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Radiological and biochemical Evaluation of 1% Bisoprolol Gel on Bone Healing in Rabbit

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ABSTRACT

Background: Beta-Adrenergic receptor antagonists (β-blockers) have been shown to reduce the incidence of cardiovascular disease. Recent evidence suggests that β-blockers may also have an effect on bone structure, metabolism and fracture healing. This study aimed to explore the effect of bisoprolol in repairing rabbit femoral bone defect.

Material and Methods: Thirty New Zealand white albino adult male rabbits weighting (2- 2.5)Kg were randomized to one of two, treated and control groups. Bone defects were produced in the femoral bone and filled with 1% Bisoprolol Gel (treated group)or Gel base only(control group).Blood samples were drawn at regular intervals .The animals were humanely killed 2, 4, 6 weeks after surgery and Postmortem radiography was undertaken to examine new bone. Serum rabbit Bone specific Alkaline Phosphatase (BALP) and total calcium concentration was measured.

Results: In present study radiography and CT scan showed significant increase in bone density in treated groups at two and four weeks as compared to control with a significant increase in BALP, (2.11+ 0.25) ng/L in treated group at the end of two weeks, while the changes in serum calcium was not significant.

Conclusion: This study demonstrated that local application of bisoprolol gel is capable of repairing femoral bone defects with good osteogenic action. Which can be suggested for beneficial use in practice of dentistry.

Key words: B blocker; Bisoprolol; bone healing; bone specific alkaline phosphatase.

INTRODUCTION

The process of bone modeling and remodeling ensures adaptation of size,shape ,micro architecture and mineral content of the skeleton as well as repair of bone damage[1]. Sympathetic nerve fiber has been detected in bone tissue and functional adrenergic receptors are present on osteoblast and osteoclast[2,3]. Adrenergic stimulation has been reported to cause both anabolic and catabolic effects on bone mediated by alpha and β adrenergic receptors, studies investigate that β adrenergic agonists could directly stimulate bone resorbing activity of mature human osteoclast[4].Further study suggested that sympathyectomy by sympathetic neurotoxic agent impaired bone resorption in rat[5]. Takeda showed that treatment of mice with the β-adrenergic receptor agonist isoproterenol decreased bone mass, bone formation and osteoblast number, on other hand treatment of mice with the β adrenergic antagonist resulted in significant increase in bone mass in vertebrae and long bone secondary to an increase in bone formation and osteoblast number[6], a possible role for β-blockers in the prevention of fracture of major clinical interest[7]. Bisoprolol fumarate is a synthetic β_1 -selective (cardio selective) adrenoreceptor-blocking agent[8]. Bone alkaline phosphatase (BALP) is an enzyme which plays an important role in the bone formation and in mineralization. It reflects the metabolic status of osteoblasts. The rates of bone production and destruction can be evaluated either by measuring predominantly osteoblastic or osteoclastic enzyme activities or by assaying bone matrix components released in the blood stream and excreted in the urine.[9].The aim of this study to evaluate the effect of topically applied1%bisoprolol gel on bone healing in the femur of rabbits.

Materials and Methods

Preparation of bisoprolol gel

The pure Bisoprolol fumarate powder was purchased (Bisoprololfumarate,united company, Jordan). Bisoprolol gel was freshly prepared by mixing 0.1gm of bisoprolol powder in 10 ml of gel base that contain (carboxymethyl

cellulose and propylene glycol) to give final concentration (1%) with continuous mixing using vortex device to prepare homogenous gel, which were kept in sterile plastic containers and stored at room temperature.

Experimental Animals

Thirty new Zealand white adult male rabbits of 6-8 months old, clinically healthy, weight (2-2.5) kg were used, all animals were housed under standard conditions with unlimited of pellet diet and water ad libitum

Surgical Procedure

Animal surgery: Rabbits were anesthetized with injection of 50mg/kg ketamine hydrochloride (Ketamine,Hameln,Germany)and xylazine 5 mg /kg (Xyla,Interchemie,Holland) respectively, and atropine sulfate injection (50) microgram / kg intra muscularly , femur was routinely asepsis , an incision was performed on left femoral bone , one cortical perforation of (3)mm in diameter and (5)mm depth with low speed hand piece of 2000rpm and profuse irrigation of distal water , the created defect was filled with 1% bisoprolol gel for treated groups, and filled with gel only for control groups, the animals received antibiotic ceftrioxone at dose (40mg/kg). I.M once daily for three days , and also received analgesic (40mg /Kg) diclofenamic acid I.M once daily to control pain. Five animals from each group were sacrificed at 2,4,and 6 weeks after the surgery. Blood samples were collected, serum calcium and BALP were estimated, sectioned femurs were fixed in 10% formaline in phosphate-buffer saline (PBS)0.1 M for 48 hours .

Radiological evaluation

The femurs were radio graphed at a standardized distance of 20 cm, with radiographs(PLANMECA W&H, USA) of the bone segments were taken. During radiographic exposure, a metal device was placed beside the tissue to obtain a radiopaque region, all radiographs were size standardized to quantify the information on rate of bone formation and eliminate the variation in radio density between radiographs. Density percentage were performed using Image J version 1.43s software (National Institutes of Health ,Bethesda, MD). Radiographic images were comparatively analyzed between the prepared bone- defect region(A) and the intact bone area around the defects (C) by correlating radiographic density using mathematical equation,[10](Figure 1).

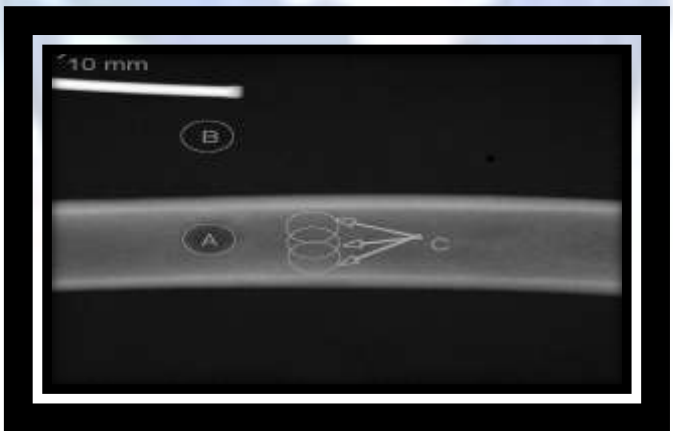

Figure 1. Radiographic bone density data using Image J software. B background area; A defect bone area; C mean of the natural bone area.

The Computerized Tomography (CT) scan, examination carried out (Philips brilliance, USA) to measure the density of callus at the site of defect**.**

Biochemical Evaluation

At the end of the experimental period(2,4,6) weeks, a blood sample was collected from the central ear vein and serum was separated by centrifugation at 1500Xg for (30) minutes, the sera were frozen ($-18C^0$) for later analysis. Serum BALP and total calcium was measured using rabbit BALP Elisa kit (My Bio Source, USA) and Calcium kit (Biolabo SA, France) respectively.

Statistical Analysis

The statistical analysis was conducted using the Statistical Package for Social Sciences for Windows, version 17.0 (SPSS).All data are expressed as the mean value ± standard error (SE). The significance of differences was evaluated by Independent-samples t-test and for repeating measures, One-way analysis of variance (ANOVA) with post hoc test and Duncan multiple range test for multiple comparisons of the mean were applied. Statistical differences between groups were accepted for p-values <0.05.

RESULTS

All animals survived to end of the study and no wound infection was observed .

Radiological Evaluation

Analysis of radiographic bone density by periods revealed that bone callus size was biggest in the group treated with1% bisoprolol gel at all time points. Furthermore, early mineralization and complete bridging of defect was seen at 4weeks after surgery in1% bisoprolol treated group, in contrast, to control group had minimal evidence of healing with a favorable significant difference (treated vs. Control, p=0.012 and p=0.003, at weeks2 and 4 respectively; table 1). Higher means of radiographic bone density were observed in the treated group at 6 weeks, but there were no statistical differences(treated vs. Control, p=0.252; table 1). However, ANOVA analysis showed no significant differences within both groups p>0.05(table 1)

Table (1): Comparison Mean ±standard error of image j level in between control and treated groups at different weeks

Time / Groups	mean ± SE			p–value=
	2nd week (No= 5)	4th week (No= 5)	6th week (No= 5)	
Control	57.43±2.51 A	45.37±6.28 A	60.56±5.81 A	.130
Treatment	72.36±3.89 A	75.99±3.60 A	68.49±2.75 A	.340
p–value=	*.012	**.003	.252	

- *, **Significant differences in two &four weeks at p<0.05 between groups
- Similar letters (A) horizontally mean out put of Duncan′s test non significant differences at p<0.05 within group

Quantitative analysis of the CT data confirmed the radiographic images (Table. 2). Two weeks after surgery, bone density in the treated group was insignificantly higher than the control (treated vs. Control, p<0. 768;table 2). By week four the treated group with 1%bisoprolol gel demonstrated a significant increase in bone density(treated vs. Control, p<0. 000;table 2), and the amount of new bone continuously increased until 6 weeks post surgery as indicated by bigger callus size (treated vs. Control, p<0. 03;table 2).In treated groups demonstrated a significant increase in bone density (2-4weeks, p=0.000). However, the control group had no significant difference in bone formation in the defects between 2nd and 4th weeks. While a statistically significant difference in bone density was observed at the 6thweeks time point (p=0.000; table 2).

Table (2): Comparison between Mean± SE of CT radiography (HU) between control and treated groups at different weeks

Time / Groups	mean ± SE			p–value=
	2nd week (No= 5)	4th week (No= 5)	6th week (No= 5)	
Control	567.0±69.32 A	450.0±160.69 A	1204.4±2.69 B	0.000

Treatment	**599.0±74.68 A**	**1345.8±55.0 B**	**1595.6±148.0 B**	**0.000**
p–value=	**0.768**	***0.000**	***0.03**	

***,*Significant differences at p<0.05 between groups
Different letters (A, B) horizontally mean out put of Duncan′s test significant differences at p<0.05 within group

Biochemical evaluation

Serum BALP activity was estimated in both groups and the results showed that at the end of 2 weeks, the serum concentration of the BALP showed a significant increase(treated vs. Control, p<0.049; table 3). At the end of four weeks the treated group with 1%bisoprolol gel demonstrated a decrease in BALP which was further decreased at the end of six week similar decline in BLAP was noticed in control group, but there were no significant differences(treated vs. Control, p<0.0929, p<0.537at 4and 6 weeks respectively table 3).). ANOVA analysis showed no significant differences within both groups control and treated p <0.05 **(table3)**

Table (3): Comparison Mean ±standard error of serum BALP level in between control and treated groups at different weeks

Time / Groups	mean ± SE			p–value=
	2nd week (No= 5)	**4th week (No= 5)**	**6th week (No= 5)**	
Control	**1.26±0.27 A**	**1.56±0.79 A**	**1.19±0.17 A**	**0.148**
Treatment	**2.11±0.25 A**	**1.64±0.52 A**	**0.9±0.26 A**	**0.829**
p–value=	***0.049**	**0.929**	**0.537**	

Serum calcium level was estimated, no significant differences were observed between both groups during different treatment periods in addition to that ANOVAs analysis showed no significant differences was observed with in both groups (p>0.05; table 4),

Table (4): Comparison Mean ±standard error of serum calcium level in between control and treated groups at different weeks

Time / Groups	mean ± SE			p–value=
	2nd week (No= 5)	**4th week (No= 5)**	**6th week (No= 5)**	
Control	**11.74±0.38 A**	**11.82±0.44 A**	**12.57±0.48 A**	**0.895**
Treatment	**12.46±0.51 A**	**11.52±0.50 A**	**12.74±0.37 A**	**0.226**
p–value=	**0.802**	**0.929**	**0.706**	

- similar letters (A) horizontally mean out put of Duncan′s test insignificant differences

Discussion

Recent data indicate that bone remodeling, remain under sympathetic control in addition to endocrine, paracrine /autocrine mechanism ([11]).Experimental studies show that the level of bone specific alkaline phosphates in osteoblast line cells and bones are proportional to the formation of collagen ([12,13]). clinical studies also show that the level of BALP in serum correlates with bone formation rate([14,15]). To our knowledge this is the first experimental study to demonstrate the local effect of beta one selective blocker-1% bisoprolol gel Rabbit(BALP) is a glycoprotein that is found on the surface of osteoblast and reflect the biosynthetic activity of bone metabolism([16]).

The results in the present study showed the BALP was significantly increased at the end of 2weeks and this increase in agreement with kurdy results during tibial fracture healing in men up to 10th week after the trauma no significant decrease([17]) also previously philipov et. al found significant higher levels of serum BAlP and osteocalcin in experimental osteomyelitis of the femur in dog([18]) . this increase of BALP accompanied by enhanced bone formation at the end of 4th week significantly. The volume of callus correlates with level of BALP([19]) and reflected the increase on defect density radio graphically using image j at the end of 2and4 weeks and at computerized tomography at the end of 4thweek.The biochemical variable total calcium showed no significant changes these findings in agreement with earlier workers([20,21]).This is evidence that bone formation exceeds bone resorption, local use of1% bisoprolol gel enhanced osteogensis at the defect area in rabbit femur , as well other beta blockers used in mice resulted in significant increase in bone mass ,in vertebrae and long bones secondary to increase in bone formation rate and number of osteoblast([6]),and also in rats([22]),Beta blocker propranolol showed increase bone formation in compact bone of tibia and inhibited bone resorption in cancellous bone of femur of ovariectomized and non ovariectomized rats([23]). in conclusion local use of 1%bisoprolol gel favorably affected bone healing and osteogenesis.

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