Design of Soymetide-4 Derivatives to Potentiate the Anti-alopecia Effect

Takahiro TSURUKI and Masaaki YOSHIKAWA

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

Received November 20, 2003; Accepted February 13, 2004

Previously, we found that soymetide-4 (MITL), an N-formyl-methionyl-leucyl-phenylalanine (fMLP) agonist peptide derived from soybean β-conglycinin α′ subunit, stimulated phagocytosis of human polymorphonuclear leukocytes, and inhibited alopecia induced by etoposide, an anticancer drug, in neonatal rats after oral administration. We found that the fMLP receptor affinity and phagocytosis-stimulating activity of soymetide-4 was potentiated by replacement of Thr3 with hydrophobic residues. Among the derivatives synthesized, [Trp]3-soymetide-4 (MIWL) was the most potent, stronger by 180 and 130 times than soymetide-4 in receptor affinity and phagocytosis-stimulating activity, respectively. The anti-alopecia effect of [Trp]3-soymetide-4 was about 3 times larger than that of soymetide-4 after oral administration.

Key words: N-formyl-methionyl-leucyl-phenylalanine (fMLP); phagocytosis; alopecia; soybean; peptide

Previously, we isolated Met-Ile-Thr-Leu-Ala-Ile-Pro-Val-Asn-Lys-Pro-Gly-Arg from the trypsin digest of soybean protein based on the phagocytosis-stimulating activity of human polymorphonuclear leukocytes. This peptide is derived from residues 173–185 of the soybean β-conglycinin α′ subunit, and named soymetide-13 because the Met residue at the N-terminus is essential for its activity. Among soymetide derivatives which were deleted amino acid residues at the C-terminus, soymetide-9 (Met-Ile-Thr-Leu-Ala-Ile-Pro-Val-Asn) showed the strongest activity, and the minimum structure required for activity was soymetide-4 (Met-Ile-Thr-Leu-Ala-Ile-Pro-Val-Asn).1) Interestingly, soymetide-4 suppressed alopecia (hair loss) induced by etoposide, an anticancer drug, after oral administration to neonatal rats.2) Soymetides showed an affinity for the chemotactic N-formyl-methionyl-leucyl-phenylalanine (fMLP) receptor, and the phagocytosis-stimulating activity was inhibited by Boc-MLP, an fMLP receptor antagonist. Soymetide is the first fMLP agonist obtained from a food source but not formylated at the N-terminus.1) In present study, we investigated structure-activity relationships of soymetide-4 in order to design potent derivatives.

We synthesized peptides in which each residue of soymetide-4 was replaced with Ala and analyzed their phagocytosis-stimulating activity (Ala-scanning). Among them, only [Ala]3-soymetide-4 was active suggesting that Thr3 can be replaced with other residues. Then we replaced Thr3 with various amino acid residues. When replaced with hydrophobic residues such as Leu, Ile, Tyr, Phe, or Trp, the derivatives exhibited more potent fMLP receptor affinity (Table 1).

### Table 1. Affinities of Soymetide Derivatives for the fMLP Receptor

<table>
<thead>
<tr>
<th>Peptides</th>
<th>fMLP receptor affinity (IC50, μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MITL (soymetide-4)</td>
<td>450</td>
</tr>
<tr>
<td>MILL</td>
<td>300</td>
</tr>
<tr>
<td>MITL</td>
<td>100</td>
</tr>
<tr>
<td>MIYL</td>
<td>50</td>
</tr>
<tr>
<td>MIFL</td>
<td>9</td>
</tr>
<tr>
<td>MIWL</td>
<td>2.5</td>
</tr>
<tr>
<td>MITLAlIPVKPGR</td>
<td>50</td>
</tr>
<tr>
<td>MIWLAlPVKPGR</td>
<td>0.2</td>
</tr>
<tr>
<td>fMLP</td>
<td>0.03</td>
</tr>
</tbody>
</table>

A binding assay was performed in the presence of [3H]-fMLP (25 nM) using human neutrophils (1.0 × 106 cell/tube), as described previously.1,9)

1) 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; FPR, formylpeptide receptor; FPRL1R, FPR like 1 receptor
They also showed stronger phagocytosis-stimulating activities than soymetide-4 (Fig. 1A). Among them, [Trp]₃-soymetide-4 (Met-Ile-Trp-Leu) exhibited the most potent and had stronger than soymetide-4 in receptor affinity and the phagocytosis-stimulating activity by 180 and 130 times, respectively. Similarly, the fMLP receptor affinity and phagocytosis-stimulating activity of soymetide-13 were also potentiated 250 and 40 times, respectively, by the replacement of Thr⁳ with Trp (Table 1, Fig. 1B).

Next, we tested the anti-alopecia effect of [Trp]₃-soymetide-4 in neonatal rat models. The experimental protocols involving laboratory animals were approved by the ethical committee of the Graduate School of Agriculture, Kyoto University. As shown in Fig. 2, soymetide-4 exhibited an anti-alopecia effect after oral administration at a dose of 300 mg/kg for 8 days.²) The anti-alopecia effect of [Trp]₃-soymetide-4 was greater than that of soymetide-4 at the same dose. The anti-alopecia effect of [Trp]₃-soymetide-4 at a dose of 100 mg/kg was almost the same as that of soymetide-4 at a dose of 300 mg/kg (Fig. 2). Thus, [Trp]₃-soymetide-4 is about 3 times more effective than soymetide-4 in anti-alopecia effect after oral administration.

There are subtypes of the fMLP receptor: formylpeptide receptor (FPR) and FPR-like 1 receptor (FPRL1R) as high and low affinity subtypes.⁵–⁷) The receptor affinities shown in Table 1 mostly represent those for FPR. Among soymetide derivatives, phagocytosis-stimulating activities were almost parallel to receptor affinities. Therefore, the phagocytosis-stimulating activities might be mediated by FPR. But, the anti-alopecia effect of [Trp]₃-soymetide-4 was only 3 times that of soymetide-4, which suggests that the anti-alopecia effects of peptides might be mediated by other receptor subtypes than FPR, such as FPRL1R.

These potentiated derivatives of soymetide can be introduced into C₁₂-conglycinin by site-directed mutagenesis.⁸)

### Acknowledgments

This work was supported in part by Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science and the PROBRAIN grant.

### References


2) Tsuruki, T., Takahata, K., and Yoshikawa, M., A soy-derived immunostimulating peptide inhibits etoposide-


