Effects of Chitosan on Experimental Abscess with Staphylococcus aureus in Dogs
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ABSTRACT. An abscess was developed experimentally by a subcutaneous inoculation of Staphylococcus (S.) aureus T-6 with a 4-cm silk suture in dogs. After draining the pus, the abscess was treated with a suspension of finely granulated chitosan (chitosan group), ampicillin (ampicillin group), or saline (control group) (Day 0). The chitosan group was further divided into 3 subgroups (0.01, 0.1, and 1.0 mg/subgroups). Similar treatment was repeated after 4 days (Day 4), followed by euthanasia on Day 8. The wound cavity contraction rate was calculated by measuring the wound cavity diameter by a sound on Days 0, 4 and 8. The wound cavity contraction rate was significantly higher in the ampicillin, 0.1 mg chitosan, and 1.0 mg chitosan groups than in the 0.01 mg chitosan and control groups on Days 4 and 8 (p<0.05). In the 0.1 and 1.0 mg chitosan groups, the abscess healed completely in 6 out of 11 (55%), and 9 out of 10 cases (90%), respectively, by Day 8. In the ampicillin group, 4 out of 10 cases (40%) healed completely by Day 8. No healing occurred in the 0.01 mg chitosan and control groups. Histologically, the granulation tissue formed had abundant vascularization in the 0.1 and 1.0 mg chitosan groups on Day 8.—KEY WORDS: abscess, canine, chitosan.


Suppurative wound infections including abscess formation are common in veterinary practice. The present concept of wound infection is mainly based on the control of systemic and local inhibitory factors, such as protein and vitamin deficiency, foreign bodies, and necrotic tissues [10, 11]. The treatment of wound infection therefore includes the use of antibiotics and antiseptics, debridement, and supplementation of deficiencies [10, 11]. Our recent studies have indicated that unmodified chitin and chitosan can accelerate wound healing in clinical applications [3–7, 9]. Chitosan was found to sometimes be more effective in healing dirty wounds such as abscesses without the concurrent use of antibiotics and antiseptics [5].

In the present study, the effects of chitosan on experimental Staphylococcus aureus- abscesses were evaluated in dogs.

Animals: Fifteen adult mongrel dogs (1–3 years old, weighing 6–15 kg), 7 males and 8 females, were used in this study.

Chitosan agent: The finely granulated chitosan used in this study was described in a previous paper by us [14]. This agent was sterilized with ethylene oxide gas before use. The finely granulated chitosan was suspended in phosphate buffer saline (PBS, pH 7.2) at a concentration ranging from 0.01 to 1.0 mg/ml, which was called Chitosine S thereafter.

Staphylococcus (S.) aureus: The S. aureus T-6 strain was originally isolated from the feces of a human patient with rheumatic fever. The strain was sensitive to ampicillin and cephalotaxons and resistant to oxytetetracycline, kanamycin, chloramphenicol, and gentamicin. The strain was suspended in PBS at a concentration of 10^7 cfu/ml.

Formation of abscesses: The dogs were anesthetized with sodium pentobarbital (25 mg/kg I.V.) after premedication with atropine sulfate (0.05 mg/kg s.c.) and buprenorphin-HCl (5 μg/kg s.c.). Skin incisions (1 cm) were made at the lumbar dorsal and lumbar sacral sites on both sides of the midline. After the subcutaneous tissue was bluntly dissected, 1 ml of the bacterial suspension was injected into the wound with a 4-cm piece of silk suture. The skin was then closed with an interrupted nylon suture. The abscess, approximately 3–5 cm in diameter, developed at the site of inoculation within 7 days after inoculation.

Experiment: Fifteen dogs with abscesses were divided into 3 groups; a chitosan group (9 dogs), an ampicillin group (3 dogs) and a control group (3 dogs). The chitosan group was further divided into 3 subgroups (0.01, 0.1 and 1.0 mg subgroups) of 3 dogs each. After draining off pus, the abscess was treated with 1 ml of Chitosine S (0.01–1.0 mg/ml), ampicillin solution (30 μg/ml) or PBS without debridement. After the initial treatment (Day 0), the wound was covered with a bandage. The bandage was changed every 2 days, and the treatment was repeated on Day 4.

Wound cavity contraction rate: The wound cavity diameter (WCD) was measured by insertion of sound into the incision in a dorsoventral direction on Days 0, 4 and 8. The contraction of the wound cavity was calculated as follows: Contraction rate (%) = (1- WCD on Day 4 or 8 / WCD on Day 0) × 100. When the contraction rate is 100%, this means that the wound cavity has contracted completely. It was judged that the abscess healed completely when the contraction rate was 100% and no bacteria were histologically observed.

Histological observation: On Day 8, the abscesses and surrounding tissues were enucleated for histological observation. The tissues were fixed in 10% phosphate-buffered formalin, embedded in paraffin, cut into 3-μm thick sections, and then stained with hematoxylin-eosin.

Effect of Chitosine S on S. aureus: S. aureus cells were confined in a triplicate Soy Agar plate. Fifty microliter lots of Chitosine S at concentrations of 0.01, 0.1, 1.0, 10 and 30 mg/ml were spotted onto the plates, incubated at 37°C for 24 hr and observed for the inhibition of bacterial growth at the spots of Chitosine S.

Statistical analysis: Statistical analysis was performed by

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Duncan's multiple range test.

**Wound cavity contraction rate:** The wound cavity contraction rate is shown in Fig. 1. The wound cavity contraction rate was significantly higher in the ampicillin, 0.1 mg chitosan, and 1.0 mg chitosan groups than in the 0.01 mg chitosan and control groups on Days 4 and 8 (p<0.05). In the 1.0 mg chitosan group, the contraction rate was higher than in the ampicillin group on Day 8, but there was no statistical difference. In the 0.1 and 1.0 mg chitosan groups, the abscess healed completely in 6 out of 11 (55%), and 9 out of 10 (90%) cases, respectively, by Day 8. In the ampicillin group, 4 out of 10 (40%) cases healed completely by Day 8. In the 0.01 mg chitosan and control groups, none of the lesions healed completely. Necrosis of the skin and white granulation tissue developed at the abscess site in the control group.

**Histological findings:** Diffuse infiltration of inflammatory cells, mainly neutrophils, was predominant in the control group (Fig. 2a), while few inflammatory cells and granulation tissue with poor neovascularization were observed in the ampicillin group (Fig. 2b). In the 0.1 and 1.0 mg chitosan groups, accumulation of inflammatory cells, mainly monocytes and polymorphonuclear leukocytes, was observed around chitosan granules (arrows) (c) and the granulation tissue which formed had abundant vascularization in the 1.0 mg chitosan group (d). H-E stain, a, b, d: ×350, c: ×700.

![Fig. 1. Wound cavity contraction rate. The wound cavity diameter (WCD) was measured by introducing sound into the incision in a dorsoventral direction on Days 0, 4 and 8. Contraction of the wound cavity was calculated as follows: Contraction rate (%) = (1-WCD on Day 4 or 8/WCD on Day 0) × 100. The reported values are the mean ± s.d. a, b, c. Mean values with different letters are significantly different (p<0.05).](image-url)
cells, mainly monocytes and polykaryocytes, was observed around the chitosan granules (Fig. 2c). The granulation tissue formed in these groups had abundant neo-vascularization (Fig. 2d) with a smaller area than that in the ampicillin group. The amount of granulation tissue was smaller in the 0.1 chitosan than in the 1.0 chitosan groups. Only a few chitosan granules and many inflammatory cells were observed in the granulation tissue in the 0.01 mg chitosan group.

Effect of Chitofine S on S. aureus: Growth of S. aureus was inhibited clearly at the spot of 30 mg/ml Chitofine S and slightly at that of 10 mg/ml, but not at other concentrations.

Conventional therapy for suppurative wound infections includes drainage of pus, debridement of devitalized tissue, and the use of antibiotics to kill bacteria [11]. In the present study, however, chitosan was observed to be effective in healing experimental S. aureus-abscess in dogs, depending upon the dose of chitosan. The wound healing was obvious at a dose of 1.0 mg per abscess, which was more effective than that of ampicillin medication. This evidence supports our previous data indicating that chitosan was sometimes more effective in healing dirty wounds such as abscesses without the concurrent use of antibiotics and antiseptics [5].

S. aureus T-6 strain was resistant to chitosan suspensions at a concentration of less than 1 mg/ml. Tanigawa et al. [15] reported that chitosan inhibited the in vitro growth of S. aureus at a concentration of 1.3 mg/ml. The effect of chitosan on abscesses was therefore not due to direct bactericidal activity. Phagocytes such as polymorphonuclear cells (PMN) and macrophages are known to be important for killing bacteria and the absorption of necrotic tissue in infected wounds [11]. Chitosan was shown to enhance the migration of PMN in our previous data [16]. Thus, the activity of PMN could be enhanced at the site of infection in the presence of chitosan. Recently, several growth factors such as epidermal growth factor, fibroblast growth factor, and transforming growth factor have been identified as promising wound healing agents and have been used in clinical practice [2]. Some studies have indicated that wound fluid tends to have a high growth factor activity [1, 12, 13]. The authors have many examples of stimulated exudation in the presence of chitosan. These lines of evidence suggest that chitosan might induce some growth factors in the healing of suppurative wound infection infected wounds.

Histologically, granulation tissue with poor neovascularization was characteristic of the ampicillin group. In contrast, granulation tissue with abundant neovascularization was observed in the chitosan group. This change was dependent on the concentration of chitosan. The chitosan group would therefore reconstitute normal tissue at earlier stages than the ampicillin group. The histological findings obtained in the presence of chitosan were similar to those observed in the presence of chitin [8].

In farm animals, the use of antibiotics is restricted in order to avoid contamination of meat. Our previous data indicated that chitosan was effective in dirty wounds in cows as well as in dogs [5]. The present study also indicated that chitosan was effective in healing experimental S. aureus-abscess in dogs. In view of this evidence, the treatment of wound infections in livestock with chitosan agents may be recommended.

REFERENCES