Inhibitory Effects of Fermented Nutraceuticals on NO Production and T Cell Proliferation in Juvenile Atopic Dermatitis

Taeheung Shim¹, Shewhan Kim², Seeun Byun³, Yonggyu Lee³, Jaeyoul Cho³, Daejung Kim³, Hyunsook Kim³ and Myeon Choe³

¹) Atopic Dermatitis Lab., B&C Care Co.
²) Dept. of Sports Science
³) School of Bioscience and Biotechnology, Kangwon National University, Korea

Abstract  As the most common inflammatory skin disease in children, atopic dermatitis begins in infancy or early childhood, with about 90% of cases appearing under age of 5. The prevalence of atopic dermatitis has rapidly increased among children in recent years. Physiological and psychological abnormalities and social impact are also well known in children with atopic dermatitis and in their families. Atopic dermatitis not only seriously affects the quality of life of the children and their families but also is leading chronic disease in children with hard-to-cure.

Recently, we found that the fermented extract of several plants had considerable potential to treat juvenile atopic dermatitis. This extract therefore is now under investigation to find the underlying immunopathological mechanism by determining its inhibitory effects on nitric oxide (NO) release and T cell proliferation.

The fermented extract dose dependently blocked NO production. In particular, the inhibitory effect of the extract was maximized up until 80-fold dilution of the original extract. This extract did not induce cytotoxic effects up to 80-fold dilution. Interestingly, doses between 320- and 80-fold dilution significantly protected cell death mediated by LPS-induced NO production. The fermented extract also significantly suppressed CD3 induced T cell proliferation in a dose dependent manner. J Physiol Anthropol 26(2): 225–227, 2007 http://www.jstage.jst.go.jp/browse/jpa2 [DOI: 10.2114/jpa2.26.225]

Keywords: children, atopic dermatitis, fermented extract of several plants, NO release, T cell proliferation

Introduction

The prevalence of atopic dermatitis has rapidly increased among children worldwide in recent years. Atopic dermatitis begins in infancy or early childhood, with about 90% of cases appearing under age of 5 (Chamlin et al., 2004). A study conducted in Korea (Lee et al., 2002) reported that 20% of under 1 year olds, 35.6% of 1 to 3 year olds, and 22.2% of over 4 year old children were diagnosed with atopic dermatitis. Laughter et al. (2000) reported that 17% of children in the United States also suffered from atopic dermatitis. The incidence of atopic dermatitis is higher in rapidly urbanizing or modernized areas than in rural or less developed areas (Woolcock and Peat, 1997; Strachan, 1989; Mutius et al. 2000).

Since physiological and psychological abnormalities and social impact are well known in children with atopic dermatitis and in their families, atopic dermatitis not only seriously affects the quality of life of the children and their families but also is leading chronic disease in children with hard-to-cure because our life style is mostly urbanized and modernized.

Recently, we found that the fermented extract of a complex of several plants has promising potential to treat juvenile atopic dermatitis (data not shown). To explore its anti-atopic dermatitis mechanism, whether it could be able to suppress NO release, T cell proliferation and the pathological parameters found in atopic dermatitis was firstly examined.

Methods

Preparation of the whole extract

Fourteen different plants, including Houttuynia cordata Thunb, Angelica gigas Nakai, Artemisia princeps var. orientalis HARA, Cnidium officinale Makino, Angelica utilis Makino, Sophora japonica L., Mentha arvensis L., Prunus armeniaca, Lonicera japonica Thunb., Zingiber officinale Roscoe, Scrophularia buergeriana Miq., Camella Sinensis L., Artemisia princes Pamp., Calendula officinalis L. and Platycodon grandiflorum (Jacq.) A. DC., were firstly fermented at 27–28°C for 15 days and were transferred to a
distillator. Distillation was indirectly conducted at 450°C for 14 hours.

**Determination of NO production**

After preincubation of RAW 264.7 cells (1×10^6 cells/ml) for 18 h, various concentrations of the fermented plant extract with lipopolysaccharide (LPS, 2.5 μg/ml) were incubated for 24 h under the same conditions as those in the previous report (Ding et al., 1988). Nitrite in culture supernatants was also measured by adding 100 ml of Griess reagent (1% sulfanilamide and 0.1% N-[1-naphthyl]-ethylenediamine dihydrochloride in 5% phosphoric acid) to 100 ml samples of medium, respectively, for 10 min at room temperature. The optical density (OD) at 550 nm was measured using a Spectramax 250 microplate reader (Molecular Devices, Sunnyvale, CA, USA). The detection limit of the assay is 0.5 mM.

**Cell viability/proliferation assay**

Effect of the fermented plant extract on cell viability was evaluated by MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl tetrazolium bromide) assay with minor modification, as reported previously (Cho et al., 2000). The cell suspension of 1×10^6 cells/ml was plated in a 96 well plate. After 18 h culture, varying concentrations of fermented plant extract and stimuli were added to each well and cultured for 24 h. Ten μl of MTT solution (10 mg/ml in phosphate buffered-saline, pH 7.4) was continuously cultured until termination. Culture was stopped by addition of 15% sodium dodecyl sulfate into each well for solubilization of formazan, and the OD at 570 nm (OD570-630) was measured by a microplate (Spectramax 250 microplate) reader. The mean value of the OD content of 4 wells was used for calculating the viability (% of control).

**T cell proliferation assay**

Splenocytes were prepared from the spleens of mice killed by cervical dislocation under the sterile conditions described previously (Cho et al., 2000). Briefly, splenocytes were released by teasing into Roswell Park Memorial Institute (RPMI) 1640 medium (GIBCO) supplemented with 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer. After removing red blood cells using 0.83% NH₄Cl-Tris buffer (pH 7.4), splenocytes were washed three times in Ca²⁺–Mg²⁺ free Hank’s balanced salt solution and resuspended to 5×10^6 cells/ml in RPMI 1640 with 100 U/ml of penicillin and 100 μg/ml of streptomycin, and 10% fetal bovine serum (FBS). Splenocytes (5×10^6 cells/ml) were cultured in 96 well plates coated with anti-CD3 (10 μg/ml) for 48 h. The proliferation assay was performed by MTT assay.

**Results**

**Effect on NO production**

To test whether the fermented plant extract was able to inhibit NO production, a macrophage cell line (RAW 264.7 cells) was employed and stimulated by LPS (2.5 μg/ml). As shown in Fig. 1, the fermented extract dose dependently blocked NO production. In particular, the inhibitory effect of the extract was maximized until 80-fold dilution of the extract.

**Effect on cytoprotective effect**

NO is one of the toxic molecules inducing cell death and inflammation. Therefore, we tested whether the fermented plant extract was capable of protecting NO-induced cell cytotoxicity. As shown in Fig. 2, the fermented plant extract did not induce a cytotoxic effect up to 80-fold dilution. Interestingly, doses between 320- and 80-fold dilution significantly protected cell death mediated by LPS-induced NO production. Up to 80-fold dilution, there was no cytotoxic effect from the fermented extract.

**Effect on T cell proliferation**

T cells are known to play an important role in both normal and pathological cell-mediated immune responses. In atopic dermatitis pathology, proliferation and T helper cell type 2 (Th2) differentiation of T cells are reported to be one of the central causes. Therefore, the effect of the fermented plant extract on T cell proliferation and Th2 differentiation should be examined to demonstrate its anti-apoptotic activity. For preliminary study, whether the fermented extract was able to modulate T cell proliferation, was first examined. As shown in
Fig. 3 Inhibitory effect on T cell proliferation of the fermented extract of the plant complex.

Fig. 3, the fermented extract significantly suppressed CD3-induced T cell proliferation in a dose dependent manner.

Discussion

Atopic dermatitis is one of the chronic allergic diseases accompanying severe inflammation and allergic reactions. Recent studies have demonstrated that atopic dermatitis is managed by inflammatory cells such as macrophages and mast cells, as well as lymphocytes such as T and B lymphocytes (Takano et al., 2006). Although IgE-mediated mast cell activation is known to be a central phenomenon in creating a severe pathological environment, including histamine and prostaglandins, secretion of numerous kinds of inflammatory mediators and cytokines from macrophages are reported to produce a severe condition, too (Steinhoff et al., 2006). Furthermore, the fundamental defect of Th1/Th2 balance is regarded as one of major basic reasons for atopic dermatitis, in which the Th2 response is much stronger than the Th1 (Hussain and Kline, 2004).

The fermented plant extract displayed a promising effect on atopic dermatitis, in particular, juvenile cases (data not shown). In this study, we therefore examined its molecular pharmacology to understand the therapeutic mechanism. As a preliminary study, we first focused on whether the extract was able to modulate T cell proliferation and NO production, which are one of main pathological phenomena in atopic dermatitis. Interestingly, the extract very strongly blocked T cell proliferation and NO production, suggesting that the suppressive effect may be one of its therapeutic mechanisms against atopic dermatitis. Due to the fact that this is just a preliminary study, further careful studies related to immunopathological phenomena, such as Th1/Th2 balancing, mast cell activation and histamine release, should follow in the near future.

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References


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Correspondence to: Myeon Choe, Functional Products R&D Laboratory, Dept. of Plant Biotechnology, College of Biotechnology, Kangwon National University, Chuncheon, 200–701, Korea
Phone: 82–33–250–8645
Fax: 82–33–250–6470
e-mail: mchoe@kangwon.ac.kr