Structure and chemotactic activity of peptides isolated from the venom sac of *Vespinae*

Yoshio Tsukamoto¹, Sachiko Fukutani¹, Yutaka Morikawa¹, Heizo Fujimoto¹,
Yasunori Tanaka¹, Masakazu Mori¹, Tadashi Yasuhara², Terumi Nakajima²,
Chieko Kitada³ and Masahiko Fujino³

¹Department of Pharmacology, Osaka Dental University,
1-47, Kyobashi, Higashi-ku, Osaka 540

²Institute for Medical Engineering, Tokyo Medical and Dental University,
Chiyoda-ku, Tokyo 101

³Central Research Division, Takeda Chemical Industries, Ltd.,
Yodogawa-ku, Osaka 532

[Accepted for publication: September 21, 1985]

Key words: Wasp venoms / peptides / chemotaxis / leucocytes

Introduction

It has been already reported that tridecapeptide amides were isolated from the venom of several species of *Vespinae*. However, the biological activities of these peptides have not yet been made clear. It was only reported that, the peptide (Crabrolin), which was isolated in U.S.A. from *Vespa crabro*, stimulate the degranulation of granulocytes, and no biological activity has been found for the peptide isolated in U.S.S.R. from *Vespa orientalis*.

Recently, it was found that one of these peptides were able to stimulate chemotaxis in leucocytes. This vespid chemotactic peptide was isolated from *Vespa tropica*¹⁾.

Therefore, we investigated all of these peptides and detected significant levels of chemotactic activity for mononuclear leucocytes (MNL) and polymorphonuclear leucocytes (PMNL).

Materials and Methods

The venom sac of several species of *Vespinae*, *Vespa tropica* (Japanese name is Himesuzumebachi, Ves-T), *Vespa mandarinia* (Ohsuzumebachi, Ves-M), *Vespa analis* (Kogatasuzumebachi, Ves-A), *Vespa xanthoptera* (Kirosuzumebachi, Ves-X), *Para-*

vespula lewisii (Jibachi, Ves-L) and *Icaria* sp. (Ves-I), were homogenized in 50% CH₃CN and the homogenates were centrifuged at 1,500×g for 10 min. From the supernatants, the peptides were isolated by LS-410 ODS column chromatography with the CH₃CN gradient (10-50%) elution system. The sequences of these peptides are shown in Table 1.

MNLs and PMNLs were separated from human peripheral blood by the Ficoll-Paque

Table 1 The sequence of vespid peptides

<i>Vespa tropica</i>
Phe-Leu-Pro-Ile-Leu-Gly-Lys-Ile-Leu-Gly-Gly-Leu-Leu-NH ₂
<i>Vespa mandarinia</i>
Phe-Leu-Pro-Ile-Ile-Gly-Lys-Leu-Leu-Ser-Gly-Leu-Leu-NH ₂
<i>Vespa analis</i>
Phe-Leu-Pro-Met-Ile-Ala-Lys-Leu-Leu-Gly-Gly-Leu-Leu-NH ₂
<i>Vespa xanthoptera</i>
Phe-Leu-Pro-Ile-Ile-Ala-Lys-Leu-Leu-Gly-Gly-Leu-Leu-NH ₂
<i>Paravespula lewisii</i>
Phe-Leu-Pro-Ile-Ile-Ala-Lys-Leu-Val-Ser-Gly-Leu-Leu-NH ₂
<i>Icaria</i> sp.
Ile-Val-Pro-Phe-Leu-Gly-Pro-Leu-Leu-Gly-Leu-Leu-Thr-NH ₂

method, and chemotaxis assay was performed by using a modification of a method described by Falk *et al.*^{2,3)}. A multi-well chemotaxis chamber (Neuro Probe) was used with a polycarbonate membrane filter (Neuro Probe) with a pore size of 3 μm for PMNLs, and 5 μm for MNLs.

After incubation, the filter was removed, fixed and stained. Leucocytes that had migrated through the filter were counted in 20 oil immersion fields at $\times 1,000$ magnification and expressed as cells per oil immersion field (cells/oif).

Results and Discussion

Vespid peptides in the varying concentrations of 0.1 to 50 nmol/ml were added to the lower wells of the chemotaxis chamber and the MNL suspension was added to the upper wells, and incubated at 37°C for 90 min. Ves-T, M, A, X, L and I showed significant levels of chemotactic activities for MNL. Although the synthetic chemoattractant, N-folmylmethionyl-leucyl-phenylalanine (10^{-8} M), induced chemotactic ratio of 138 cells/oif at concentration of 10 nmol/ml and 4 cells/oif by the buffer control, most of

vespid peptides elicited more than 150 cells/oif at concentrations of 1.0 to 20 nmol/ml. Especially, at 2.0 to 10 nmol/ml, activities of Ves-T, M, X and A were notable (Fig. 1).

The experiments were performed to determine whether leucocyte migration was merely representative of hyperkinesis or was indeed a directional migration towards these peptides⁴⁾, however, the result of these experiments showed that MNLs and PMNLs migration occurred only in the presence of a positive stimulatory gradient.

Moreover, several peptides were synthesized from the amino sequence assay of vespid peptides, and tested for leucocyte chemotaxis. Some of them, such as Phe-Leu-Pro-Ile-Leu-Gly-Lys-Ile-Leu-Gly-Gly-Leu-Leu-NH₂, Lys-Ile-Leu-Gly-Gly-Leu-Leu-NH₂, Gly-Leu-Leu-NH₂, Leu-Gly-Gly-Leu-Leu-NH₂ and Lys-Ile-Leu-Gly, induced varying degrees of migration in PMNLs or MNLs.

Thus, tridecapeptide amides isolated from the venom sac of several species of *Vespinae* can cause directional migration of PMNLs and MNLs. Further, synthetic peptides and their fragments have active effect on the migration of leucocytes, equal to or higher than vespid peptides.

Fig. 1 Dose dependent monocytic chemotaxis to vespid peptides. Data represent the mean number of migrated cells per oil immersion field (20 fields counted).

References

- 1) Yasuhara, T., Nakajima, T., Fukuda, K., Tsukamoto, Y., Mori, M., Kitada, C. and Fujino, M.: Structure and activity of chemotactic peptide from the venom sac of vespinae. *Peptide Chem. Proc. 21st Symp. Peptide Chem.* 185–190, 1983.
- 2) Falk, W., Goodwin Jr., R. H. and Leonard, E. J.: A 48-well micro chemotaxis assembly for rapid and accurate measurement of leukocyte migration. *J. Immunol. Methods* 33: 239–247, 1980.
- 3) Harvath, L., Falk, W. and Leonard, E. J.: Rapid quantitation of neutrophil chemotaxis: Use of a polyvinylpyrrolidone-free polycarbonate membrane in a multiwell assembly. *J. Immunol. Methods* 37: 39–45, 1980.
- 4) Tsukamoto, Y., Helsel, W. E. and Wahl, S. M.: Macrophage production of fibronectin, a chemoattractant for fibroblasts. *J. Immunol.* 127(2): 673–678, 1981.