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Effect of Different Concentrations of Mg Incorporated Hydroxyl Apatite Crystal on Bone Healing of Femur Fracture in Rats

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ABSTRACT

Hydroxy apatite HA is an essential component of bone and teeth, it's responsible for their rigidity. It substitutes some human tissues cause of the structure similarity. The present study aimed to make the hydroxyl apatite crystal as a carrier for the magnesium ions to improve the HA crystal properties and enhance the absorption of calcium leading to improve bone mineral density. Preparing pure HA apatite and Mg loaded HA of different magnesium concentrations. Hematological and Histopathological examination were applied on the blood and bone tissues .HA -Mg considered to be a safe biomaterial, it didn't cause any toxicity or leukocytosis effect and the best histological picture for bone healing at which the bone defects treated with Hydroxyapatite + 3 gm Mg so it is recommended based on our study.

Keywords: *Hydroxyapatite, stoichiometric , pathological, Hematological.*

INTRODUCTION

Biomaterials considered to be one of the most important tools in the scientific applications owing to its use in replacing parts in human bodies. Boceramic is a type of biomaterials composed of calcium and phosphates like hydroxyl apatite which one of the most famous bioceramic. It is an essential component of bone and teeth, it's responsible for their rigidity. Hydroxyapatite is the preferred choice for substituting human bone tissues because, although it is a stoichiometric, crystalline form with generally larger crystal sizes, is chemically closest to the naturally occurring mineral in one. Synthetic hydroxyapatite, a10 (PO4)6(OH) 2, HA, can directly bond to bones without infection and fibrous encapsulation, thus is regarded as bioactive and biocompatible. [1] However, its brittleness limit its application as load bearing implants. One solution is to incorporate another a biomedical metal into the HA crystal, so that the bioactivity and biocompatibility are utilized along with the good mechanical properties of the metal [2]

In the last decade many substances have been used for doping hydroxypatite like F, Mg, CO3, Mn, Zn, Bi and Na. The influence of magnesium doping on hydroxypatite represents an important research topic because of the enhancements it brings without affecting the biocompatibility. Improve the properties of hydroxyl apatite consider a target to increase HA hardness and functions.

Just over half the body's magnesium is found in bone, where it forms a surface constituent of the hydroxyapatite mineral component, and a further third is found in muscles and soft tissues [3].

The presence of magnesium in the HA crystal improve the formation and structure of the crystal, thus Mg+2 used to increase strength of HA. Magnesium substituted phase has better performance than the unsubstituted HA.

The in vivo bone response of 3D periodic hydroxyapatite (HA) scaffolds is investigated. Observations suggest that HA rods are first coated with a layer of new bone followed by subsequent scaffold infilling via outward and inward radial growth of the coated regions. Direct-write assembly of 3D periodic scaffolds composed of micro-porous HA rods arrayed to produce macro-pores that are size-matched to trabecular bone may represent an optimal strategy for bone repair and replacement structures. [4]

Groups of Wistar rats (5/sex/dose) received a paste of nano-hydroxyapatite in water at 0, 25, 50 and 100 mg/kg bw/d up to 6 hr/day for 7d/week for 28 days. At the end of the exposure period, significant reduction in haemoglobin, platelets and mean corpuscular volume (MCV) at the highest dose, with no other statistically significant differences between control and treated animals. [5]

MATERIALS AND METHOD

Preparation of the Samples

As our previous study [6], preparing pure HA apatite (S1) and Mg loaded HA (S2,S3 and S4) of different magnesium concentrations [0.004 ,0.01 ,0.02mole] by wet precipitation method . Diamonium hydrogen phosphate[(NH4)2HPO4] is added in droplet manner(1-3 mL per min.) to a mixture of [(NH4)2 HPO4] solution was fed from a burette, in a dropwise manner (2-5 ml/min), into a stirred solution of 0.16 M [CaCl2.2H2O] and Magnesium Nitrate Mg(NO3)2.6H2O with magnetic stirring at constant temp. 60 C0 and pH at 10

Medical Application

This study was carried out at the Surgery, Anasethiology & Radiology Department, Faculty of Veterinary Medicine, Suez Canal University. Thirty-six healthy male albino Wistar rats aged 5-6 months, clinically healthy with weight ranged from200 to 250gm were used in this study. The animals were subjected to neuroleptanalgesia, using i.M injection xylazine 8mg/kg and ketamine 40 mg/kg[7].The site of operation (lateral aspect of the left thigh) was disinfected with povidon iodine solution (Betadine®). The rats were divided into 6 groups, each one 6 rates.

As in fig (1), The left femur of all animals except 6 (control negative group) was drilled to create a defect in the mid-diaphysisusing a dental drill of 1.6 mm diameter, adapted to a dental micromotor at a speed of 3500 rpm [7].After induction of bone defects, the defects were packed with Hydroxyapatite in Group III and the other groups (IV,V,VI) filled with hydroxyl apatite with different concentrations of Mg as in fig (2).

Figure1. *Induction of femoral diaphysial defect*

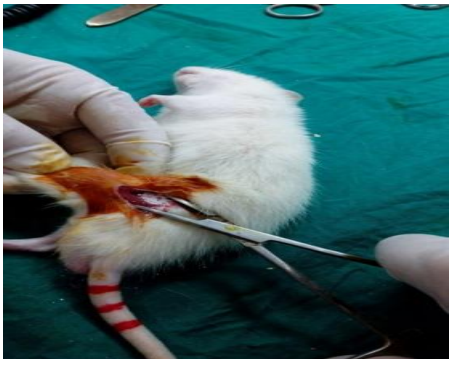

Figure2. *Packing of the defect with the selected material*

Random 3 rats were selected from each group at 2 and 4 weeks from the beginning of the experiment. Two blood samples were obtained from the medial canthus of the eye of each rat. The samples were put in an inclined position for 20 minutes at room temperature, and, put in for clot retraction, and then the samples were centrifuged at 3000 rpm for 10 minutes and the clear serum was separated carefully and was stored in epindorf tubes at -20 °C until estimation of serum biochemical.

MEDICAL EXAMINATION

Hematological Examination

Erythrocytic and Leucocytic Count:

Erythrocytes,red blood cell count (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and total leukocytic

count (T.L.C) were performed by manual method using improved neubauerhemocytometer and diluting fluids of erythrocyte and leukocyte according to Feldman, B. F.et.al [8].

Histopathological Examination

The bone specimens were taken from each group week postoperative after euthanasia of rats using overdose of thiopental sodium. The bone specimens will be fixed in 10% neutral buffered formalin for 4 days, then decalcified for 15 days in trichloroacetic acid. After inclusion in paraffin, serial sections of 5 µm thickness were performed. The histological slides were then stained by Goldner`s Trichrome method [9]

Results and Discussion

The Hematological Analysis

From the hematological results, the present study revealed that after 4 weeks, as in (table 1) there were non-significant changes in red blood cell count (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) in all groups. Meanwhile, all treated groups showed significant decline in the Hb concentration in comparison with the control one. In contrast, the T.L.C. in the control positive group was significantly increased and this may be due to the inflammation. It was noticed that (L.T.C.) concentrations became normal at the groups of animals that treated with HA and HA doped with different Mg concentrations compared with the control + ve group. This indicates that the presence of HA-Mg help in accelerating the healing process and Mg may reduce the inflammation making the L.T.C. ratio return to normal range compared with the control - ve group. Thus the reduction in magnesium cause inflammatory response [10].

Table1. *Hemogram (Mean ± S.E.) in rats for 4weeks.*

Group	RBCs 10^6/µl	Hb g/dl	PCV %	MCV fl	MCH pg	MCHC %	T.L.C 10^3/µl
Control – ve	6.53^{a} ±0.45	15.30^{a} ±0.15	39.10^{a} ±1.95	60.87^{a} ±3.36	23.53^{a} ±1.45	39.30^{a} ±1.63	6.47^{b} ±0.29
Control +ve	6.93^{a} ±0.34	14.30^{bc} ±0.15	33.83^{a} ±1.65	48.93^{a} ±1.50	20.73^{ab} ±0.96	42.50^{a} ±2.45	11.27^{a} ±0.52
HA	6.67^{a} ±0.26	13.93^{c} ±0.26	32.60^{a} ±1.42	48.97^{a} ±2.26	20.97^{ab} ±1.22	42.93^{a} ±2.26	7.40^{b} ±0.21
HA+ 1gmMg	6.93^{a} ±0.19	12.57^{d} ±0.14	34.50^{a} ±2.72	49.93^{a} ±4.37	18.17^{b} ±0.63	36.83^{a} ±2.47	7.20^{b} ±0.42
HA+ 3gmMg	6.87^{a} ±0.29	12.47^{d} ±0.23	33.83^{a} ±1.94	49.27^{a} ±3.06	18.13^{b} ±0.41	37.07^{a} ±2.14	7.43^{b} ±0.60
HA+ 5gmMg	7.20^{a} ±0.17	14.73^{ab} ±0.17	35.73^{a} ±2.55	49.80^{a} ±4.14	20.50^{ab} ±0.26	41.67^{a} ±3.18	6.23^{b} ±0.30

Histopathological Results

Based on pathological investigations it could be concluded that the animals groups in which HA and HA doped with Mg samples will have better healing than the control positive groups. This is may be due to the dissolution of Ca+2 ions from the HA crystal ,after that the Calcium ions would interact with the carbonate group from the blood forming a scaffold of calcium carbonate which acts as a bridge where new bone tissues is formed above it as in fig (3,4).

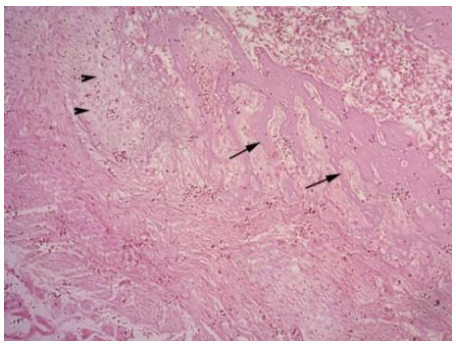

Figure3. *Showing thin compact bone spicules anastomosing each other with some cartilaginous cells among the connective tissue, group II, 8 weeks postoperative, H&E, X100.*

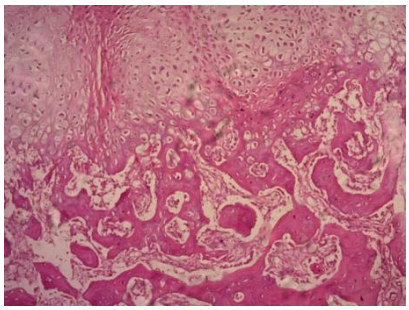

Figure4. *Showing fair arrangement of bone trabeculae that joining each other and intermingled with mature granulation tissue, group V (HA+Mg3gm), 8 weeks postoperative, H&E, X100.*

CONCLUSION

From the hematological analysis it was clearly noticed that there is no toxicity or leukocytosis effect or any abnormal results which indicated that HA and HA doped with Mg samples are safe biomaterials and their implantation in the animal for 8 weeks didn't cause hazards.

The bone defects which treated with Hydroxyapatite + 3 gm Mg and 5 gm Mg showed the best histological picture for bone healing and this indicated that Mg increased the calcium absorption by the body causing complete fracture healing.

As there is no clear difference between the 3 and 5 gm of Mg in the process of healing, so Hydroxyapatite +3 gm Mg is recommended based on our study.

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