CLINICAL EVALUATION OF CHITIN AND CHITOSAN IN THE MANAGEMENT OF FRACTURES

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ABSTRACT

Chitin and chitosan have evolved into one of the most promising biomaterials due to their multivarious properties as biodegradable bioimplants in surgery. The present study was conducted on dogs to assess their biocompatibility and efficacy in fracture healing. Chitin and. chitosan were used to fill the defects in fractured segments of radius and ulna of dogs after stabilizing with dynamic compression plates. Haematobiochemical parameters and radiological evaluation were recorded. Histomorphological studies of the bone biopsy were also examined. The study revealed that the fracture healing was better in chitosan group of dogs

Key Words: Chitin; Chitosan-fracture healing.

INTRODUCTION

Chitin, commonly found as the tough polymer of crab and shrimp shells has a special combination of biological activity as physical properties, Chitin-B-(I - 4)-poly-N-acetyl-D- glucose amine is present as a protein complex along with minerals and requires deprotenisation and demineralization to obtain chitin. Deacetylation of chitin yields chitosan. Both have unusual combination of properties having wide applications in medical field opened a new vista for recycling the waste by product of prawn industry.

The raw material, exoskeleton of fresh prawn for preparation of chitin and chitosan was obtained from local market. (*Paneus Indicus*). The shells and heads of fresh prawn were thoroughly and repeatedly washed in water and sun dried. The raw material was completely immersed (steel container) in three percent sodium hydroxide and boiled for thirty minutes for deprotenisation. After cooling, the alkali is drained off and washed repeatedly and finally with ionized water to obtain neutral pH. The contents were transferred to a plastic container and five percent hydrochloric acid added and allowed to act for thirty minutes. The acid was decanted and repeatedly washed with water and then with ionized water. The excess water from the chitin obtained is removed by squeezing in a sterile lint cloth and air-dried. Chitin was immersed in forty percent sodium hydroxide and heated up to 90 degree Celsius for 90 minutes. The sodium hydroxide is quickly drained off and the content washed repeatedly with water and finally with ionized water and the chitosan obtained was air dried after removing the excess water as above. The prepared chitin and chitosan was sterilized with ethylene oxide and packed in pre autoclaved polythene containers.

MATERIALS AND METHODS

A total of 18 dogs divided into three groups were subjected to fracture healing studies to study the effect of chitin and chitosan. Chitin and chitosan was packed in the fracture defect in two groups and the defect was left as such in the control group.

The operative site was aseptically prepared for surgery. The dogs were premedicated with xylazine l mg/kg and atropine sulphate 0.04 mg/kg and anaesthetized with ketamine l0 mg/kg body weight. Skin incision was made on the cranio-lateral border of the fore arm and continued through the fascia, muscles and the tendon of the common digital extensor carpi radialis. Separating the muscles exposed the fractured bone. The radius was stabilized with a suitable dynamic compression plate. The defect was either packed with chitin or chitosan and in the control group the defect was left unfilled. The muscles and skin were closed as per standard procedure. The surgical wound was dressed regularly and the skin sutures were removed on the 8th postoperative day. All the animals were subjected to clinical evaluation and the following parameters were studied: Weight bearing pattern, haematobiochemical parameters and radiological evaluation. The parameters were recorded on 0, 3, 7, 14 and 21" clay. Bone biopsies were taken for histomorphological studies.

RESULTS AND DISCUSSION

From the 3rd post operative day in all the three groups dogs were able to bear weight on the operated limb which may be due to the support afforded by the DCP. Hence there was no significant deviation in the weight bearing pattern in any of the groups. The findings concurred with that of Moore et. al., (1987) who had used other biomaterials for fracture healing.

A significant increase in the total leucocyte count was observed on the 3rd post operative day. The increase might be due to the surgical stress and trauma (Wagne, 1991). In all the three groups neutropenia and lymphocytosis was observed from 3rd to 14th post operative day which could be attributed to increased margination and migration into tissues to modulate inflammatory response (Walker and Willenize, 1980).

In all the three groups serum calcium reduced up to 14"' post operative day and this concurred with the findings of Deka et al. 1994. The low level of serum calcium could be due to injury to the bone tissue as reported by Henderson and Noble (1996). The significant reduction in serum Calcium level in chitin and chitosan treated groups could be as a result of stimulation of osteoblast line cell present in endosteal, periosteal and bone marrow by growth factors entrapped in coagulum-chitosan mixture which gave rise to intramembranous bone formation promoted by simultaneous proliferation and angiogenetic event which a...re characteristic of the osteoconductive properties of' chitin and chitosan (Muzzarelli et. al., 1993). Baird and Ling (1987) stated that N-acetyl glucosamine units present in chitosan was similar to glycosaminoglycons and could bind fibroblast growth factor in the same way as heparin which is known to stimulate angiogenesis and osteoblast line cell proliferation. Serum inorganic phosphorous significantly increased from 3rd day to 14th postoperative day in all the dogs. The increase could be due to cellular disintegration at the fracture site. There was a gradual increase in serum alkaline phosphatase up to 14th postoperative day which could be attributed to the proliferation of fibrous tissue at the fracture site. The increase in SAP activity observed in Chitin and Chitosan treated dogs on the 3 and the 14th postoperative day suggested superior activity. The increase might be due to N-acetyl glucosamine which builds fibroblast growth factor which in turn stimulate angiogenesis and osteoblasts-like cell proliferation (Nishimura et.al 1985). Chitosan treated group showed radiopaque callous with less density and mild periosteal reaction on the 21st postoperative day. Early formation and radiopaque callus with less density could be attributed to osteoinduction property of chitosan (Muzzarelli et. al., 1994.). Osteoinduction property of chitosan could be

attributed to its cationic nature and chelating ability of chitosan which results in endosteal, periosteal and bone marrow osteoblast like precursors stimulated by growth factors entrapped in the coagulum-polysaccharide mixture which gives rise to intra membranous bone formation promoted by simultaneous proliferation and angiogenetic events (Muzzarelli et al., 1993).

Histological examination of chitosan group revealed increased number of osteoblasts with blood vessels replacing woven bone still present and gradual conversion of fibro vascular -matrix to fibro-osseous matrix was also noticed. These changes may be attributed to the osteoinduction properties of chitosan (Muzzarelli et. al., 1994).

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