Mechanism of the Protective Effect of Intraperitoneally Administered Agonists for Formyl Peptide Receptors against Chemotherapy-Induced Alopecia

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Previously, we found that an intraperitoneally administered chemotactic peptide, N-formyl-Met-Leu-Phe (fMLP), and MMK-1, a selective agonist of formyl peptide receptor-like 1 (FPRL1) receptor, the low affinity subtype of the fMLP receptor, prevented the alopecia in neonatal rats induced by the anticancer agent etoposide.1) Intraperitoneal administration of MMK-1 (Leu-Glu-Ser-Ile-Phe-Arg-Ser-Leu-Leu-Phe-Arg-Val-Met, 10 mg/kg for 4 d), a synthetic agonist of the formylpeptide receptor-like 1 (FPRL1) receptor, a low affinity subtype of fMLP receptors in humans, had a similar effect.2)

In this study, we sought to specify the receptor and signaling mechanism mediating the anti-alopecia effect of fMLP and MMK-1. In humans, three receptor subtypes of formylpeptide receptors (FPRs) exist, FPR, FRPL1 receptor, and FPRL2 receptor.3) FPR has high affinity and FRPL1 receptor low affinity for fMLP. FPRL2 receptor does not bind fMLP.4) Boc-Phe-Leu-Phe-Leu-Phe (Boc-FLFLF), recently obtained by screening a peptide library,5) is an antagonist of FPRL1 receptor and FPRL2 receptor.6) Although the classification of fMLP receptor subtypes in rats is unclear, we examined the effects of these antagonists on the anti-alopecia effect of fMLP and MMK-1.

Materials and Methods

Reagents. fMLP and Boc-FLFLF were purchased from Sigma Chemical (St. Louis, MO) and pyrrolidine dithiocarbamate (PDTC), an inhibitor of NF-κB activation, was from Nacalai Tesque (Kyoto, Japan).

Synthesis of peptides. MMK-1, WRW4, and Lys-d-Pro-Thr (K(D)PT, an interleukin-1 inhibitor) were prepared with a solid phase peptide synthesizer (PS3, Protein Technologies, Tuscon, AZ) according to the 9-fluoroenyl methoxycarbonyl (Fmoc) method, and purified by reverse-phase high performance liquid chromatography (HPLC) using an ODS column (Cosmosil SC18-AR-II, 20 × 250 mm, Nacalai Tesque).

Key words: alopecia; etoposide; formyl peptide receptor-like 1 (FPRL1); interleukin-1; nuclear factor-κB (NF-κB)
Evaluation of the anti-alopecia effect.

Experimental protocols involving laboratory animals were approved by the ethical committee of the Graduate School of Agriculture of Kyoto University. Lactating Sprague-Dawley (SD) rats were purchased from Japan SLC. Ten 10-d-old male and female rats nursed by one lactating mother rat were used in the experiments. The test materials were dissolved in saline and administered intraperitoneally in volumes of 10 ml/kg body weight. Etoposide (Nippon Kayaku, Tokyo) was administered at a dose of 1.5 mg/kg i.p. for 3 d starting when the neonates became 11 d old. In this neonatal rat model, almost total alopecia is induced about 7 d after the last injection. Alopecia is prevented by an intraperitoneal injection of fMLP (30 mg/kg) or MMK-1 (10 mg/kg) for 4 d beginning 1 d before the first etoposide injection. Antagonists or inhibitors are administered simultaneously with fMLP or MMK-1. Photographs were taken 7 d after the last etoposide injection. Analyzing the digital images by NIH image, the hair area, which is the percentage of hair-covered area of the total back area, was calculated. The hair-covered area was defined as that with whiteness above a certain threshold value.

Results

Effect of antagonists selective for FPR and FPRL1 receptor on the anti-alopecia effects of fMLP or MMK-1

The FPR selective antagonist Boc-FLFLF has been
reported to inhibit the protective action of fMLP against myocardial ischemia-reperfusion injury in a rat model.\(^7\) Hence, we tested the effect of this antagonist on the anti-alopecia effect of fMLP to clarify the involvement of the rat FPR homolog. The anti-alopecia effect of fMLP was not blocked by Boc-FLFLF (1 mg/kg i.p. for 4 d) (Fig. 1A), suggesting that it is not mediated by FPR. WRW\(^4\), an antagonist of FPRL1 receptor and FPRL2

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**Fig. 3.** The Effects of an Inhibitor of IL-1 on the Anti-Alopecia Effects of Intraperitoneally Administered fMLP and MMK-1.
Etoposide was administered into 11-d-old rats at a dose of 1.5 mg/kg intraperitoneally for 3 d. (A) fMLP (30 mg/kg) or (B) MMK-1 (10 mg/kg) was administered intraperitoneally for 4 d beginning 1 d before the first etoposide injection. KdoPT (10 mg/kg i.p. for 4 d) was administered simultaneously with fMLP or MMK-1. The photographs were taken 7 d after the last etoposide injection. Values are expressed as mean ± SEM (**P < 0.001, ***P < 0.005 vs. etoposide-injected group, ###P < 0.001 vs. etoposide + MMK-1-injected group).**

**Fig. 4.** The Effects of an Inhibitor of NF-κB on the Anti-Alopecia Effects of Intraperitoneally Administered fMLP and MMK-1.
Etoposide was administered to 11-d-old rats at a dose of 1.5 mg/kg intraperitoneally for 3 d. (A) fMLP (30 mg/kg) or (B) MMK-1 (10 mg/kg) were administered intraperitoneally for 4 d beginning 1 d before the first etoposide injection. PDTC (100 mg/kg i.p. for 4 d) was administered simultaneously with fMLP or MMK-1. The photographs were taken 7 d after the last etoposide injection. Values are expressed as mean ± SEM (**P < 0.001 vs. etoposide-injected group, ###P < 0.001 vs. etoposide + MMK-1-injected group).
receptor, is known to block the intracellular calcium mobilization in human neutrophils induced by FPRL1 receptor agonists such as MMK-1, but not by the FPR agonist.\textsuperscript{5) Hence, we next tested the effect of WRW\textsuperscript{4} on the anti-alopecia effect of fMLP. WRW\textsuperscript{4} (30 mg/kg i.p. for 4 d) partially inhibited the anti-alopecia effect of fMLP (Fig. 1B), suggesting that the anti-alopecia effect of fMLP is mediated at least partly by the FPRL1 receptor homolog in rats and not by the FPR homolog.

MMK-1 is a highly selective agonist of the FPRL1 receptor.\textsuperscript{8,9) Hence, we examined the effect of the FPRL1 receptor antagonist WRW\textsuperscript{4} on the anti-alopecia effect of intraperitoneally administered MMK-1, and found that the effect of MMK-1 was completely blocked by simultaneous injection of WRW\textsuperscript{4} (Fig. 2). WRW\textsuperscript{4} had no effect on saline-administered rats. Hence, the anti-alopecia effect of MMK-1 in rats appears to be mediated by their FPRL1 receptor homolog.

**Effect of an IL-1 inhibitor on the anti-alopecia effects of fMLP and MMK-1.**

It is known that human recombinant interleukin-1\(\beta\) (IL-1\(\beta\)) inhibits alopecia induced in neonatal rats by the anticancer agent cytosine arabinoside,\textsuperscript{10) and that fMLP stimulates human phagocytic leukocytes, leading to their production of IL-1\(\alpha\) and \(\beta\).\textsuperscript{11) Hence, we investigated whether the anti-alopecia effect of fMLP or its agonist is mediated by IL-1 release. K\((\text{d})\)PT has been reported to inhibit the nociceptive effect of IL-1\(\beta\) in mice,\textsuperscript{12) and we found that the protective effect of fMLP and that of MMK-1 against etoposide-induced alopecia was inhibited by intraperitoneal injection of K\((\text{d})\)PT (10 mg/kg i.p. for 4 d) (Fig. 3A, B), but K\((\text{d})\)PT itself did not induce alopecia. These results suggest that IL-1 is involved in the anti-alopecia effects of fMLP and MMK-1.

**Effect of NF-\(\kappa\)B on the anti-alopecia effects of fMLP or MMK-1.**

Alopecia associated with cancer chemotherapy is induced by the apoptosis of hair follicle cells. NF-\(\kappa\)B is an apoptosis-suppressing translational factor activated by proinflammatory mediators like IL-1.\textsuperscript{13) Hence, we assessed whether the anti-alopecia effect of fMLP agonists would be influenced by an NF-\(\kappa\)B inhibitor. PDTC (100 mg/kg i.p. for 4 d), which blocks NF-\(\kappa\)B activation by preventing I-\(\kappa\)B degradation,\textsuperscript{14) inhibited the anti-alopecia effect of fMLP and that of MMK-1 (Fig. 4A, B), although it did not induce alopecia when given alone. Thus, the activation of NF-\(\kappa\)B appears to be involved in the anti-alopecia effects of fMLP and MMK-1.

**Discussion.**

fMLP is bound to FPR and FPRL1 receptor, but not to FPRL2 receptor.\textsuperscript{9) The anti-alopecia effect of fMLP was only partially inhibited by WRW\textsuperscript{4}, an antagonist of FPRL1 receptor and FPRL2 receptor, while that of MMK-1 was completely abolished by the antagonist. The anti-alopecia effect of fMLP was not blocked by Boc-FLFLF, an FPR antagonist, at all. These results suggest that FPRL1 receptor homolog is involved only partly in the anti-alopecia effect of fMLP in rats, while the effect of MMK-1 is mediated only by the receptor. Another receptor, neither FPR nor FPRL2 receptor, might be involved in the anti-alopecia effect of fMLP.

We have found that the anti-alopecia effects of fMLP and MMK-1 were inhibited by pyrilamine and cimetidine, which antagonize histamine H\(_1\) and H\(_2\) respectively.\textsuperscript{21) Once distributed into the blood stream after intraperitoneal injection, fMLP and its agonists are thought to stimulate basophiles in the blood, thereby inducing them to release histamine.\textsuperscript{15}) Taking into account a documented report that murine macrophages are stimulated by histamine to release IL-1,\textsuperscript{16}) it is probable that histamine released from basophiles by fMLP and its agonist enhance IL-1 levels. The released IL-1 might then activate TAK1, which is responsible for activating AP-1 and NF-\(\kappa\)B.\textsuperscript{17}) It is possible that NF-\(\kappa\)B blocks the etoposide-induced apoptosis of hair follicle cells, thus alleviating alopecia. In conclusion, fMLP and also MMK-1 might stimulate basophiles to release histamine, which leads to the production of IL-1 and then to activation of NF-\(\kappa\)B, which turns off the etoposide-induced pro-apoptotic mechanisms in the hair follicle.

Recently, we found that the anti-alopecia mechanism of orally administered MMK-1 is different from that reported here.\textsuperscript{18) Fragmental peptides released from MMK-1 might be working in this case (unpublished results).

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**References.**


