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Compressive strength of Phenytoin and Sodium Fluoride effects on cancellous bone defect in rats

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ABSTRACT

Bone rebuilding was one of the surgeons' motivations from the past. The purpose of this study was biomechanics evaluate the effect of sodium fluoride and phenytoin on cancellousbone defect healing in rat model.The experiment was conducted on 40 male adult SD rats which were divided into eight groups of control and experiments. After induction of general anesthesia, a hole in size of 2×3 mm in diameter and depth was made using a dental bit in the inner aspect of the between condyles of right femor. In all groups, defect was left untreated. Control group was given water and group II received daily 5 mg/kg/IP of Phenytoin and groups III, IV and V were exposed to fluoridated water at different concentrations (8, 30 and 60 mg F¯/L), group VI, VII and VIII received together of daily 5 mg/kg/IP of Phenytoin and fluoridated water at different concentrations (8, 30 and 60 mg F¯/L). After 45 days all rats were sacrificed and biomechanical penetration tests of the distal femoral bone were performed. Data were submitted to statistical analysis by variance analysis (ANOVA) at a significance level of 5% ($p<0.05$).The results show positive effect of phenytoincompared to the control group. Also in the groups receiving fluoride and phenytoin, the best result is related to the group receiving 30 mg. And increase the amount of fluoride causes a negative impact the compressive strength of the bone healing.The results of this study show that fluoride level at 30 mg F¯/L in drinking water and 5 mg/kg phenytoin increases compressive strength of cancellous bone defect in healing process and could stimulate osteogenesis in femoral cancellous bone defect in rats.

Key words: Sodium fluoride, Phenytoin, cancellous bone, Compressive strength, rats

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INTRODUCTION

The factor time is most important effect on healing of bone fractures, whatever, the case, which takes the form of faster healing of bone defect will be have greater consistency and strength and possibility of delay in union or non-union will be decreased and occurrence of infection and fail in orthopedic surgery will be decreased [1-3].Sodium fluoride is effective in bone formation and increase in cancellous bone volume[4-6]. Sodium fluoride in clinical use for treatment of osteoporosis and vertebral fractures[7, 8].Previous studies showed that the positive effects of phenytoin in bone healing. In these studies, the positive effects of phenytoin on bone metabolism have been mentioned[9]. Use of phenytoin in low-doses resulted in improvement of histomorphic parameters of bone formation and has osteogenic characteristics under in-vivo conditions [10].The findings show thatdaily injections of phenytoin significantly increase ALP and Osteocalcinand also have positive effects on bone healing[11].The purpose of the present studywas to investigate the healing effect of Phenytoin and Sodium fluoride on cancellousbone defect in rats that is a biomechanical evaluation.

MATERIALS AND METHODS

Investigations using experimental animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care, and our ethical committee on animal care approved the protocol. The experiment was performed on 40 male adult Sprague-Dawley rats, 250–300 gram. Rats were obtained from the central animal laboratory of veterinary medicine faculty of Islamic Azad University-Tabriz Branch and were housed in colony rooms with 12/12 hr light/dark cycle at 21±2°C for 2 weeks before initiation of the study, fed with laboratory pellet chow and drinking water was given ad libitum.

Animals were anesthetized with Ketamine hydrochloride (Ketamine 10%, Alfasan, Woerden-Holland, 50 mg/kg) and Xylazine (Xylazin 2%, Alfasan, Worden-Holland, 5mg/kg) intraperitoneally. The right hind limb was routinely prepared for surgery. A 2-cm skin incision was made on the medial aspect of the distal femoral condyles. The muscle and articular capsule were dissected bluntly to expose the lateral and medial condyles. The distal femoral condyles were exposed anda confined cancellous defect was created in between of lateral and medial condyles using a low-speed dental bit, saline-cooled in a stepwise fashion. A hole in size of 2×3 mm in diameter and depth was made and left untreated. The muscle attachment was sutured with simple stitches using Vicryl 4.0 suture with nontraumatic needle, and skin was sutured with silk suture 4.0, being removed at seven days after surgery. The animals received antibiotics Enrofloxacin 2.5% intramuscular in the dose of 2.5ml/Kg of body weight for five days after surgery, and anti-inflammatory Banamine (FlonexinMeglumine) injected in the dose of 1.1ml/ Kg of body weight for three days after surgery by applications in the muscle.After surgery rats divided into eight groups of 5 animals each, according to the procedure performed:

(1): control group (Group I), (2): 5 mg/kg/day/IP Phenytoin (Group II), (3): 8 mg/L NaFin drinking water (Group III), (4): 30 mg/L NaFin drinking water (Group IV),(5): 60 mg/L NaFin drinking water (Group V), (6): 5 mg/kg/day/IP Phenytoin and 8 mg/L NaFin drinking water (Group VI), (7): 5 mg/kg/day/IP Phenytoin and 30 mg/L NaFin drinking water (Group VII), (8): 5 mg/kg/day/IP Phenytoin and 60 mg/L NaFin drinking water (Group VIII). Animals were sacrificialed after 45 days postoperatively under general anesthesia, with an injection of over dosage of Thiopental sodium (60 mg/kg). The distal of right femurs include osseous defect were harvested, stripped of soft tissues and wrapped in saline-soaked gauze, frozen and stored. Specimens were thawed at room temperature in a saline bath prior to mechanical testing. All mechanical testing were performed using a biomechanics measurement device (Zwick/Roell Z010, TI-PR010TH.A50 model) with a crosshead speed of 0.01 mm/sec. A load-distance curve was recorded to determine mechanical properties. The central load support was applied to the lateral and medial condyles of femur. Maximum stress and maximum strain were calculated based on standard engineering equations for three-point bending [12].

Statistical evaluation of data was performed using the software package SPSS version 18 (SPSS Inc., Chicago, IL). Data are reported as mean±standard deviations (SD). The significant level was set at $p<0.05$. Statistical comparisons were used analysis of variance (ANOVA). Tukey HSD multiple comparison testing was used to determine experimental defects with normal bone.

RESULTS AND DISCUSSION

Survey results show that, Sodium fluoride increases bone strength in group III than control group.Alsothe results of the group IV shows increases bone strength in this group compared with the control group and group III. But results of group V shows a decrease in bone strength in this than groups III, IV and control group.It should be noted that, reduce the amount of strength that occurs in Group V, indicative increase the negative effects of sodium fluoride and slow healing in defects of this group. Survey data indicates the significance of the results, as group 30 mg sodium fluoride compared to other groups that have been treated with sodium fluoride show increase the compressive strength.Phenytoin injection in group II has caused bone strength increased compared to the control group and represents positive effect of phenytoin.Used together of 5 mg phenytoin and sodium fluoride in groups VI, VIIand VIII show with theincreasing dose of sodium fluoride of 8 mg to 30 mg increases bone strength, but with increasing reload sodium fluoride to 60 mg reduced bone strength.Results of mechanical tests obtained in this study show that the mean load for fracturing in group VII is significantly higher than other groups. The mean±SD of mechanical test results for each group are provided in table 1.

Table 1.Mean±STD of biomechanical results

	Control	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
Max Stress (Force N)	173 ±23.05	175.20 ±22.4	177.31 ±36.81	181.90 ±36.81	168.51 ±54.35	180.05 ±21.05	201.13 ±22.31	175.21 ±12.11

Survey results show that feeding of sodium fluoride cause increases bone strength in group III (8 mg/L NaF) to the control group.Also, with increasing doses of sodium fluoride to 30 mg/L in group IV causeincreases bone strength compared with the group III and control group.But increasing the dose of sodium fluoride to 60 mg/L in group V, reduced bone strength in these groups, which is less than the groups III and IV and control group.The compressive strength decreased in groupV, can attribute to the increase negative impact of sodium fluoride and created slow healing of the defect.The statistics of these results indicate that significance.As in previous studies has been shown, daily injections of phenytoin (5mg/kg) causes positive effect on bone healingand bone strength in group II compared with control group.Phenytoin injection (5mg/kg/day) in group VI with 8 mg/L sodium fluoride in drinking water was significantly increased bone strength compared to the control group and group II, and compared with group 3 that was only8 mg/L sodium fluoride in drinking water have greater bone strength.With increasing doses of sodium fluoride to 30 mg/Lin drinking water with daily injections of 5 mg/kg phenytoin, significant increases in bone strength than the other groupswas created, and compared with the group IV that was used only 30 mg/L in drinking water sodium fluoride, there was also a significant increase in bone strength.However, increasing doses of sodium fluoride to 60 mg/L in drinking water with daily injections of 5 mg/kg phenytoin reduced bone strength than other groups. Only had more strength than the control group and had similar bone strength with group II. Compared with group 5, that was used only 60 mg/L in drinking water sodium fluoride, bone strength was greater, that is the effect of phenytoin.

Mousavi et al. have recently reported that fluoride increased cancellous bone formation at defect area in the rat at 30 mg F^-/L [13]. Bohatyrewicz et al. have reported the anabolic action of fluoride demonstrated in the form of increased bone mineral mass of whole femoral bones [14]. Ohta et al. reported fluoride can act as a mitogen enhancer in osteoblast [11]. Fluoride prevents dephosphorylation protein phosphatase activity in osteoblast cells and fluoride prevents dephosphorylation of phosphotyrosyl proteins believed to be key in mediating proliferation and differentiation of cells and it causes increasing themitogenic signals generated by endogenous growth factors [15, 16].Sklans et al., it demonstrated that use of phenytoin may improve healing of mandibular fractures in rabbits. In that study, the positive effects of phenytoin on bone metabolism have been mentioned [17]. Ohta et al., 1995 showed that daily injection of phenytoin at the 5 mg/kg result in significance increase in values of 2 important factors, ALP and Osteocalcin, in bone formation and also has positive effects on bone histomorphic parameters [11].It seems that a low dose of phenytoin has osteogenic properties and can stimulate the ossification [10].

CONCLUSION

We have recently obtainedthat together use of 5mg/kg Phenytoin and 30mg/L Sodium Fluorideincreased cancellous bone formation at defect area.

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