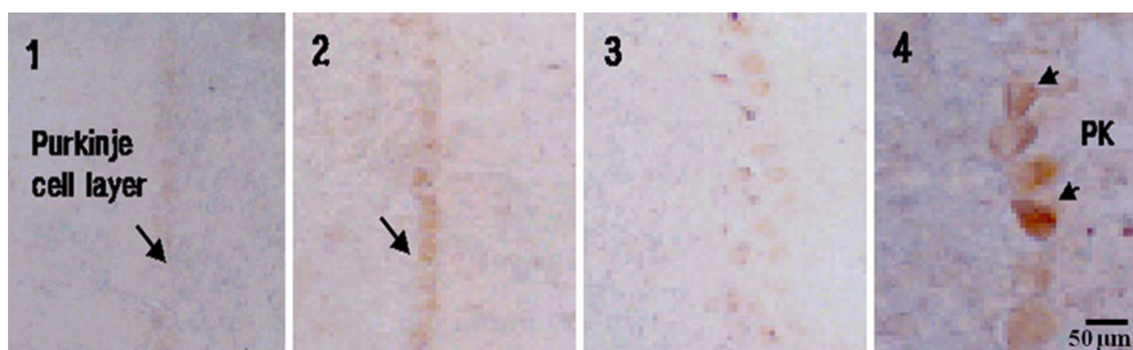

Fig. 4 Photomicrographs of IL-18-immunostained sagittal sections through the cerebellum in controls (*1*) and PTE (*2*, *3*, and *4*)-treated animals. PTE (20 mg/kg) was intraperitonially injected into rats. After 4 h, sagittal sections through the cerebellum were incubated with IL-18 antibodies and visualized with diaminobenzidine (DAB) reaction products. Photographs were captured with an inverted microscope (Eclipse TE 2000-U; Nikon) at magnifications of ×100, ×200, and ×400. Note the increased IL-18 immunoreactivity found in the animals treated with PTE. *Arrows in 1 and 2* show purkinje cell layer; *arrowheads in 4* show purkinje cells. *PK* Purkinje cells. *Bar* 50 µm

stimulation of the IL-18 promoter and its gene expression. This view is supported by the findings that *Cordyceps militaris* extract induced IL-18 mRNA expression via enhancement of the P1 promoter region, and this resulted in the activation of IFN-γ production (Kim et al. 2008). Moreover, LPS and *Cordyceps militaris* extract induced IL-18 gene expression in rat brain (Kim et al. 2000, 2008). In agreement with these reports, our data once again demonstrated that LPS increased IL-18 expression in rat brain, and a further increase was observed with the use of PTE.

IL-18 is an important modulator of immune responses and has been shown to be pleiotrophic. It has been reported that IL-18 can be expressed in the central nervous system (CNS), where it not only influences homeostasis and behavior, but also participates in neuroinflammatory and neurodegenerative processes (Alboni et al. 2010). The most interesting finding in the present study is the identification of IL-18 immunoreactive neurons in the Purkinje cells of the cerebellum. Although the implication of this effect is unclear, previous studies suggest an autocrine effect of IL-18 on the CNS (Alboni et al. 2010). Moreover, it has been shown that intracerebral administration of IL-18 can modulate scrapie pathogenesis, possibly through a microglia-mediated pattern (Pasquali et al. 2006). Additionally, it was reported that PTE stimulated T-cell immune responses and enhanced myeloid growth factors such as GM-CSF in cultured Peyer's patches cells in response to concanavalin A (Takano et al. 2005). GM-CSF has been used clinically in cancer chemotherapy, for leucopenia (Danova and Aglietta 1997). Interestingly, IL-18 has been shown to be essential for IFN-γ production, and it induces GM-CSF from peripheral blood mononuclear cells (PBMCs) (Ushio et al. 1996).

In conclusion, the present study demonstrates that PTE upregulates the expression of IL-18 in PC12 cells and rat brain. This finding may have profound implications, and *Paecilomyces tenuipes* may be a potential agent for the treatment of numerous inflammatory-associated disorders including rheumatoid arthritis and autoimmune diseases, as well as cancer.

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