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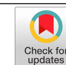

The Effect of FK 506 on the Reepithelialization of Superficial Skin Wound

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Dear Editor:

It has been well known that FK 506 could decrease the atopic inflammation through controlling T-cells and their cytokines[1]. However, the effect of FK 506 on wounds has scarcely been investigated although FK 506 has been widely used in treating moderate to severe atopic dermatitis, where various minor wound occurs because of scratch owing to itchy. Therefore, we investigated the effect of FK 506 on wound healing with an *in vitro* study of outer root sheath keratinocytes (ORSKs) because ORSK is a key factor in cutaneous wound healing[2].

Human ORSKs were prepared using a method described previously with minor modifications[3]. We used the Ez-cytox® assay kit (ITSBIO, Seoul, Korea) to measure the cell proliferation. Cultured ORSKs were incubated for 4 days in 96-well microtiter plates in a final volume of 100 μl/well culture medium and various concentrations of FK 506. And then tetrazolium salt (10 μl) was added to each well and the absorbance was checked using a microtiter plate reader at 420～480 nm. For migration assay, cell monolayer in culture dish divided by scratching with a p200 pipet tip. We added 10^4 nM FK 506 to the medium and created 10 different markings to be used as reference points. An initial photograph was taken, and additional images were obtained after 48 hours incubation. The width of the gap was measured using the TOMORO ver. 3.5 Scope Eye image analyzer program (Techsan Digital Imaging, Seoul, Korea). Cytokeratins 6, 16, and 17, as well as transforming growth factor-beta 1, 2 (TGF-β1, 2) have an important role in proliferation, and migration of keratinocytes. We assayed the cytokines using reverse transcription-polymerase chain reaction after treatment of FK 506. Data were evaluated using Student's t-test. ORSK proliferation in FK 506-treated groups (10^2 and 10^4 nM) increased to 103.7%±3.8% and 110%±9.0% of the control group (100%), respectively. It was not significant statistically in eight independent experiments (Fig. 1A). ORSK migration increased significantly in response to FK 506 ($p<0.05$). ORSKs treated with FK 506 occupied up to 75.8%±5.5% of the initial gap, whereas the control cells were about 9.4%±1.6% in three independent experiments (Fig. 1B).

The TGF-β1 expression increased 48 hours after treatment with 10^4 nM FK 506 (Fig. 2A). Cytokeratin 6, 16, and 17 mRNA levels did not change in any of the FK 506-treated groups (Fig. 2B).

10～10^4 nM FK 506 did not inhibit proliferation of normal human epidermal keratinocytes (NHEK)[4,5]. In the present study, FK 506-treated ORSK proliferation was not significant statistically. Cytokeratin 6, 16, and 17 are highly expressed in the hyperproliferative epidermis in wound healing[6]. However their levels did not increase in FK 506-treated ORSKs, which was consistent with the finding that cell proliferation did not increase in our study. Thus, it appears that FK 506 had no effect on ORSK or NHEK proliferation.

ORSK migration increased significantly in response to 10^4 nM FK 506. Keratinocyte migration, rather than pro-

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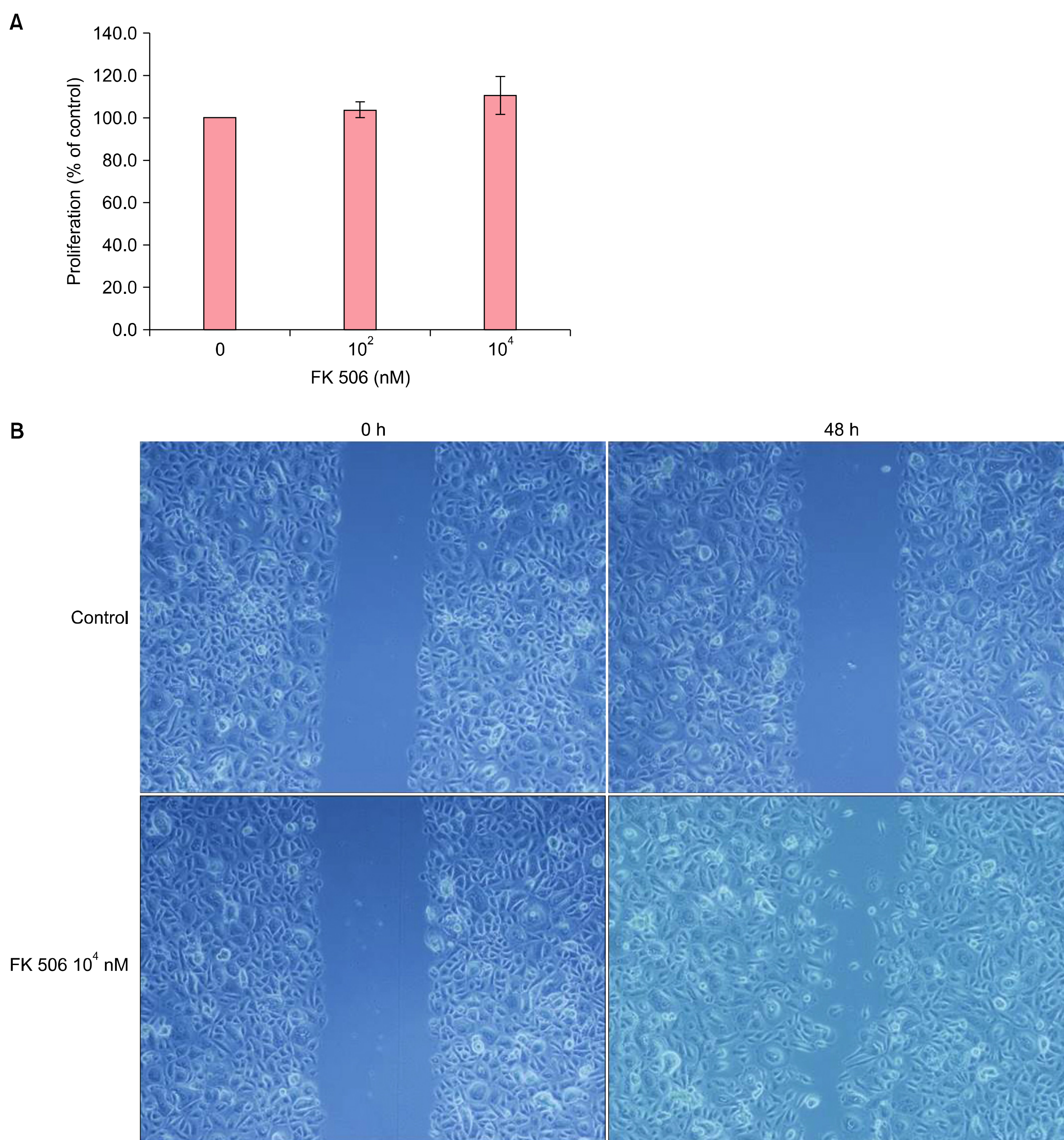

Fig. 1. (A) Proliferation assay for tacrolimus (FK 506)-treated human outer root sheath keratinocytes (ORSKs) using Ez-cytox®. A dose dependent increase was detected but it was not statistically significant. Results are shown as mean ± standard deviation (SD) of eight independent experiments. (B) ORSKs treated with FK 506 occupied up to 75.8% ± 5.5% of the initial gap, whereas the control cells were about 9.4% ± 1.6% (p<0.05). Results are shown as mean ± SD of three independent experiments.

liferation, plays a major role during re-epithelialization of epidermal defects[7]. In this study, TGF-β1 was detected 48 hours after treating the ORSKs with 10^4 nM FK 506. TGF-β1 plays a critical role in keratinocyte migration[7,8]. Thus, the increase in ORSK migration in our study may be partially due to TGF-β1 expression. The cytoskeleton generally provides the strength for keratinocytes move and lamellopodial crawling during re-epithelialization. In particular, cytokeratin 6, 16, and 17 were induced in ORSKs at the wound's edge[8]. However, cytokeratin 6, 16, and 17 expression did not change in FK 506-treated ORSKs.

In conclusion, we showed that FK 506 did not inhibit

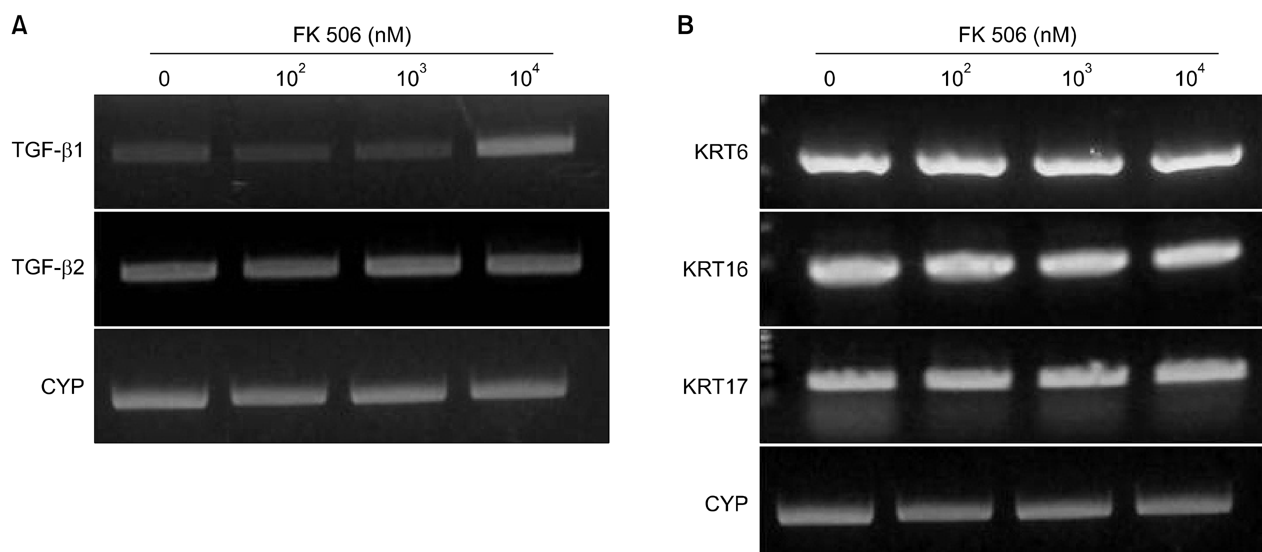

Fig. 2. (A) Transforming growth factor-beta (TGF-β1) was highly expressed 48 hours after treatment of human outer root sheath keratinocytes (ORSKs) with 10^4 nM tacrolimus (FK 506), whereas no change was detected in the control, 10^2 or 10^3 nM FK 506-treated groups. (B) No change in cytokeratin 6, 16, and 17 mRNA expression after ORSKs were treated with FK 506. CYP: cyclophilin A, KRT: cytokeratin.

ORSK proliferation but increased ORSK migration, which could have been partially due to TGF-β1 expression.

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CONFLICTS OF INTEREST

The authors have nothing to disclose.

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