The Effects of Roxithromycin and Minocycline on Melanin Synthesis

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Introduction

Both roxithromycin and minocycline have been used to treat acne vulgaris. However, minocycline occasionally causes cutaneous hyperpigmentation. We examined the effects of roxithromycin and minocycline on melanogenesis in cultured human melanoma cells and keratinocytes.

Materials and Methods

Roxithromycin and minocycline at doses of 10 and 30 μg/mL were added for 5 days to cultured HM3KO human melanoma cells. Cell number, melanin content, the activities of the enzymes tyrosine hydroxylase and DOPAchrome tautomerase, and the amounts of tyrosinase and of tyrosinase-related protein (TRP) -1 and -2 were examined. Roxithromycin and minocycline were added to cultured human keratinocytes at doses of 10 μg/mL for 0.5 and 24 hours, and then ultraviolet B (UVB) irradiation was applied at 25 mJ/cm^2. After another 15 hours of incubation, RNA was extracted, and the reverse-transcriptase polymerase chain reaction was performed to evaluate the messenger (m) RNA expression of pro-opiomelanocortin (POMC) and endothelin (ET) 1.

Results

Roxithromycin inhibited cell growth 43.6% at a dose of 30 μg/mL but did not significantly inhibit cell growth at a dose of 10 μg/mL. Minocycline at doses of 10 and 30 μg/mL inhibited cell growth by 25.5% and 51.8%, respectively. Roxithromycin inhibited melanin synthesis; in contrast, minocycline increased melanin synthesis dose-dependently.

Roxithromycin at a dose of 30 μg/mL significantly inhibited tyrosine hydroxylase activity; however, minocycline at a dose of 30 μg/m increased tyrosine hydroxylase activity. An *in vitro* tyrosinase assay using human crude tyrosinase extracted from the melanosome fraction of HM3KO cells showed that neither roxithromycin nor minocycline directly inbibited tyrosinase. Furthermore, DOPAchrome tautomerase activity was not affected by roxithromycin or minocycline at any dose.

Western blotting showed that roxithromycin did not induce any significant changes in the expression of tyrosinase, TRP-1, or TRP-2. Minocycline increased the expression of glycosylated tyrosinase but did not affect the expression of TRP-1 or TRP-2.

The addition of roxythromycin to keratinocytes did not affect the mRNA expression of POMC or ET-1. However, minocycline increased the mRNA expression of POMC but not that of ET-1. UVB irradiation increased POMC expression, which was also synergistically increased by minocycline.

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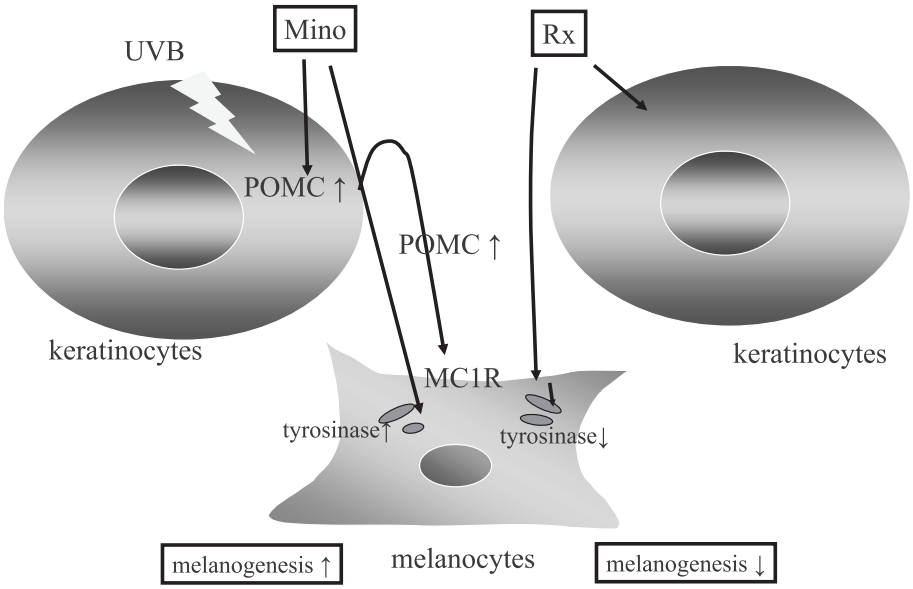

Fig. 1. Roxythromycin inhibits tyrosinase and results in the suppression of melanin synthesis. Minocycline increases melanin synthesis by acting on both keratinocytes and melanocytes. By acting directly on melanocytes, minocycline increases tyrosinase activity. In keratinocytes, minocycline enhances the mRNA expression of POMC synergistically with UVB.
UVB: ultraviolet B, Rx: roxythromycin, Mino: minocycline, POMC: pro-opiomelanocortin, MC1R: melanocortin 1 receptor

Discussion and Conclusion

Both melanocytes and keratinocytes determine skin color. Our *in vitro* study using melanoma cells and keratinocytes showed that minocycline increases pigmentation via the induction of POMC synergistically with UVB irradiation as well as direct activation of tyrosinase. On the other hand, roxythromycin activity inhibited tyrosine hydroxylase. The schematic summary is shown in **Figure 1**. Our results suggest that avoidance of UVB exposure is necessary to prevent skin hyperpigmentation during the usage of minocycline.