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LETTER TO THE EDITOR

FK506 inhibits the enhancing effects of TGF-β on wound healing in a rabbit dermal ulcer model

KEYWORDS
FK-506; TGF-β; Fibroblasts; Wound healing

To the Editor,

The wound healing process consists of inflammation, granulation tissue formation, and wound contraction followed by re-epithelialization. Transforming growth factor (TGF)-β is an important cytokine that affects the process of wound healing at various phases [1]. For instance, the injection of TGF-β directly to the wound at the time of wounding increased the healing rate accompanied by an increased influx of mononuclear cells and fibroblasts and by marked increases in collagen deposition at the site of application of TGF-β in rats [2].

FK506 is an immunosuppressive macrolide that binds to FKBP12 [3]. The FK506/FKBP12 complex recruits and thereby inactivates the serine/threonine phosphatase calcineurin, resulting in the blockade of the signaling pathway mediated by

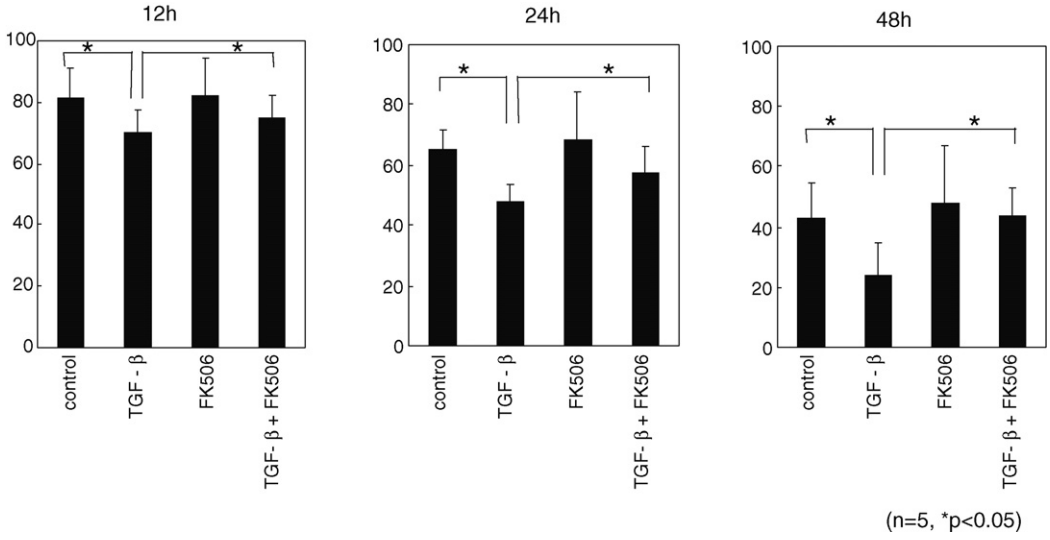

Fig. 1 Effects of FK506 on *in vitro* wound healing model. A mechanical wound defect was created in a cell monolayer on reaching confluence, and treated with 10 ng/ml recombinant human TGF-β1 (R&D Systems, Minneapolis, MN, USA) in the presence or absence of 0.2 nM FK-506 (CALBIOCHEM, Darmstadt, Germany). The images of cell migration in the leading edge of the wound to fill the wound space were compared. The results were expressed as a rate of un-repopulated area at 12, 24, and 48 h after wounding. The rate of fibroblast migration into the wound defect was enhanced by the presence of TGF-β1, which was suppressed by FK-506. Data are summarized as mean ± S.D. The statistical analysis of the results was performed by using an unpaired Student's *t*-test. $p < 0.05$ was considered to be significant.

Abbreviations: TGF-β, transforming growth factor-β; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum α-SMA, α-smooth muscle actin; VEGF, vascular endothelial growth factor

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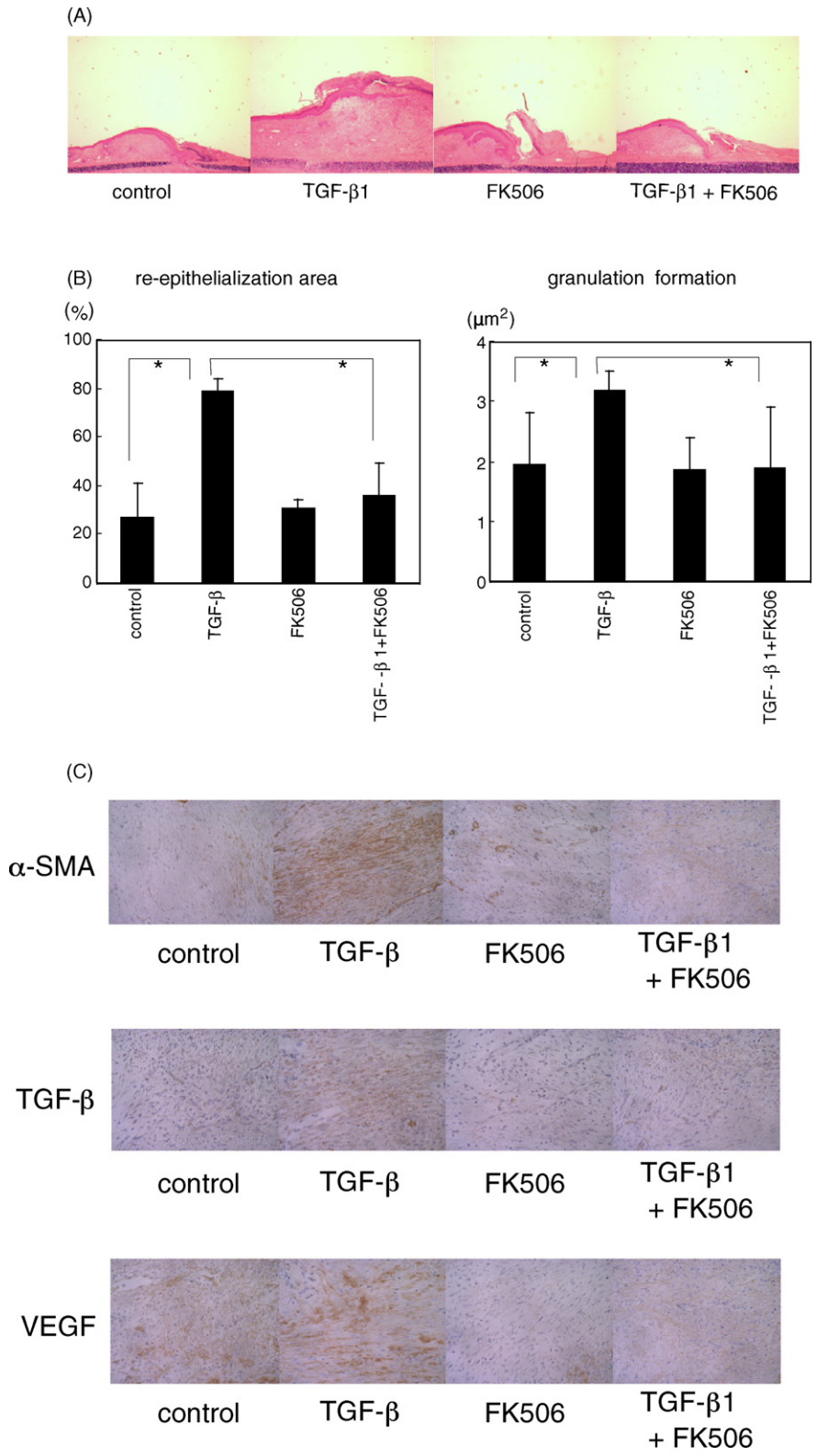

Fig. 2 Effects of FK506 on wound healing in a rabbit dermal ulcer model. Four full-thickness round wounds were prepared on the injected area using a calibrated 6 mm trephine (Acu Punch, Acuderm Inc., Lauderdale, Florida) under sterile conditions (day 1). The perichondrium was kept undamaged. The wounds were covered with a sterilized transparent dressing (Tegaderm, 3 M, Tokyo, Japan), and 20 μl PBS or 20 μl recombinant human TGF-1 (R&D, Minnesota) solution (25 ng/μl: 500 ng/one ulcer) was applied to each wound using a syringe once every other day until the fifth day (on days 1, 3, and 5; total 1.5 μg/one ulcer) with or without 20 μl FK506 solution (2.5 ng/μl: 50 ng/one ulcer on days 1, 3,

calcineurin [3]. FKBP12 has been also shown to bind to TGF-β type I receptor and inhibits its signaling function [4]. FK506 prevents the binding of FKBP12 to TGF-β type I receptor and affects TGF-β signaling [4].

Based on these backgrounds, we investigated whether FK506 affects the enhanced effects of TGF-β on wound healing by using both *in vitro* and *in vivo* wound healing model. Normal dermal fibroblasts were prepared from fresh skin tissues obtained at the time of surgical excision after informed consent as previously described [5]. A small scrape wound was made across a monolayer of the cultured human dermal fibroblasts in the presence or absence of 10 ng/ml TGF-β1 and/or 0.2 nM FK506 to examine the effect of FK506 on the wound-healing process *in vitro*. Our preliminary experiments showed that pretreatment with 0.4 mg/ml mitomycin C, which inhibits cell proliferation, did not inhibit the TGF-β-enhanced wound closure (data not shown), suggesting that this assay is primarily dependent on fibroblast migration, not proliferation. As shown in Fig. 1, human fibroblasts cultured in the presence of TGF-β1 promoted wound closure, which was inhibited by the addition of FK506. Thus, FK506 inhibited the enhanced effects of TGF-β on wound closure *in vitro*.

We next examined the effect of FK506 on tissue repair after full-thickness wounding on rabbit ear as previously reported by us [6]. Four full-thickness round wounds were made on the inner side of each ear using a punch-biopsy instrument (6 mm diameter) in rabbits. The wounds were covered with a sterilized transparent dressing and TGF-β and/or FK506 applied to each ear. The ear was bandaged and kept covered throughout the experiments. The rabbits were sacrificed on the seventh day after the wounding and then the amount of granulation tissue area and the degree of re-epithelialization were evaluated as previously described [6]. Consistent with the *in vitro* findings (Fig. 1), TGF-β1 enhanced the granulation formation and re-epithelialization in the rabbit ear, both of which were blocked by the addition of FK506 (Fig. 2A and B).

Because histological evaluation revealed that FK506 inhibited the accelerated effects of TGF-β on tissue repair in a rabbit ulcer model, we then examined the effects of FK506 on cellular and molecular events associated with wound healing. Again, FK506 inhibited the enhanced effects of TGF-β on the number of α-smooth muscle (α-SMA) positive-cells (myofibroblasts) and expression of TGF-β1 and VEGF in the wounded skin (Fig. 2C). We found that FK506 (0.02—2 nM) did not affect the viability of both keratinocytes and fibroblasts *in vitro* (data not shown).

In summary, we showed that FK506 inhibited the enhanced effects of TGF-β on wound healing both *in vitro* and *in vivo*. Our results thus suggest a novel role of FK-506 and this drug may be useful for the treatment of skin diseases associated with excessive expression or activity of TGF-β such as keloid. Rao et al. previously reported that FK506 rather enhanced TGF-β expression in a rat liver transplanted model [7], which may be opposite to the current findings. Responses to FK506 may therefore differ in various organs or depend on experimental conditions. Further studies are needed to investigate this issue.

Acknowledgments

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and 5; total 250 ng/one ulcer) (Asteras, Tokyo, Japan). The ear was bandaged and kept covered throughout the experiment. The rabbits were killed on the seventh day after the wounding by intravenous administration of pentobarbital solution. The wounded areas were excised and fixed in 10% buffered formalin solution. All animal experiments were performed according to the approved manual of the Institutional Review Board of Juntendo University. The section with the widest original wound margin was used for assessment. (A) A histological cross-section stained with haematoxyline and eosin. (B) The parameters measured were degree of re-epithelialization, area of granulation tissue. The wounds treated with TGF-β1, had significant stimulatory effects on granulation tissue formation and re-epithelialization. FK-506 suppressed both TGF-β-induced granulation formation and re-epithelialization. (C) Immunohistochemical findings of granulation tissue stained for α-SMA, TGF-β1 and VEGF. α-SMA, TGF-β1 and VEGF were enhanced with TGF-β1 treatment, which were efficiently suppressed by FK-506.

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Toshio Hasegawa[a,b,*]
Koji Sumiyoshi[a,b]
[a]*Department of Dermatology, Juntendo University School of Medicine, Tokyo 113-8421, Japan*
[b]*Atopy (Allergy) Research Center, Juntendo University School of Medicine, Tokyo 113-8421, Japan*

*Corresponding author at:
Department of Dermatology, Juntendo University School of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo 113-8421, Japan.
Tel.: +81 3 5802 1226; fax: +81 3 3813 9443
E-mail address: t-hase@med.juntendo.ac.jp
(T. Hasegawa)

Hitoshi Tsuchihashi
Shigaku Ikeda
Department of Dermatology, Juntendo University School of Medicine, Tokyo 113-8421, Japan

Atsuhito Nakao[a,b,*]
[a]*Atopy (Allergy) Research Center, Juntendo University School of Medicine, Tokyo 113-8421, Japan*
[b]*Department of Immunology, Faculty of Medicine, University of Yamanashi, Yamanashi 409-3898, Japan*

Hideoki Ogawa[a,b]
[b]*Department of Dermatology, Juntendo University School of Medicine, Tokyo 113-8421, Japan*
[b]*Atopy (Allergy) Research Center, Juntendo University School of Medicine, Tokyo 113-8421, Japan*

*Corresponding author at:
Department of Immunology, Faculty of Medicine, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi 409-3898, Japan
E-mail address: anakao@yamanashi.ac.jp
(A. Nakao)

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