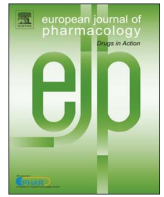
European Journal of Pharmacology 791 (2016) 331–338

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar



Endocrine pharmacology

Aspirin prevents bone loss with little mechanical improvement in high-fat-fed ovariectomized rats

Sien Lin [a,b,c,1], Wayne Y.W. Lee [b,c,1], Meiling Huang [d,1], Ziwei Fu [a], Yanlong Liang [a], Haiyou Wu [a], Liangliang Xu [b,c], Chun Wai Suen [b], Jianping Huang [e], Tie Wu [a], Liao Cui [a,*], Gang Li [b,c,**]

[a] Department of Pharmacology, Guangdong Key Laboratory for Research and Development of Natural Drugs, Guangdong Medical University, Zhanjiang, Guangdong, China
[b] Department of Orthopaedics and Traumatology and Li Ka Shing Institute of Health Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong, China
[c] The CUHK-ACC Space Medicine Centre on Health Maintenance of Musculoskeletal System, Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen, Guangdong, China
[d] Department of Rehabilitation, The Second People's Hospital of Shenzhen, Shenzhen, Guangdong, China
[e] Department of Stomatology, Guangdong Medical University, Zhanjiang, Guangdong, China

ARTICLE INFO

Article history:
Received 22 June 2016
Received in revised form
8 September 2016
Accepted 8 September 2016
Available online 9 September 2016

Keywords:
Aspirin
Osteoporosis
Ovariectomy
High fat diet
Biomechanics
Inflammation

Chemical compounds studied in this article:
Aspirin (PubChem CID: 2244)
Calcein (PubChem CID: 65079)
Cholesterol (PubChem CID: 5997)
Sodium cholate (PubChem CID: 23668194)
Propylthiouracil (PubChem CID: 657298)
Methyl methacrylate (PubChem CID: 6658)

ABSTRACT

Obesity and osteoporosis are often concurrently happened in the menopausal women. Obesity in menopausal women is not only related to a high risk of cardiovascular disease, but also results in a detrimental effect on bone health. This study aimed to investigate the effects of aspirin, a popular antithrombosis drug, on bone quantity and quality in the high-fat-fed animal model. Adult female rats were subjected to either sham operations or ovariectomized operations. The ovariectomized rats were orally administered with deionized water or standardized high fat emulsion with or without aspirin. All rats were injected with calcein before killed for the purpose of double *in vivo* labeling. Biochemistry, histomorphometry, micro-computed tomography analysis, mechanical test, and component analysis were performed after 12 weeks. *In vitro* cell culture was also performed to observe the effect of aspirin in osteogenesis. We found that high fat remarkably impaired bone formation and bone biomechanics. Aspirin treatment significantly prevented bone loss by increasing bone formation. *In vitro* studies also validated the enhancement of osteogenic differentiation. However, aspirin presented no significant improvement in bone mechanical properties. Component analysis shown aspirin could significantly increase the content of mineral, but had limited effect on the content of collagen. In conclusion, aspirin is beneficial for the prevention of bone loss; meanwhile, it may cause an imbalance in the components of bone which may weaken the mechanical properties. The current study provided further evidence that aspirin might not be powerful for the prevention of fracture in osteoporotic patients.

© 2016 Published by Elsevier B.V.

1. Introduction

Aspirin is one of the nonsteroidal anti-inflammatory drugs (NSAIDs), and it has been used over one hundred and fifty years for the treatment of acute pain, inflammation, and fever. Aspirin is also recommended for the prevention of cardiovascular events such as acute myocardial infarction (Lewis et al., 1983), ischemic shock (Diener et al., 2004) and peripheral vascular diseases (Hirsch, 2001) by the antiplatelet effect. It is believed that the imbalance in bone turnover after menopause may be related to the imbalance in immune system (Arron and Choi, 2000; Lorenzo, 2000). Considering the close relationship between inflammation and osteoporosis, people have attempted to manage the progression of osteoporosis by using aspirin. Actually, the relationship of using aspirin and postmenopausal osteoporosis in women has been studied in the past two decades. A finding shown that regular use of aspirin in a small dose can improve bone mineral density (BMD) in postmenopausal women (Carbone et al., 2003). However, studies of the relationship between the use of aspirin on BMD and fracture risk are conflicting in postmenopausal women (Bauer

* Correspondence to: Guangdong Key Laboratory for Research and Development of Natural Drugs, Guangdong Medical University, Zhanjiang 524023, China.
** Correspondence to: Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong Special Administrative Region.
E-mail addresses: cuiliao@163.com (L. Cui), gangli@cuhk.edu.hk (G. Li).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.ejphar.2016.09.018
0014-2999/© 2016 Published by Elsevier B.V.

et al., 2009; Vestergaard et al., 2012). It appeared to have no clinical significance in the reduction of subsequent risk of fractures by regular use of aspirin (Bauer et al., 2009; Vestergaard et al., 2012). However, the reasons of the inconsistency between bone mass and bone biomechanics in these clinical studies still have not been elucidated.

Obesity and osteoporosis are often concurrently happened in the menopausal women, both of which are becoming major public health concerns (Reid, 2008; Zhao et al., 2007). Obesity in menopausal women is not only related to a high risk of cardiovascular disease (Mathieu et al., 2010), but also results in a detrimental effect on bone health (Reid, 2008; Zhao et al., 2007). Our previous study has shown that bone mechanical properties could be significantly impaired by high fat diet in the ovariectomized (OVX) rats (Lin et al., 2015). We then hypothesized that aspirin which designed for the prevention of cardiovascular disease might also show protective effect on bone health. Then we design this study to observe the effects of aspirin, a popular anti-inflammation and anti-thrombosis drug, on bone quantity and quality in the high-fat-fed animal model. And this study is also designed to explore the underlying mechanisms of aspirin acting on bone health.

The dose of aspirin (9 mg/kg/d) used in this study was equivalent to that (100 mg/d) prescribed for preventing humans cardiovascular events by clinicians (Huang et al., 2004; Lin et al., 2015; U.S. Food and Drug Administration, 2005). Blood biochemistry, bone histomorphometry, mechanical test, micro-computed tomography (micro-CT), component analysis, and *in vitro* cell culture were performed in this study.

2. Material and methods

2.1. Animal model and treatments

All procedures involving animals were in accordance with the ethical requirements of the Laboratory Animal Ethical Committee of the Guangdong Medical University. 3-month-old Sprague-Dawley female rats were acclimated to local vivarium conditions (temperature 24–26 °C, humidity 67%) and allowed free access to water and diets containing 1.11% calcium, 0.74% phosphorus. All rats received four subcutaneous injections with calcein (10 mg/kg, Sigma-Aldrich, St. Louis, MO, USA) on day 14, day 13, day 4 and day 3 before sacrifice.

The rats were subjected to either sham operation (n=8) or bilaterally OVX operation (n=24) as previous described (Cui et al., 2004, 2001). Both the sham-operated rats (CON, n=8) and the OVX rats (OVX, n=8) were orally administered deionized water. The remaining OVX rats (n=16) were orally administered 5 ml/kg/d standardized high fat emulsion containing 50% lard oil (local fresh market), 10% cholesterol (China Xinxing Chemical Institute, Shanghai, China), 2% sodium cholate (China Xinxing Chemical Institute, Shanghai, China) and 1% propylthiouracil (Jinghua Pharmaceutical Co., Nantong, Jiangsu, China) without addition (n=8, OVX+Fat) or with the addition of 9.0 mg/kg/d aspirin (Bayer AG, Leverkusen, Germany; n=8, OVX+Fat+As). All rats were treated for 12 weeks post operation.

2.2. Sample collection and applications

Rats were weighed weekly. At the endpoint, the rats were killed by cardiac puncture under anesthesia using overdose of 20% sodium pentobarbital. The serum was collected for biochemical assays. The 4th lumbar vertebras (LV4s) were dissected for trabecular micro-CT analysis. The left proximal tibial metaphyses (PTMs) were performed in undecalcified sections for analysis of bone histomorphometry. The right femora and 5th lumbar vertebras (LV5s) were dissected for measurement of biomechanics. The right femora were then digested for component analysis.

2.3. Serum markers assays

Blood was collected in specimen tubes and kept at 25 °C for 40–50 min in a vertical position until completely clotting. And then the serum was separated by centrifuging at 1000*g* for 10 min and stored at −80 °C for biochemical markers assays. Serum levels of osteocalcin (Immunodiagnostic System, Tyne and Wear, UK), tartrate-resistant acid phosphatase 5b (TRACP-5b, Biomedical technologies, Stoughton, MA, USA), and IL-6 (Boster bioengineering, Wuhan, Hubei, China) were determined by enzyme-linked immunosorbent assay (ELISA) with commercial kits using ELX800 Microplate Reader (Bio-Tek Instruments, Winooski, VT, USA) according to the protocols attached.

2.4. Microcomputed tomography (micro-CT) scanning

The LV4 were scanned using a desktop preclinical specimen micro-CT (μCT-40, Scanco Medical, Bassersdorf, Switzerland). Briefly, the vertebral bodies were aligned perpendicularly to the scanning axis for a total scanning length of 6.0 mm at custom isotropic resolution of 8-μm isometric voxel size with a voltage of 70 kV p and a current of 114 μA. Three dimensional (3D) reconstructions of mineralized tissues were performed by an application of a global threshold (211 mg hydroxyapatite/cm^3), and a Gaussian filter (sigma=0.8, support=2) was used to suppress the noise. A volume of interest (VOI) containing only the trabecular bone within the vertebral body extracted from the cortical bone with 1.80 mm thick (150 slices) was acquired 1.0–1.2 mm from both cranial and caudal growth plate-metaphyseal junctions. The three dimensional reconstructed images were used directly to quantify microarchitecture, the morphometric parameters including bone volume fraction (BV/TV), bone mineral density (BMD, g/mm^2), trabecular thickness (Tb.Th, mm), trabecular separation (Tb.Sp, mm), and connective density (Con. D, 1/mm^3) were calculated with the image analysis program of the micro-CT workstation (Image Processing Language v4.29d, Scanco Medical, Switzerland) (Campbell et al., 2011; Lin et al., 2014).

2.5. Bone histomorphometry

The PTMs were collected and trimmed with IsoMet® precision bone saw (Buehler, Lake Bluff, IL, USA), and then were fixed in 10% buffered formalin for 24 h, followed by gradient alcohol dehydration, xylene defatting, and embedded in methyl methacrylate. The frontal PTM tissue was cut into 9 μm and 5 μm thick sections with the RM2155 hard tissue microtome (Leica, Wetzlar, Germany), respectively. The unstained 9 μm sections were used for dynamic histomorphometric analysis. The 5 μm sections were stained with Masson-Goldner trichrome for static histomorphometric measurements. A semi-automatic digitizing image analysis system (OsteoMetrics, Atlanta, GA, USA) was used for quantitative bone histomorphometry (Cui et al., 2012; Dempster et al., 2013).

The measurement region of PTM was cancellous bone between 1 and 4 mm distal to the growth plate-epiphyseal junction. The quantitative analysis was performed on each sample, with one section each. Dynamic histomorphometry and bone cell measurement were performed under magnifications of 100× and 400×, respectively. The histomorphometric measurements were done on the cancellous bone only. The abbreviations of the bone histomorphometric parameters used were recommended by the ASBMR Histomorphometric Nomenclature Committee (Dempster et al., 2013). Structural parameters were tissue volume (TV), bone volume (BV), adipocyte volume (AV), and bone surface (BS).

Download English Version:

https://daneshyari.com/en/article/8530394

Download Persian Version:

https://daneshyari.com/article/8530394

Daneshyari.com