Migration of Canine Neutrophils to Chitin and Chitosan

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ABSTRACT. Suspension of chitin and chitosan particles (mean size of 1 μm) were found to attract canine neutrophils chemotactically as determined by a checkerboard assay through polycarbonate filter with 5 μm pore size in Blind well chamber. Suspension of chitin induced chemokinetic migrations of the neutrophils. These evidences might reflect accumulation of neutrophils to chitin- and chitosan-implanted regions in dogs.—KEY WORDS: canine neutrophils, chemotaxis, chitin and chitosan.


Chitin and chitosan, polymers of N-acetyl-D-glucosamine and D-glucosamine, are widely distributed in nature as the skeletal material of crustaceans and insects and cell walls of fungi and bacteria [4]. Wound healing was found to be accelerated in the presence of chitin and chitosan agents in our previous studies [1-3, 11, 13]. Histologically, polymorphonuclear cells (PMN), mainly neutrophils, were highly accumulated to the chitin- and chitosan-implanted regions in an early phase of wound repair in cows and dogs [3, 12]. From these evidences, chitin and chitosan were expected to induce chemotaxis of neutrophils in these animals. In the present study, chitin and chitosan particles were confirmed to induce chemotaxis of canine neutrophils by a checkerboard assay.

Animal: Four mongrel dogs (1 year old), a male and three females, were used in the present study. They were in health without hematological and biochemical disorder.

Cell preparation: One ml of peripheral blood was collected with a plastic syringe containing heparin (10 units per ml of blood) from a jugular vein, diluted with 2 ml of Hank’s balanced salt solution (HBSS), placed on 3 ml of Ficoll-Conray solution (specific gravity of 1.082), and centrifugated at 700 × g for 30 min at room temperature to obtain PMN-rich cell suspension. The cell suspension was treated with 0.83% ammonium chloride (NH₄Cl) to hemolyze, washed twice with HBSS, and then suspended in HBSS at a cell density of 2 × 10⁶ per ml. Purity and viability of canine PMN in the resting cell suspension were more than 90 and 95%, respectively. The PMN obtained was almost neutrophil.

Chitin and chitosan: Chitin (less than 30% deacetylated) and chitosan (more than 80% deacetylated) were supplied by Sunfite Co., Ltd. (Tottori, Japan). Chitin and chitosan particles, 1 μm on mean size (range from 0.2 to 3.0 μm), were suspended in HBSS at concentration of 0.1 and 1.0 mg/ml. Ranges of size of these particles were measured by SK laser Micron Sizer 7000S (Seisın K. K., Japan).

Measurement of canine neutrophil migration: Migration of canine neutrophils was measured in Blind well chambers 200-187 (Neuro Probe Co., Ltd., U.S.A.), as described previously [15]. A checkerboard assay was performed by method of Zigmond and Hirsch [16]. Statistical analysis was performed by Student’s t-test. Experiment was carried out in duplicate determinations and repeated at three times.

Checkboard assays on migrations of canine neutrophils to chitin and chitosan suspension are shown in Table 1. Canine neutrophils incubated in the presence of positive chitin and chitosan gradients, as shown in the right side of the diagonal line of the table, exhibited markedly increased migration. The maximum migratory activity of canine neutrophils, obtained when concentrations of chitin and chitosan in lower and upper wells were 1.0 and zero mg/ml, respectively, were about fifteen- and five-fold than that of canine neutrophils obtained when chitin and chitosan were absent in both wells. However, canine neutrophils incubated in the presence of negative chitin and chitosan gradients, as shown in the left side of the diagonal line of the table, exhibited less migration. From these evidences, canine neutrophils were confirmed to be attracted to chitin and chitosan suspension chemotactically.

When concentrations of chitin in upper and lower wells were same, migratory activity of canine neutrophils significantly increased with increasing concentration of

Table 1. A checkerboard assay on migration of canine neutrophils to chitin and chitosan suspension

<table>
<thead>
<tr>
<th></th>
<th>Concentration in upper well (mg/ml)</th>
<th>Concentration in lower well (mg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>chitin(mg/ml)</td>
<td>0.0</td>
<td>35±32</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>34±4</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>13±4</td>
</tr>
<tr>
<td>chitosan(mg/ml)</td>
<td>0.0</td>
<td>40±9</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>25±6</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>2±2</td>
</tr>
</tbody>
</table>

a) Upper well contains canine neutrophils at a density of 2×10⁶ cells/ml.

b) Chitin and chitosan particles were suspended in HBSS at concentration of 0.1 or 1.0 mg/ml.

c) Migrated canine neutrophils per mm² lower surface of the membrane (mean±SD) after incubation at 37°C for 60 min. Experiment was carried out in duplicate determinations and repeated at three times.

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chitin (p<0.01). The maximum migratory activity of canine neutrophils, obtained when concentrations of chitin in both wells were 1.0 mg/ml, was about four-fold than that of canine neutrophils obtained when chitin was absent in both wells. When concentration of chitosan in both wells were same, however, the migratory activity of canine neutrophils at the concentration of 1.0 mg/ml was lower than that of 0.1 mg/ml, though they were higher than that in the absence of chitosan (p<0.05). These evidences show that chitin could induce chemokinesis of canine neutrophils, though the chemokinesis with chitosan was not clear.

From the present study, chitin and chitosan suspension were found to induce chemotaxis of canine neutrophils. Furthermore, chitin suspension was found to induce chemokinesis of canine neutrophils. These evidences support our in vivo observations that the neutrophils were highly accumulated at chitin- and chitosan-administered regions at early stages of wound healing in domestic animals [3, 12]. The accumulation of neutrophils to the regions would reflect their chemotactic and chemokinetic responses to these agents.

Random migration of canine neutrophils in the presence of chitosan at the concentration of 1.0 mg/ml was lower than that of 0.1 mg/ml. Many neutrophils were seen to phagocytize more particles in 1.0 mg/ml chitosan suspension than in 0.1 mg/ml suspension (data not shown). From the facts that chitosan is cationic agent but chitin is neutral [10] and that cationic agent is phagocytized more easily by neutrophils than neutral one [14], neutrophils might phagocytize many chitosan particles and destroy themselves easily in the upper chamber.

Chemotactic activity of canine neutrophils to the chitin suspension was approximately twice as high as that to chitosan suspension. Chemically modified chitin such as 30, 70 and 80%-deacetylated chitin were reported to enhance biological activities including anti-tumor activity [5, 6], adjuvant activity [6] and production of several cytokines from macrophages [7-9], while unmodified chitin and chitosan showed none of these responses [5-7]. Chemotactic activity of canine neutrophils to chitosan, more than 80% deacetylated chitin, was lower in level than that to chitin in the present study, which might be due to the rate of deacetylation.

Chitin and chitosan examined in the present study are macromolecules, which give us a question that active agents to induce chemotaxis of canine neutrophils might be unknown contaminated substances, not the chitin and chitosan particles themselves. In order to eliminate the possibility, migratory activity of the neutrophils were measured to the supernatants obtained from centrifuga-

REFERENCES