

Inhibition of Experimental Atopic Dermatitis by Rhubarb (Rhizomes of *Rheum tanguticum*) and 5-Lipoxygenase Inhibition of its Major Constituent, Emodin

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The antiallergic activity of rhubarb and its constituents, anthraquinones, has been reported previously. For further evaluation of the antiallergic activity, a 70% ethanol extract of the rhizomes of *Rheum tanguticum* (RTE) was prepared and its inhibitory activity on an animal model of atopic dermatitis (AD) was examined for the first time. Oral administration of RTE (30–300 mg/kg/day) for 5 weeks significantly inhibited hapten-induced dermatitis in NC/Nga mice based on the skin severity score. In addition, treatment with RTE at 100 mg/kg/day also reduced the numbers of white blood cells, neutrophils and eosinophils in the blood, and led to a significant reduction in the IgE concentration in the serum. In rat basophilic leukemia (RBL)-1 cells, RTE inhibited 5-lipoxygenase (5-LOX)-catalysed leukotriene production ($IC_{50} = 43.6 \mu\text{g/mL}$). Among the anthraquinone derivatives isolated, emodin strongly inhibited this parameter ($IC_{50} = 4.3 \mu\text{M}$). Taken together, these findings suggest that rhubarb exerts inhibitory activity against AD, and that the 5-LOX inhibitory activity of its major constituent, emodin, may contribute to this inhibitory action. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: *Rheum tanguticum*; rhubarb; emodin; atopic dermatitis; lipoxygenase; antiallergy.

INTRODUCTION

Atopic dermatitis (AD) is a chronic allergic disorder that is characterized by frequent itching with flare, hemorrhage, hyperplasia and other symptoms on the affected area of skin (Leung, 1995; Rudikoff and Lebwohl, 1998). New therapeutic agents are needed urgently for the treatment of AD since it is still difficult to control and cure atopic dermatitis with currently used drugs. Although the etiology is not fully understood, many pathological molecules involved in this disease have been identified (Novak, 2009). For example, several chemokines and receptors such as CCR3 are important in this disease (Elsner *et al.*, 2004). In addition, NO is an abrogating factor of AD (Taniuchi *et al.*, 2001) and 5-lipoxygenase (5-LOX) products such as leukotrienes (LT) are also involved (Rubin and Mollison, 2007). Therefore, inhibition of the production of these pathological molecules may ameliorate the symptoms of atopic dermatitis.

Rhubarb is the rhizome of *Rheum* species including *R. tanguticum*, *R. palmatum*, *R. officinale* and *R. undulatum*, which have long been used to enhance blood circulation, as a purgative agent and for the treatment of inflammatory/allergic disorders in China, Korea and Japan (Bae, 2000). Previously, an aqueous ethanol extract of *R. palmatum* showed antiinflammatory and antiallergic activity against tetradecanoylphorbol 13-acetate-, arachidonic

acid-induced ear edema and oxazolone-induced delayed hypersensitivity following topical application (Cuellar *et al.*, 2001). As to its constituents, anthraquinone derivatives such as emodin are major ingredients. Emodin was demonstrated previously to inhibit phytohaematoglutinin-induced mononuclear cell proliferation (Huang *et al.*, 1992) and to inhibit calcium mobilization in human T lymphocytes (Kuo *et al.*, 2001). Another anthraquinone, chrysophanol, also exhibited antiallergic action (Kim *et al.*, 2000). These findings demonstrate that rhubarb and some of its major constituents possess antiallergic activity. These results also indicate that rhubarb may exert an inhibitory effect on the chronic allergic skin disorder, atopic dermatitis. Therefore, this study was conducted to evaluate the inhibitory activity of rhubarb against experimental atopic dermatitis for the first time in a hapten-induced dermatitis model using NC/Nga mice. Additionally, to evaluate the cellular action mechanism, the 5-LOX inhibitory action of rhubarb and its anthraquinone constituents was evaluated.

MATERIALS AND METHODS

Chemicals. A23187 was obtained from Biomol (Plymouth Meeting, PA). Nordihydroguaiaretic acid (NDGA, LOX inhibitor) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St Louis, MO). DMEM and other cell culture reagents including FBS were products of Gibco BRL (Grand Island, NY). A protein assay kit was

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purchased from Bio-Rad (Hercules, CA). Clobetasol-17-propionate ointment (0.05% w/w, Dermovate®) was obtained from GlaxoSmithKline Korea.

Animals. NC/Nga mice (male, 4 weeks old) were obtained from SLC (Japan). The animals were fed with standard laboratory chow and water was freely available. The animals were maintained in the animal facility (KNU) at 20–22 °C under 40–60% relative humidity and a 12 h/12 h (light/dark) cycle for at least 7 days prior to the experiment. The experimental design using the animals was approved by the local committee for animal experimentation of KNU (KIACUC-09-0029). The animals were handled according to the guidelines described in the Food and Drug Administration (Korea) Guide for the Care and Use of Laboratory Animals throughout the experiments.

Plant materials. The rhizomes of *Rheum tanguticum* Maxim. (Polygonaceae) were purchased from Yuseong herbal drug market, Daejeon, Korea and identified by Dr J. H. Lee (College of Oriental Medicine, Dongguk University, Korea). A voucher specimen (CNU-1345) has been deposited in the herbarium of the College of Pharmacy, Chungnam National University, Korea.

Preparation of the 70% ethanol extract and isolation of the anthraquinones from the rhubarb. The dried and milled rhizomes of rhubarb (1 kg) were extracted with 5 L 70% ethanol, three times. The ethanol extract was combined and concentrated to yield a residue (108 g, RTE). The RTE was used throughout this study. From RTE, chrysophanol, emodin, physcion and chrysophanol-8-*O*- β -glucopyranoside were isolated and structurally identified, essentially following the procedures described previously (Ngoc *et al.*, 2008) (Fig. 1).

Hapten-induced atopic dermatitis in NC/Nga mice. Hairs were removed from the backs of NC/Nga mice. After 24 h, 200 μ L of 1% dinitrochlorobenzene (DNCB) solution in acetone:olive oil (3:1) was applied to the hair-removed area as described previously (Matsuda *et al.*, 1997; Suto *et al.*, 1999). Three days later, DNCB solution was applied again. One week after the initial treatment, 0.4% DNCB solution (150 μ L) was applied three times per week for 4 weeks to induce dermatitis. On the final day, the skin severity was scored based on the skin

clinical symptoms, dry skin and lichenification, edema and excoriation, and erythema, according to the slightly modified criteria of the previously described procedure (Leung *et al.*, 1990; Suzuki *et al.*, 2002). After scoring, the mice were killed and blood was withdrawn by heart puncture. From the whole blood, the numbers of white blood cells, neutrophils and eosinophils were counted. Additionally, the IgE concentration of the serum was measured using an ELISA kit (Shibaya Co., Japan). For histological observation, the skin was removed and stored in formalin solution. RTE was administered orally at the indicated doses for 5 weeks during the experiment. Clobetasol-17-propionate ointment was applied topically as a reference drug.

Rat basophilic leukemia-1 (RBL-1) cell culture and 5-lipoxygenase (5-LOX)-catalysed leukotriene (LT) production. To evaluate the effect on 5-LOX-catalysed LT production, RBL-1 cells purchased from the American Type Culture Collection (ATCC, Rockville, VA) were cultured in RPMI 1640 with 10% FBS, 2 mM glutamine and 1% antibiotics under 5% CO₂ at 37 °C. The cells were then incubated in 96-well plates for 2 h. The test compounds were dissolved in DMSO and diluted to appropriate concentrations with serum-free DMEM. The final concentration of DMSO was adjusted to 0.1% (v/v). A-23187 (ionophore, 3 μ M) was then added to activate the 5-LOX and the cells were incubated for 15 min as described previously, with slight modification (Tries *et al.*, 2002). The media was then collected and the concentration of the 5-LOX product, cysteinyl-leukotrienes (LTC₄/D₄/E₄), was measured using an ELISA kit (Cayman Chemicals) as recommended by the manufacturer. The cells were then pre-incubated with the test compounds for 10 min. The cell viability was assessed using an MTT assay as described previously (Mossman, 1983). No significant reduction of cell viability was found in all tested groups at the indicated concentrations (data not shown).

Statistical analysis. All data are represented as an arithmetic mean $\pm$ SD. One-way analysis of variance (ANOVA), followed by Dunnett's test was used to determine the statistical significance.

RESULTS

NC/Nga mice developed human AD-like symptoms in response to application of the hapten, DNCB, for 5 weeks. As shown in Fig. 2a and 2b, DNCB application led to a drastic increase in the skin severity score and induced characteristic symptoms of dermatitis. The histology of the affected skin area showed the same results. In the blood, the numbers of WBC, neutrophils and eosinophils increased by 51.0%, 86.9% and 167.7%, respectively (Fig. 3a–3c). The IgE concentration in the serum also increased by approximately 4-fold in response to hapten treatment (Fig. 3d). Under these conditions, RTE reduced these parameters when administered orally at 30–300 mg/kg/day. The RTE also ameliorated the skin lesions as determined by the skin severity score. Interestingly, RTE showed the strongest inhibition of dermatitis when administered at a dose of 100 mg/kg/day (74.5% inhibition) when compared with doses of 30 and

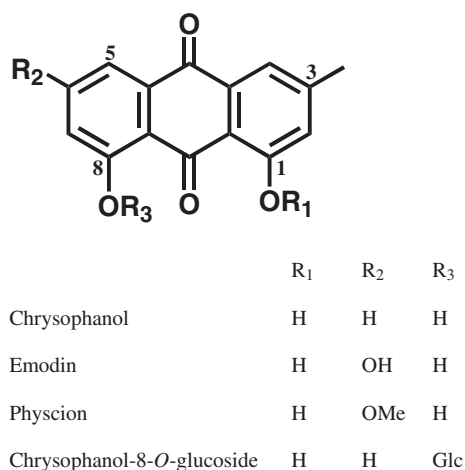


Figure 1. The chemical structures of anthraquinones isolated from rhubarb in this study.

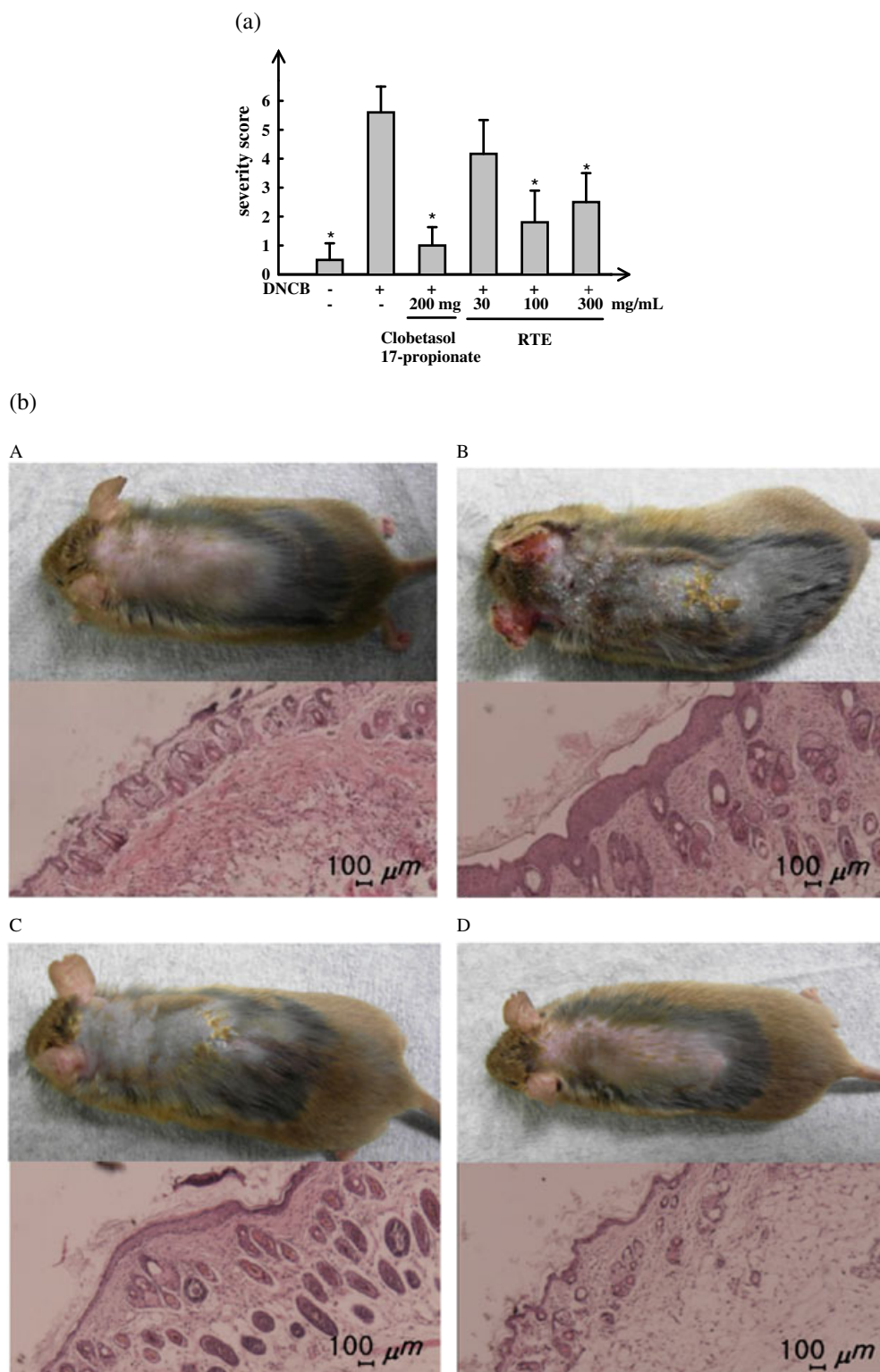


Figure 2. Inhibitory effect of rhubarb against experimental atopic dermatitis in NC/Nga mice. (a) Skin severity score: three categories were checked on the skin of NC/Nga mice (dry skin and lichenification, edema and excoriation, erythema), and each category was scored based on the severity as follows: no symptom (0), weak (1), medium (2), severe (3). The scores were combined in each animal. Data shown are the arithmetic mean $\pm$ SD ($n = 5$). * $p < 0.05$, significantly different from the DNCB-treated control group. (b) Histology of the skin lesions of DNCB-treated NC/Nga mice. Note: many inflammatory cells were infiltrated in the dermal area, but epidermal and dermal hyperplasia were not observed (control, A), while massive epidermal and dermal hyperplasia along with many inflammatory cells infiltrated in the dermal area was observed (DNCB-treated control, B). Oral administration of RTE (100 mg/kg/day, C) strongly reduced epidermal and dermal hyperplasia, whereas inflammatory cell infiltration was moderately reduced. Almost complete reduction of these two parameters was seen in the clobetasol-17-propionate-treated group (D) (magnification, $\times 40$). This figure is available in colour online at wileyonlinelibrary.com/journal/ptr

300 mg/kg/day (28.1% and 60.8% inhibition). When the cell numbers and IgE concentration were examined, RTE (100 mg/kg/day) reduced the WBC (21.5%), neutrophil (191.1%) and eosinophil (86.4%) levels when compared

to those of the DNCB-treated control group. RTE (100 mg/kg/day) led to a particularly strong reduction in IgE concentration (106.6% inhibition). As expected, clobetasol-17-propionate, which was used as a reference

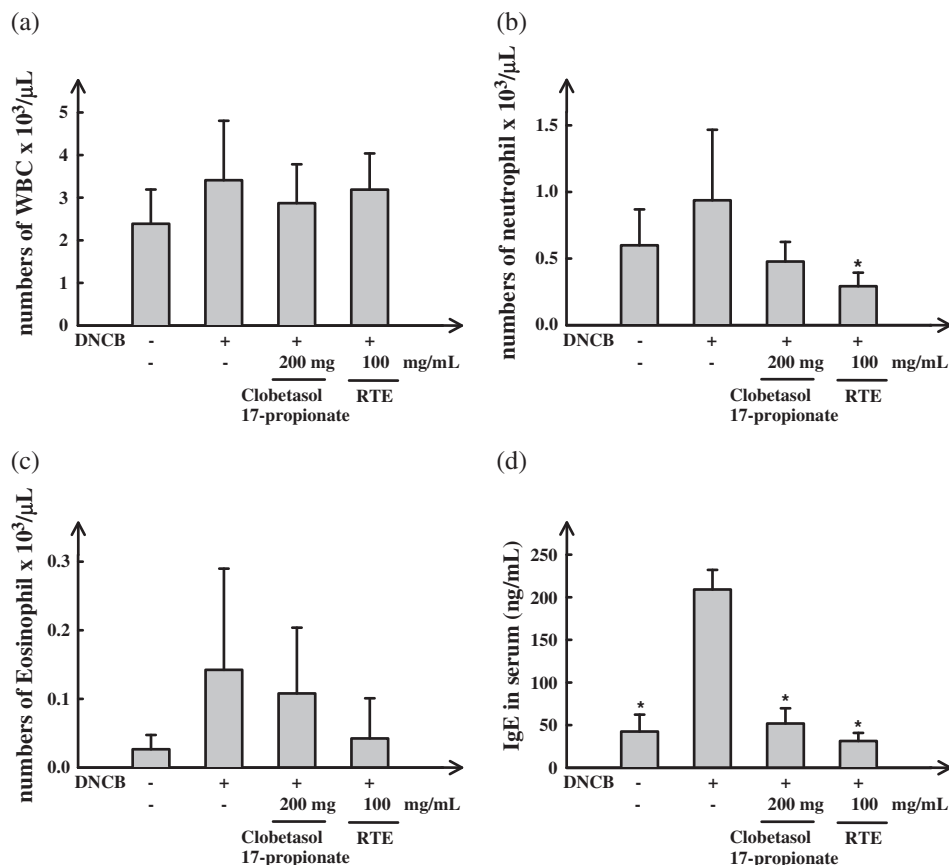


Figure 3. Effects of rhubarb on blood cell counts and serum IgE concentration in NC/Nga mice. (a) Effects on the number of white blood cells in the blood, (b) Effects on the number of neutrophils, (c) Effects on the number of eosinophils, (d) Effects on the IgE concentration in the serum. Data are presented as the arithmetic mean $\pm$ SD ($n = 5$). * $p < 0.05$, significantly different from the DNCB-treated control group.

drug, showed potent inhibition of all parameters measured when topically applied. Specifically, the percent inhibitions of clobetasol-17-propionate were 90.2%, 52.6%, 136.2%, 29.8% and 94.3% for the skin severity score, and the WBC, neutrophil and eosinophil numbers, and IgE concentrations, respectively.

For elucidation of some action mechanism(s), the 5-LOX inhibitory activity was examined. When RBL-1 cells were treated with A23187, they produced large amounts of LTs by activated 5-LOX. In the present study, cysteinyl-LTs of 1.34 ± 0.15 ng/mL were produced during the 15 min incubation with A23187 from the basal level of 0.08 ± 0.03 ng/mL ($n = 3$). Under these conditions, RTE and emodin significantly inhibited 5-LOX (Table 1), with emodin showing especially strong inhibitory activity.

Table 1. Inhibition of 5-LOX-catalysed LT production in A23187-treated RBL-1 cells

Compound	% inhibition ^a	IC ₅₀ (μM)
RTE	72.7	43.6 ^b
Chrysophanol	- ^c	
Emodin	86.0	4.3
Physcion	-	
Chrysophanol-8- <i>O</i> -glucoside	-	
NDGA	99.5	0.1 – 1

^aArithmetic mean ($n = 3$). All compounds were tested at 10 μM, except NDGA (1 μM) and rhubarb extract (30 μg/mL).

^bμg/mL.

^cNot active.

DISCUSSION

NC/Nga mice are used to evaluate the effect of many drug candidates against human AD. These mice spontaneously develop AD-like symptoms under conventional environments or in response to topical application of hapten in shorter time periods. In addition to skin lesions, IgE concentration is elevated and eosinophilia is observed in NC/Nga mice (Matsuda *et al.*, 1997; Suto *et al.*, 1999). Since patients with AD frequently show high titers of IgE for various allergens and typical eosinophilia, these molecules may be used as biomarkers of AD (Habif, 1996). In the present study, animals that developed dermatitis in response to hapten treatment showed these typical reactions: elevated IgE and increased numbers of neutrophils and eosinophils in the blood. The rhubarb extract considerably inhibited each of these parameters, suggesting its inhibitory potential against human AD.

Among the small chemical mediators, LTs synthesized by the 5-LOX reaction are believed to play an important role in AD (Chari *et al.*, 2001). In the present study, the rhubarb extract and one of its major constituents, emodin, are found to possess a 5-LOX inhibitory action. Although detailed structure–activity relationships could not be determined in this study due to the limited numbers of the derivatives examined, it has been suggested that C-6 OH (emodin vs chrysophanol, physcion) is important among the anthraquinone derivatives for exerting 5-LOX inhibition.

It was demonstrated previously that anthraquinone derivatives, including emodin, inhibited soy lipoxygenase (Ngoc *et al.*, 2008). In the present study, it was demonstrated that the rhubarb extract and emodin are 5-LOX inhibitors. In addition to its suppressive effect on lymphocyte activation (Kuo *et al.*, 2001; Kim *et al.*, 2000), it is strongly suggested that the inhibitory action of emodin on 5-LOX contributes, at least in part, to the inhibitory activity of rhubarb against AD. To the best of our knowledge, this is the first report to demonstrate the inhibitory activity of rhubarb and its constituent, emodin, on 5-LOX-catalysed LT production and the inhibitory action of rhubarb in an animal model of atopic dermatitis.

CONCLUSION

The present investigation demonstrated that rhubarb inhibited an animal model of atopic dermatitis when

administered orally. Rhubarb and its major constituent, emodin, were found to inhibit 5-LOX-mediated LT production from mast cells. The 5-LOX inhibitory action of emodin may, at least in part, contribute to the inhibitory action of rhubarb against AD.

Acknowledgements

This study was financially supported by the research fund of Studies on the Identification of the Efficacy of Biologically Active Components from Oriental Herbal Medicines from Korean Food and Drug Administration (2009–2011) and the post-BK21 project from the Ministry of Education, Science and Technology (Korea). We would like to thank the Institute of Pharmaceutical Science (KNU) for use of the bioassay facilities.

Conflict of Interest

The authors have declared that there is no conflict of interest.

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