Effect of 14-Membered-Ring Macrolides on Production of Interleukin-8 Mediated by Protease-Activated Receptor 2 in Human Keratinocytes

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Received 31 January 2007/Returned for modification 10 April 2007/Accepted 10 January 2008

The production of interleukin-8 induced by the activation of protease-activated receptor 2 and its synergism with interleukin-1β were modulated by 14-membered-ring macrolides, namely, roxithromycin, erythromycin, and clarithromycin, in cultured normal human epidermal keratinocytes. Those macrolides may attenuate the protease-activated receptor 2–interleukin-8 axis and thereby modulate proinflammatory responses in the skin.

Besides their antibiotic actions, 14-membered-ring macrolides have immunomodulatory activities (24) that are beneficial for patients with inflammatory skin disorders (10, 11, 23) or with chronic inflammatory airway diseases (12, 15), possibly due to their inhibitory actions on neutrophils and macrophages (15) and/or on inflammatory cytokines from regional epithelial cells (4, 21). Recent findings have suggested that protease-activated receptor 2 (PAR2) is involved in various aspects of skin inflammation, such as allergic and toxic contact dermatitis (18), via the up-regulation of intercellular cell adhesion molecule 1 expression (2) and interleukin-8 (IL-8) in keratinocytes (6); therefore, PAR2 might play a pivotal role in modulating the inflammatory processes of the skin. Here we show that 14-membered-ring macrolides suppress the keratinocyte PAR2–IL-8 axis, which might be a therapeutic target for the control of cutaneous inflammation.

Normal human epidermal keratinocytes (NHEK) plated at a density of 1.0 × 10⁴ cells per cm² were incubated for 72 h, after

FIG. 1. (A) Effect of a PAR2-specific siRNA on the expression of the PAR2 gene in NHEK. The indicated concentration of the PAR2-specific siRNA was transfected into NHEK. The expression of the PAR2 gene was inhibited by the PAR2-specific siRNA in a dose-responsive manner. (B) Effect of the PAR2-specific siRNA on the production of IL-8 induced by the agonist peptide SLIGKV-NH₂ in NHEK. The SLIGKV-NH₂-induced production of IL-8 was completely suppressed by 100 nM PAR2-specific siRNA. An analysis of variance followed by Scheffe’s test was performed for the statistical analysis of data. *, P < 0.001 (versus the control).

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³ Published ahead of print on 22 January 2008.
which they were transfected with a \textit{PAR2}-specific small interfering RNA (siRNA) (Qiagen, Inc., Hilden, Germany). After 24 h of incubation, the cells were harvested for analysis by real-time quantitative reverse transcription-PCR. An analysis of variance followed by Scheffe’s test was performed for the statistical analysis of data. *, \( P < 0.001; **, P < 0.01; ***, P < 0.05; \text{ns}, \text{not significant.} \)

**FIG. 2.** Effect of the 14-membered-ring macrolide RXM (A), EM (B), or CAM (C) on the SLIGKV-NH\(_2\)-induced production of IL-8 in NHEK. Empty bars, control; filled bars, SLIGKV-NH\(_2\)-treated cells. An analysis of variance followed by Scheffe’s test was performed for the statistical analysis of data. *, \( P < 0.001; **, P < 0.01; ***, P < 0.05; \text{ns}, \text{not significant.} \)

In NHEK transfected with the \textit{PAR2}-specific siRNA, \textit{PAR2} transcript levels were decreased in parallel with the transfected amounts of siRNA (Fig. 1A). At 100 nM siRNA, \textit{PAR2} transcripts were as little as about 25% of the control, and the SLIGKV-NH\(_2\) induction of IL-8 was decreased to the control level (Fig. 1B). This indicated that the increase of IL-8 elicited by SLIGKV-NH\(_2\) depends on the expression of the \textit{PAR2} gene in NHEK. When NHEK were treated with 14-membered-ring macrolides, the SLIGKV-NH\(_2\)-induced production of IL-8 was decreased by roxithromycin (RXM) at 10 and 100 \( \mu \text{M} \) or by erythromycin (EM) at 5, 10, and 100 \( \mu \text{M} \) (Fig. 2A and B). However, clarithromycin (CAM) showed weaker effects than RXM or EM at concentrations up to 100 \( \mu \text{M} \) (Fig. 2C). In contrast, 16-membered-ring macrolides (spiramycin, oleandomycin, and josamycin) did not suppress the SLIGKV-NH\(_2\)-induced production of IL-8 (data not shown). When NHEK were treated with 10 or 100 \( \mu \text{M} \) RXM, EM, or CAM, those drugs did not decrease the cell viability as assessed with a colorimetric assay kit (Fig. 2D) or the levels of \textit{PAR2} transcripts (data not shown). Therefore, it is unlikely that the effects of 14-membered-ring macrolides on the SLIGKV-NH\(_2\)-induced production of IL-8 are due simply to their cytotoxic effects or to the down-regulation of the \textit{PAR2} gene. As shown
in Fig. 3, the treatment of NHEK with both IL-1β and SLIGKV-NH₂ synergistically increased IL-8 levels. In contrast, IL-18, a cytokine in the IL-1 family, did not show such activities. The control peptide VKGILS-NH₂ did not significantly increase the production of IL-8, regardless of the presence of IL-1β. In NHEK treated with both IL-1β and SLIGKV-NH₂, 10 μM RXM or EM reduced the synergistic increase of IL-8 production (Fig. 4A and B). CAM also significantly decreased the synergistic effect but was less effective than RXM or EM (Fig. 4C).

For several cases of inflammatory skin disorders, eruptions and/or symptoms are improved by treatment with macrolides. Especially in psoriasis vulgaris, the activation of PAR2 may contribute to the pathological condition of the disease, in which IL-8 recruits neutrophils into the epidermis to form microabscesses (16). The activation mechanism of PAR2 in the psoriatic epidermis is unknown, but it is possible that serine proteinases released from keratinocytes or from infiltrating inflammatory cells could activate PAR2 in the epidermis (7). It is interesting that itchy sensations are well controlled by RXM, but not by CAM, for patients with psoriasis vulgaris (personal communication from Kunihiko Tamaki, Tokyo University Graduate School of Medicine). The clinical findings of Tamaki et al. correlate with our in vitro results that CAM is not as effective as RXM or EM for inducing the production of IL-8 in NHEK, although the reason for that is unknown. The biological effects of those macrolides seem not to necessarily be coordinated as has been shown for other types of cells (14, 17).

In a case with chronic prurigo nodularis, the combination of RXM with tranilast was effective (5). That case implies that the action of RXM is not so strong that it completely reduces the disease activity. When therapeutic doses of those macrolides are taken, their peak concentrations in human skin range from 0.5 to 30 μM (1, 3, 22). The mild anti-inflammatory effect of macrolides on those skin diseases seems to be reflected in our results; the suppressive effect of RXM or EM on IL-8 remains 25 to 30% at pharmacological concentrations ranging up to 10 μM. The effect of those macrolides on the PAR2 system in other types of cells has not been tested, but we speculate that RXM and EM might show similar effects on the PAR2 system, at least in some epithelial cells, because the release of IL-8 from tracheal or nasal epithelial cells is decreased by the administration of those macrolides (8, 19).

As shown in this study and in a previous report (7), the combination of IL-1β with a PAR2 agonist, SLIGKV-NH₂, shows a synergistic induction of IL-8 in NHEK. Possibly, this synergistic effect boosts inflammation in the epidermis in vivo. RXM, EM, and CAM significantly decrease the synergistic

![FIG. 3. Effect of IL-1β or IL-18 on the SLIGKV-NH₂-induced production of IL-8 in NHEK. A synergistic increase in IL-8 production was induced by the combination of SLIGKV-NH₂ with IL-1β but not with IL-18. No significant changes were observed in the presence of the control peptide VKGILS-NH₂. Empty bars, control; filled bars, SLIGKV-NH₂-treated cells; gray bars, VKGILS-NH₂-treated cells. Student’s two-tailed t test was performed for the statistical analysis of data. ns, not significant.](http://aac.asm.org/)

![FIG. 4. Effect of macrolides on the synergistic production of IL-8 induced by both IL-1β and SLIGKV-NH₂ in NHEK. The synergistic induction of IL-8 was suppressed by 10 μM RXM (A), EM (B), or CAM (C). Empty bars, control; filled bars, SLIGKV-NH₂-treated cells. Student’s two-tailed t test was performed for the statistical analysis of data. *, P < 0.005; **, P < 0.05.](http://aac.asm.org/)
induction of IL-8. It has not been examined whether signals elicited by the activation of PAR2 cross talk with those originating from IL-1β in keratinocytes, but at least some signals might activate nuclear factor κB (2, 13), which is a possible target of RXM as well as p38 and AP-1 in keratinocytes (9, 20). Hence, it is likely that signals from PAR2 might be mediated by those signaling molecules and transcription factors.

RXM was kindly provided by Eisai Pharmaceutical Co., Ltd., and CAM was from Taisho Toyama Pharmaceutical Co., Ltd. (Tokyo, Japan). We thank Shen-Chun Shen and members of the Joint-Use Research Facilities of the Hyogo College of Medicine for their technical assistance. This work was partially supported by a Ministry of Education, Science, Sports, and Culture Grant-in-Aid for Scientific Research and for Young Scientists; by a High-Tech Research Center grant; by a Grant-in-Aid for Scientific Research and for Research Facilities of the Hyogo College of Medicine (TARC); and by a Grant-in-Aid from the Japan Foundation for Applied Enzymology; and by a Grant-in-Aid for Researchers, Hyogo College of Medicine.

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