Communications to the Editor

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ENHANCING EFFECTS OF N-ACETYL-CHITO-OLIGOSACCARIDES ON THE ACTIVE OXYGEN-GENERATING AND MICROBICIDAL ACTIVITIES OF PERITONEAL EXUDATE CELLS IN MICE

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N-Acetyl-chito-oligosaccharides, tetra-N-acetyl-chitotetraose, penta-N-acetyl-chitopentaose, and hexa-N-acetyl-chitohexaose were found to enhance active oxygen-generating and candidacidal effects of peritoneal exudate cells in BALB/c male mice given 50 mg/kg of each oligosaccharide intraperitoneally.

KEYWORDS——tetra-N-acetyl-chitotetraose; penta-N-acetyl-chitopentaose; hexa-N-acetyl-chitohexaose; chitin; peritoneal exudate cells; active oxygens; candidacidal effect

In the previous papers of this series,[1,2] we reported the immunopotentiating effect of chitin and chitosan using mice challenged with tumor cells or with pathogenic microbes. Because of the insolubility of these polysaccharides in water, it was necessary to investigate these effect using the lower molecular weight, water-soluble analogues, N-acetyl-chito- and de-N-acetyl-chito-oligosaccharides. Here we report that the N-acetyl-chito-oligosaccharides administered intraperitoneally enhance the active oxygen-generating and candidacidal effects of peritoneal exudate cells (PEC) of mice.

Table I. Chemiluminescence Response of PEC in Mice Given N-Acetyl-chito-oligosaccharides, Chitin, and Casein

Substance	Chemiluminescence (CPM)
Saline	7493 ± 1174[a]
Tetra-N-acetyl-chitotetraose	40948 ± 11083 ($p < 0.05$)[b]
Penta-N-acetyl-chitopentaose	141162 ± 55098 ($p < 0.01$)[c]
Hexa-N-acetyl-chitohexaose	443649 ± 135766 ($p < 0.01$)
Chitin	121720 ± 23354 ($p < 0.01$)
Casein	9237 ± 1025 (N.S.)[d]

a) Mean ± standard errors of eight mice.
b) Significantly different from the control by Student's t test, $p < 0.05$.
c) Significantly different from the control by Student's t test, $p < 0.01$.
d) Not significantly different from the control by Student's t test.

The N-acetyl-chito-oligosaccharides, tetra-N-acetyl-chitotetraose, penta-N-acetyl-chitopentaose, and, hexa-N-acetyl-chitohexose[3,4] were supplied by Ihara Chemical Industry Co., Ltd., Tokyo, Japan. Each oligosaccharide, 50 mg/kg, was injected ip into BALB/c male mice of 4 to 6 weeks. Chitin and casein were used as positive controls. Three hours after administration, the PEC were collected by washing the peritoneal cavity with Hanks' balanced salt solution (HBSS), then the cells were resuspended in the same buffer, 1×10^6 cells/ml.

Table I shows the results of active oxygen generation assay of 1×10^6 cells achieved by the method described in the preceding paper.[2] It is evident that the hexaose has the strongest active oxygen-generating effect of the three oligosaccharides.

Table II. Candidacidal Activity of PEC in Mice Given N-Acetyl-chito-oligosaccharides and Chitin.

Sample	Candidacidal acivity (%)[a]
Saline	36.3 ± 6.0[b]
Tetra-N-acetyl-chitotetraose	71.3 ± 2.7 ($p < 0.01$)[c]
Penta-N-acetyl-chitopentaose	80.6 ± 3.1 ($p < 0.01$)
Hexa-N-acetyl-chitohexaose	94.0 ± 3.4 ($p < 0.01$)
Chitin	73.5 ± 3.1 ($p < 0.01$)

a) Killing (%) = 200-Number of Colonies/200 x 100.
b) Mean ± standard error of eight mice.
c) Significantly different from the control by Student's t test, $p < 0.01$.

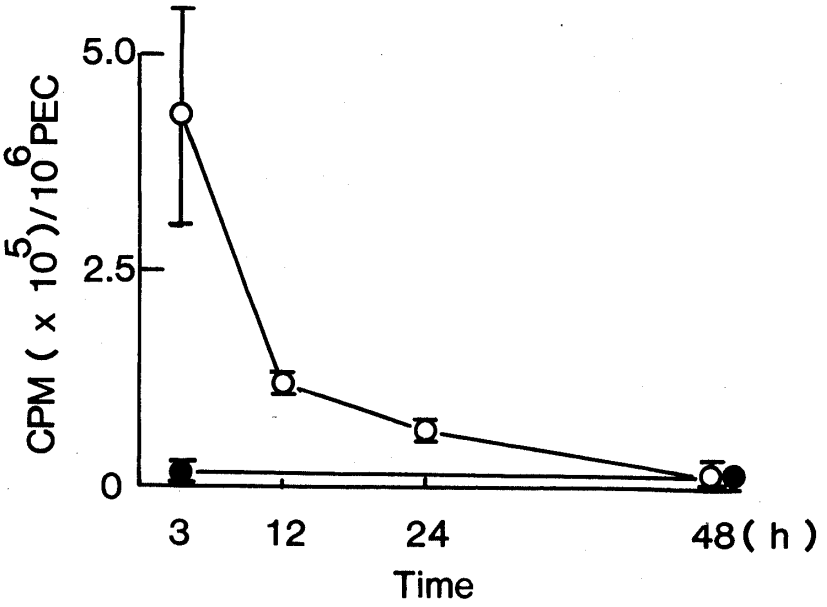

Fig. 1. Time-Course of Active Oxygen Generation from Peritoneal Exudate Cells in Mice Given Hexa-N-acetyl-chitohexaose

Open circle indicates hexa-N-acetyl-chitohexaose and closed circle saline control. Bar: Mean ± standard error.

Table II summarizes the results of the candidacidal assay with 1 x 10^5 PEC against 2 x 10^2 viable cells of Candida albicans NIH A-207 strain conducted by essentially the same method described in the preceding study.[2] The result shows that all oligosaccharides display fairly strong candidacidal effect.

Next, a time-course study of the active oxygen-generating effect of the hexaose was conducted with 1 x 10^6 PEC. As Fig. 1 shows, the hexaose responded very rapidly, within 3 h after ip administration.

All findings obtained in the present study indicate the following conclusions: water-soluble N-acetyl-chito-oligosaccharides, from tetraose to hexaose, are able to enhance the host-mediated microbicidal effect by stimulating the active oxygen-generating system of PEC.

REFERENCES AND NOTES

1) S. Suzuki, Y. Okawa, Y. Okura, K. Hashimoto, and M. Suzuki, "Immunoadjuvant Effect of Chitin and Chitosan, in Chitin and Chitosan," Proceedings of the Second International Conference on Chitin and Chitosan, eds. S. Hirano and S. Tokura, July 12-14, 1982, Sapporo, Japan, p.210, Japanese Society of Chitin and Chitosan, Tottori Univ., Tottori 680, Japan.

2) K. Suzuki, Y. Okawa, K. Hashimoto, S. Suzuki and M. Suzuki, "Protecting Effect of Chitin and Chitosan on Experimentally Induced Murine Candidiasis," Microbiol. Immunol., 28, 903 (1984).

3) Abbreviations: Tetra-N-acetyl-chitotetraose; O-β-2-acetamido-2-deoxy-D-glucopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose, Penta-N-acetyl-chitopentaose; O-β-2-acetamido-2-deoxy-D-glucopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose. Hexa-N-acetyl-chitohexaose; O-β-2-acetamido-2-deoxy-D-glucopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-O-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose.

4) Specific Rotation Values of N-Acetyl-chito-oligosaccharides

Oligosaccharide	$[\alpha]_D^{20}$ (°) [a]
Tetra-N-acetyl-chitotetraose	-4.0
Penta-N-acetyl-chitopentaose	-12.0
Hexa-N-acetyl-chitohexaose	-15.0

a) c = 1.0, l = 1.0, water.

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