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Original Article

Generation and evaluation of three rat models with alcoholic osteoporosis

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Abstract: Background: Osteoporosis is a skeletal disorder which could be influenced by diet. Chronic and excessive alcohol consumption results in increasing risk of osteoporosis. Methods: In the present study, we compared three osteoporosis models of rats, which were generated by gastric tube feeding, intraperitoneal injection and fluid forage feeding with ethanol. All of the Sprague-Dawley rats were randomly divided into four groups, including control group which were fed basal diet, intragastric injection group, intraperitoneal injection group and fluid forage group. After six months' treatment, we determined the bone mineral density (BMD), blood and uric biochemical examinations, pathological examinations and bone biomechanics. Results: Our results revealed that chronic alcoholic consumption significantly reduced the BMD in femur and enhanced the expressions of serum calcium, serum phosphorus and urinary calcium. In addition, induction of ethanol resulted in increases of alkaline phosphatase (ALP), tartrate resistant acid phosphatase (TRACP), and decreases of bone glaprotein (BGP), the maximum load and stiffness of femur. Among the three alcoholic osteoporosis models of rats, intraperitoneal injection presented to be an optimization. Conclusion: Based on our results, the alcohol-induced osteoporosis rat model constitutes a tool of great value for future pre-clinic researches.

Keywords: Alcohol consumption, osteoporosis, rat

Introduction

Osteoporosis is a bone debilitating disease characterized by low bone mass and deteriorated microarchitecture leading to skeletal fragility, which affecting more than 10 million people around the world. As the incidence of osteoporosis is raising every year, fractures have been the third highest cause of becoming bedridden [1]. It has been reported that a reduction of bone mass was found in alcoholics, especially in femoral neck, which contains a high proportion of trabecular bone [2]. Alcohol abuse is an independent risk factor for osteoporosis. And, the prevalence of alcohol-induced osteoporosis is increasing. There are three different extents of alcohol intake: light, moderate, and binge drinking. As pointed out, long-term and heavy alcohol intake causes damage to multiple organs, including bones, and induces secondary osteoporosis [3, 4]. Published studies demonstrated that alcohol mainly affects bone metabolism via suppressing osteoblast activity and new bone formation [5].

The major animal models for osteoporosis are postmenopausal osteoporosis, disuse osteoporosis and glucocorticoid-induced osteoporosis [6, 7]. Till the date, several animal models of alcohol-induced osteoporosis have been suggested. Rat is frequently used as a popular animal model because the bone metabolism of rat is similar to that of human. Nevertheless, the rat models for alcoholic osteoporosis induced by different supply methods showed difference in osteocyte functions, which needs to be further clarified.

In this regard, to identify the optimized rat model, three alcoholic osteoporosis models of alcohol-induced Sprague-Dawley rats were generated in this experiment. In the present study, we demonstrated that a high level of long-term alcohol intake induced osteoporosis in rats. We assessed the BMD, biomechanical testing, histological analysis and bone biomechanics of three animal models for alcoholic osteoporosis.

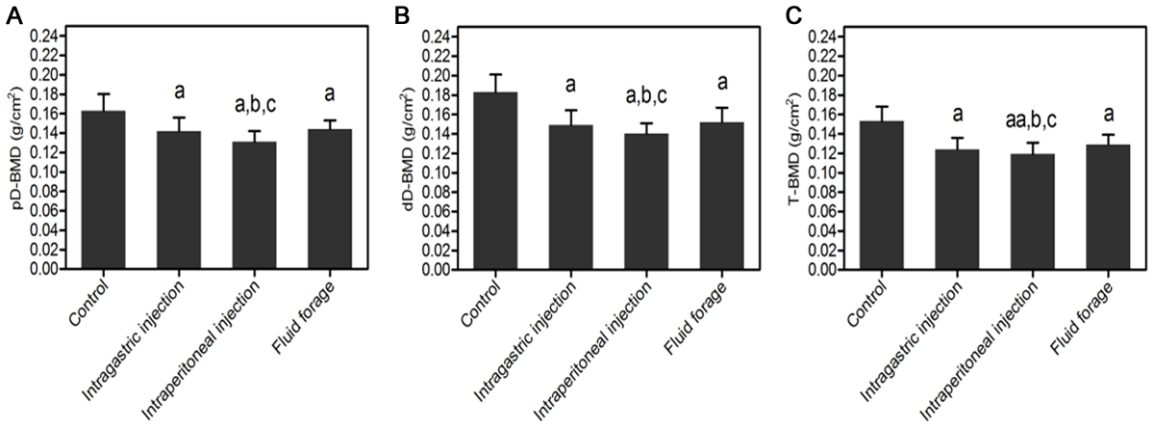

Figure 1. Bone mineral density at the end of the experiments. A. Proximal diaphyseal BMD (pD-BMD). B. Distal diaphyseal BMD (dD-BMD). C. Total BMD (T-BMD). Values are presented as mean ± SD, n=10 per group. a, $p<0.05$, compared to control group. aa, $p<0.01$, compared to control group. b, $p<0.05$, compared to intragastric injection group. c, $p<0.05$, compared to fluid forage group.

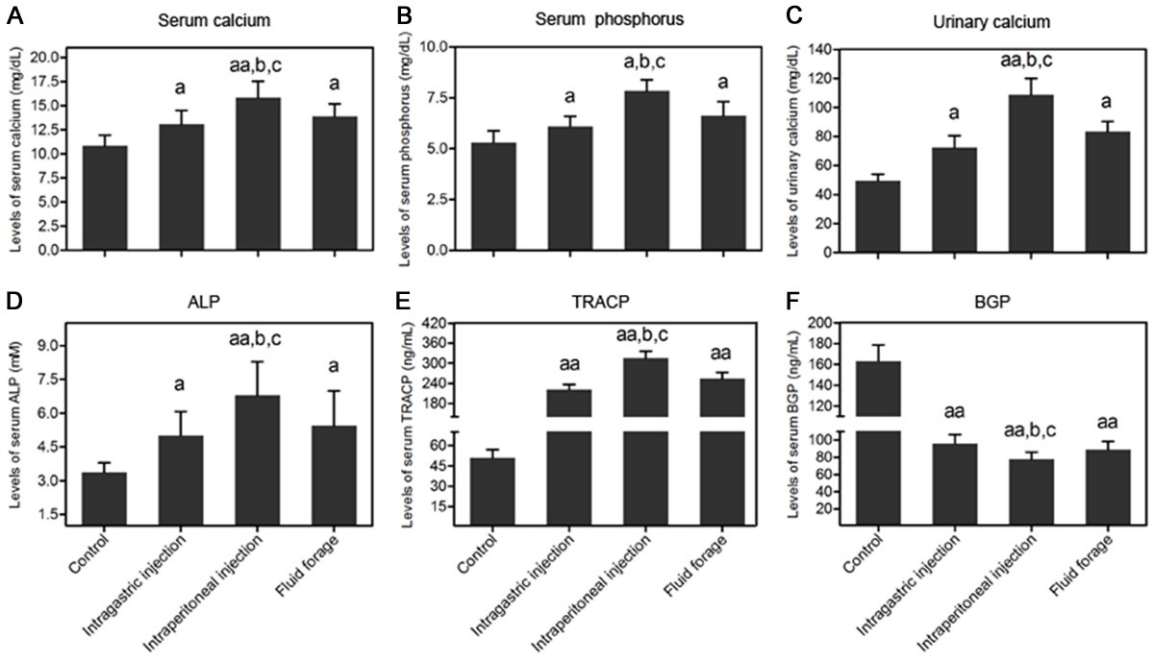

Figure 2. Biochemical analyses of serum and urine samples. The data represent mean ± SD. a, $p<0.05$, compared to control group. aa, $p<0.01$, compared to control group. b, $p<0.05$, compared to intragastric injection group. c, $p<0.05$, compared to fluid forage group.

Materials and methods

Experimental animals and treatments

Four-month-old Sprague-Dawley rats were purchased from Shanghai Laboratory Animal Corporation (n=40; mass, 200-250 g; half male and half female). All rats were maintained under controlled temperature at 23°C±1°C and humidity at 45%-65%, and were kept in cages under specific pathogen-free conditions with 12 h light/12 h dark cycle. Food and water was available freely. This research was approved by the ethical committee (Shanghai 6th People's Hospital Animal Care and Use Committee, SYXJ 2011-0128).

After an acclimation period of a week, all rats were randomly assigned to four groups. Grouping was as follows: (1) Control group, which received basal diet for 6 months. (2) Intragastric injection group, which was given ethanol at a

daily dose of 1 ml/k body weight by intragastric tube feeding, five times a week. (3) Intraperitoneal injection group, which dosed with 0.1 ml/k/d ethanol by intraperitoneal injection, five times a week. (4) Fluid forage group, which consumed diet with 1 ml/k/d ethanol, five times a week. The rats were euthanized after being maintained on their respective treatment for 6 months.

Measurements

BMD assay: The rats were euthanized for bones. The right femurs of all rats were dissected from muscle and fascia, soaked into 0.9% saline water, and then scanned with dual-energy X-ray absorptiometry (Model pDEXA Sabre; Norland, USA) with the resolution of 0.1 cm × 0.1 cm and a scan area of 2.0 cm × 2.0 cm to assess the BMD.

Serum and uric biochemical: Peripheral blood samples and 24 h urine were collected before the rats were euthanized. Serum was harvested after clotting at 4°C for 2 h and centrifuged at 3000 rpm for 15 min at 4°C. Serum and urine was stored at -80°C until assay. Biochemical examinations of serum samples were undertaken by an automatic clinical chemistry analyzer (Labospect 008; Hitachi, Japan). The expressions of calcium, phosphate, ALP, TRACP, and BGP were determined.

Histological analysis of the right femur: The right femurs of rats were fixed with 10% buffered formaldehyde solution, decalcified with EDTA (pH 7.4), and then embedded in paraffin, for histological analysis. The specimens were cut into 4 μm thick sections and stained with hematoxylin and eosin (H&E). The histopathological observation was performed on digital microphotographs (40 ×) of the sections under the microscope (BX53; OLYMPUS CORPORATION, Japan).

Biomechanical testing: The left mid-diaphyseal femur was tested for mechanical strength with three-point bending, using a universal testing machine (Electromechanical Material Testing Machine; Avalon Technologies, USA) as described previously [8]. The bone strength was tested at a span length of 20 mm and a loading speed of 1.5 mm/min. The maximum load (N) and the ultimate stiffness (N/mm) were obtained.

Statistical analysis

The data in all cases are presented as mean ± standard error. Statistical analyses of the data were performed by using Graphpad prism 6.0 software. Independent two-tailed student's t-tests were conducted to compare two groups. One-way analysis of variance (ANOVA) was used to compare more than two groups. P values < 0.05 were considered significantly different.

Results

BMD analysis of right femur

No death was observed in all rats in this study. To evaluate the effect of alcohol on bone loss in three different rat models, the BMD of the right femur was measured. As shown in **Figure 1**, we observed a significantly lower femoral pD-BMD, dD-BMD and T-BMD in intragastric injection group compared to control group (p<0.05). Intraperitoneal injection group presented a significantly lower femoral pD-BMD, dD-BMD and T-BMD than control group (p<0.05). Compared to control group, consumption of fluid forage with alcohol significantly decreased the femoral pD-BMD, dD-BMD and T-BMD (p<0.05). We also observed a remarkable decline of femoral pD-BMD, dD-BMD and T-BMD in intraperitoneal injection group, compared to intragastric injection and fluid forage group (p<0.05).

Biochemical analyses of serum and urine samples

After 6-month administration period, the levels of serum calcium, serum phosphorus, urinary calcium, ALP, TRACP and BGP were detected. The levels of serum calcium, serum phosphorus, urinary calcium, ALP and TRACP were significantly higher in the intragastric injection, intraperitoneal injection and fluid forage group than in control group (**Figure 2**, p<0.05). In the same way, levels of serum calcium, serum phosphorus, urinary calcium, ALP and TRACP in intraperitoneal injection group increased significantly, compared to intragastric injection and fluid forage group (p<0.05). A significant decrease of BGP level was found in intragastric injection, intraperitoneal injection and fluid forage group, compared to control group (p<0.05). Also, a remarkable reduction of BGP level was found in intraperitoneal injection group, compared to intragastric injection and fluid forage group (p<0.05).

Histological analyses of the femur

To illuminate the effect of treatment to rats, histological analyses were carried out by HE staining. As shown in **Figure 3**, completed trabecular structure and ordered arrangement of trabecu-

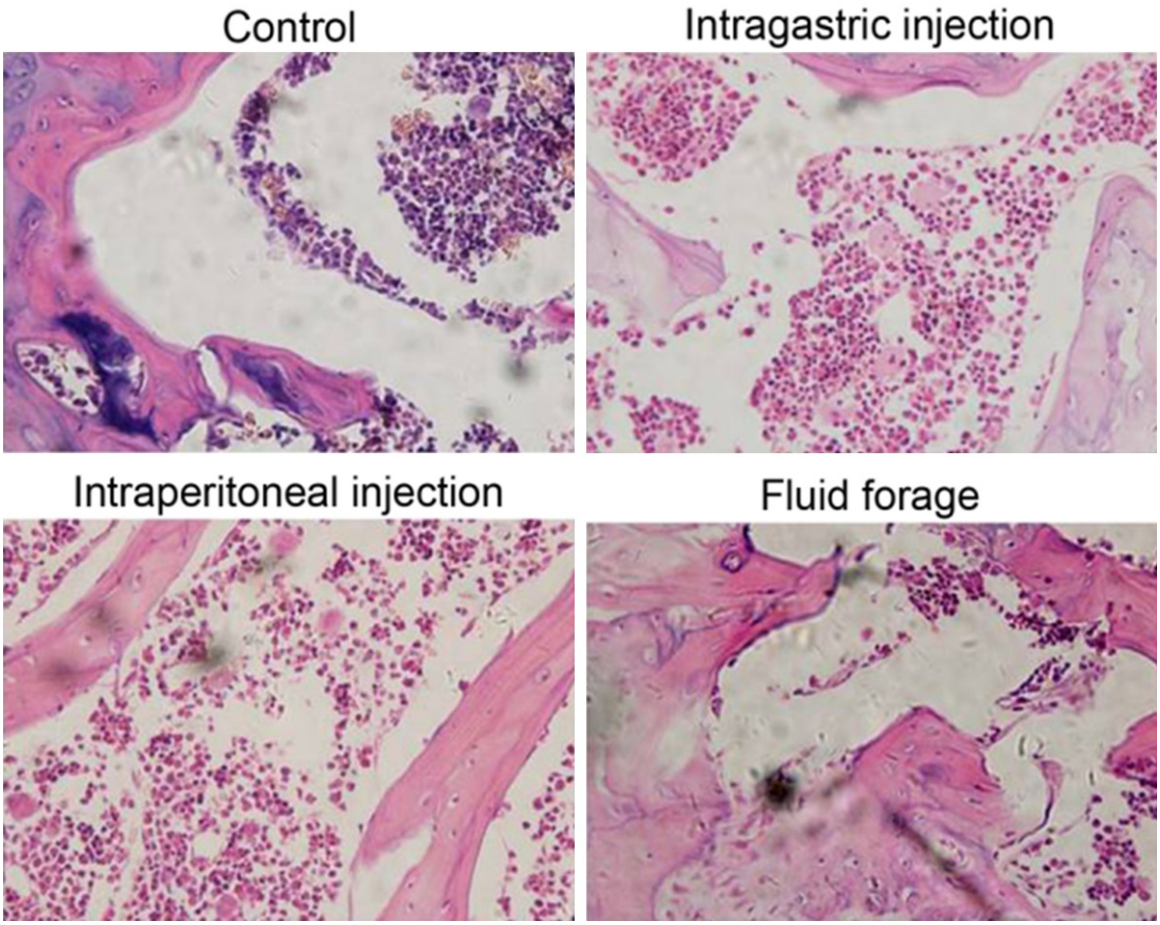

Figure 3. Representative images of HE staining sections. The morphological changes were evaluated under an optical microscopy (× 40).

Table 1. Bone biomechanical properties of femur

Group	Maximum load (N)	Stiffness (N/mm)
Control	133.87±12.45	251.04±21.96
Intragastric injection	95.01±8.06[aa]	180.72±26.16[aa]
Intraperitoneal injection	72.34±6.70[aa,b,c]	140.96±16.37[aa,b,c]
Fluid forage	90.44±4.55[aa]	163.03±19.78[aa]

The data expressed mean ± SD. [aa]$p<0.01$, compared to control group. [b]$p<0.05$, compared to intragastric injection group. [c]$p<0.05$, compared to fluid forage group.

lar was found in control group. Alcohol intake for 6 months in the intragastric injection, intraperitoneal injection and fluid forage group led to thinning of bone trabecular, decreased osteocytes and enlarged medullary cavity.

Bone biomechanical analyses

Osteoporosis is characterized as compromised bone strength. Treatment of alcohol declined maximum load and stiffness of the femur, compared to control group (**Table 1**, $p<0.05$). The maximum load and stiffness of intraperitoneal injection showed marked decrease compared to intragastric injection and fluid forage group ($p<0.01$).

Discussion

Osteoporosis is a chronic systemic bone disease which results in the loss of bone mass. Excessive alcohol consumption is generally associated with bone loss and plays an important role in disease prevalence. Studies indicated that alcohol may suppress osteoblast activity and stimulate osteoblast activity [9-13]. At this time, suitable animal models for osteoporosis are of great value and essential. Till date, there are several animal models for three major types of osteoporosis. However, for the un-going osteoporosis research, there are no perfect animal models for alcohol-induced

osteoporosis. Small animal model such as rat model is preferred because of faster metabolism and aging rates [14]. Moreover, adult ovariectomized rats as osteoporosis have been well studied [15-17].

In this study, we established and evaluated three alcoholic osteoporosis models of rats. To our knowledge, the BMD was considered as the gold standard to evaluate the incidence of osteoporosis. The reduction of femoral pD-BMD, dD-BMD and T-BMD following long-term detrimental alcohol consumption was observed in our study. Among the three model groups, BMD of intraperitoneal injection group presented significant difference compared to other two alcohol-treated groups.

Calcium is an essential nutrient for bone. Also, phosphorus plays a vital role in bone development and maintenance. In the clinic, ALP and BGP were most commonly evaluated as bone formation indicators, while TRACP was commonly used as bone resorption indicator. In our experiment, we found an elevation of serum calcium, serum phosphorus and urinary calcium levels in the intragastric injection, intraperitoneal injection and fluid forage group, especially in intraperitoneal injection group, suggesting that alcohol intake affected the bone and energy metabolism of rats.

Furthermore, inferior bone strength was considered as one of the standard criteria of osteoporotic bone. Values of maximum load and stiffness are indicative of bone strength. We demonstrated that maximum load and stiffness of the femur in the intragastric injection, intraperitoneal injection and fluid forage group declined significantly, especially in intraperitoneal injection group.

Conclusion

We were able to affect the bone metabolism in rats and cause pronounced systemic bone loss and trabecular structure deterioration by intragastric, intraperitoneal injection and fluid forage feeding with chronic excessive alcohol. The above findings not only indicated that alcohol-induced osteoporosis rat models were generated successfully but also implied that intraperitoneal injection of alcohol might be the optimization. Based on our results, the alcohol-induced osteoporosis rat model constitutes a tool of great value for future pre-clinic researches.

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Disclosure of conflict of interest

None.

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References

[1] Ishimi Y. Osteoporosis and lifestyle. J Nutr Sci Vitaminol (Tokyo) 2015; 61 Suppl: S139-141.
[2] Nilsson BE and Westlin NE. Changes in bone mass in alcoholics. Clin Orthop Relat Res 1973; 229-232.
[3] Clark MK, Sowers MF, Dekordi F and Nichols S. Bone mineral density and fractures among alcohol-dependent women in treatment and in recovery. Osteoporos Int 2003; 14: 396-403.
[4] Do SH, Jeong WI, Jeong DH, Ki MR, Lee IS, Kwak DM, Kim TH, Kim YK, Kim SB and Jeong KS. Alcohol-induced bone degradation and its early detection in the alcohol-fed castrated rats. Mol Cell Biochem 2006; 282: 45-52.
[5] Turner RT, Kidder LS, Kennedy A, Evans GL and Sibonga JD. Moderate alcohol consumption suppresses bone turnover in adult female rats. J Bone Miner Res 2001; 16: 589-594.
[6] Ochi H and Takeda S. [Animal models for bone and joint disease. The genetically-modified mice as a tool for osteoporosis research]. Clin Calcium 2011; 21: 226-232.
[7] Oheim R, Schinke T, Amling M and Pogoda P. Can we induce osteoporosis in animals comparable to the human situation? Injury 2016; 47 Suppl 1: S3-9.
[8] Han N, Xu J, Xu F, Liu Z and Yin J. The in vivo effects of a fraction from Dioscorea spongiosa on glucocorticoid-induced osteoporosis. J Ethnopharmacol 2016; 185: 53-59.
[9] Alvisa-Negrin J, Gonzalez-Reimers E, Santolaria-Fernandez F, Garcia-Valdecasas-Campelo E, Valls MR, Pelazas-Gonzalez R, Duran-Castellon MC and de Los Angeles Gomez-Rodriguez M.

Osteopenia in alcoholics: effect of alcohol abstinence. Alcohol Alcohol 2009; 44: 468-475.
[10] Bauer NB, Khassawna TE, Goldmann F, Stirn M, Ledieu D, Schlewitz G, Govindarajan P, Zahner D, Weisweiler D, Schliefke N, Bocker W, Schnettler R, Heiss C and Moritz A. Characterization of bone turnover and energy metabolism in a rat model of primary and secondary osteoporosis. Exp Toxicol Pathol 2015; 67: 287-296.
[11] Chakkalakal DA. Alcohol-induced bone loss and deficient bone repair. Alcohol Clin Exp Res 2005; 29: 2077-2090.
[12] Diez-Ruiz A, Garcia-Saura PL, Garcia-Ruiz P, Gonzalez-Calvin JL, Gallego-Rojo F and Fuchs D. Bone mineral density, bone turnover markers and cytokines in alcohol-induced cirrhosis. Alcohol Alcohol 2010; 45: 427-430.
[13] Gaddini GW, Turner RT, Grant KA and Iwaniec UT. Alcohol: a simple nutrient with complex actions on bone in the adult skeleton. Alcohol Clin Exp Res 2016; 40: 657-671.
[14] Quinn R. Comparing rat's to human's age: how old is my rat in people years? Nutrition 2005; 21: 775-777.
[15] Francisco JI, Yu Y, Oliver RA and Walsh WR. Relationship between age, skeletal site, and time post-ovariectomy on bone mineral and trabecular microarchitecture in rats. J Orthop Res 2011; 29: 189-196.
[16] French DL, Muir JM and Webber CE. The ovariectomized, mature rat model of postmenopausal osteoporosis: an assessment of the bone sparing effects of curcumin. Phytomedicine 2008; 15: 1069-1078.
[17] Yoon KH, Cho DC, Yu SH, Kim KT, Jeon Y and Sung JK. The change of bone metabolism in ovariectomized rats: analyses of MicroCT scan and biochemical markers of bone turnover. J Korean Neurosurg Soc 2012; 51: 323-327.