



Glutamine suppresses dinitrophenol fluorobenzene-induced allergic contact dermatitis and itching: Inhibition of contact dermatitis by glutamine

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ABSTRACT

Background: Cytoplasmic phospholipase A₂ (cPLA₂) is importantly implicated in a variety of inflammatory diseases by liberating arachidonic acid from phospholipids. The increased cPLA₂ activities as well as increased levels of cPLA₂ metabolites are associated with pathogenesis of many inflammatory skin disorders including atopic dermatitis. The non-essential amino acid L-glutamine (Gln) has been reported to have an anti-inflammatory activity. Regarding the molecular mechanism of Gln, we have recently shown that Gln effectively inhibits cPLA₂ phosphorylation and activity.

Objective: To examine whether Gln could suppress allergic contact dermatitis (CD) induced on mouse ears by dinitrophenol fluorobenzene (DNFB).

Methods: Mice were sensitized five times on their ears with a 0.15% solution of DNFB in a 3 day interval. To examine Gln effects, Gln solution (4% in saline) was applied three times a day onto both sides of DNFB-applied ears from the last day of DNFB application. The inflammatory reactions of ears were evaluated by measuring ear thickness and hematoxylin and eosin (H&E) staining. Mouse scratching behavior was objectively evaluated using a MicroAct apparatus. cPLA₂ phosphorylation and activity were analyzed using Western blotting and a cPLA₂ assay kit, respectively.

Results: Topical application of Gln significantly attenuated inflammatory symptoms (ear thickness, histological inflammatory skin reactions) as well as itching. Gln inhibited cPLA₂ phosphorylation and enzymatic activity. Arachidonyl trifluoromethyl ketone (AACOCF₃) inhibited cPLA₂ activity in DNFB-challenged ears and attenuated DNFB-induced ear inflammation and itching.

Conclusion: The results indicate that Gln suppresses DNFB-induced dermatitis and itching, at least in part, by inhibiting cPLA₂ activity.

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Abbreviations: AACOCF₃, arachidonyl trifluoromethyl ketone; Ala, alanine; cPLA₂, cytosolic phospholipase A₂; DNFB, dinitrophenol fluorobenzene; ESR, ear swelling response; Gln, L-glutamine; H&E, hematoxylin and eosin; 12-HPETE, 12-hydroperoxyeicosatetraenoic acid; LT, leukotriene; PAR, proteinase-activated receptor; PMNL, polymorphonuclear leukocytes; siRNA, small interfering RNA; TRPV-1, transient receptor potential vanilloid-type 1.

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1. Introduction

Phospholipase A₂ (PLA₂) comprises a diverse family of enzymes that cleave the *sn*-2 fatty acyl ester bond of glycerophospholipids to yield a free fatty acid and a lysophospholipid [1,2]. Among the PLA₂s, a 85-kDa cytoplasmic PLA₂ (cPLA₂) is constitutively expressed in all cells and specifically releases arachidonic acid from the *sn*-2 position of glycerophospholipids to provide the precursor to various eicosanoids, such as leukotrienes (LTs), prostaglandins, and platelet-activating factor [3,4]. The increased cPLA₂ activity as well as increased levels of cPLA₂ metabolites are associated with pathogenesis of a variety of inflammatory skin disorders including atopic [5–11].

The non-essential amino acid L-glutamine (Gln) is abundant in plasma [12] and is an energy substrate for most cells [13,14].

Consequently, Gln is important in multiple ways in the nitrogen- and carbon-skeleton exchange among different tissues [15]. Human clinical trials with several patient populations have demonstrated that Gln treatment decreases infectious complications, shortens hospitalization, and decreases hospital costs [16]. Additionally, extensive research in animal models of endotoxin shock, including severe injury models, has demonstrated that supplementation with Gln improves survival, enhances immune and gut barrier function, decreases bacteremia, and inhibits gut mucosal atrophy [17,18]. Regarding the molecular mechanism of Gln, we have recently shown that Gln effectively protects animals from endotoxin shock as well as inhibit bronchial asthma by inhibiting cPLA₂ activity [19,20].

The present study investigated whether Gln could suppress allergic contact dermatitis (CD) induced on mouse ears by dinitrophenol fluorobenzene (DNFB). The results demonstrate that the topical application of Gln inhibits cPLA₂ phosphorylation and enzymatic activity in DNFB-challenged ears, but also suppresses DNFB-induced CD and itching.

2. Materials and methods

2.1. Animals

Specific pathogen-free female C57BL/6J mice were purchased from the Korean Institute of Chemistry Technology (Daejeon, Republic of Korea). The mice were housed in a laminar flow cabinet and were maintained on a standard laboratory chow ad libitum. Mice were 7–8-weeks-old at the start of each experiment. All animals were cared for under a protocol approved by the Institutional Animal Care and Use Committee of the Chonbuk National University Medical School.

2.2. Reagents

DNFB, L-Gln (biotechnology performance certified, G-8540), and alanine (Ala) were purchased from Sigma–Aldrich (St. Louis, MO). DNFB was dissolved in a mixture of acetone and olive oil (4:1). Gln and Ala were dissolved in saline. The cPLA₂ inhibitor AACOCF₃ (BIOMOL, Plymouth Meeting, PA) was dissolved in dimethylsulfoxide and was administered by intraperitoneal (i.p.) injection 30 min before challenge. Antibodies against cPLA₂ α , phospho-cPLA₂ α were purchased from Cell Signaling Technology (Danvers, MA).

2.3. Induction of CD

Mice were sensitized by topical application with 100 μ l of 0.15% DNFB to the shaved back of skin. Five days later, mice were challenged three times on their right ears with 30 μ l of 0.15% DNFB on days 0, 3, and 6. Vehicle (acetone and olive oil mixture) was applied to the left ears.

2.4. Measurement of ESR

The ear thickness before and after the application of DNFB was measured with a model 7326 dial thickness gauge (Mitutoyo, Tokyo, Japan). Percent ESR change was expressed as [(value at post-DNFB application – value at pre-DNFB application) $\div$ value at pre-DNFB application] $\times$ 100.

2.5. Histological examination

At the indicated times, animals were sacrificed and both ears of each animal was excised. Specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, cut in 4 μ m-thick sections using a SM2000R microtome (Leica, Jena, Germany). The sections

were stained with Hematoxylin and eosin (H&E) for leukocytes and with Congo red solution for eosinophils. The total number of leukocytes and eosinophils per unit area (mm²) was counted by a single observer who was unaware of the individual sections.

2.6. Measurement of cPLA₂ activity

Ears were dissected and homogenized in 50 mM HEPES (pH 7.4) containing 1 mM EDTA and protease inhibitor. Homogenates were centrifuged at 13,200 rpm for 15 min at 4 °C, and supernatants were used for measurement of cPLA₂ α enzymatic activity using a cPLA₂ assay kit purchased from Cayman Chemical (Ann Arbor, MI) following the manufacturer's instructions.

2.7. Evaluation of scratching behavior

Mouse scratching behavior was automatically detected and objectively evaluated using a MicroAct apparatus (Neuroscience, Tokyo, Japan) as described elsewhere [21]. Briefly, a small Teflon-coated magnet, 1 mm in diameter and 3 mm in length, was inserted subcutaneously into both hind paws under anesthesia 7 days before the start of the experiment. It was confirmed that the operation and magnet did not affect behavior of the mice. The mice were individually placed in an observation chamber and characteristic waves corresponding to scratching behavior, defined as the incidence of consecutive scratching behavior in an indicated period of time after application of DNFB, were detected using a computer. On the basis of our repeated preliminary experiments, we established the following parameters for the detection of consecutive scratching behavior: threshold p–p limit, 0.125 V; threshold minimum duration, 0.2 s; maximum amplitude range, 0.5 V; minimum amplitude range, 0.05 V; maximum frequency, 20 Hz; minimum frequency, 5 Hz. Under our experimental conditions, the apparatus detected consecutive scratching behavior consisting of 3 or more beats.

2.8. Immunoblotting analysis

Mice were sacrificed by cervical dislocation and the ears were collected immediately thereafter and briefly washed with cold PBS and then dried with blotting paper. The ears were frozen in liquid nitrogen and were stored in –70 °C until analysis. Ear specimens were homogenized in the PhosphoSafe Extraction Reagent (Novagen, Madison, WI), and were subjected to immunoblotting analysis as described previously [17]. Signal intensity of specific bands was analyzed quantitatively using Fluor-STM Imager (Bio-Rad) and plotted as relative intensity.

2.9. RNA interference

Small interfering RNA (siRNA) strands for cPLA₂ α was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). In vivo delivery of siRNA was performed using in vivo-jet polyethylene imine (PEI, Polyplus-transfection), according to the instructions of manufacturer. In brief, MKP-1 siRNA dissolved in 5% glucose were mixed in a volume of 50 μ l or 200 μ l at room temperature for 20 min and the mixture was intravenously administered 24 h before the final DNFB challenge. The mixture containing control siRNA and PEI dissolved in 5% glucose without siRNA were used as controls. To confirm that the MKP-1 siRNA used really block the synthesis of its target, an immunoblotting analysis was performed. We determined the optimum concentration of the siRNA (0.4 nmol) in a preliminary experiment.

2.10. Measurement of LTB₄

The levels of LTB₄ were quantified in ear tissues using an enzyme-linked immunosorbent assay (ELISA) performed

according to the protocol of the manufacturer (R&D Systems, Minneapolis, MN). Sensitivity for LTB₄ was 3.7 pg/ml.

2.11. Statistical analysis

Data are presented as mean $\pm$ standard error of mean (SEM). Student's unpaired *t*-test was applied to determine significant differences between corresponding treated groups and control groups. A value of *p* < 0.05 was considered statistically significant.

3. Results

3.1. Gln inhibits DNFB-induced ear swelling response (ESR) and inflammatory reactions of the ear

Challenge of DNFB three times increased ESR by approximately 120%. To determine the effect of Gln on ESR, Gln was topically applied onto the ears once, twice or three times a day from the day of the last challenge of DNFB. Gln significantly suppressed DNFB-induced ESR as early as from day 2 in an application frequency-dependent manner (Fig. 1A). The inhibition was not evident in the Ala control mice. We also determined the effects of varying concentrations (1, 2, and 4%) of Gln. Gln exerted its suppression in a dose-dependent manner (Fig. 1B). Leukocyte infiltration was

determined on ear sections as a cellular mechanism underlying ESR. The ears were obtained from the mice received 4% Gln three times a day. H&E staining of the sections revealed the development of strong inflammatory reactions at the DNFB-challenged sites, which were characterized by marked edema with an extensive cellular infiltration in the swollen dermis (Fig. 1C). The extent of infiltration of total leukocytes and eosinophils was significantly lower in Gln-treated mice than that in vehicle- or Ala-treated mice for the entire period of observation (Fig. 1D).

3.2. Gln inhibits scratching behaviors

Gln was topically applied onto the ears (1) once at 5 min, (2) twice at 5, and 15 min, or (3) three times at 5, 15, and 25 min after the last challenge of DNFB. Scratching behavior was recorded for 4 h beginning 30 min following the last challenge of DNFB. A high incidence of scratching behavior was observed in the first 1 h, and the incidence declined thereafter (Fig. 2A). Gln significantly suppressed the scratching behavior in an application frequency-dependent manner. Application of 4% solution of Ala three times did not show such suppression. We also determined effects of varying concentrations (1, 2, and 4%) of Gln. Gln suppressed the scratching behavior in a dose-dependent manner (Fig. 2B).

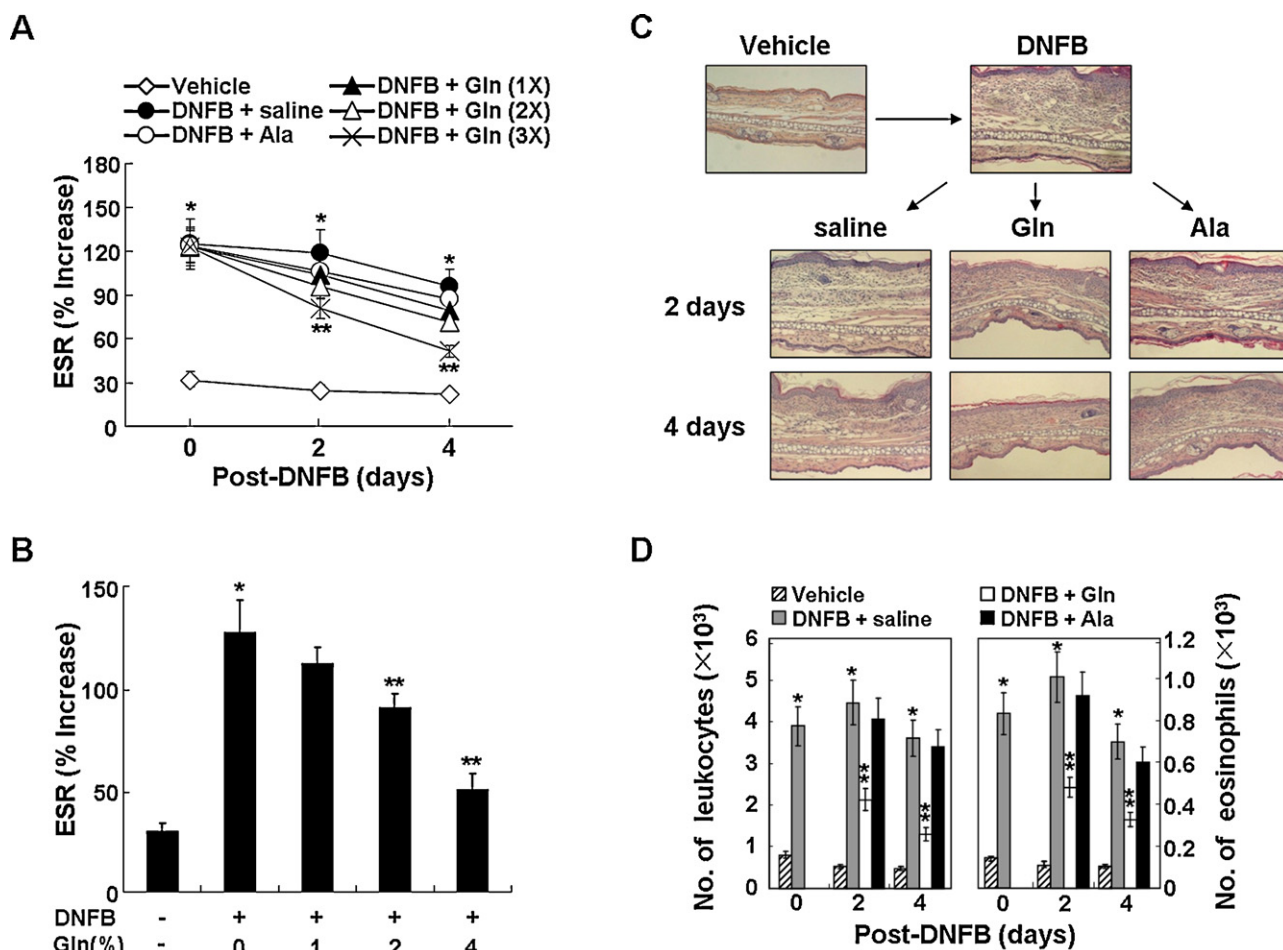


Fig. 1. Gln inhibits DNFB-induced inflammation. (A) Gln (4% in saline) was applied onto the ears (1) once a day (1×) (at 9 a.m.), (2) twice a day (2×) (at 9 a.m. and 9 p.m.), or (3) three times a day (3×) (at 9 a.m., 3 p.m., and 9 p.m.) for 4 days from the day of the last challenge of DNFB. DNFB was challenged at 8 a.m. Ala solution (4% in saline) was applied three times a day. (B) Varying concentrations of Gln were applied three times a day for 4 days. As a DNFB control group, saline was applied. (C) After measurement of ESR, the mice were sacrificed, and the ear sections were stained with H&E. Original magnification: 100×. (D) Total leukocytes and eosinophils were counted as described in Materials and Methods. Data represent the mean $\pm$ SEM of three independent experiments with five mice each per time point. **p* < 0.01 vs. vehicle group. ***p* < 0.05 vs. DNFB + saline group.

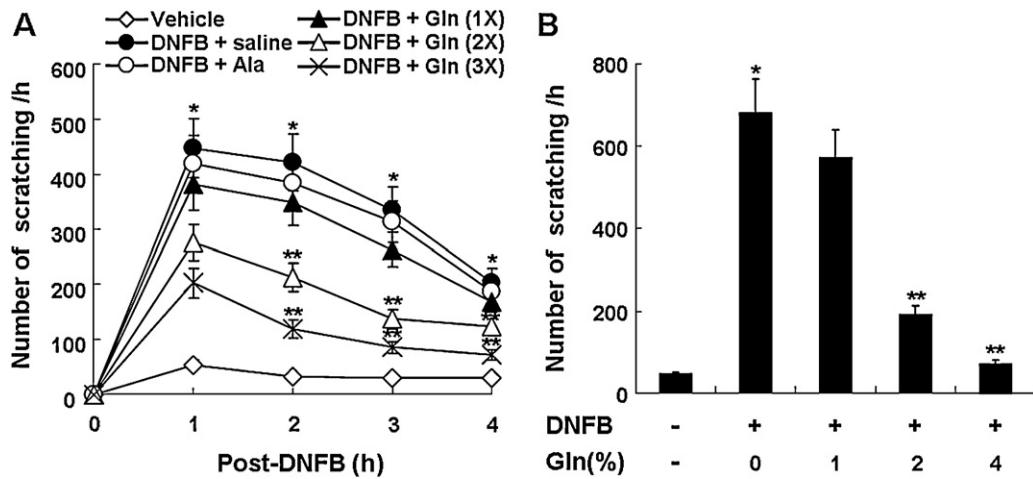


Fig. 2. Gln inhibits DNFB-induced scratching behavior. (A) Gln (4% in saline) was topically applied onto the ears (1) once at 5 min, (2) twice at 5 and 15 min, or (3) three times at 5, 15, and 25 min after the last challenge of DNFB. Ala (4% in saline) was applied three times. (B) Varying concentrations of Gln were topically applied onto the ears three times. From 30 min after the last DNFB challenge, scratching behavior was recorded for the next 1 h. Data represent the mean $\pm$ SEM of three independent experiments with five to seven mice each per group. * $p < 0.01$ vs. vehicle group. ** $p < 0.05$ vs. DNFB + saline group.

3.3. Gln inhibits cPLA₂ phosphorylation and enzymatic activity in DNFB-challenged ears

cPLA₂ phosphorylation assessed by Western blotting increased after DNFB challenge for 4 days. The last DNFB challenge increased the phosphorylation, and it was sustained at relatively high levels for up to 3 days (Fig. 3A). Enzymatic activity of cPLA₂ also increased compared with the levels after vehicle challenge (Fig. 3B). Application of 4% Gln solution onto the ears three times after the final challenge of DNFB significantly inhibited DNFB-induced cPLA₂ phosphorylation (Fig. 3A) and enzymatic activity (Fig. 3C). Treatment with Ala failed to inhibit cPLA₂ phosphorylation and enzymatic activity (Fig. 3A and C).

3.4. cPLA₂ inhibitor attenuates DNFB-induced CD and itching

To establish the linkage between cPLA₂ and DNFB-induced dermatitis and itching, we examined whether the cPLA₂ inhibitor, arachidonyl trifluoromethyl ketone (AACOCF₃), and cPLA₂ siRNA could inhibit DNFB-induced CD and itching. AACOCF₃ and cPLA₂, but not control, siRNA inhibited the DNFB-induced increased cPLA₂ activity (Fig. 4A), scratching behavior (Fig. 4B), ESR (Fig. 4C) and inflammatory cell infiltration (Fig. 4D). We verified the efficacy of cPLA₂ siRNA by demonstrating its activity to inhibit cPLA₂ protein expression and DNFB-mediated cPLA₂ phosphorylation (Fig. 4E).

Activated cPLA₂ is required for generation of various eicosanoids, including LTs [3,4]. LTB₄ acts as a potent mediator in

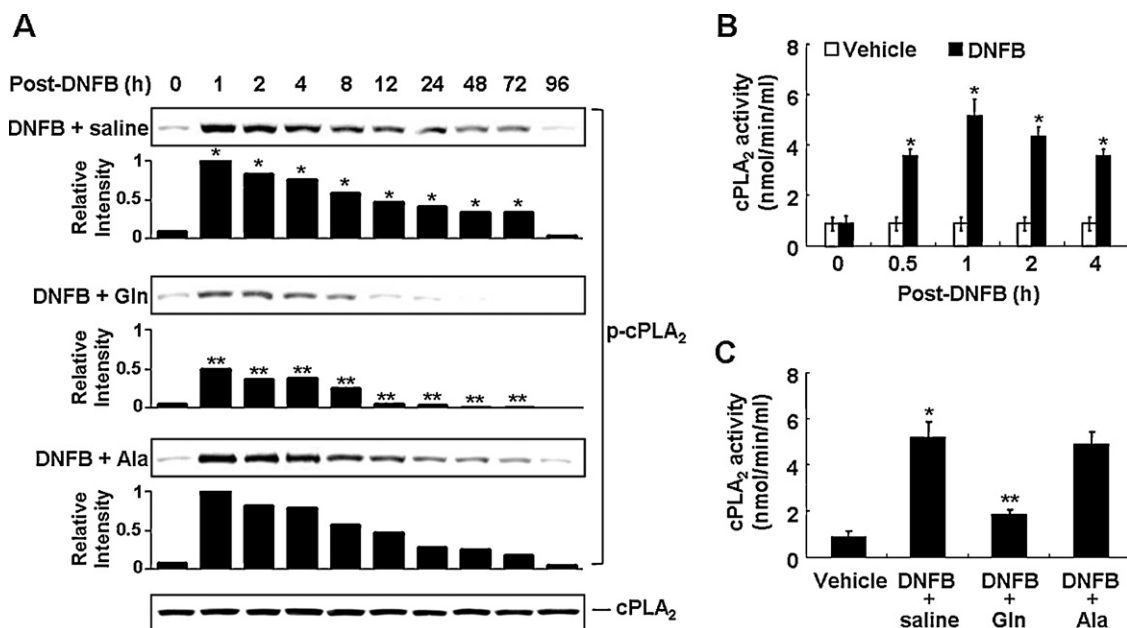


Fig. 3. Gln inhibits phosphorylation and enzymatic activity of cPLA₂. (A) After the last DNFB challenge, 4% Gln or Ala solution was applied three times onto the ears of different groups of mice. Ears were removed at the indicated time points after the last DNFB application, and time courses of cPLA₂ phosphorylation and densitometric analysis were assessed. A representative of three independent experiments with three mice per time point is shown. (B) Time courses of cPLA₂ activity were assessed after the last DNFB challenge. (C) Gln or Ala solution (4%) was applied three times onto the ears of different groups of mice, and the ears were removed 1 h after the last DNFB application to assess cPLA₂ activity. Data represent the mean $\pm$ SEM of three independent experiments with five mice each per group. * $p < 0.01$ vs. vehicle group. ** $p < 0.01$ vs. DNFB + saline group.

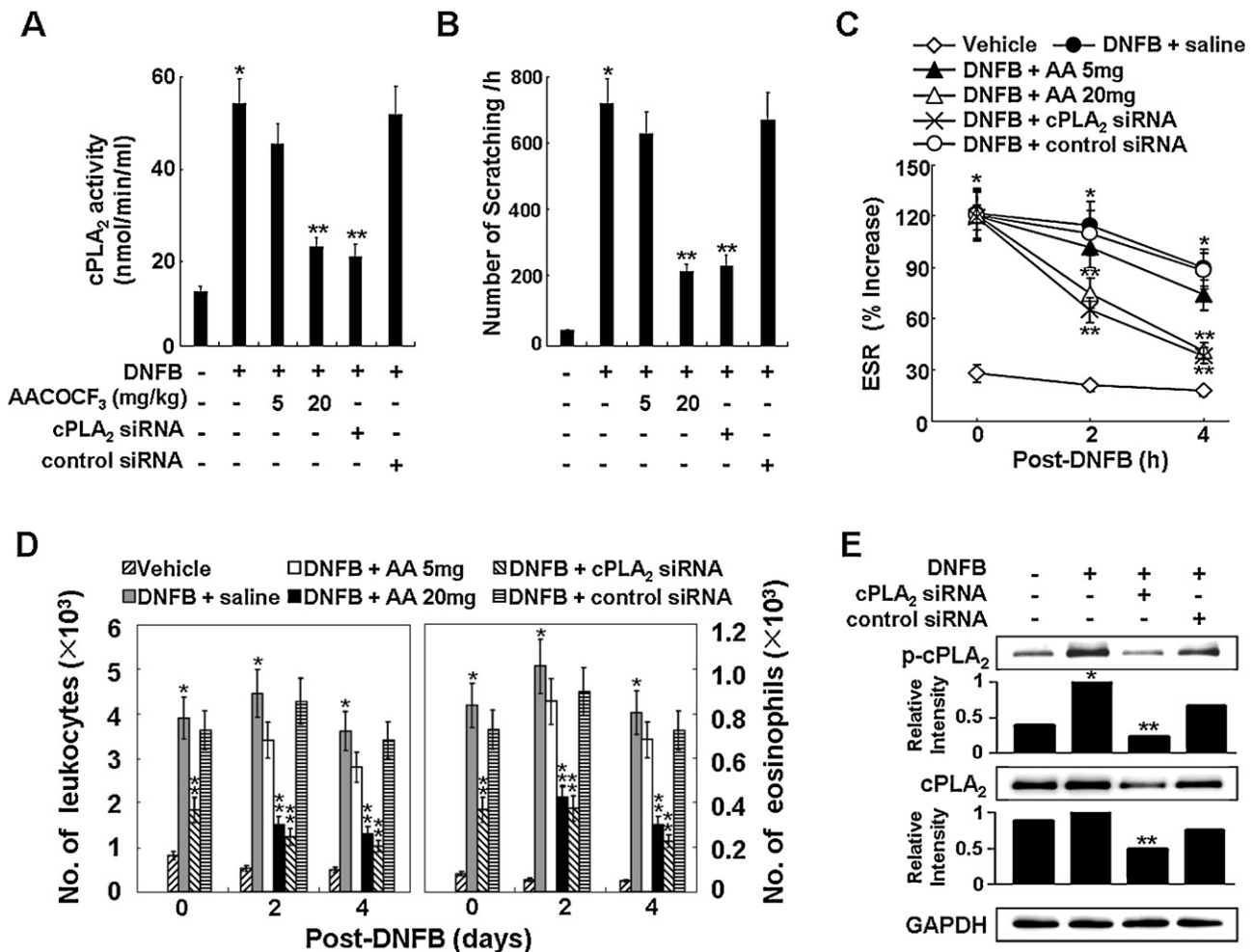


Fig. 4. cPLA₂ inhibitor inhibits DNFB-induced dermatitis and itching. From the day of the last DNFB challenge, varying concentrations of AACOCF₃ were administered i.p. 30 min before the last DNFB challenge or 0.4 nM of cPLA₂ siRNA or control siRNA was administered i.v. 24 h before the last DNFB challenge. We measured cPLA₂ activity (A), scratching behavior (B), ESR (C), and leukocyte infiltration into the ears (D). (E) 0.4 nM of cPLA₂ siRNA or control siRNA was administered i.v. 24 h before the last DNFB challenge. The ears were removed 30 min after DNFB challenge. Data represents the mean $\pm$ SEM of two or three independent experiments with five mice each per group. * $p < 0.01$ vs. vehicle group. ** $p < 0.01$ vs. DNFB + saline group.

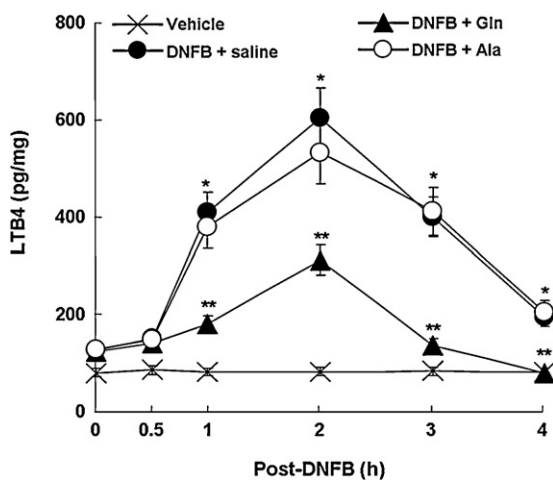


Fig. 5. Gln inhibits the levels of LTB₄ in DNFB-challenged ears. 4% Gln or Ala solution was applied three times onto the ears. The ears were removed at the indicated time points after the last DNFB application, and LTB₄ was measured. Data represents the mean $\pm$ SEM of two or three independent experiments with five mice each per group. * $p < 0.01$ vs. vehicle group. ** $p < 0.01$ vs. DNFB + saline group.

inflammatory skin diseases [5–8] and even in inducing itching [19]. Therefore, we examined the involvement of LTB₄ in DNFB-induced CD. The levels of LTB₄ in the ear tissues were increased upon DNFB challenge, reached a peak at 1 h and declined thereafter, which was inhibited by Gln (Fig. 5).

4. Discussion

Grounded on the observation that Gln inhibits cPLA₂ activity [20,21], this study investigated the therapeutic efficacy of Gln in DNFB-induced CD in ears of a mouse model. To gain clinical relevancy, Gln was topically applied after the establishment of dermatitis. This route of application of Gln three times daily significantly attenuated the established ESR, including inflammatory skin reactions such as leukocyte infiltration. The effects of Gln were seen as early as 2 days after its application. Gln exerted its effect, at least in part, by inhibiting cPLA₂ activity. cPLA₂ phosphorylation and activity were elevated in DNFB-treated ear tissue, but were diminished by the application of Gln. Likewise, a specific cPLA₂ inhibitor, AACOCF₃ inhibited cPLA₂ activity and suppressed inflammatory skin reactions to levels comparable to those of Gln.

cPLA₂ plays a key role in many signal transduction cascades by hydrolyzing arachidonic acid from membrane phospholipids. Tight

control of cPLA₂ activity by a number of regulatory mechanisms is essential to its cellular function. The physiological effect of the enzyme is probably attributable to its capability to generate various eicosanoids. In human skin, eicosanoids are generated and exert proinflammatory and immunoregulatory actions through their effects on blood vessels and inflammatory cells; eicosanoids are also involved in the regulation of growth and differentiation of the epidermis [22,23]. Therefore, eicosanoids have been suspected to contribute to the pathogenesis of a number of skin diseases such as atopic dermatitis [8,9,12,13], urticaria [10], psoriasis [7,11] and ultraviolet dermatitis [24]. The present data demonstrates a beneficial effect of a cPLA₂ inhibitor in DNFB-induced dermatitis, which further supports the linkage of cPLA₂ in the pathogenesis of skin inflammatory diseases.

Another important finding was that Gln significantly attenuated DNFB-induced scratching behavior. A similar inhibition was also achieved by cPLA₂ inhibition, suggesting that cPLA₂ activity is involved in the pathogenesis of DNFB-induced itching, and that Gln exerts its inhibitory effect on itching, at least in part, by inhibiting cPLA₂. The role of cPLA₂ in the induction of itching has not been hitherto well-documented. However, cPLA₂ and its metabolites have been implicated in signaling pathway involving the transmission of itch sensation to spinal cord. Proteinase-activated receptor (PAR) is a family member of G-protein-coupled receptors [25]. Recently, it has been suggested that PAR-2 may be a receptor on sensory nerve fibers involved in the induction of pruritus during inflammatory skin diseases [26]. Activation of PARs leads to stimulation of the intracellular protein kinase C pathway, resulting in the sensitization of the transient receptor potential vanilloid-type 1 (TRPV1) by phosphorylation of the intracellular C terminus (heterologous sensitization). Sensitization of TRPV1 leads to the release of neuropeptides (activation of mast cell degranulation) from nerve terminals of sensory nerves as well as stimulation of activation potentials, resulting in transmission of pruritus to the spinal cord [26]. TRPV1 is activated by metabolic products of 5-lipoxygenase, most notably by 12-hydroperoxyeicosatetraenoic acid (12-HPETE) [27]. Furthermore, TRPV1 also plays a key role in mediating the pruritogenic action of histamine via the PLA₂/LO pathway [28]. Additionally, the cPLA₂ metabolite LTB₄ has been implicated in inducing itching in mice [19], and recent studies have revealed an antipruritic potency of the LT receptor antagonists zafirlukast and zileuton [29]. The data provide evidence that cPLA₂ is involved in the underlying mechanism of itching. Therefore, it is possible that Gln suppresses itching by inhibiting the generation of cPLA₂ metabolites, either by directly inducing itching or by involvement in the TRPV1-associated signaling pathway. Recently, we demonstrated that Gln, in addition to DNFB-induced itching, effectively suppresses the itching induced by many pruritogenic agents, such as histamine, serotonin, and compound 48/80 in mice. The itching-suppression activity of Gln can be seen as early as within 10 min after Gln application (data not shown), suggesting that Gln suppresses itching by blocking a signaling pathway involving the transmission of the itch sensation rather than the suppression of generation of cPLA₂ metabolites. Further studies are required to address this issue.

At present, it is unclear how glutamine inhibits cPLA₂ phosphorylation. We previously reported that Gln deactivates p38 and JNK MAPKs by a rapid induction of MAPK phosphatase (MKP)-1 protein, which preferentially dephosphorylates p38 and JNK [30]. This suggests that Gln indirectly inhibits cPLA₂ phosphorylation via blocking MAPK phosphorylation. However, we recently found that Gln deactivates cPLA₂ by dephosphorylating it only when cPLA₂ was phosphorylated (This is not an action of kinase), and MKP-1 siRNA abrogates Gln inhibition of cPLA₂ phosphorylation (data not shown). This strongly suggests that a "phosphatase" rather than MAPKs is involved in Gln-mediated

dephosphorylation of cPLA₂. Further studies are required to address this issue.

In summary, this study demonstrates that Gln attenuates the established DNFB-induced dermatitis and itching by inhibiting cPLA₂ activity. Given that even the parenteral administration of Gln as a component of supportive nutritional therapy is safe [31,32], topical use of Gln in patients with a variety of skin diseases accompanying inflammation and pruritus in which cPLA₂ plays an important role is probably more beneficial than the use of other compounds or biological agents.

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References

- [1] Vadas P, Pruzanski W. Role of secretory phospholipases A₂ in the pathobiology of disease. *Lab Invest* 1986;55:391–404.
- [2] Arita H, Nakano T, Hanasaki K. Thromboxane A₂: its generation and role in platelet activation. *Prog Lipid Res* 1989;28:273–301.
- [3] Sharp JD, White DL, Chiou XG, Goodson T, Gamboa GC, McClure D, et al. Molecular cloning and expression of human Ca²⁺-sensitive phospholipase A₂. *J Biol Chem* 1991;266:14850–53.
- [4] Clark JD, Milona N, Knopf JL. Purification of a 110-kilodalton cytosolic phospholipase A₂ from the human monocytic cell line U937. *Proc Natl Acad Sci U S A* 1990;87:7708–12.
- [5] Ikai K, Imamura S. Role of eicosanoids in the pathogenesis of atopic dermatitis. *Prostaglandins Leukot Essent Fatty Acids* 1993;48:409–16.
- [6] Ikai K. Psoriasis and the arachidonic acid cascade. *J Dermatol Sci* 1999;21:135–46.
- [7] Sabroe RA, Greaves MW. The pathogenesis of chronic idiopathic urticaria. *Arch Dermatol* 1997;133:1003–8.
- [8] Fogh K, Kragballe K. Eicosanoids in inflammatory skin diseases. *Prostaglandins Other Lipid Mediat* 2000;63:43–54.
- [9] Yamamoto M, Haruna T, Imura K, Hikita I, Furue Y, Higashino K, et al. Inhibitory effect of a potent and selective cytosolic phospholipase A₂ inhibitor RSC-3388 on skin inflammation in mice. *Pharmacology* 2008;81:301–11.
- [10] He R, Oyoshi MK, Wang JY, Hodge MR, Jin H, Geha RS. The prostaglandin D₂ receptor CRTH2 is important for allergic skin inflammation after epicutaneous antigen challenge. *J Allergy Clin Immunol* 2010;126:784–90.
- [11] Roebrock K, Wolf M, Bovens S, Lehr M, Sunderkötter C. Inhibition of benzalkonium chloride-induced skin inflammation in mice by an indol-1-ylpropan-2-one inhibitor of cytosolic phospholipase A₂α. *Br J Dermatol* 2012;166:306–16.
- [12] Häussinger D. Hepatic glutamine transport metabolism. *Adv Enzymol* 1998;72:43–86.
- [13] Fox RE, Hopkins IB, Cabacungan ET, Tildon JT. The role of glutamine and other alternate substrates as energy sources in the fetal rat lung type II cell. *Pediatr Res* 1996;40:135–41.
- [14] Encarnacion S, Calderon J, Gelbard AS, Cooper AJL, Mora J. Glutamine biosynthesis and the utilization of succinate and glutamine by *Rhizobium etli* and *Sinorhizobium meliloti*. *Microbiology* 1998;144:2629–38.
- [15] Kovacevic Z, McGivan JD. Mitochondrial metabolism of glutamine and glutamate and its physiological significance. *Physiol Rev* 1983;63:547–605.
- [16] Griffiths RD, Jones C, Palmer TE. Six-month outcome of critically ill patients given glutamine supplemented parenteral nutrition. *Nutrition* 1997;13:295–302.
- [17] Newsholme EA, Crabtree B, Ardawi MSM. Glutamine metabolism in lymphocytes: its biochemical, physiological, and clinical importance. *Q J Exp Physiol* 1985;70:473–89.
- [18] Wischmeyer PE, Kahana M, Wolfson R, Ren H, Musch MM, Chang EB. Glutamine reduces cytokine release, organ damage, and mortality in a rat model of endotoxemia. *Shock* 2001;16:398–402.
- [19] Andoh T, Kuraishi Y. Intradermal leukotriene B₄, but not prostaglandin E₂, induces itch-associated responses in mice. *Eur J Pharmacol* 1998;353:93–6.
- [20] Kim YS, Kim GY, Kim JH, You HJ, Park YM, Lee HK, et al. Glutamine inhibits lipopolysaccharide-induced cytoplasmic phospholipase A₂ activation and protects against endotoxin shock in mouse. *Shock* 2006;25:290–4.
- [21] Ko HM, Kang NI, Kim YS, Lee YM, Jin ZW, Jung YJ, et al. Glutamine preferentially inhibits Th2 cell-mediated airway inflammation and late airway hyperresponsiveness through the inhibition of cytosolic phospholipase A₂ activity in a murine asthma model. *Clin Exp Allergy* 2007;38:357–64.
- [22] Ruzicka T. Eicosanoids and the skin. Boca Raton: CRC Press; 1990.
- [23] Ikai K. Proinflammatory mediators of the arachidonic acid cascade. In: Kydonieus AF, Wille JJ, editors. *Biochemical modulation of skin reactions in dermal and transdermal delivery of drugs*. Boca Raton, FL: CRC Press; 1999.

- [24] Craig CM, Pamela H, Alice PP, Ultraviolet B. injury increases prostaglandin synthesis through a tyrosine kinase-dependent pathway. *J Biol Chem* 1994;269:3529–33.
- [25] Macfarlane SR, Seatter MJ, Kanke T, Hunter GD, Plevin R. Proteinase-activated receptor. *Pharmacol Rev* 2001;53:245–82.
- [26] Paus R, Schmelz M, Bíró T, Steinhoff M. Frontiers in pruritus research: scratching the brain for more effective itch therapy. *J Clin Invest* 2006;116(5):1174–86.
- [27] Hwang SW, Cho H, Kwak J, Lee SY, Kang CJ, Jung JY, et al. Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. *Proc Natl Acad Sci U S A* 2000;97:6155–60.
- [28] Shim WS, Tak MH, Lee MH, Kim MJ, Kim MK, Koo JY, et al. TRPV1 mediates histamine-induced itching via the activation of phospholipase A₂ and 12-lipoxygenase. *J Neurosci* 2007;28:2331–7.
- [29] Carucci JA, Washenik K, Weinstein A, Shupack J, Cohen DE. The leukotriene antagonist zafirlukast as a therapeutic agent for atopic dermatitis. *Arch Dermatol* 1998;134:785–6.
- [30] Ko HM, Oh SH, Bang HS, Kang NI, Cho BH, Im SY, et al. Glutamine protects mice from lethal endotoxic shock via a rapid induction of MAPK phosphatase-1. *J Immunol* 2009;182:7957–62.
- [31] Wischmeyer PE, Lynch J, Liedel J, Wolfson R, Riehm J, Gottlieb L, et al. Glutamine administration reduces Gram-negative bacteremia in severely burned patients: a prospective, randomized, double-blind trial versus isonitrogenous control. *Crit Care Med* 2001;29:2075–80.
- [32] Novak F, Heyland DK, Avenell A, Drover JW, Su X. Glutamine supplementation in serious illness: a systematic review of the evidence. *Crit Care Med* 2002;30:2022–9.