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European Journal of Pharmacology 518 (2005) 56 – 62

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# Prostaglandin $D_2$ and prostaglandin $E_2$ accelerate the recovery of cutaneous barrier disruption induced by mechanical scratching in mice

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Received 10 January 2005; received in revised form 24 May 2005; accepted 7 June 2005

**Abstract**

The role of prostaglandins in mechanical scratching-induced cutaneous barrier disruption in mice was investigated. Skin prostaglandins contents were measured after cutaneous barrier function was disrupted by scratching using a stainless-steal wire brush (mechanical scratching), then effects of prostanoids on recovery of cutaneous barrier functions were examined. This mechanical scratching increased transepidermal water loss and skin prostaglandins (prostaglandin $D_2$, prostaglandin $E_2$, 6-keto-prostaglandin $F_{1\alpha}$ and prostaglandin $F_{2\alpha}$) contents, count-dependently. Topical application of indomethacin immediately after cutaneous barrier disruption delayed the recovery period of cutaneous barrier disruption. We examined effects of several prostanoids (prostaglandin $D_2$, prostaglandin $E_2$, prostaglandin $F_{2\alpha}$, prostaglandin $I_2$ and U46619) on delay of the recovery process of mechanical scratching-induced cutaneous barrier disruption with treatment of indomethacin. Topically applied prostaglandin $D_2$ and prostaglandin $E_2$ accelerated the recovery of cutaneous barrier disruption and topical application of prostaglandin $J_2$, limaprost, sulprostone and ONO-4819, but not 13,14-dihydro-15-keto-prostaglandin $D_2$, 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$, 17-phenyl-trinor-prostaglandin $E_2$ or butaprost had effects on recovery of the cutaneous barrier. These results suggest that prostaglandin $D_2$ and prostaglandin $E_2$ accelerate the recovery process of cutaneous barrier disruption caused by mechanical scratching, via specific prostanoid $DP_1$, $EP_3$ and $EP_4$ receptors.


*Keywords:* Cutaneous barrier disruption; Prostaglandin; Transepidermal water loss; Mechanical scratching

## 1. Introduction

The cutaneous barrier prevents foreign matter from getting into the interior and from excessive moisture loss from the body. This disruption is characterized by transepidermal water loss, which represents water evaporation from the skin surface and reflects integrity of the stratum corneum water barrier (Baker and Kligman, 1967; Grubauer et al., 1989; Ozawa and Takahashi, 1994). Scratching behavior in patients with atopic dermatitis can cause physical damage to their skin, and the relationship between increment of transepidermal water loss, and the severity of atopic dermatitis symptoms has been reported (Watanabe et al., 1991).

An experiment model of cutaneous barrier disruption has been demonstrated using treatment with an organic solution of surfactant (Matoltsy et al., 1968; Fredriksson, 1969), diet without essential fatty acid (Prottey et al., 1976; Elias et al., 1980) and physical disruption by tape stripping (Elias et al., 1981; Frodin and Skogh, 1984). These models involved mainly removal of lipid components in the stratum corneum such as ceramides, cholesterol and free fatty acids, which play an important role in cutaneous barrier functions (Elias, 1983; Grubauer et al., 1989). A new murine model of cutaneous barrier disruption with itch-associated response has been designed with repeated treatment using an organic solvent and water (Miyamoto et al., 2002).

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doi:10.1016/j.ejphar.2005.06.006

Prostaglandins are the major arachidonic acid metabolite released from phospholipids membrane by phospholipase $A_2$, and different molecular species are produced by respective synthetase after conversion to prostaglandin $H_2$ by cyclooxygenase (Larsen and Henson, 1983; Goodwin, 1989). The actions of prostaglandin $D_2$, prostaglandin $E_2$, prostaglandin $F_{2\alpha}$, prostaglandin $I_2$ and thromboxane A2 are mediated by stimulation of prostanoid DP, $EP_{1\sim4}$, FP, IP and TP receptors, respectively (Coleman et al., 1994a). Prostaglandins are notably present in inflammatory tissue, and are generally recognized to be a potent mediator which enhances pain and inflammation, but the actual roles in various inflammatory diseases are unclear. We reported that several prostaglandins inhibit the pruritic activity in NC/Nga mice, a model of atopic dermatitis (Arai et al., 2004). In the present study, we examined the role of prostaglandins on cutaneous barrier function, after the barrier has been disrupted in mice using a wire-brush.

## 2. Materials and methods

### 2.1. Animals

Male BALB/c mice purchased from SLC Japan (Shizuoka, Japan) were all housed under conditions of controlled temperature (23±3 °C), humidity (55±20%) and lighting (lights on from 7:00 to 19:00), then used at 8 weeks of age. Food and tap water were provided ad libitum to all mice. All studies reported here have been reviewed by the Taisho Pharmaceutical Animal Care Committee and have met the Japanese Experimental Animal Research Association Standards as defined in the Guidelines for Animal Experiments (1987).

### 2.2. Materials

Indomethacin was obtained from Sigma-Aldrich (St. Louis, Missouri, USA), prostaglandin $D_2$, prostaglandin $E_2$, prostaglandin $F_{2\alpha}$, prostaglandin $I_2$, prostaglandin $J_2$ (prostanoid $DP_1/DP_2$ receptor agonist), 13,14-dihydro-15-keto-prostaglandin $D_2$ (prostanoid $DP_2$ receptor agonist), 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ (peroxisome proliferators-activated receptor γ (PPARγ) agonist), 17-pheny-trinor-prostaglandin $E_2$ (prostanoid $EP_1$ receptor agonist), Sulprostone (prostanoid $EP_3$ receptor agonist) and U-46619 (prostanoid TP receptor agonist) were obtained from Cayman Chemical (Ann Arbor, Michigan, USA). Butaprost (prostanoid $EP_2$ receptor agonist), limaprost (prostanoid EP receptor agonist) and ONO-4819 (prostanoid $EP_4$ receptor agonist) were synthesized in our research center. These drugs were dissolved in ethanol (Kokusan Kagaku, Tokyo, Japan) and applied to the rostral part of the back of mice.

### 2.3. Treatment of cutaneous barrier disruption

Hair on the skin of the rostral part of the back was shaved off using an electric razor with the mice put under diethyl ether anesthesia the day before the experiment to avoid damage to the barrier. The next day, the cutaneous barrier function on the back was disrupted by scratching with a stainless-steal wire brush (diameter; 0.175 mm, length; 15 mm, strength; 60±10 g/cm$^2$, Paock Co., SCWS-005P) (mechanical scratching) with the mice under diethyl ether anesthesia.

### 2.4. Measurement of transepidermal water loss

Transepidermal water loss was measured using a skin evaporative water recorder (Tewameter® TM210, Courage and Khazawa, Germany). The probe consisted of a cylindrical chamber, 12 mm in diameter. Measurements were recorded when transepidermal water loss readings were stabilized at approximately 30 s after the probe had been placed on the skin.

### 2.5. Measurement of the skin prostaglandins contents

Five minutes after the cutaneous barrier disruption by mechanical scratching, mice were injected with indomethacin (10 mg/kg) to prevent further production of prostaglandins. Five minutes later, mice were decapitated and the back skin of each mouse (about 100 mg) was removed. The skin was minced and homogenized in ice-cold phosphate buffered saline (PBS) containing 10 μM indomethacin with a Polytron tissue homogenizer for 30 s on ice. Four milliliters of acetone was added to the sample and vortexed then the precipitate was removed by centrifugation at 3000 *g* for 10 min at 4 °C. The supernatant was carefully poured into a test tube and evaporated to dryness under a stream of nitrogen and resuspended in enzyme-immunoassay buffer. The amounts of prostaglandin $D_2$, prostaglandin $E_2$ and 6-keto-prostaglandin $F_{1\alpha}$ were measured for each prostaglandin using specific enzyme-immunoassay (EIA) kits (Cayman Chemical, Michigan, USA), and the amounts of prostaglandin $F_{2\alpha}$ were measured using prostaglandin $F_{2\alpha}$ EIA kits (R and D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's instruction. Data are presented as tissue weight (picograms per milligram).

### 2.6. Drug treatment

#### 2.6.1. Effect of indomethacin on recovery of cutaneous barrier

Mice were disrupted of the cutaneous barrier so that transepidermal water loss of the disrupted area was about 20 g/m$^2$/h by mechanical scratchings 50±10 times. A 0.1 w/v% (percent) indomethacin dissolved in ethanol in a volume of 0.1 ml/mouse was topically applied to the shaved area just after mechanical scratching to block production of endogenous prostaglandins by mechanical scratching. Transepidermal water loss was measured 24 h after topical application of indomethacin. Then, indomethacin was topically applied after measurement of transepidermal water loss once a day for 5 days.

#### 2.6.2. Effect of several prostanoids and prostanoids receptor agonists on recovery of cutaneous barrier

After cutaneous barrier disruption by mechanical scratching and topical application of indomethacin described above, several prostanoids and prostanoids receptor agonists (their related compounds) dissolved in ethanol in a volume of 0.1 ml/mouse were topically applied to shaved areas 10 min after topical application of indomethacin. Transepidermal water loss was measured 24 h after the topical application of them. Then, these

drugs were topically applied after measurement of transepidermal water loss once a day for 4 days.

### 2.7. Data analysis

Experimental values are given as the mean±S.E.M. Statistical significance was analyzed using Dunnett's test after Bartlett's test, Student's *t*-test or Welch's *t*-test after *F*-test. $*P<0.05$, $**P<0.01$ and $***P<0.001$ values were considered significant.

## 3. Results

### 3.1. Changes of transepidermal water loss by mechanical scratching

Transepidermal water loss of normal skin was 1.70±0.14 g/m$^2$/h. The skin on the rostral part of the back was mechanical scratched 10, 20, 30, 40 and 50 times with a wire-brush, and transepidermal water loss increased to 2.97±0.37, 5.12±1.21, 10.08±2.19, 15.93±2.63 and 21.28±3.04 g/m$^2$/h count-dependently. Transepidermal water loss of mechanical scratching counts over 40 times to the skin had a significant increase compared to that of normal skin (Fig. 1).

### 3.2. Changes of the skin prostaglandins contents by mechanical scratching

Basal values for prostaglandin $D_2$, prostaglandin $E_2$, 6-keto-prostaglandin $F_{1\alpha}$ (stable metabolite of prostaglandin $I_2$) and prostaglandin $F_{2\alpha}$ were 8.09±1.33, 2.96±0.71, 1.02±0.12 and 0.18±0.08 pg/mg tissue weight, respectively. By mechanical scratching from 20 to 50 times, the skin prostaglandins (prostaglandin $D_2$, prostaglandin $E_2$, 6-keto-prostaglandin $F_{1\alpha}$ and prostaglandin $F_{2\alpha}$) contents increased count-dependently. Prostaglandin $D_2$, prostaglandin $E_2$, 6-keto-prostaglandin $F_{1\alpha}$ and prostaglandin $F_{2\alpha}$ contents of mechanical scratching counts 50 times were 186.94±18.90, 163.11±21.40, 7.97±1.22 and 17.33±2.20 pg/mg tissue weight, respectively (Fig. 2A–D). Prostaglandin $D_2$ and prostaglandin $E_2$ contents exceeded 6-keto-prostaglandin $F_{1\alpha}$ and prostaglandin $F_{2\alpha}$ contents.

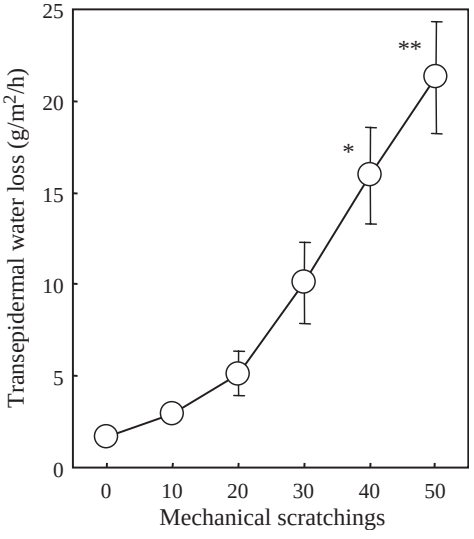


Fig. 1. Change in transepidermal water loss by mechanical scratching. The skin of the rostral part of the mouse back was mechanically scratched 10, 20, 30, 40 and 50 times. Values represent the mean±S.E.M. from 6 mice. $*P<0.05$, $**P<0.01$ when compared with mechanical scratchings 0 (Student's *t*-test or Welch's *t*-test).

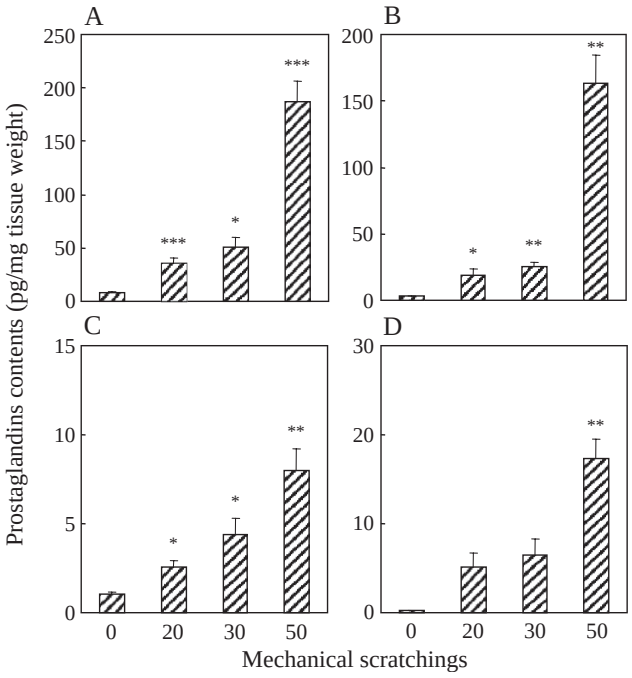


Fig. 2. Changes in skin prostaglandins contents by mechanical scratching. The amounts of prostaglandin $D_2$ (A), prostaglandin $E_2$ (B), 6-keto-prostaglandin $F_{1\alpha}$ (C) and prostaglandin $F_{2\alpha}$ (D) were measured 10 min after the treatment of cutaneous barrier disruption by mechanical scratching for 20, 30 and 50 times. Values represent the mean±S.E.M. pg/mg tissue weight from 6 mice. $*P<0.05$, $**P<0.01$, $***P<0.001$ when compared with mechanical scratchings 0 (Student's *t*-test or Welch's *t*-test).

### 3.3. Effect of indomethacin on recovery of cutaneous barrier

Transepidermal water loss of indomethacin-applied mice was significantly higher than that of vehicle-applied mice for all experimental periods and delay in recovery on transepidermal water loss was observed (Fig. 3). Compared to treatment of cutaneous barrier disruption (transepidermal water loss was about 20 g/m$^2$/h) by mechanical scratching of about 50 times and skin prostaglandins contents were significantly increased, then 0.1% indomethacin was topically applied to the scratched area in a volume of 0.1 ml/mouse 10 min before scratching, the skin prostaglandins contents were completely inhibited (Table 1).

### 3.4. Effects of several prostanoids on recovery of the cutaneous barrier

We examined the effect of prostanoids on delay of cutaneous barrier recovery induced by indomethacin. On day 1, transepidermal water loss of vehicle-applied mice was 21.49±0.68 g/m$^2$/h, and transepidermal water loss of 0.1% prostaglandin $D_2$ and prostaglandin $E_2$-applied mice was 13.60±0.73 and 17.51±0.84 g/m$^2$/h respectively, hence both groups had a significant decrease and recovered the delay of cutaneous barrier recovery induced by

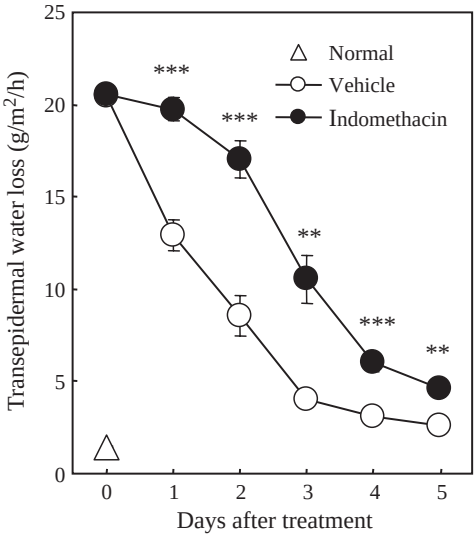


Fig. 3. Effect of indomethacin on recovery of the cutaneous barrier. Mice were subjected to cutaneous barrier disruption by mechanical scratching about 50 times and 0.1 mL of 0.1% indomethacin (●) was then topically applied once a day for 5 days. Transepidermal water loss was measured in 24 h after topically application of indomethacin. Values represent the mean±S.E.M. from 8 mice. Normal represents the value of mice given no treatment (△). $^{**}P<0.01$, $^{***}P<0.001$ when compared with vehicle (○) (Student's *t*-test).

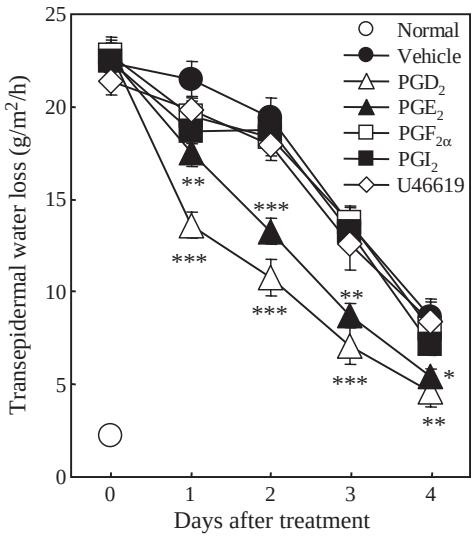


Fig. 4. Effects of several prostanoids on recovery of the cutaneous barrier. Mice were subjected to cutaneous barrier disruption by mechanical scratching about 50 times and then inhibition of production of endogenous prostaglandins by topical application of indomethacin. A 0.1 mL of 0.1% several prostanoids (prostaglandin $D_2$ ($PGD_2$;△), prostaglandin $E_2$ ($PGE_2$;▲), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$;□), prostaglandin $I_2$ ($PGI_2$;■) and U46619 (◇) were topically applied once a day for 4 days 10 min after topical application of indomethacin. Transepidermal water loss was measured at 24 h after topically application of each prostanoid. Values represent the mean±S.E.M. from 8 mice. Normal represents the value of mice given no treatment (○). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ when compared with vehicle (●) (Dunnett's test).

indomethacin. Although both groups showed a significant difference compared to the vehicle group from day 1, transepidermal water loss of 0.1% prostaglandin $D_2$-applied mice was always lower than that of 0.1% prostaglandin $E_2$-applied mice during examinations. However, transepidermal water loss of 0.1% prostaglandin $F_{2\alpha}$, prostaglandin $I_2$ and U46619-applied mice showed no significant difference compared to the vehicle group (Fig. 4).

### *3.5. Effects of a low concentration of prostaglandin $D_2$ and prostaglandin $E_2$ on recovery of the cutaneous barrier*

Topical application of prostaglandin $D_2$ and prostaglandin $E_2$ recovered the delay of cutaneous barrier induced by indomethacin, in a dose-dependent manner. On day 2, topical application of 0.001%, 0.01% and 0.1% prostaglandin $D_2$ and prostaglandin $E_2$, but neither 0.00001% nor 0.0001% prostaglandin $D_2$ nor prostaglandin $E_2$ were significantly lower than transepidermal water loss of the vehicle group (Fig. 5A,B). Suppressive effects of prostaglandin $D_2$ and prostaglandin $E_2$ reached the plateau at 0.01% concentration.

Table 1
Effect of indomethacin on the mechanically induced barrier disruption of the skin on prostaglandins contents

| | $PGD_2$ | $PGE_2$ | 6-keto-$PGF_{1\alpha}$ | $PGF_{2\alpha}$ |
|---|---|---|---|---|
| Non-treatment | 5.35±0.36 | 5.88±0.92 | 1.11±0.10 | 0.80±0.23 |
| VH+MS | 38.42±31.20[a] | 285.60±36.69[a] | 16.79±1.98[a] | 39.16±4.26[a] |
| IM+MS | 3.10±0.56[b] | 3.30±0.92[b] | 0.94±0.10[b] | 0.17±0.05[b] |

Value represents the means±S.E.M. pg/mg tissue weight from 6 mice. MS; Mice were subjected to cutaneous barrier disruption by mechanical scratching (MS) about 50 times using a wire-brush, 10 min before the measurement of the skin prostaglandins (prostaglandin $D_2$ ($PGD_2$), prostaglandin $E_2$ ($PGE_2$), 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)) contents. IM; 0.1 mL of 0.1% indomethacin (IM) was topically applied to the mouse skin of the back, 10 min before MS.

[a] $P<0.001$ compared with non-treatment of each prostanoid content.

[b] $P<0.001$ compared with vehicle (VH) of each prostaglandin content.

### *3.6. Effects of prostaglandin $D_2$ metabolites on recovery of the cutaneous barrier*

We examined which receptors were involved in the effect of cutaneous barrier recovery of prostaglandin $D_2$. On day 2, topical application of 0.1% prostaglandin $J_2$, but not 0.1% 13,14-dihydro-15-keto-prostaglandin $D_2$ and 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ were lower than transepidermal water loss of the vehicle group. However, compared with 0.01% prostaglandin $D_2$ which had a sufficient effect in cutaneous barrier recovery, transepidermal water loss of 0.1% prostaglandin $J_2$ at a ten-fold higher concentration was high (Fig. 6).

### *3.7. Effects of prostanoids EP agonist on recovery of the cutaneous barrier*

We examined which receptors were involved in the effect of cutaneous barrier recovery of prostaglandin $E_2$. On day 2, topical application of 0.001% limaprost, sulprostone and ONO-4819, but not 0.001% 17-phenyl-trinor-prostaglandin $E_2$ and butaprost were lower than transepidermal water loss of the vehicle group (Fig. 7). At 0.00001% concentration, ONO-4819

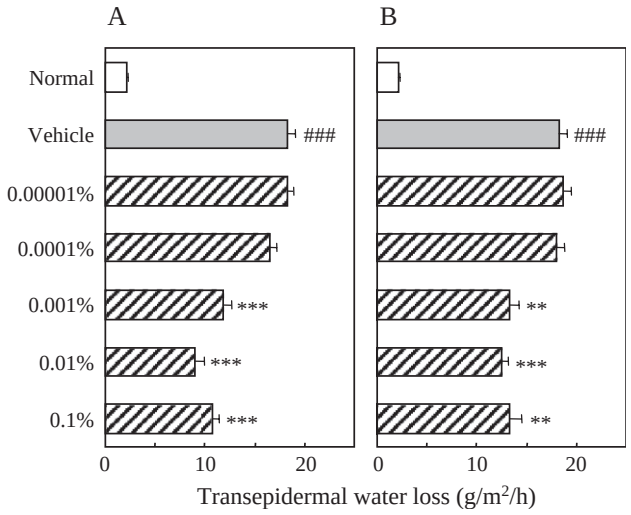


Fig. 5. Effects of a low concentration of prostaglandin $D_2$ and prostaglandin $E_2$ on recovery of the cutaneous barrier. Mice were subjected to cutaneous barrier disruption by mechanical scratching about 50 times then inhibition of production of endogenous prostaglandins by topical application of indomethacin. A 0.1 mL of 0.00001%, 0.0001%, 0.001%, 0.01% and 0.1% prostaglandin $D_2$ (A) and prostaglandin $E_2$ (B) were topically applied once a day for 2 days 10 min after topical application of indomethacin (hatched columns). Transepidermal water loss was measured at 24 h after the second topically application of each prostanoid. Values represent the mean ± S.E.M. from 8 mice. Normal represents the value of mice given no treatment (open column). $^{###}P<0.001$ when compared with normal (Student's *t*-test). $^{**}P<0.01$, $^{***}P<0.001$ when compared with vehicle (shadowed column) (Dunnett's test).

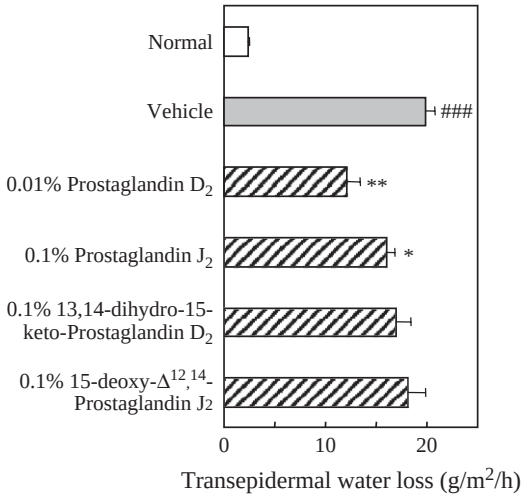


Fig. 6. Effects of the prostaglandin $D_2$ metabolites on recovery of the cutaneous barrier. Mice were subjected to cutaneous barrier disruption by mechanical scratching about 50 times and inhibition of production of endogenous prostaglandins by topical application of indomethacin. A 0.1 mL of 0.01% prostaglandin $D_2$, 0.1% prostaglandin $J_2$, 13,14-dihydro-15-keto-prostaglandin $D_2$ and 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ were topically applied once a day for 2 days 10 min after topical application of indomethacin (hatched columns). Transepidermal water loss was measured at 24 h after the second topically application of each prostaglandin $D_2$ metabolite. Values represent the mean ± S.E.M. from 8 mice. Normal represents the value of mice given no treatment (open column). $^{###}P<0.001$ when compared with normal (Student's *t*-test). $^{*}P<0.05$, $^{**}P<0.01$ when compared with vehicle (shadowed column) (Dunnett's test).

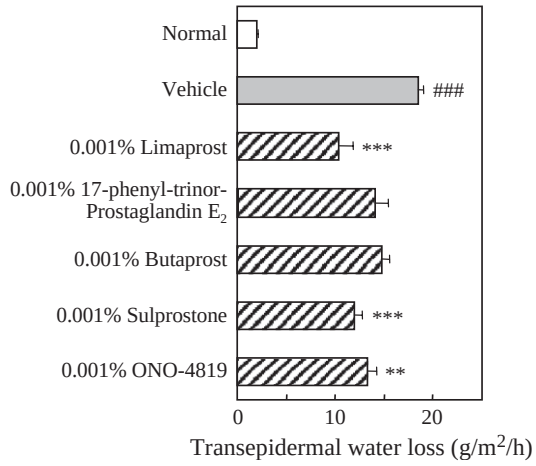


Fig. 7. Effects of the prostanoid EP receptor agonists on recovery of the cutaneous barrier. Mice were subjected to cutaneous barrier disruption by mechanical scratching about 50 times then inhibition of production of endogenous prostaglandins by topical application of indomethacin. A 0.1 mL of 0.01% limaprost, 17-phenyl-trinor-prostaglandin $E_2$, butaprost, sulprostone and ONO-4819 were topically applied once a day for 2 days 10 min after topical application of indomethacin (hatched columns). Transepidermal water loss was measured at 24 h after the second topically application of each prostanoid EP agonist. Values represent the mean ± S.E.M. from 6 mice. Normal represents the value of mice given no treatment (open column). $^{###}P<0.001$ when compared with normal (Welch's *t*-test). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ when compared with vehicle (shadowed column) (Dunnett's test).

only had a significant difference compared to the vehicle group (data not shown).

## 4. Discussion

In the present study, we found for the first time the innovative physiological function of prostaglandins on cutaneous barrier disruption. Itching is a sensation, which causes a strong desire to scratch, and is one of the most important symptoms in various dermatitis such as atopic dermatitis (Williams, 1994). Scratching aggravates lesions of the skin in patients with atopic dermatitis (Kimura and Miyazawa, 1989) and in NC/Nga mice (Hashimoto et al., 2004), which have been recognized as a model of atopic dermatitis (Suto et al., 1999). It is well known that scratching behavior with finger nails cause physical damage to their skin, and relationship of an increment of transepidermal water loss and the severity of dermatitis in patients with atopic dermatitis (Watanabe et al., 1991) and in NC/Nga mice (Hashimoto et al., 2004) was reported. Therefore, in the present study, this scratching with a nail was replaced by scratching with a mechanical instrument to set up a model of cutaneous barrier disruption.

The relationship between cutaneous barrier disruption and prostaglandins production in the skin was examined. Transepidermal water loss and the skin prostaglandin $D_2$, prostaglandin $E_2$, 6-keto-prostaglandin $F_{1\alpha}$ (stable metabolite of prostaglandin $I_2$) and prostaglandin $F_{2\alpha}$ contents

increased by mechanical scratching, count-dependently. These results suggest that the increment of prostaglandins in the skin had an effect when cutaneous barrier was disrupted. Prostaglandins are generally recognized as mediators of enhancing pain, regulating vascular function, regulating the production of inflammatory cytokines and so on. Indomethacin, as a nonsteroidal anti-inflammatory drug, blocks endogenous prostaglandins biosynthesis by inhibiting cyclooxygenase activity and decreases inflammation (Vane, 1971, 1976). Unexpectedly, the topical application of indomethacin to the mouse skin delayed spontaneous recovery of the cutaneous barrier after mechanical scratching in our study, which means that prostaglandins help to recover the cutaneous barrier.

Next, the effects of several prostanoids on recovery of the cutaneous barrier were examined. Enhancement of endogenous prostaglandins in the skin was inhibited by the topical application of indomethacin after cutaneous barrier disruption by mechanical scratching. Application of high concentrations (0.1%) of prostaglandin $D_2$ and prostaglandin $E_2$, but not prostaglandin $F_{2\alpha}$, prostaglandin $I_2$ nor U46619 (thromboxanes $A_2$ agonist), significantly accelerated recovery of cutaneous barrier disruption. Moreover, there was a tendency that prostaglandin $D_2$ acted at the early phase and had a potent effect compared with prostaglandin $E_2$. Prostaglandin $D_2$ is the major cyclooxygenase product from arachidonic acid that shows various pharmacological activities such as inhibition of platelet aggregation (Whittle et al., 1978), bronchoconstriction (Patterson et al., 1980), sleep induction (Ueno et al., 1983), and hypothermia (Ueno et al., 1982), under various physiological and pathological conditions. Moreover, prostaglandin $D_2$ is also produced by allergen-activated mast cells and has been implicated in various allergic diseases as a proinflammatory lipid mediator (Lewis et al., 1982), but the actual roles in various inflammatory diseases have been unclear. On the contrary, prostaglandin $E_2$ enhances inflammation as an important modulator, and induces transient wheal and flare response (Archer et al., 1987). Now, we found a novel innovative physiological function in that prostaglandin $D_2$ and prostaglandin $E_2$ have effects on recovery of a broken cutaneous barrier.

PPARγ is a member of the nuclear receptor superfamily that includes steroid, thyroid, and retinoid hormones (Evans, 1988). PPARγ activation is mediated exclusively by metabolites of prostaglandin $D_2$, the most active of which is 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ as a natural PPARγ ligand (Barry et al., 1995). On the other hand, prostanoid $DP_2$ receptor (also known as chemoattractant receptor-homologous molecule expressed on $Th_2$ cells ($CRTH_2$)) is a seven-transmembrane G protein-coupled receptor structurally related to members of the *N*-formyl peptide receptor subfamily (Nagata et al., 1999a). Prostanoid $DP_2$ receptor can mediate intercellular $Ca^{2+}$ mobilization in response to a factor released from activated mast cells, suggesting that prostanoid $DP_2$ may be closely involved in mast cell-madiated allergic inflammation (Nagata et al., 1999b). One of the prostaglandin $D_2$ metabolites, 13,14-dihydro-15-keto-prostaglandin $D_2$ is a highly selective agonist for the prostanoids $DP_2$ receptor (Hirai et al., 2001). In the present study, PPARγ agonist (15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$) and prostanoid $DP_2$ receptor agonist (13,14-dihydro-15-keto-prostaglandin $D_2$) had no apparent effect on recovery of the cutaneous barrier. Although prostaglandin $J_2$ activated both prostanoid $DP_1$ and $DP_2$ receptors, selectivity to the prostanoid $DP_1$ receptor attenuates as prostaglangin $D_2$ is metabolited to prostaglandin $J_2$, consequently the effect on recovery of the cutaneous barrier was attenuated. These findings indicate that the suppressive effect of prostaglandin $D_2$ is mediated by the prostanoid $DP_1$ receptor.

On the other hand, there are four known subtypes ($EP_1$, $EP_2$, $EP_3$ and $EP_4$) of prostanoid EP receptor (Coleman et al., 1994b; Ushikubi et al., 1995; Narumiya et al., 1999). In our study, topical application at 0.001% concentration of limaprost (prostanoid $EP_{1\sim4}$ receptor agonist), sulprostone (prostanoid $EP_3$ receptor agonist) and ONO-4819 (prostanoid $EP_4$ receptor agonist), but neither 17-phenyl-trinor-prostaglandin $E_2$ (prostanoid $EP_1$ receptor agonist) nor butaprost (prostanoid $EP_2$ receptor agonist) affected recovery of the cutaneous barrier. Moreover, at 0.00001% concentration, ONO-4819 had only a significant difference compared to the vehicle group. It seems likely that the suppressive effect of prostaglandin $E_2$ is mediated by prostanoids $EP_3$ and $EP_4$ receptors, in particular prostanoid $EP_4$.

Our results reported here suggest that prostaglandins, especially prostaglandin $D_2$ and prostaglandin $E_2$, in the skin markedly increase when the cutaneous barrier is disrupted and that these prostaglandins act on recovery of the cutaneous barrier. In our previous study, we found that indomethacin significantly enhanced the spontaneous scratching behavior by NC/Nga mice and several prostaglandins (prostaglandin $D_2$, prostaglandin $E_1$, prostaglandin $E_2$ and prostaglandin $I_2$) inhibited the spontaneous scratching behavior in these mice, in particular the effect of prostaglandin $D_2$ is dramatically potent as compared to others (Arai et al., 2004). All these results taken together suggest that scratching behavior increases skin prostaglandins contents to reduce itch sensation and it follows that the disrupted cutaneous barrier will recover with the enhanced skin prostaglandins contents.

In conclusion, we found the physiological function of prostaglandin $D_2$ and prostaglandin $E_2$ for accelerating the recovery process of cutaneous barrier disruption caused by mechanical scratching, and we attribute this to specific prostanoid $DP_1$, $EP_3$ and $EP_4$ receptors.

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