Biosci. Biotechnol. Biochem., **69** (8), 1633–1635, 2005

Communication

Anti-Alopecia Effect of Gly-Leu-Phe, an Immunostimulating Peptide Derived from α-Lactalbumin

Takahiro TSURUKI and Masaaki YOSHIKAWA†

Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Uji, Kyoto 611-0011, Japan

Received April 12, 2005; Accepted June 25, 2005

Gly-Leu-Phe (GLF), an immunostimulating peptide derived from α-lactalbumin, prevented alopecia induced by an anticancer agent etoposide in a neonatal rat model after intraperitoneal injection at a dose of 100 mg/kg for 4 d or oral administration at a dose of 300 mg/kg for 6 d. By microscopic analysis of skin sections, GLF proved to inhibit etoposide-induced loss of hair, thickening of the epidermis, and thinning of the adipocyte layer. The anti-alopecia effect of GLF was inhibited by pyrilamine, a histamine H_1 receptor antagonist, suggesting that the anti-alopecia effect is mediated by histamine release.

Key words: alopecia; Gly-Leu-Phe (GLF); peptide; phagocytosis; α-lactalbumin

We have reported that soymetide-4 (Met-Ile-Thr-Leu), an immunostimulating peptide derived from soybean β-conglycinin, prevented alopecia induced by anticancer agents, etoposide and cytosine arabinoside (ARA-C), in neonatal rats.[1–4] Soymetide-4 promoted phagocytosis of human polymorphonuclear leukocytes (PMN) and had weak affinity for chemotactic peptide formyl-Met-Leu-Phe (fMLP) receptor.[1,5,6] fMLP itself exhibited an anti-alopecia effect after intraperitoneal injection, but was inactive after oral administration.[2] It has been reported that fMLP stimulated histamine release.[7] We found that histamine H_1 receptor antagonist pyrilamine inhibited the anti-alopecia effects of intraperitoneally injected fMLP, suggesting that histamine is involved in the anti-alopecia effects.[8]

In this study, we tested the effect of Gly-Leu-Phe (GLF) on etoposide-induced alopecia in neonatal rats to investigate whether the immunostimulating peptide acting through a receptor other than fMLP exhibits an anti-alopecia effect. Originally, GLF was reported to be derived from human casein and to potentiate phagocytosis of human or murine phagocytic cells,[9–11] but in fact it is derived from human or bovine α-lactalbumin. GLF binds to specific binding sites on human PMN and monocytes, and the binding is inhibited by a complement protein, C1q, but not by fMLP.[12]

Experimental protocols involving laboratory animals were approved by the ethical committee of the Graduate School of Agriculture, Kyoto University. In this study, reagents were dissolved in saline and intraperitoneally injected in a volume of 10 ml/kg. GLF was purchased from Bachem AG (Bubendorf, Switzerland), and pyrilamine, a histamine H_1 receptor antagonist was from Sigma Chemical (St. Louis, MO). Etoposide (Nippon Kayaku, Tokyo) was injected at a dose of 1.5 mg/kg or 1.2 mg/kg i.p. for 3 d, and alopecia was induced about 1 week after the last injection in a neonatal rat model. Seven d after the last injection of etoposide, photographs were taken and skin was dissected from neck area of the dorsal skin. After fixing with 4% phosphate buffered paraformaldehyde for 24 h, the tissues were embedded in paraffin wax. Skin sections (4 μm) were stained with hematoxilin-eosin.

GLF injected intraperitoneally at a dose of 100 mg/kg for 4 d consecutively completely suppressed alopecia induced by etoposide, but was less effective at a dose of 30 mg/kg for 4 d (Fig. 1). Orally administered GLF at a dose of 300 mg/kg for 6 d exerted a weak anti-alopecia effect, but was inactive at a dose of 100 mg/kg (Fig. 2).

Next, we analyzed histochemically the dorsal skin of etoposide-treated rats in which alopecia was prevented with intraperitoneally injected GLF. The loss of hair, thinning of the adipocyte layer, and thickening of the epidermis induced by etoposide were suppressed by GLF at a dose of 100 mg/kg intraperitoneally for 4 d (Fig. 3). The shape of the hair follicles in rats given both etoposide and GLF was normal, as it was in control rats injected only with saline. GLF did not cause inflammatory infiltration of macrophages or granulocytes into skin tissues under these conditions.

We tested the effect of the histamine antagonist on the anti-alopecia effect of intraperitoneally injected GLF. After intraperitoneal injection at a dose of 10 mg/kg for 4 d, a histamine H_1 receptor antagonist, pyrilamine, blocked the anti-alopecia effects of GLF (Fig. 4). Pyrilamine itself did not induce alopecia (8). These results suggest that GLF prevents etoposide-induced

† To whom correspondence should be addressed. Fax: +81-774-38-3774; E-mail: yosikawa@kais.kyoto-u.ac.jp

Abbreviations: PMN, polymorphonuclear leukocytes; fMLP, *N*-formyl-methionyl-leucyl-phenylalanine

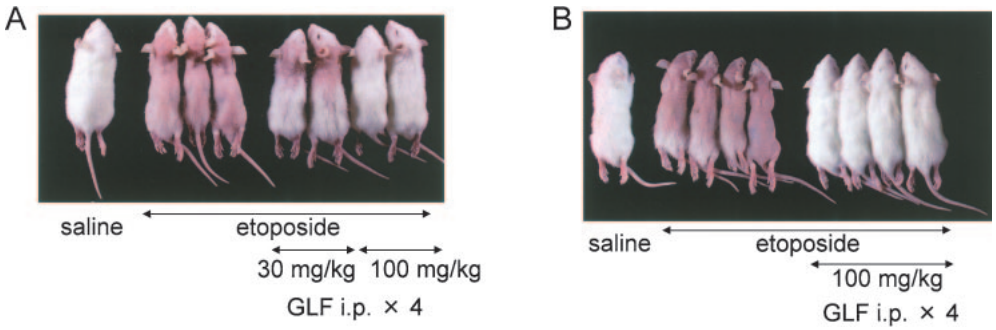

Fig. 1. Protective Effects of Intraperitoneally Administered GLF against Etoposide-Induced Alopecia.

Etoposide was injected at a dose of 1.5 mg/kg intraperitoneally for 3 d consecutively into 11-d-old rats. GLF was administered intraperitoneally at doses of (A), 30 and 100 mg/kg, and (B), 100 mg/kg for 4 d consecutively beginning 1 d before the first etoposide injection. Pictures were taken 7 d after the last etoposide injection.

Fig. 2. Protective Effects of Orally Administered GLF against Etoposide-Induced Alopecia.

Etoposide was injected at a dose of 1.2 mg/kg intraperitoneally for 3 d consecutively into 11-d-old rats. GLF (100 and 300 mg/kg) were administered orally for 6 d consecutively beginning 3 d before the first etoposide injection. Pictures were taken 7 d after the last etoposide injection.

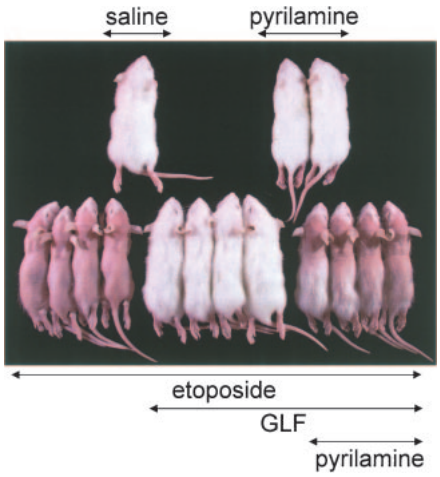

Fig. 4. Inhibition of the Anti-Alopecia Effect of GLF by a Histamine H_1 Antagonist.

Etoposide was injected at a dose of 1.5 mg/kg intraperitoneally for 3 d consecutively into 11-d-old rats. GLF (100 mg/kg i.p. for 4 d) was injected intraperitoneally for 4 d consecutively beginning 1 d before the first etoposide injection. Pyrilamine (10 mg/kg i.p. for 4 d) was injected simultaneously with GLF. Pictures were taken 7 d after the last etoposide injection.

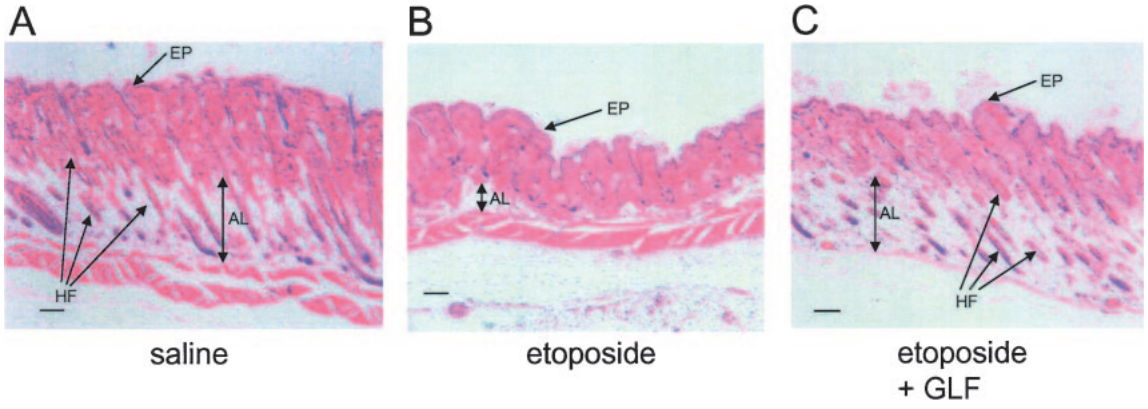

Fig. 3. Light Micrographs of Hematoxilin-Eosin Stained Dorsal Skin Sections from Neonatal Rats Treated with Saline (A), Etoposide (1.5 mg/kg, i.p. × 3) (B), or Etoposide (1.5 mg/kg, i.p. × 3) + GLF (100 mg/kg, i.p. × 4) (C).

EP, epidermis; HF, hair follicle; AL, adipose layer. Scale bars: 100 μm.

alopecia by stimulating histamine release. Thus fMLP and GLF share a common post-receptor mechanism, even though their receptors are different.

GLF is the second example of a food-derived peptide that exerts an anti-alopecia effect. It is an interesting problem whether other immunostimulating or histamine-releasing peptides show anti-alopecia effects.

Acknowledgments

This work was supported in part by a PROBRAIN grant.

References

1) Tsuruki, T., Kishi, K., Takahashi, M., Tanaka, M., Matsukawa, T., and Yoshikawa, M., Soymetide, an immunostimulating peptide derived from soybean β-conglycinin, is an fMLP agonist. *FEBS Lett.*, **540**, 206–210 (2003).
2) Tsuruki, T., Takahata, K., and Yoshikawa, M., A soy-derived immunostimulating peptide inhibits etoposide-induced alopecia in neonatal rats. *J. Invest. Dermatol.*, **122**, 848–850 (2004).
3) Tsuruki, T., and Yoshikawa, M., Design of soymetide-4 derivatives to potentiate the anti-alopecia effect. *Biosci. Biotechnol. Biochem.*, **68**, 1139–1141 (2004).
4) Tsuruki, T., Takahata, K., and Yoshikawa, M., Anti-alopecia mechanisms of soymetide-4, an immunostimulating peptide derived from soy β-conglycinin. *Peptides*, **26**, 707–711 (2005).
5) Showell, H. J., Freer, R. J., Zigmond, S. H., Schiffman, E., Aswanikumar, S., Corcoran, B., and Becker, E. L., The structure-activity relations of synthetic peptides as chemotactic factors and inducers of lysosomal secretion for neutrophils. *J. Exp. Med.*, **143**, 1154–1169 (1976).
6) Williams, L. T., Snyderman, R., Pike, M. C., and Lefkowitz, R. J., Specific receptor sites for chemotactic peptides on human polymorphonuclear leukocytes. *Proc. Nat. Acad. Sci. U.S.A.*, **74**, 1204–1208 (1977).
7) Hook, W. A., Schiffmann, E., Aswanikumar, S., and Siraganian, R. P., Histamine release by chemotactic, formyl methionine-containing peptides. *J. Immunol.*, **117**, 594–596 (1976).
8) Tsuruki, T., Ito, A., Takahata, K., and Yoshikawa, M., FPRL1 receptor agonist peptides prevent etoposide-induced alopecia in neonatal rats. *J. Invest. Dermatol.*, **123**, 242–243 (2004).
9) Migliore-Samour, D., Floc'h, F., and Jollès, P., Biologically active casein peptides implicated in immunomodulation. *J. Dairy Res.*, **56**, 357–362 (1989).
10) Parker, F., Migliore-Samour, D., Floc'h, F., Zerial, A., Werner, G. H., Jolles, J., Casaretto, M., Zahn, H., and Jolles, P., Immunostimulating hexapeptide from human casein: amino acid sequence, synthesis and biological properties. *Eur. J. Biochem.*, **145**, 677–682 (1984).
11) Gattegno, L., Migliore-Samour, D., Saffar, L., and Jolles, P., Enhancement of phagocytic activity of human monocytic-macrophagic cells by immunostimulating peptides from human casein. *Immunol. Lett.*, **18**, 27–31 (1988).
12) Jaziri, M., Migliore-Samour, D., Casabianca-Pignede, M. R., Keddad, K., Morgat, J. L., and Jolles, P., Specific binding sites on human phagocytic blood cells for Gly-Leu-Phe and Val-Glu-Pro-Ile-Pro-Tyr, immunostimulating peptides from human milk proteins. *Biochim. Biophys. Acta*, **1160**, 251–261 (1992).