Study on the mechanisms of wound healing acceleration by chitosan

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Chitosan is well known as a wound healing accelerator and used as a wound dressing in veterinary medicine. Granulation is enhanced with chitosan treatment. However the precise mechanisms of the action is unknown. The scope of the present work was to determine the detailed mechanisms with chitosan treatment on wound healing.

In the first section, in order to evaluate the effect of chitosan as an accelerator of wound healing, we made experimental open skin wounds on the dorsal side in normal beagles. Chitosan was applied for 15 days, and the wounds were evaluated histologically and immunohistochemically. On the 3rd day, the chitosan-treated wounds showed histologically severe infiltration of polymorphonuclear (PMN) cells and an increase in effusion compared with that in the control. Migration of macrophages and foreign giant cells was also accelerated by the chitosan-treatment on the 3rd day. Granulation was more accelerated by the chitosan-tratment on the 9th and 15th day, and the amount of collagen was more rich than that of the control. Immunohistochemical typing of collagen Ⅰ, Ⅲ and Ⅳ showed an increase of type Ⅲ collagen in the chitosan group. The number of mitotic cells increased in the control group on the 3rd day, and on the chitosan group on the 6th day. From these results, chitosan was suggested to accelerate the infiltration of PMN cells at the early stage of wound healing, followed by an increase in macrophages migration, and the production of collagen by fibroblasts.

In the second section, PMN in the granulation tissue treated with chitosan in canine experimental wound was studied and osteopontin (OPN) was strongly positive in PMN immunohistochemically. OPN is a glycosylated phosphoprotein and may play a role in granulomatous inflammation. Production of OPN in PMN was therefore investigated *in vitro* using human PMN using reverse transcriptase polymerase chain reaction (RT-PCR) and enzyme immunoassay (EIA) in this study. PMN stimulated with granulocyte-colony stimulating factor (G-CSF) and chitosan accumulated OPN messenger ribonucleic acid (mRNA) expression, and released OPN protein into their culture supernatants. These findings suggested that OPN was synthesized by migrating PMN and had a significant role in the evolution of wound healing with chitosan treatment at early phase of healing.

Histological findings of previous reports indicated that chitosan accelerated the reformation of connective tissue, however the mechanism has not been cleared in detail.

In the third section, firstly L 929 mouse fibroblasts were cultured with chitosan and the production of extracellular matrix (ECM) was evaluated *in vitro*. Type I and Ⅲ collagen and fibronectin were secreted by L 929 with or without chitosan, however there was no significant difference in the amount of

ECM between the control and the chitosan groups. Secondly, macrophages were stimulated with chitosan, and then transforming growth factor-beta 1 (TGF-b1) and platelet-derived growth factor (PDGF) mRNA expressions and productions of their proteins were assayed *in vitro*. As a result, chitosan promoted the production of TGF-b1 and PDGF. These results indicate that chitosan does not directly accelerate ECM production by fibroblast, and the ECM production may be increase by the growth factors.

The results of these studies showed the mechanism of accelerated wound healing by chitosan as follows ; (1) Chitosan accelerates the migration of PMN at inflammation phase of healing. (2) Migrated PMN cells produce OPN which accelerates the migration macrophages. (3) The production of growth factor (TGF-b1 and PDGF) is enhanced by chitosan stimulation. (4) These growth factors promote the proliferation of fibroblast and the production of ECM. (5) As a result, chitosan accelerates the granulation and wound healing.

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Apical delta formation of the root apex and efficacy of enamel matrix proteins in periodontal regeneration for periodontal disease in dogs

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Veterinary small animal dentistry has recently developed rapidly and is now respected as a veterinary medical discipline. Usually, the therapeutic techniques in human dentistry have been applied to small animal dentistry without any special considerations. However, various differences such as morphology, food, etc. between humans and animals are evident, and understanding these differences is essential to protect animal teeth from disease and to select the correct technique for treatment.

In dogs, apical lesions (apical abscesses) frequently form dental fistulae at ventral sites of the orbit and the root apex of the canine eminence. The apical delta has a complex structure of the root apex in adult dogs, and this may be one of the factors causing the apical lesion of the tooth. The apical delta is an intricate system of cavities in which numerous passages of blood vessels and nerves branch from the pulp cavity to the root apex. This complicated structure makes complete removal of infected pulp by routine root canal treatment difficult. There are various reports on the rate of existence of the apical delta, but the root apexes immediately after the eruption of the permanent teeth in dogs are immature and have not yet closed. The eruption time also varies depending on