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ORIGINAL ARTICLE

# Novel anti-acne actions of nadifloxacin and clindamycin that inhibit the production of sebum, prostaglandin $E_2$ and promatrix metalloproteinase-2 in hamster sebocytes

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## ABSTRACT

**Acne vulgaris is characteristic of excess sebum production and the induction of inflammatory reactions, for example, the augmentation of cytokine, prostaglandin (PG) and matrix metalloproteinase (MMP) production in sebaceous glands and pilosebaceous units. As *Propionibacterium acnes* is considered to be involved in the aggravation of acne vulgaris, antimicrobial agents have been found to be effective for treating acne leading to the remission of inflammation. However, it is not fully understood whether antimicrobial agents influence sebum production and/or the inflammatory reactions in sebaceous gland cells (sebocytes). In the present study, topical antimicrobial agents such as nadifloxacin (NDFX) and clindamycin (CLDM) decreased the production of triacylglycerols (TG), which are a major component of sebum in insulin-differentiated hamster sebocytes. These antibiotics also suppressed insulin-augmented gene expression and the production of perilipin, by which intracellular lipid droplet formation was concomitantly inhibited. On the other hand, peptidoglycan (PGN) from Gram-positive bacteria dose-dependently increased TG production in hamster sebocytes. The augmented TG production was decreased by treating NDFX or CLDM. Furthermore, NDFX and CLDM inhibited the PGN-augmented $PGE_2$ production in the sebocytes. Moreover, NDFX, but not CLDM, suppressed the PGN-augmented gene expression and production of pro-MMP-2/progelatinase A in hamster sebocytes. Therefore, these results provide novel evidence that NDFX and CLDM exhibit anti-lipogenesis and anti-inflammatory activities against insulin- or PGN-activated sebocytes which at least partly mimic acne pathology *in vitro*. Moreover, NDFX for acne therapy is likely to be effective in not only inhibiting microbial proliferation but also in preventing the onset of acne scar formation.**

**Key words:** acne vulgaris, antimicrobial agents, matrix metalloproteinase, prostaglandin $E_2$, sebum production.

## INTRODUCTION

Acne vulgaris, a common inflammatory skin disease,[1–3] has the following characteristics: (i) excess sebum production and hyperplasia of sebaceous glands; (ii) formation of microcomedones, which is closely associated with the hyperkeratinization of the follicular wall and infundibulum; and (iii) the induction of inflammatory reactions such as the acceleration of cytokine production and the biosynthesis of arachidonic acid metabolites in keratinocytes, sebocytes and invaded inflammatory cells.[4] In addition, the aggravation and duration of the inflammation have been closely associated with the formation of acne scars, a severely disfiguring and permanent sequel.[5] Furthermore, acne scarring may result in a psychological and social impact that affects the patient's quality of life. Moreover, recent studies have reported abnormal extracellular matrix (ECM) remodeling in sebaceous and pilosebaceous units in the skin of acne patients, under which matrix metalloproteinases (MMP) play important roles in the onset of acne scarring.[6–9]

*Propionibacterium acnes*, a Gram-positive anaerobic microbial species, is considered to play a role in the aggravation of acne.[1] *P. acnes*, as well as peptidoglycan (PGN) from Gram-positive bacteria, has been reported to potentially augment the production of cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-8 in human keratinocytes, human monocytic THP-1 and U937 cells, and peripheral blood mononuclear cells from acne patients.[10–14] We recently reported that *P. acnes* directly augments sebum production in hamster sebaceous glands *in vivo* and *in vitro*.[15] In addition, *P. acnes* has been reported to augment the expression of pro-MMP-1/procollagenase-1 and pro-MMP-9/progelatinase B in human monocytes,[8] and that of pro-MMP-2/progelatinase A in human dermal fibroblasts.[9] We have also reported that *P. acnes* and PGN augment the expression of pro-MMP-2 in hamster sebocytes.[16]

Topical and systemic antibiotics have been used for the treatment of inflammatory acne with mild to moderate conditions.[17] Nadifloxacin (NDFX) and clindamycin (CLDM), fluoroquinolone and

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lincosamide antibiotics, respectively, have been reported to be topically effective for acne patients with multiple inflamed acne lesions due to their antimicrobial activities against *P. acnes*.[18,19] In addition, it has been reported that NDFX or CLDM inhibits the production of pro-inflammatory cytokines, such as γ-interferon, IL-1β and IL-12p70, in the heat-killed *P. acnes*-stimulated human peripheral blood mononuclear cells.[20] Furthermore, NDFX has been reported to exert other anti-inflammatory activities, for example, the inhibition of reactive oxygen species generation and cell migration in keratinocytes and neutrophils.[21] However, it is not well understood whether NDFX or CLDM directly improves abnormal lipogenesis and the inflammatory reactions in *P. acnes*-stimulated sebaceous glands. We here examined the effects of NDFX and CLDM on the production of triacylglycerols (TG), which is a major component of sebum,[22,23] prostaglandin $E_2$ ($PGE_2$) and pro-MMP-2 in insulin- or PGN-stimulated hamster sebocytes.

## METHODS

### Cell culture and treatment

Hamster sebocytes were cultured in DMEM/F12 supplemented with 6% heat-denatured fetal bovine serum (JRH Bioscience, Tokyo, Japan), 2% human serum (ICN Biochemicals, Costa Mesa, CA, USA), 0.68 mmol/L L-glutamine (Invitrogen, Carlsbad, CA, USA), and recombinant human epidermal growth factor (10 nmol/L) (Progen Biotechnik GmbH, Heidelberg, Germany) as previously described.[22] For $PGE_2$ and pro-MMP-2 production, the cells were treated for 24 h with NDFX (10–50 μg/mL) (kindly provided by Otsuka Pharmaceutical Co., Tokyo, Japan) or CLDM hydrochloride (10–50 μg/mL) (AppliChem GmbH, Darmstadt, Germany) in the presence or absence of PGN (5–50 μg/mL) from *Staphylococcus aureus* (Sigma Chemical, St Louis, MO, USA) in Dulbecco's modified Eagle's medium (DMEM)/F12 supplemented with 0.2% lactalbumin hydrolysate (Sigma Chemical, St Louis, MO, USA).[16,24] In this series of experiments, hamster sebocytes were used as far as the third passage level.

### TG measurement

Hamster sebocytes ($1.8 \times 10^5$ cells) in the 35-mm diameter culture dish were treated every 3 days for up to 9 days with NDFX or CLDM (10–50 μg/mL) in the presence or absence of insulin (10 nmol/L) (Sigma Chemical) or PGN (5–50 μg/mL) in DMEM/F12 supplemented with heat-denatured fetal bovine serum, human serum and L-glutamine. The harvested cells were subjected to TG quantification using Liquitech TG-II (Roche Diagnostics, Tokyo, Japan) as previously described.[15] The amounts of intracellular TG were calculated using an authentic trioleinate-standard solution (0.6 mg/mL). Intracellular DNA contents were measured using salmon sperm DNA (6.25–100 μg/mL) and 3,5-diaminobenzoic acid dihydrochloride (Sigma Chemical) as previously described.[15]

### Western blot analysis

The harvested cell lysate (50 μg protein) or culture medium (1.5 mL) was subjected to western blot analysis using 12.5% acrylamide gel as previously described.[16,24] The membrane was reacted with rabbit anti-human perilipin IgG, which was customized by Operon Biotechnologies (Tokyo, Japan), or sheep anti-human pro-MMP-2 IgG (graciously provided by Professor H. Nagase, the Kennedy Institute of Rheumatology, The Imperial College London, London, UK). Immunoreactive perilipin and pro-MMP-2 were visualized with Amersham enhanced chemiluminescence western blotting detection reagents (GE Healthcare Bio-Sciences, Tokyo, Japan) according to the manufacturer's instructions. Relative amounts of perilipin and pro-MMP-2 protein were quantified by densitometric scanning using an Image Analyzer LAS-1000 Plus (GE Healthcare Bio-Sciences), and the relative expression level was expressed as the mean value of the control as 100%.

### Enzyme immunoassay for $PGE_2$

The amounts of $PGE_2$ in the harvested culture media were measured using enzyme immunoassay kits for $PGE_2$ (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions.

### Real-time polymerase chain reaction

For the quantification of *perilipin*, diacylglycerol acyltransferase 1 (*DGAT-1*) and *pro-MMP-2* mRNA, total RNA was isolated from cells using ISOGEN (Nippon Gene, Toyama, Japan) and then subjected to reverse transcriptase reaction for the synthesis of cDNA using a PrimeScript RT reagent Kit (Takara Bio, Shiga, Japan) according to the manufacturer's instructions. Aliquots (an equivalent of 2.5 ng of total RNA) of the transcript were subjected to real-time polymerase chain reaction using SYBR Premix Ex Taq II (Takara Bio) and following specific primers: hamster *perilipin* (AB091681),[25] 5′-ACCTTGCTGGATGGAGACC-3′ (sense) and 5′-CCAGGACCTTGTCTGAAGT-3′ (antisense); human *DGAT-1* (NM_012079),[15] 5′-TCTACAAGCCCATGCTTCGAC-3′ (sense) and 5′-GGACGCTCACCAGGTACT-3′ (antisense); hamster *pro-MMP-2* (AF260254),[16] 5′-TATCCCAAACCACTGACC-3′ (sense) and 5′-GTATCTCCAGAACTTGTCTCC-3′ (antisense); and hamster glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (X52123),[16] 5′-CAGAACATCATCCCTGCAT-3′ (sense) and 5′-TAGGAACACGGAAGGCCAT-3′ (antisense). The amplification cycle was performed at 94°C for 5 s and 60°C for 30 s using a Thermal Cycler Dice Real Time System TP-800 (Takara Bio). The obtained threshold cycle ($C_T$) value for *pro-MMP-2* was normalized by that for *GAPDH*, and the relative expression level was expressed as the mean value of the control as 100%.

### Statistical analysis

A one-way ANOVA was used for the statistical analysis, and then Fisher's exact test was applied when multiple comparisons were performed.

## RESULTS

### Identification of anti-lipogenic activity of NDFX and CLDM in hamster sebocytes

To evaluate the regulation of sebaceous lipogenesis by NDFX and CLDM, we first examined the effects of NDFX and CLDM on the production of a major component of sebum, TG, in hamster sebocytes.[22,23] As hamster sebocytes spontaneously differentiated to accumulate the intracellular TG,[22] NDFX (10–50 μg/mL) was found

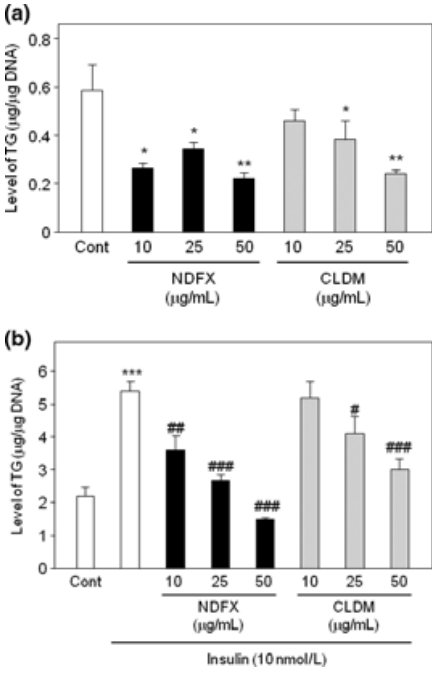


**Figure 1.** Effects of NDFX and CLDM on insulin-augmented TG production in hamster sebocytes. Hamster sebocytes at the third passage were treated every 3 days for 9 days with or without NDFX (10–50 μg/mL) and CLDM (10–50 μg/mL) in the presence or absence of insulin (10 nmol/L) (b and a, respectively). Levels of TG in the harvested cell lysates were measured as described in the Methods. Data are shown as mean ± standard deviation of three dishes. *,**,***Significantly different from untreated cells (Cont) ($P < 0.05$, 0.01 and 0.001, respectively). #,##,###Significantly different from insulin-treated cells ($P < 0.05$, 0.01 and 0.001, respectively). CLDM, clindamycin; NDFX, nadifloxacin; TG, triacylglycerols.

to decrease the spontaneous TG accumulation (45.0 ± 4.2% at 10 μg/mL) (Fig. 1a). In addition, CLDM did not influence the intracellular level of TG at less than 10 μg/mL, but dose-dependently suppressed it at more than 25 μg/mL (41.5 ± 1.8% at 50 μg/mL) (Fig. 1a). Because insulin has been reported to augment sebaceous lipogenesis in humans and hamsters,[25–27] we further examined the effects of NDFX and CLDM on the insulin-augmented lipogenesis in hamster sebocytes. Figure 1(b) shows that the insulin-augmented TG production was dose-dependently suppressed by NDFX and CLDM (27.7 ± 0.8 and 55.6 ± 6.3% at 50 μg/mL, respectively). However, we confirmed that there was no cytotoxicity in either of the antibiotic-treated hamster sebocytes (data not shown). These results suggest that NDFX and CLDM exhibit inhibitory activities against spontaneous and insulin-augmented TG production in hamster sebocytes.

### Suppression of *perilipin* and *DGAT-1* gene expression by NDFX and CLDM in insulin-differentiated hamster sebocytes

We previously reported that the expression of a lipid droplet associated protein, perilipin and DGAT-1 activity are augmented along with an increase in the intracellular levels of TG in insulin-differentiated hamster sebocytes.[24,25] As shown in Figure 2(a), NDFX was found to inhibit the insulin-augmented production of perilipin at 10 μg/mL (18.2 ± 1.1% vs untreated cells) and completely did so at more than 25 μg/mL. In addition, CLDM dose-dependently decreased the insulin-augmented perilipin production (12.6 ± 1.9% at 50 μg/mL vs untreated cells). The suppression of insulin-induced perilipin production by both NDFX and CLDM was found to be due to the inhibition of *perilipin* mRNA expression (Table 1). Furthermore, oil-red O staining revealed a decrease in lipid droplet formation in the NDFX- and CLDM-treated differentiated hamster sebocytes (data not shown). Moreover, the insulin-augmented gene expression of *DGAT-1*, a rate-limiting enzyme of TG synthesis,[28] was found to be suppressed by NDFX and CLDM (Table 1). Thus, these results suggest for the first time that NDFX and CLDM inhibit lipogenesis by transcriptionally suppressing the production of perilipin and DGAT-1 in insulin-differentiated hamster sebocytes.

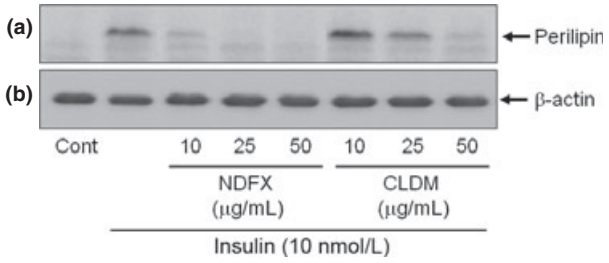


**Figure 2.** Suppression of insulin-augmented perilipin production by NDFX and CLDM in hamster sebocytes. Hamster sebocytes at the third passage were treated every 3 days for 9 days with or without NDFX and CLDM in the presence of insulin (10 nmol/L) as described in Figure 1. The cell lysates were subjected to western blot analysis for perilipin (a) and β-actin (b) as described in the Methods. CLDM, clindamycin; NDFX, nadifloxacin.

**Table 1.** NDFX and CLDM inhibit mRNA expression of *perilipin* and *DGAT-1* in insulin-stimulated hamster sebocytes

| | Relative mRNA expression (% of control) | |
|---|---|---|
| Treatment | *Perilipin* | *DGAT-1* |
| Control | 100.0 | 100.0 |
| Insulin (10 nmol/L) | 162.7 ± 26.7** | 414.0 ± 75.2** |
| Insulin (10 nmol/L) + NDFX (50 μg/mL) | 45.9 ± 3.9### | 138.4 ± 7.2## |
| Insulin (10 nmol/L) + CLDM (50 μg/mL) | 66.8 ± 7.1## | 147.0 ± 8.9## |
| NDFX (50 μg/mL) | 61.7 ± 9.5** | 62.1 ± 13.7* |
| CLDM (50 μg/mL) | 60.1 ± 4.9** | 76.5 ± 8.2 |

*,**Significantly different from untreated cells (control) ($P < 0.05$ and 0.01, respectively). ##,###Significantly different from insulin-treated cells ($P < 0.01$ and 0.001, respectively). CLDM, clindamycin; DGAT-1, diacylglycerol acyltransferase 1; NDFX, nadifloxacin.

### NDFX and CLDM inhibit PGN-augmented TG production in hamster sebocytes

The activation of Toll-like receptor 2 (TLR2) by *P. acnes*-derived factors including PGN has been reported to facilitate inflammatory reactions such as pro-inflammatory cytokine and MMP production in sebaceous glands, pilosebaceous units and immune cells infiltrated into acne lesions.[1–3] However, it is not well understood whether or not PGN stimulates sebum production in sebocytes. As shown in Figure 3(a), PGN was found to dose-dependently augment the production of TG in hamster sebocytes. In addition, the PGN-augmented TG production was inhibited with the addition of NDFX or CLDM (48.5 ± 4.9 and 66.8 ± 16.8%, respectively, vs PGN-treated cells at 50 μg/mL) (Fig. 3b). Furthermore, both antibiotics were found to suppress the gene expression of *DGAT-1* in the PGN-treated hamster sebocytes (Table 2). Thus, these results suggest that NDFX and CLDM exhibit inhibitory activities against DGAT-1-mediated TG synthesis in hamster sebocytes.

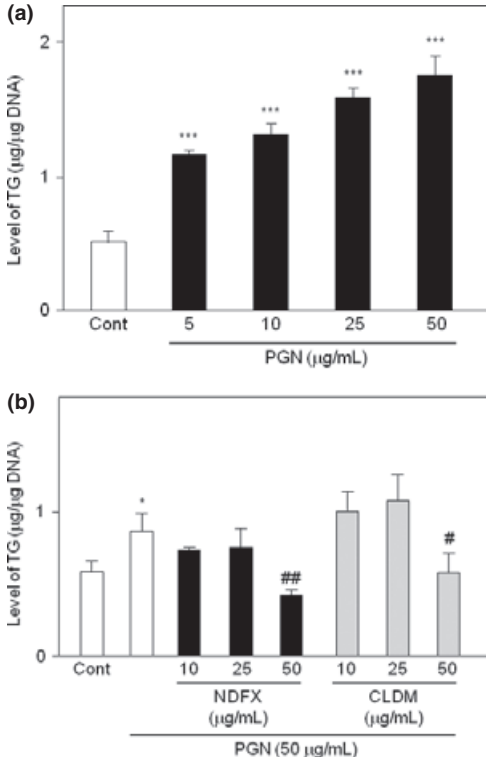


**Figure 3.** Inhibition of PGN-augmented TG production by NDFX and CLDM in hamster sebocytes. Hamster sebocytes at the 3rd passage were treated every 3 days for 9 days with or without PGN (5–50 μg/mL) (a) or with NDFX and CLDM (10–50 μg/mL) in the presence of PGN (50 μg/mL) (b). The intracellular level of TG was measured as described in the Methods. Data are shown as mean ± standard deviation of three dishes. *,***Significantly different from untreated cells (Cont) ($P < 0.05$ and 0.001, respectively). #,##Significantly different from PGN-treated cells ($P < 0.05$ and 0.01, respectively). CLDM, clindamycin; NDFX, nadifloxacin; PGN, peptidoglycan; TG, triacylglycerols.

**Table 2.** Effects of NDFX and CLDM on mRNA expression of *DGAT-1* and *pro-MMP-2* in PGN-stimulated hamster sebocytes

| Treatment | Relative mRNA expression (% of control) | |
|---|---|---|
| | *DGAT-1* | *Pro-MMP-2* |
| Control | 100.0 | 100.0 |
| PGN (10 μg/mL) | 155.4 ± 21.2* | 301.0 ± 26.0*** |
| PGN (10 μg/mL) + NDFX (50 μg/mL) | 114.2 ± 9.9# | 74.9 ± 5.6### |
| PGN (10 μg/mL) + CLDM (50 μg/mL) | 106.6 ± 6.7## | 369.2 ± 65.6 |
| NDFX (50 μg/mL) | 57.2 ± 12.8** | 62.0 ± 10.5* |
| CLDM (50 μg/mL) | 65.3 ± 7.5** | 100.4 ± 25.5 |

*,**,***Significantly different from untreated cells (control) ($P < 0.05$, 0.01, and 0.001, respectively). #,##,###Significantly different from PGN-treated cells ($P < 0.05$, 0.01, and 0.001, respectively). CLDM, clindamycin; DGAT-1, diacylglycerol acyltransferase 1; MMP, matrix metalloproteinase; NDFX, nadifloxacin; PGN, peptidoglycan.

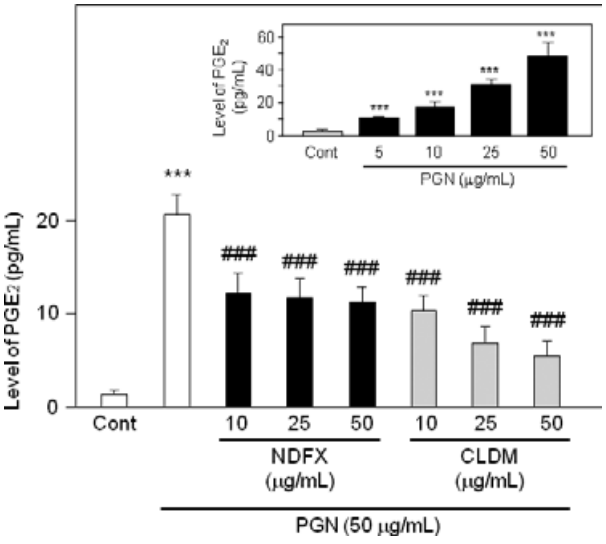


**Figure 4.** NDFX and CLDM inhibit PGN-augmented production of $PGE_2$ in hamster sebocytes. Hamster sebocytes at the third passagewere treated for 24 h with or without NDFX and CLDM (10–50 μg/mL) in the presence of PGN (50 μg/mL). The harvested culture media were subjected to measurement of $PGE_2$ as described in the Methods. Inset indicates that PGN dose-dependently augmented $PGE_2$ production in hamster sebocytes. Data are shown as mean ± standard deviation of four wells. ***Significantly different from untreated cells (Cont) ($P < 0.001$). ###Significantly different from PGN-treated cells ($P < 0.001$). CLDM, clindamycin; NDFX, nadifloxacin; $PGE_2$, prostaglandin $E_2$; PGN, peptidoglycan.

### Anti-inflammatory activities of NDFX and CLDM in PGN-treated hamster sebocytes

The augmented production of PG, particularly $PGE_2$, has been reported to initiate and/or aggravate inflammatory acne.[29–31] Therefore, to evaluate the anti-inflammatory activities of NDFX and CLDM

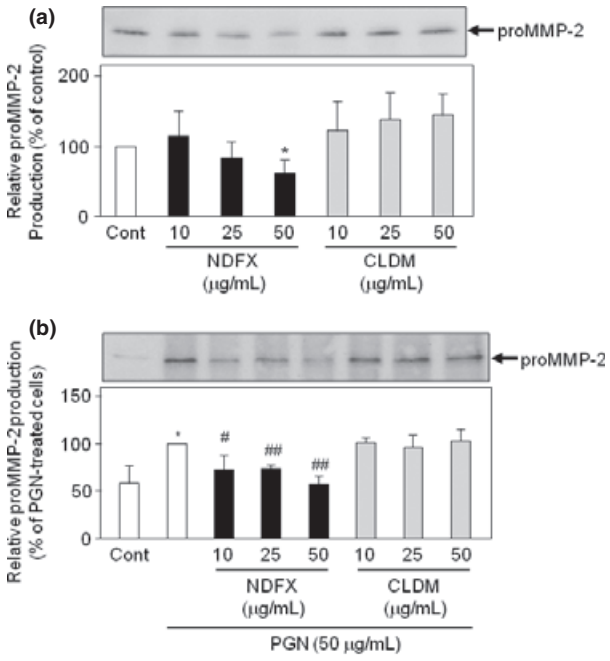


**Figure 5.** NDFX, but not CLDM, decreases the production of pro-MMP-2 in hamster sebocytes. The harvested culture media as shown in Figure 4 were subjected to western blot analysis for pro-MMP-2 as described in the Methods. The relative amounts of pro-MMP-2 were quantified by densitometric scanning and expressed by taking untreated (a) and PGN-treated cells (b) as 100%. Data are shown as mean ± standard deviation of four independent experiments. *Significantly different from untreated cells (Cont) ($P < 0.05$). #,##Significantly different from PGN-treated cells ($P < 0.05$ and 0.01, respectively). CLDM, clindamycin; MMP, matrix metalloproteinase; NDFX, nadifloxacin; PGN, peptidoglycan.

against inflamed sebaceous glands, we examined the effects of both antibiotics on the production of $PGE_2$ in PGN-treated hamster sebocytes. As shown in Figure 4, the production of $PGE_2$ was dose-dependently augmented by PGN in hamster sebocytes (inset). In addition, the augmented $PGE_2$ production was decreased by administrating NDFX or CLDM to the cells (Fig. 4). However, there was no change in hamster sebocytes treated with NDFX or CLDM alone (data not shown). On the other hand, we demonstrated that the constitutive production of pro-MMP-2 was dose-dependently decreased by NDFX (62.2 ± 19.0% at 50 μg/mL) (Fig. 5a). In addition, PGN-augmented pro-MMP-2 production was suppressed by NDFX in a dose-dependent manner (57.7 ± 7.8% at 50 μg/mL). Furthermore, the inhibition of *pro-MMP-2* mRNA expression by NDFX was observed in the PGN-treated and untreated hamster sebocytes (Table 2). However, there were no changes in either the protein or mRNA levels of pro-MMP-2 in the CLDM-treated hamster sebocytes in the presence or absence of PGN (Fig. 5 and Table 2). These results suggest that NDFX exhibits anti-inhibitory activities against PGN-augmented $PGE_2$ and pro-MMP-2 production, whereas CLDM is effective for decreasing PGN-induced $PGE_2$ production in sebocytes.

## DISCUSSION

Acne is a common skin disorder characterized by non-inflammatory (e.g. comedones) and inflammatory lesions (e.g. papules, pustules and nodules).[1–3] In the former, comedogenesis has been reported to result from an abnormality in ductal keratinocyte proliferation and differentiation,[32] which is closely associated with the disorder of sebaceous lipid metabolisms by the activation of insulin/insulin growth factor-1 signalings.[26,33] In the present study, we found that NDFX and CLDM inhibited the constitutive and insulin-augmented biosynthesis of TG by decreasing DGAT-1 expression in hamster sebocytes. In addition, both antibiotics were found to suppress the insulin-induced production and gene expression of perilipin, which is involved in lipid-droplet formation in sebocytes as well as adipocytes.[25] We have also confirmed that NDFX and CLDM inhibit lipid-droplet formation in insulin-treated sebocytes in hamsters and humans (data not shown). Therefore, these results provide novel evidence that NDFX and CLDM exhibit anti-lipogenic activity in sebaceous glands under non-inflammatory acne conditions (e.g. comedone formation).

It has been reported that steroidogenesis locally exists in sebaceous glands and its activation causes the augmentation of sebum production leading to acne development.[2,3] We previously reported that 5α-dihydrotestosterone (5α-DHT) augments lipid-droplet formation by increasing the production of TG and perilipin in hamster sebocytes.[22,25] In addition, we have preliminarily confirmed the constitutive gene expression of *5α-reductase type-1* and *-3*, and production of testosterone and 5α-DHT in hamster sebocytes (T. Sato *et al.*, unpubl. data, 2011). Furthermore, Inui *et al.*[34] have reported that NDFX inhibits the transcriptional activity of androgen receptor in CV-1 monkey kidney cells. Although the molecular mechanisms of NDFX- and CLDM-mediated anti-lipogenic activities remain to be elucidated, we hypothesize that the anti-androgenic effect of NDFX is at least partially involved in the suppression of TG and perilipin production in hamster sebocytes.

We previously reported that *P. acnes*-derived factor(s) facilitate sebum production by increasing DGAT-1-dependent TG synthesis in hamster sebocytes *in vivo* and *in vitro*.[15] In the present study, we demonstrated that PGN from *Staphylococcus aureus* directly augmented TG production in hamster sebocytes. Regarding the usage of the PGN, Romics *et al*.[35] reported that PGN from *S. aureus* as well as heat-killed *P. acnes* augments nuclear factor-κB activation and IL-8 production in Chinese hamster ovary cells and human keratinocytes overexpressed human TLR-2, respectively. However, there is no change in IL-6 production in peritoneal macrophages from TLR-2-knockout mice.[35] Therefore, PGN from *S. aureus* is likely to mimic *P. acnes*-dependent cellular responsiveness in acne lesions *in vitro.* In our *in vitro* acne-like model, moreover, we demonstrated that both NDFX and CLDM decreased PGN-augmented TG production. Thus, our findings strongly suggest that NDFX and CLDM exhibit anti-lipogenic actions toward *P. acnes*-stimulated sebaceous glands in acne lesions.

Some antibiotics including NDFX and CLDM have been reported to show not only antimicrobial but also anti-inflammatory activities in acne lesions.[17–21] Kuwahara *et al.*[20] reported the inhibition of γ-interferon, IL-1β and IL-12p70 production by NDFX or CLDM in heat-killed *P. acnes*-stimulated human peripheral blood

mononuclear cells. In addition, it has been reported that NDFX decreases the generation of reactive oxygen species and cell migration in keratinocytes and neutrophils.[21] In the present study, we found that PGN dose-dependently augmented $PGE_2$ production and both NDFX and CLDM inhibited the augmented production of $PGE_2$ in hamster sebocytes. As an inflammatory mediator, $PGE_2$, has been reported to increase in sebaceous glands in mild and moderate acne patients,[29] PGN from *P. acnes* is likely to be a direct causal factor that induces $PGE_2$ production leading to the exacerbation of acne lesions.[1–3] Moreover, we hypothesize that NDFX and CLDM exert anti-inflammatory activities due to the suppression of $PGE_2$ production in sebaceous glands in acne patients. As we previously reported that the production of cyclooxygenase 2/prostaglandin endoperoxide H synthase-2 is augmented by the activation of TLR-4 in hamster sebocytes,[15] further experiments will be needed to clarify the molecular mechanisms of the downregulation of sebaceous $PGE_2$ production by PGN, NDFX and/or CLDM.

Exacerbation of the inflammation in acne has been reported to cause the destruction of the integrity in sebaceous glands and pilosebaceous units, resulting in the formation of intractable acne scars.[3] In addition, scar formation has been reported to be associated with aberrant ECM production and degradation.[5] Regarding ECM degradation, MMP play crucial roles in abnormal ECM remodeling in various diseases, for example, scleroderma, keloid formation, and tumor development and metastasis.[36,37] Papakonstantinou *et al.*[6] reported the existence of MMP-9 and MMP-13/collagenase 3 in sebum obtained from acne patients. In addition, Trivedi *et al.*[7] reported the upregulation of *MMP-1* mRNA expression and production in the skin from acne patients. Furthermore, we reported that PGN, as well as *P. acnes*-derived factor(s), transcriptionally augment pro-MMP-2 production in hamster sebocytes,[16] suggesting that sebocytes play important roles for sebaceous and pilosebaceous ECM degradation in acne by increasing production of different sets of MMP in concert with keratinocytes, dermal fibroblasts and infiltrated immune cells.[6,7,9] In the present study, we have provided novel evidence that NDFX, but not CLDM, inhibits constitutive and PGN-augmented gene expressions and production of pro-MMP-2 in hamster sebocytes. Because MMP-2 has a higher substrate specificity to type IV collagen, a structural element of the basement membrane,[38] of which destruction between the epithelium and dermis subsequently may lead to dermal ECM degradation for acne scarring,[5] our findings allow us to hypothesize that the topical application of NDFX is effective for preventing the onset of acne scaring via the suppression of sebaceous pro-MMP-2 expression. Our hypothesis of antibiotic-mediated anti-scarring is supported by the report of Choi *et al.*[9] that the tetracycline antibiotic, doxycycline, decreases *P. acnes*- and TNF-$\alpha$-augmented pro-MMP-2 production in human dermal fibroblasts.

In conclusion, we have demonstrated that NDFX and CLDM inhibit the production of TG and $PGE_2$ in insulin- or PGN-stimulated hamster sebocytes. Furthermore, NDFX, but not CLDM, suppresses the constitutive and PGN-augmented expression of pro-MMP-2 in hamster sebocytes. Therefore, these results provide novel evidence that NDFX and CLDM exhibit anti-lipogenesis and -inflammatory activities against insulin- or PGN-activated sebocytes that at least partly mimic acne pathology *in vitro*. Moreover, NDFX for acne therapy is likely to be effective in not only inhibiting microbial proliferation but also in preventing the onset of acne scar formation.

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