



Contents lists available at ScienceDirect

International Immunopharmacology

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

Preliminary report

Effects of topically applied rapamycin and mycophenolic acid on TNCB-induced atopic dermatitis-like skin lesions in NC/Nga mice

Kyung Eun Jung^a, Ye Jin Lee^b, Yun Hee Ryu^b, Jung Eun Kim^c, Hei Sung Kim^d, Beom Joon Kim^e, Hoon Kang^c, Young Min Park^{b,*}^a Department of Dermatology, Eulji University Hospital, College of Medicine, Eulji University, 95 Dunsanse-ro, 302-799 Daejeon, Republic of Korea^b Department of Dermatology, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 505 Banpo-daero, 137-701 Seoul, Republic of Korea^c Department of Dermatology, St. Paul's Hospital, Seoul, College of Medicine, The Catholic University of Korea, 620-56 Jeonnon-dong, 130-709, Seoul, Korea^d Department of Dermatology, Incheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Dongsuro, Bupyeong-gu, 150-713 Incheon, Republic of Korea^e Department of Dermatology, Chung-Ang University Hospital, College of Medicine, Chung-Ang University, 102 Heukseok-ro, 156-755 Seoul, Republic of Korea

ARTICLE INFO

Article history:

Received 31 July 2014

Received in revised form 3 March 2015

Accepted 4 March 2015

Available online xxxx

Keywords:

Atopic dermatitis

Mycophenolic acid

Rapamycin

Topical administration

ABSTRACT

Rapamycin (RPM) and mycophenolic acid (MPA) are immunosuppressive drugs approved for use in preventing transplant rejection. These drugs have also been used in the field of dermatology as glucocorticoid sparing agents for autoimmune and inflammatory disorders such as atopic dermatitis (AD). The aim of this study was to investigate the therapeutic effect of topically applied RPM and/or MPA on AD-like skin lesions in NC/Nga mice. RPM (0.04% – 4%), MPA (0.2% – 5%), and formulations of both agents at various ratios were administered topically to NC/Nga mice with 2-chloro-1,3,5-trinitrobenzene (TNCB)-induced AD-like skin lesions. The therapeutic effects of topical RPM, MPA, and the mixed formulations in TNCB-treated NC/Nga mice were assessed by measuring skin severity scores, ear thickness, and histological changes in the lesioned skin including mast cell count and total serum IgE levels. Expression of interleukin (IL)-4, and interferon (IFN)- γ was also assessed. Topical 4% RPM and/or 1% MPA treatment significantly improved clinical signs of AD such as erythema, edema, excoriation, and dryness on day 29 ($P < 0.05$). In addition, 4% RPM, 1% MPA, and the mixed formulations significantly decreased epidermal thickening, dermal edema, and cellular infiltration into the dermis compared with the vehicle. RPM (4%) and/or MPA (1%) significantly reduced the expression of IL-4 and IFN- γ mRNA and protein levels compared with the vehicle ($P < 0.05$). No significant change in the levels of total serum IgE was induced by topical 4% RPM and/or 1% MPA. The present results demonstrated that topical 4% RPM and/or 1% MPA improved TNCB-induced AD-like lesions of NC/Nga mice by suppressing expression of Th2-related cytokines (IL-4) and Th1-related cytokines (IFN- γ). These findings suggest that RPM and/or MPA may be promising topical therapeutic candidates for the treatment of AD.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disorder characterized by recurring flare-ups, or flares [1]. In most patients, AD is successfully managed with topical glucocorticoids and/or calcineurin inhibitors (CnIs). However, topical glucocorticoids have several well-known side effects including skin atrophy, striae, easy bruising, telangiectasia, tachyphylaxis and rebound phenomenon, whereas use of topical CnIs is associated with cutaneous irritation. As such, there have

been a number of efforts aimed at developing new safe and effective topical agents for AD [2–6].

Rapamycin (RPM) and mycophenolic acid (MPA) are immunosuppressive drugs usually used to prevent acute graft rejection. RPM inhibits the response to interleukin (IL)-2, thereby blocking activation of T and B lymphocytes. RPM also inhibits G1 to S phase cell-cycle progression [7]. On the other hand, MPA inhibits lymphocyte proliferation by inhibiting inosine monophosphate dehydrogenase, which is required for proliferation of T and B lymphocytes [8].

Recently, systemic treatment with MPA has been shown to be effective in treating AD [9,10]. MPA delays the clinical response of AD compared with cyclosporine by arresting the cell cycle of T lymphocytes at the G0/G1 phase, but not by inhibiting IL-2 synthesis in lymphocytes that have already been activated.

Both RPM and MPA regulate dendritic cell maturation and T cell activity, and thus their ability to improve immunologic skin disorders is

* Corresponding author at: Department of Dermatology, Seoul St. Mary's Hospital, The Catholic University of Korea, #505 Banpo-daero, Seocho-gu, Seoul 137-701, Republic of Korea. Tel.: +82 2 2258 6223; fax: +82 2 594 3255.

E-mail address: yminpark6301@hotmail.com (Y.M. Park).

not unexpected [11]. Indeed, based on existing pharmacodynamic data, topical RPM and MPA are both potential candidates to manage AD. To date, there have been several attempts to use RPM and MPA topically as therapeutics for inflammatory skin diseases [12–16]. Recently, Yang et al. reported that topical administration of 0.2% RPM on AD-like skin lesions for 4 weeks improved AD symptoms and histologic features of NC/Nga mice as well as decreased IL-4 and IL-13 expression levels in the lesional skin [14]. The anti-allergic and anti-inflammatory effects of MPA have also been confirmed through a reduction in visual skin reaction as well as suppression of hyperkeratosis and inflammatory cell infiltration in the epidermis and dermis in experimental allergic contact dermatitis induced by dinitrofluorobenzene [15]. Lastly, Wohlrab et al. reported that 2% topical mycophenolate mofetil (MMF), the prodrug of MPA, improves chronic plaque psoriasis lesions compared with 0.1% betamethasone-17-valerate cream [16]. Taken together, these reports of the anti-inflammatory properties of RPM and MPA strongly suggest their use as anti-atopic agents.

MMF may be combined with other traditional immunosuppressive agents to take advantage of potential synergistic effects; however, such approaches may increase the risk infection and neoplasia [17]. Along these lines, Nguyen et al. suggested that the combination of RPM and MMF may better conserve the inhibition of T cell proliferation compared to cyclosporine [18]. The combination of RPM and MMF may also interfere with early immune responses by targeting essential functions of dendritic cells [19]. Therefore, the combination of these agents is assumed to offer safer and more complementary immunosuppressive effects with reduced side effects compared with either drug alone.

Despite the potential promising effects of topical administration of RPM or MPA alone, the effects of a combined formulation on AD have not been fully studied. In addition, there have been very few experimental trials concerning the topical use of this combination in animal models of AD. Thus, this study aimed to investigate the effects of topically applied RPM, MPA, and their combination, on 2-chloro-1,3,5-trinitrobenzene (TNCB)-induced AD-like skin lesions in NC/Nga mice.

2. Materials and methods

2.1. Animals

4-week-old female NC/Nga mice were purchased from Japan SLC (Shizuoka, Japan). Mice were maintained under SPF conditions at $24 \pm 1^\circ\text{C}$ with a humidity of $50 \pm 10\%$ under a 12-h light/12-h dark cycle. Food and tap water were provided ad libitum. Animal care, handling, and experimental procedures were performed in accordance with a protocol approved by the Animal Care and Use Committee of The Catholic University of Korea.

2.2. Reagents

RPM, MPA, and the vehicle used in this study were kindly provided by Iksu Co., Seoul, Korea. RPM (0.04–4%) and MPA (0.2–5%) were prepared by first dissolving each in ethanol followed by further dilution into the vehicle. Specifically, RPM was prepared first as a 28% (w/v, 4 g) solution in ethanol, and then as 10 g of 4% RPM (8.6 g vehicle and 1.4 g of the 28% solution). MPA was prepared in a similar fashion by preparing a 30% solution (w/v, 3 g) in ethanol, and then as 10 g of 1% MPA (9.833 g vehicle and 0.167 g of the 30% solution). Combined RPM and MPA formulations (RM) were prepared at ratios of 2:8, 5:5, and 8:2. The vehicle included glycerin (5%), xanthan gum (0.1%), 1,3-butylene glycol (6%), carbomer (0.3%), beeswax (2%), cetanol (2%), white petrolatum (1.5%), lecithin (0.5%), arlacel 165 (2%), tween 60 (0.2%), arlacel 60 (0.2%), liquid paraffin (6%), dimethicone (1%), triethanolamine 0.3%, preservative (0.3%), and water (up to 100%). Among them, some ingredients such as beeswax (2%) and dimethicone (1%) are known to have an effect in inhibiting inflammation. For the positive control,

0.03% tacrolimus (Protopic®, Astellas Pharma Inc., Tokyo, Japan) was used.

2.3. Induction of AD-like skin lesions

TNCB-induced AD-like skin lesions in NC/Nga mice were produced as previously described [3]. Briefly, the abdomens and ears of mice were sensitized epicutaneously by applying 150 μl of 5% TNCB dissolved in ethanol/acetone mixture (4:1 vol/vol) once. One week after sensitization, the dorsal surface of the ears was challenged each with 20 μl of 1% TNCB dissolved in acetone/olive oil (4:1 vol/vol). The treatment with 1% TNCB was applied repeatedly to the ears every other day for 2 weeks, and then twice a week for a further 2 weeks along with topical treatments.

2.4. Topical administration of RPM, MPA, and mixed formulations on NC/Nga mice

After AD-like lesions were fully developed during the first 2-week challenge, RPM (0.04–4%), MPA (0.2–5%), three mixed formulations of RPM and MPA (2:8, 5:5, 8:2), vehicle, and 0.03% tacrolimus were applied topically to the dorsal side of ears (0.05 g per ear) of each group once per day, five times a week, for a total of 2 weeks. The design of the study is summarized in Fig. 1. In the first experiment, NC/Nga mice were allocated into 9 groups (TNCB only; vehicle; 0.04%, 0.4%, and 4% RPM; 0.2%, 1%, and 5% MPA; and 0.03% tacrolimus, with 6 mice per group. In the second experiment, NC/Nga mice were divided equally into 8 groups (TNCB only, vehicle, 4% RPM, 1% MPA, 4% RPM and 1% MPA mixed formulations (2:8, 5:5, 8:2), and 0.03% tacrolimus), with 5 mice per group.

2.5. Measurement of skin severity score, ear thickness, and scratching behavior

The severity of dermatitis was assessed macroscopically and photographed in a blinded fashion once a week beginning after the first challenge using the following scoring system. The skin severity score was defined as the sum of individual scores for each of the following 4 signs: erythema (hemorrhage), edema (swelling), excoriation (erosion), and dryness. Each of these items was scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe) as previously described [3]. The degree of therapeutic effects of each trial agent was expressed as a relative fold increase ((D29 (post-treatment) – D15 (pre-treatment)) / D15 (pre-treatment) skin severity score + 1). Ear thickness was measured once per week using dial calipers (Kori Seiki MFG, Tokyo, Japan). Two separate investigators who were blinded to the grouping of the animals performed the assessments independently.

2.6. Histopathology

Ear samples were obtained on day 29, fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at a thickness of 5 μm . Tissue sections were then stained with hematoxylin and eosin (H&E) and toluidine blue. The number of mast cells was counted independently by two separate researchers in 5 randomly selected sites at $\times 400$ magnification.

2.7. Quantitative reverse transcription–polymerase chain reaction (RT-PCR)

Total RNA was isolated using Trizol® reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Equal amounts of RNA (1 μg) were reverse transcribed into cDNA using Prime Script™ RT reagent Kit with gDNA Eraser® (Takara Bio, Otsu, Japan). Quantitative RT-PCR was performed using a CFX96™ Real-Time PCR Detection System® (Bio-Rad, Hercules, CA) with SYBR® premix Ex Taq™ (TaKaRa

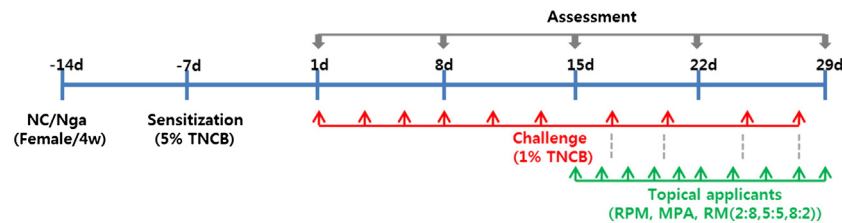


Fig. 1. Study design.

BIO) and specific primers for IFN- γ , IL-4, and β -actin. The primer sequences are shown Table 1. The cycling conditions consisted of an initialization step for 10 s at 95 °C followed by two-step PCR for 45 cycles of 95 °C for 5 s (denaturation) and 58 – 60 °C for 30 s (annealing/extension). Fluorescence intensity was measured in real time using the optical module. Melt curves were used to determine product specificity. Results were normalized to the level of β -actin gene expression.

Table 1
Primers utilized in this study.

Gene	Forward primer 5' → 3'	Reverse primer 5' → 3'
IFN- γ	CGGCACAGTCATTGAAAGCCTA	GTTGCTGATGGCTGATTGTC
IL-4	TCTCGAATGTACCAGGAGCCATATC	AGCACCTTGAAGCCCTACAGA
β -actin	TGTGATGGTGGGAATGGGTCAGAA	TGTGGTGCCAGATCTTCTCCATGT

Analysis of relative gene expression data was calculated using the $2^{-\Delta\Delta CT}$ method. All experiments were repeated twice.

2.8. Enzyme-linked immunosorbent assay (ELISA)

IL-4 and INF- γ protein levels of skin lesions were measured by commercial ELISA kits® (eBioscience, San Diego, CA) according to the manufacturer's instructions. Total IgE in the serum was measured using an ELISA kit® (Bethyl Laboratories Inc., Montgomery, TX) according to the manufacturer's instructions.

2.9. Statistical analysis

All data were expressed as the mean $\pm$ standard error of the mean (SEM). One-way analysis of variance followed by Tukey's multiple comparison test was used for statistical analysis. The Kruskal–Wallis test was used for comparisons of the three groups. In all cases, P values <0.05 were considered significant.

3. Results

3.1. Therapeutic effect of topical RPM and MPA on TNCB-induced AD-like skin lesions according to concentration

To identify the most effective concentrations of RPM and MPA for treating TNCB-induced AD-like skin lesions, we investigated RPM at 0.04%, 0.4%, and 4% and MPA at 0.2%, 1%, and 5% based on previous data [12–16]. To evaluate the therapeutic effect of RPM and MPA on AD-like skin lesions, we scored the clinical signs of the ears according to criteria for 4 major clinical signs of AD, namely, erythema/hemorrhage, edema, excoriation/erosion, and scaling/dryness. The skin severity scores of all TNCB-treated groups increased progressively with time during the 2-week challenge period. However, after initiating treatment,

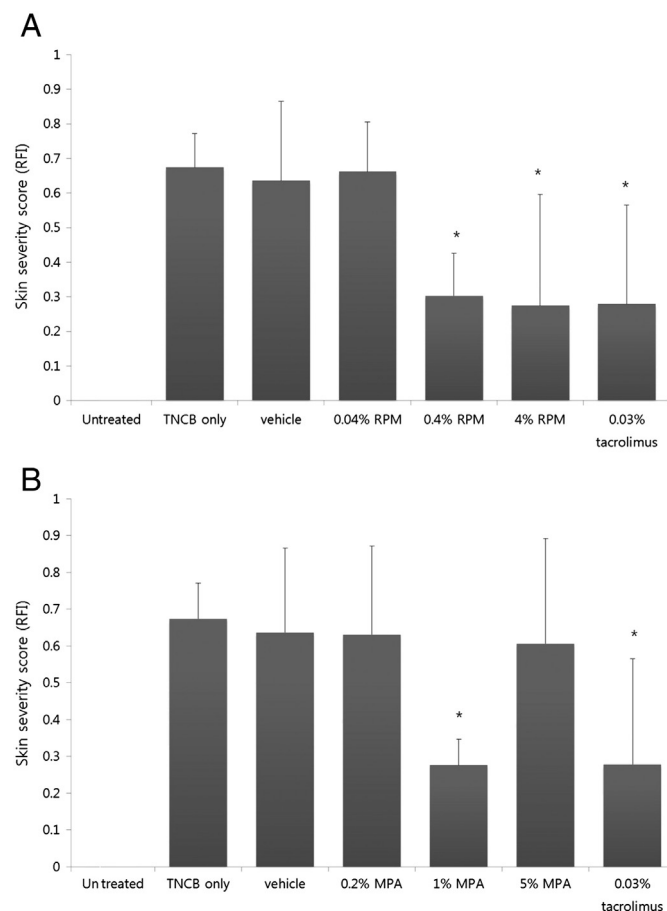


Fig. 2. Changes in skin severity scores according to RPM and MPA treatments as a function of concentration. Changes in skin severity scores after 2 weeks of treatment with RPM or MPA are shown as the relative fold increase (RFI). Each experimental group consisted of six mice. * $P < 0.05$ compared with the vehicle. RPM; rapamycin, MPA; mycophenolic acid.

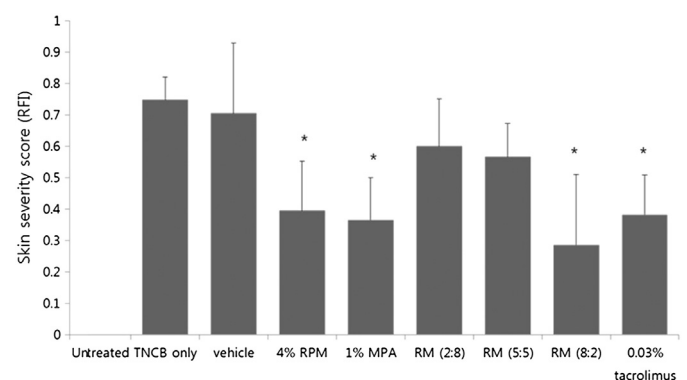


Fig. 3. Changes in skin severity scores according to treatment with 4% RPM, 1% MPA, and several mixed formulations (2:8, 5:5, 8:2). Changes in skin severity scores after 2 weeks of treatment with RPM and MPA are shown as the relative fold increase (RFI). Each experimental group consisted of five mice. * $P < 0.05$ compared with the vehicle. RPM; rapamycin, MPA; mycophenolic acid, RM; rapamycin + mycophenolic acid.

4% RPM was found to have the most significant effect on reducing the skin severity score compared with the vehicle ($P < 0.05$) (Fig. 2A). Among the MPA treated groups, administration of 1% MPA resulted in the largest decrease in skin severity score ($P < 0.05$) (Fig. 2B).

3.2. Therapeutic effect of topical 4% RPM, 1% MPA, and mixed formulations on TNCB-induced AD-like skin lesions

We conducted the next experiments using the mixed formulations containing 4% RPM and 1% MPA. First, in order to determine whether

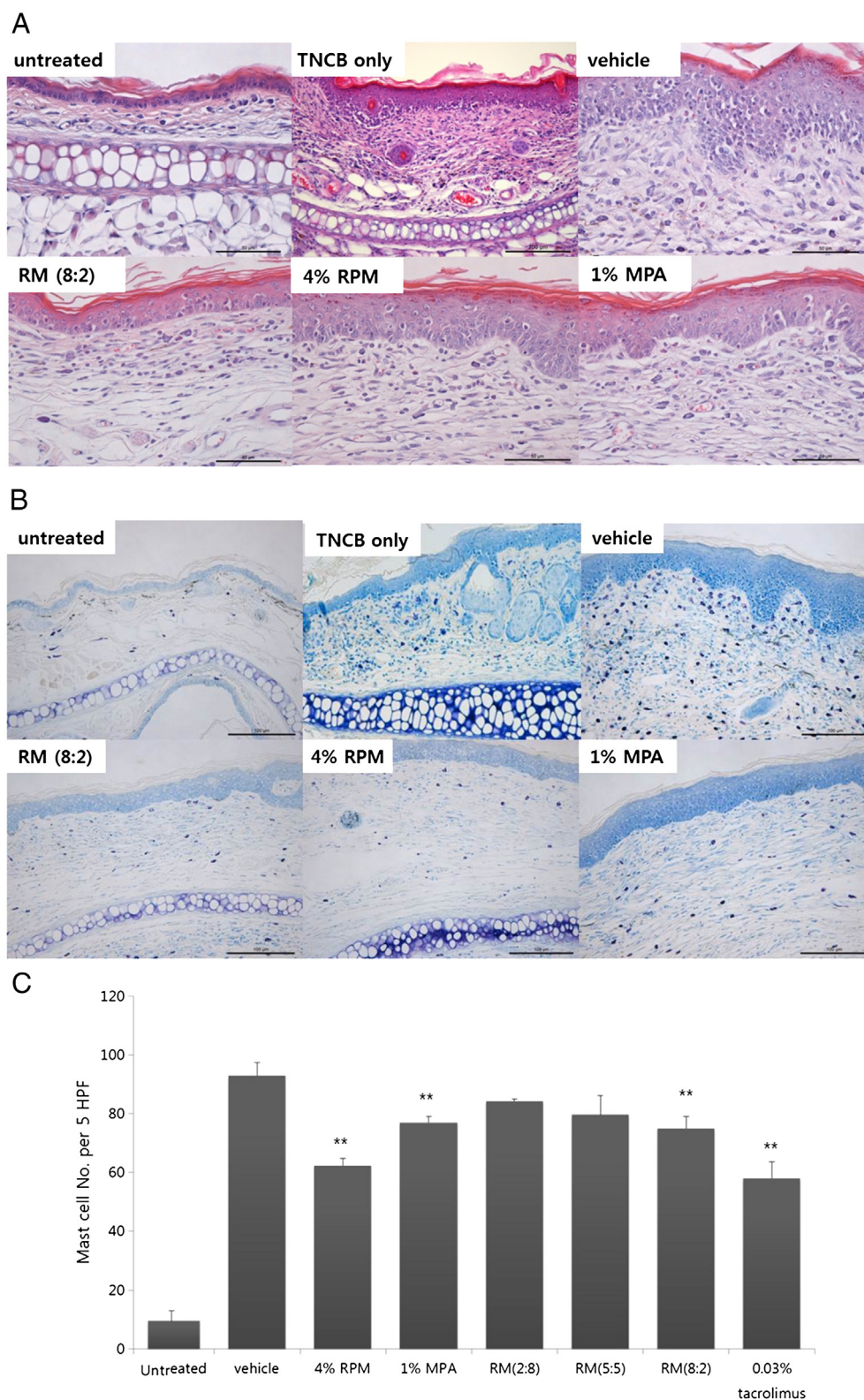


Fig. 4. Histological features of AD-like skin lesions after a 2 weeks of treatment with RM (8:2), 4% RPM, and 1% MPA. (A) H&E; original magnification, $\times 100$ (B) toluidine blue; original magnification, $\times 100$ (C) Number of mast cells in 5 randomly chosen visual fields at $\times 400$ magnification. HPF indicates high-power fields. Each experimental group consisted of five mice. All values are expressed as the mean $\pm$ SEM. ** $P < 0.01$ compared with the vehicle. RPM; rapamycin, MPA; mycophenolic acid, RM; rapamycin + mycophenolic acid.

the mixed formulation of 4% RPM and 1% MPA was superior to the effects of each drug alone, we tested various mixed formulations (2:8, 5:5, 8:2) with those of the single agent. Although there were no statistically significant differences between the various treatment groups, the largest decrease in skin severity score was achieved with the combination of 4% RPM and 1% MPA (RM, 8:2) ($P < 0.05$) (Fig. 3).

3.3. Effect of topical RM (8:2), 4% RPM, and 1% MPA on TNCB-induced epidermal thickening and inflammatory cell infiltration in the ear skin

Histologically, epidermal hyperplasia and inflammatory cell infiltration including eosinophils and mast cells in the dermis represented the largest decrease following treatment with the RM (8:2) formulation (Fig. 4A, B). Specifically, the mast cell number increased by TNCB was significantly reduced in groups treated with RM (8:2), 4% RPM, and 1% MPA compared to the vehicle ($P < 0.01$), although there were no significant differences between RM (8:2) and single agents (Fig. 4C).

3.4. Effect of topical RM (8:2), 4% RPM, and 1% MPA on mRNA expression of cytokines in TNCB-induced ear skin

The mRNA expression of IL-4 and IFN- γ was constitutively absent or minimal in the untreated group. However, TNCB-induced mRNA expression of IL-4 and IFN- γ was significantly reduced in the RM (8:2)

treatment group compared to the vehicle ($p < 0.05$). Expression of IL-4 and IFN- γ mRNA was also significantly reduced in the 4% RPM treatment group compared to the vehicle ($p < 0.05$). With respect to the 1% MPA treated group, the TNCB-induced mRNA expression of both IL-4 and IFN- γ was significantly reduced compared to the vehicle ($p < 0.05$) (Fig. 5).

3.5. Effect of topical RM (8:2), 4% RPM, and 1% MPA on protein levels of cytokines in TNCB-induced ear skin

The protein expression of IL-4 and IFN- γ was significantly increased in TNCB-induced AD-like skin lesions. The increase of IL-4 and IFN- γ was significantly reduced in the RM (8:2) treatment group and the 4% RPM treatment group compared to the vehicle ($p < 0.05$). With regard to the 1% MPA treated group, the TNCB-induced protein expression of IL-4, but not of IFN- γ was significantly reduced compared to the vehicle ($p < 0.05$) (Fig. 6).

3.6. Effect of topical RM (8:2), 4% RPM, and 1% MPA on TNCB-induced total serum IgE levels

RM (8:2), 4% RPM, and 1% MPA all failed to reduce total serum IgE levels (data not shown).

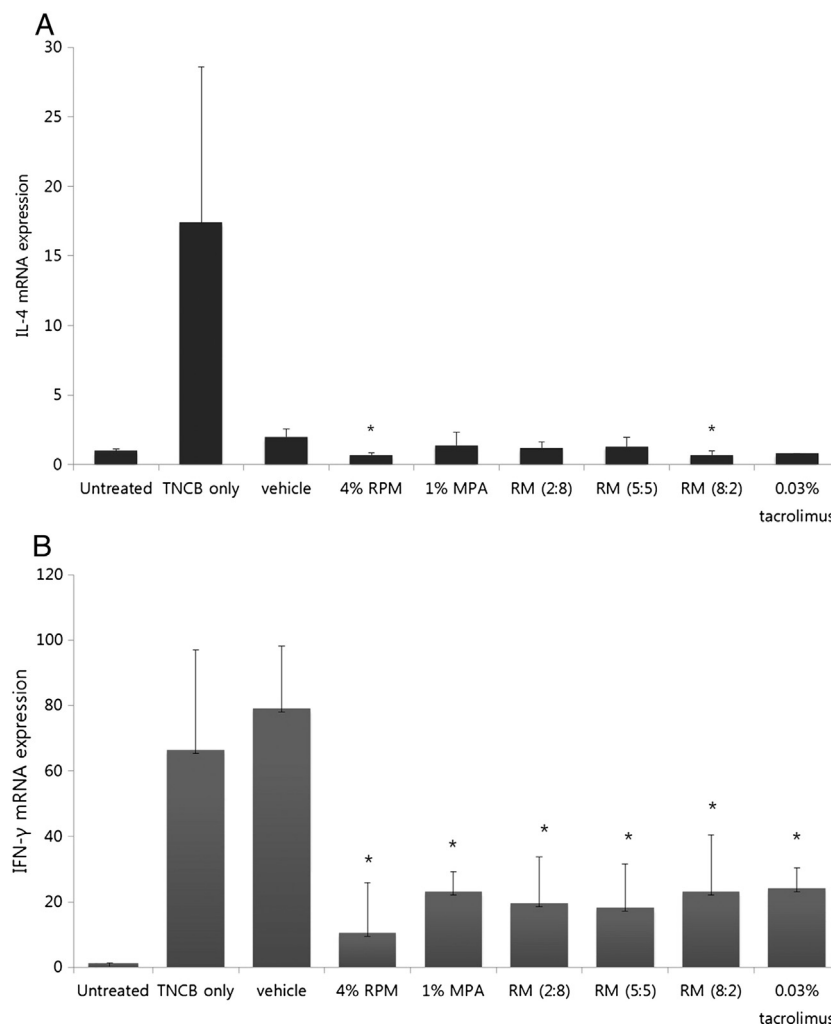


Fig. 5. Effect of topical RM (8:2), 4% RPM, and 1% MPA on mRNA expression of (A) IL-4, (B) IFN- γ in TNCB-induced ears of NC/Nga mice. Each experimental group consisted of five mice. Data are presented as the mean $\pm$ SEM. * $P < 0.05$ compared with the vehicle. IL-4; interleukin-4, IFN- γ ; interferon γ , RPM; rapamycin, MPA; mycophenolic acid, RM; rapamycin + mycophenolic acid.

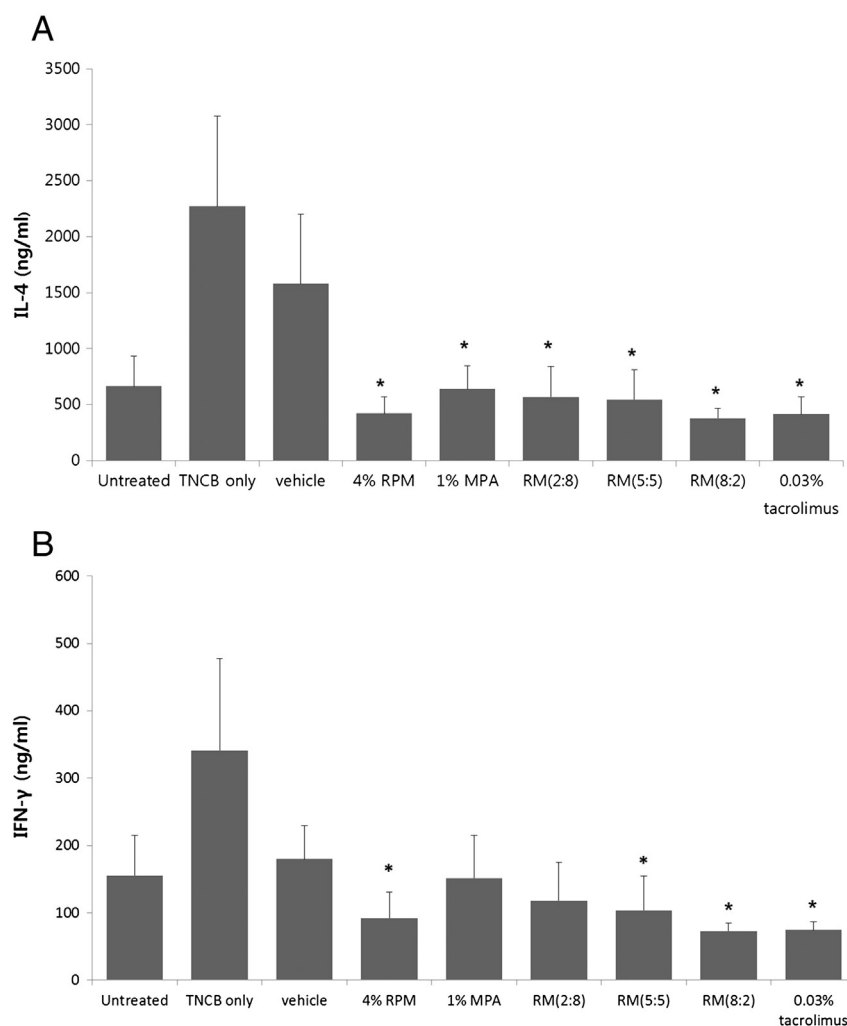


Fig. 6. Effect of topical RM (8:2), 4% RPM, and 1% MPA on protein expression of (A) IL-4, (B) IFN- γ in TNCB-induced ears of NC/Nga mice. Each experimental group consisted of five mice. Data are presented as the mean $\pm$ SEM. * $P < 0.05$ compared with the vehicle. IL-4; interleukin-4, IFN- γ ; interferon γ , RPM; rapamycin, MPA; mycophenolic acid, RM; rapamycin + mycophenolic acid.

4. Discussion

We demonstrated here that a combined mixture of topical RPM and MPA markedly improves AD-like skin lesions in NC/Nga mice. Clinical analysis revealed that topical 4% RPM, 1% MPA, and the combination of 4% RPM and 1% MPA (RM, 8:2) significantly decreased skin severity scores encompassing erythema/hemorrhage, edema, excoriation/erosion and scaling/dryness compared to the vehicle group. Although there were no statistically significant differences between the various treatment groups, treatment with the RM combination resulted in the largest decrease in skin severity score. These findings were supported by histopathologic analysis. Our present data was different from Yang et al.'s result that 0.2% RPM was most effective. It has been estimated that our base is cream formulation, not ointment which usually is known to be better penetration than cream. Further studies are needed with various base formulations of RPM. As for MPA, 5% MPA has been ineffective both clinically and histologically. The lack of effectiveness of 5% MPA is perhaps due to skin irritation where it is higher in concentration than that previously reported to be effective. We would need to refine the effective range of MPA in future experiments.

Leung and Bieber reported that Th2 cytokines are dominant during the acute phase of AD, and that Th1 cytokines are also highly expressed and contribute to the pathogenesis of chronic AD [20]. Th2-type cytokines, especially IL-4, are predominantly produced by T cells and mast

cells, and might also play an important role in the pathogenesis of AD [21]. Furthermore, the presence of IFN- γ -producing T cells correlates with the chronification and severity of AD skin lesions [22]. In the present study, the mixed RM formulation significantly suppressed TNCB-induced elevation of IL-4 and/or IFN- γ mRNA and protein expression compared to the vehicle group. Thus, our results suggest that the suppressive effect of topically administered mixed RM formulation, as well as 4% RPM and 1% MPA as single agents, on AD-like skin lesions in NC/Nga mice was possibly mediated by a local tissue Th2 and Th1 response. Therefore, it might be possible that 4% RPM and/or 1% MPA are effective for both acute and chronic stage AD. IL-4 mRNA and protein expression as well as INF- γ protein level in the vehicle group were also reduced compared to TNCB only. These results might be due to anti-inflammatory effects of beeswax (2%) and dimethicone (1%) in the vehicle [23,24].

Serum IgE levels are elevated in approximately 70 – 80% of AD patients. Although serum IgE levels are not necessary to diagnose AD, they do appear to correlate with disease severity [25]. In addition, itch intensity in AD patients is closely related to IgE [26]. Contrasting reports of intranasal and intraperitoneal administration of RPM reducing IgE in experimental allergic asthma suggest that the effects of RPM are largely dependent on the route of administration [27]. In addition to these findings, Chang et al. demonstrated that systemic MPA treatment inhibits IgE production in vitro [28]. In our study, topical administration of 4%

RPM and/or 1% MPA failed to reduce TNCB-induced elevation of total serum IgE levels. These results may suggest that topical administration of 4% RPM and/or 1% MPA does not have systemic effects.

This preliminary study had some limitations. Specifically, we were unable to measure the skin permeability of the individual agents. In addition, we were unable to measure the skin infection which may follow the use of immunosuppressive drugs (PRM and MPA). To compare the therapeutic effect of topical RPM, MPA, and their mixed formulation and demonstrate their safety, additional studies using an additional animal model of AD are underway.

In conclusion, our results demonstrated that topical administration of 4% RPM, 1% MPA, and a mixture of these two agents suppressed TNCB-induced AD-like skin lesions in NC/Nga mice, and that 4% RPM contributed a greater antipruritic effect. Taken together, RPM and/or MPA may be considered as potential topical therapeutic options for managing AD in the near future.

Conflicts of interest

None declared.

Acknowledgments

This study was supported by a grant of the Korean Health Technology R&D Project, Ministry for Health & Welfare, Republic of Korea (HI09C1555).

References

- [1] D.Y. Leung, M. Boguniewicz, M.D. Howell, I. Nomura, Q.A. Hamid, New insights into atopic dermatitis, *J. Clin. Invest.* 113 (2004) 651–657.
- [2] H.S. Kim, D.H. Kim, B.K. Kim, S.K. Yoon, M.H. Kim, J.Y. Lee, et al., Effects of topically applied Korean red ginseng and its genuine constituents on atopic dermatitis-like skin lesions in NC/Nga mice, *Int. Immunopharmacol.* 11 (2011) 280–285.
- [3] M.J. Kang, J.Y. Eum, S.H. Park, M.H. Kang, K.H. Park, S.E. Choi, et al., Pep-1 peptide-conjugated elastic liposomal formulation of taxifolin glycoside for the treatment of atopic dermatitis in NC/Nga mice, *Int. J. Pharm.* 402 (2010) 198–204.
- [4] E. Guttman-Yassky, N. Dhingra, D.Y. Leung, New era of biologic therapeutics in atopic dermatitis, *Expert. Opin. Biol. Ther.* 13 (2013) 549–561.
- [5] I.S. Kim, G.Y. Song, D.H. Kim, S.H. Cho, C.Y. Yun, J.S. Lee, Effect of (E)-2-(3,4-dimethoxyphenyl)-4-oxo-4H-chromen-7-yl-3-(3,4-dimethoxyphenyl) acrylate on the development of atopic dermatitis-like lesions, *Life Sci.* 91 (2012) 338–344.
- [6] R. Bissonnette, Y. Poulin, Y. Zhou, J. Tan, H.C. Hong, J. Webster, et al., Efficacy and safety of topical WBI-1001 in patients with mild to severe atopic dermatitis: results from a 12-week, multicentre, randomized, placebo-controlled double-blind trial, *Br. J. Dermatol.* 166 (2012) 853–860.
- [7] S.G. Van Velsen, I.M. Haec, C.A. Bruijnzeel-Koomen, Severe atopic dermatitis treated with everlimus, *J. Dermatolog. Treat.* 20 (2009) 365–367.
- [8] J.T. Ransom, Mechanism of action of mycophenolate mofetil, *Ther. Drug Monit.* 17 (1995) 681–684.
- [9] W.T. Waxweiler, R. Agans, D.S. Morrell, Systemic treatment of pediatric atopic dermatitis with azathioprine and mycophenolate mofetil, *Pediatr. Dermatol.* 28 (2011) 689–694.
- [10] D. Simon, Systemic therapy of atopic dermatitis in children and adults, *Curr. Probl. Dermatol.* 41 (2011) 156–164.
- [11] W. Bäumer, B. Sülzle, H. Weigt, V.C. De Vries, M. Hecht, T. Tschernig, et al., Cilomilast, tacrolimus and rapamycin modulate dendritic cell function in the elicitation phase of allergic contact dermatitis, *Br. J. Dermatol.* 153 (2005) 136–144.
- [12] J.G. Meingassner, A. Stütz, Anti-inflammatory effects of macrophilin-interacting drugs in animal models of irritant and allergic contact dermatitis, *Int. Arch. Allergy Immunol.* 99 (1992) 486–489.
- [13] A.D. Ormerod, S.A. Shah, P. Copeland, G. Omar, A. Winfield, Treatment of psoriasis with topical sirolimus: preclinical development and a randomized, double-blind trial, *Br. J. Dermatol.* 152 (2005) 758–764.
- [14] F. Yang, M. Tanaka, M. Wataya-Kaneda, L. Yang, A. Nakamura, S. Matsumoto, et al., Topical application of rapamycin ointment ameliorates *Dermatophagoides farinae* body extract-induced atopic dermatitis in NC/Nga mice, *Exp. Dermatol.* 23 (2014) 568–572.
- [15] Y. Shoji, T. Fukumura, M. Kudo, A. Yanagawa, J. Shimada, Y. Mizushima, Effects of topical preparation of mycophenolic acid on experimental allergic contact dermatitis of guinea-pigs induced by dinitrofluorobenzene, *J. Pharm. Pharmacol.* 46 (1994) 643–646.
- [16] J. Wohlrab, K. Jahn, M. Plaetzer, R. Neubert, W.C. Marsch, Topical application of mycophenolatemofetil in plaque-type psoriasis, *Br. J. Dermatol.* 144 (2001) 1263–1264.
- [17] Jeffrey P. Callen, Immunosuppressive and immunomodulatory drugs, in: L.A. Goldsmith, S.I. Katz, B.A. Gilchrist, A.S. Paller, D.J. Leffell, K. Wolff (Eds.), *Fitzpatrick's Dermatology in General Medicine*, 8th ed. McGraw-Hill, New York, 2012, pp. 2807–2808.
- [18] V.H. Nguyen, R. Zeiser, R.S. Negrin, Role of naturally arising regulatory T cells in hematopoietic cell transplantation, *Biol. Blood Marrow Transplant.* 12 (2006) 995–1009.
- [19] H. Hackstein, A.G. Thomson, Dendritic cells: emerging pharmacological targets of immunosuppressive drugs, *Nat. Rev. Immunol.* 4 (2004) 24–34.
- [20] D.Y. Leung, T. Bieber, Atopic dermatitis, *Lancet* 11 (2003) 361.
- [21] S. Sehra, Y. Yao, M.D. Howell, E.T. Nguyen, G.S. Kansas, D.Y. Leung, et al., IL-4 regulates skin homeostasis and the predisposition toward allergic skin inflammation, *J. Immunol.* 184 (2010) 3186–3190.
- [22] B. Homey, M. Steinhoff, T. Ruzicka, D.Y. Leung, Cytokines and chemokines orchestrate atopic skin inflammation, *J. Allergy Clin. Immunol.* 118 (2006) 178–189.
- [23] D. Carbajal, V. Molina, S. Valdés, M.L. Arruzazabala, R. Más, J. Magraner, Anti-inflammatory activity of D-002: an active product isolated from beeswax, *Prostaglandins Leukot. Essent. Fatty Acids* 59 (1998) 235–238.
- [24] D. Beeckman, S. Verhaeghe, T. Defloor, L. Schoonhoven, K. Vanderwee, A 3-in-1 perineal care washcloth impregnated with dimethicone 3% versus water and pH neutral soap to prevent and treat incontinence-associated dermatitis: a randomized, controlled clinical trial, *J. Wound Ostomy Continence Nurs.* 38 (2011) 627–634.
- [25] M. Ogawa, P.A. Berger, O.R. McIntyre, W.E. Clendenning, K. Ishizaka, IgE in atopic dermatitis, *Arch. Dermatol.* 103 (1971) 575–580.
- [26] C.H. Lee, H.Y. Chuang, C.C. Shih, S.B. Jong, C.H. Chang, H.S. Yu, Transepidermal water loss, serum IgE and beta-endorphin as important and independent biological markers for development of itch intensity in atopic dermatitis, *Br. J. Dermatol.* 154 (2006) 1100–1107.
- [27] E.M. Mushaben, E.L. Kramer, E.B. Brandt, G.K. Khurana Hershey, T.D. Le Cras, Rapamycin attenuates airway hyperreactivity, goblet cells, and IgE in experimental allergic asthma, *J. Immunol.* 187 (2011) 5756–5763.
- [28] C.C. Chang, G. Aversa, J. Punnonen, H. Yssel, J.E. de Vries, Brequinar sodium, mycophenolic acid, and cyclosporin A inhibit different stages of IL-4- or IL-13-induced human IgG4 and IgE production in vitro, *Ann. N. Y. Acad. Sci.* 696 (1993) 108–122.