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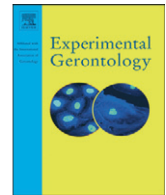

Advanced oxidation protein products accelerate bone deterioration in aged rats

Ji-Huan Zeng [a,1], Zhao-Ming Zhong [a,*,1], Xiao-Dan Li [b], Qian Wu [a], Shuai Zheng [a], Jian Zhou [a], Wen-Bin Ye [a], Fang Xie [c], Xiu-Hua Wu [a], Zhi-Ping Huang [a], Jian-Ting Chen [a,*]

[a] Department of Spinal Surgery, Nanfang Hospital, Southern Medical University, China
[b] Department of Nursing, Guangdong General Hospital, China
[c] Department of Gastroenterology, Nanfang Hospital, Southern Medical University, China

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ABSTRACT

Advanced oxidation protein products (AOPPs) are novel markers of oxidation-mediated protein damage, and accumulation of AOPPs is involved in many pathophysiological conditions. Our previous studies demonstrated that the serum level of AOPPs negatively correlated with the age-related change in bone mineral density (BMD) in rats and that AOPPs inhibited rat osteoblast-like cell proliferation and differentiation in vitro. However, whether AOPPs are involved in senile osteoporosis is still largely unknown. The present study aimed to test the hypothesis that accumulation of AOPPs might accelerate bone deterioration in aged rats. Seventy 18-month-old male Sprague Dawley (SD) rats were randomized to intravenous injection of vehicle, native rat serum albumin (RSA), AOPPs-modified RSA (AOPPs-RSA) with or without oral administration of apocynin (a nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor), or apocynin alone. After treatment for 8 weeks or 16 weeks, seven rats in each group were sacrificed. Bone and blood samples were harvested for BMD measurement, micro-computed tomographic (micro-CT) imaging, and biochemical analysis of circulating bone biomarkers. Compared to RSA- or vehicle-treated rats, AOPPs-RSA-treated animals displayed significantly decreased total vertebral BMD and deteriorated microstructure in both the tibias and the lumbar vertebral bodies, which were associated with down-regulated plasma bone-specific alkaline phosphatase concentration and up-regulated tartrate-resistant acid phosphatase 5b concentration. These AOPPs-induced perturbations in aged rats could be prevented by the oral administration of apocynin. However, no significant differences in BMD were detected in the femurs or the biomechanical parameters tested between the different treatment groups. These data suggest that accumulation of AOPPs accelerates bone deterioration in aged rats, likely via the activation of NADPH oxidase. This study provides new information toward understanding the pathogenic basis of senile osteoporosis and may provide targets for intervention.

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1. Introduction

Osteoporosis is a disease characterized by low bone mass and microstructure deterioration of bone tissue with a concurrent increase in bone fragility and fracture risk. It is estimated that in white populations, approximately 50% of women and 20% of men older than 50 years will have a fragility fracture in their remaining lifetime (Sambrook and Cooper, 2006). Therefore, osteoporosis is considered a major public health problem (Cummings and Melton, 2002). Numerous studies employing various design methods have dealt with the pathophysiology of postmenopausal osteoporosis (Raisz, 2005). In contrast, senile osteoporosis (SOP) has been less studied. SOP is thought to be a type of low turnover osteoporosis, resulting from age-related calcium deficiency and an imbalance between bone breakdown and formation (Duque and Troen, 2008). Over the past 30 years, many therapies, such as calcium, vitamin D and bisphosphonates, have been developed to treat SOP (Mosekilde et al., 2013). Despite significant advances in management, there remain a number of key unresolved issues regarding the pathogenesis of SOP.

An imbalance between peroxidation and antioxidant defense systems results in the accumulation of reactive oxygen species (ROS, e.g., superoxide anion radicals, hydrogen peroxide and hydroxyl radicals) and oxidative stress (OS). Then OS may cause damage to proteins, lipids, and DNA and even lead to cell death. Advanced oxidation protein products (AOPPs) are dityrosine-containing, cross-linking protein products and are considered novel markers of oxidant-mediated protein damage (Witko-Sarsat et al., 1996). Increased plasma levels of AOPPs have been found in many diseases, such as diabetes, uremia, obesity, coronary artery disease and inflammatory bowel diseases (Klima et al., 2012; Kocak et al., 2007; Krzystek-Korpacka et al., 2008; Piwowar

* Corresponding authors at: Department of Spinal Surgery, Nanfang Hospital, Southern Medical University, 1838 North Guangzhou Avenue, Guangzhou 510515, China.
E-mail addresses: zhongzm@smu.edu.cn (Z.-M. Zhong), chenjt99@tom.com (J.-T. Chen).

[1] Contributed equally to this work.

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et al., 2007; Witko-Sarsat et al., 1996). AOPPs were also demonstrated to be important agents in the progress of chronic renal failure, diabetic kidney and atherosclerosis (Liu et al., 2006; Shi et al., 2008; Witko-Sarsat et al., 1998). Furthermore, the pathological roles of AOPPs in vascular endothelial cell activation, mesangial cell perturbation and podocyte apoptosis have been well documented (Guo et al., 2008; Wei et al., 2009; Zhou et al., 2012).

OS plays a central role in human aging. Oxidative damage enhances and accumulates during the aging process (Finkel and Holbrook, 2000; Wei and Lee, 2002). As a class of protein-oxidation products, plasma AOPPs levels increase with age (Komosinska-Vassev et al., 2012). Our previous studies confirmed that there was a negative correlation between plasma AOPPs levels and the age-related changes in bone mass in male rats (Zhang et al., 2011), and AOPPs inhibited proliferation and differentiation of rat osteoblast-like cells by triggering ROS generation (Zhong et al., 2009). However, whether accumulation of AOPPs is actually involved in SOP is an urgent question to be further clarified. In the current study, aged male Sprague Dawley (SD) rats were used as an age-related osteoporosis model. Experimental rats were treated with or without AOPPs-rat serum albumin (RSA), plasma AOPPs levels, bone turnover markers, bone mineral density, the trabecular microstructure, and biomechanical quality were assessed to test the hypothesis that AOPPs may play a causal role in SOP in vivo.

2. Materials and methods

2.1. AOPPs-RSA preparation and determination

AOPPs-RSA was prepared in vitro as described previously (Witko-Sarsat et al., 1996; Zhong et al., 2009). In brief, RSA (Sigma, USA) solution (20 mg/ml) was incubated with 40 mM HCLO in phosphate buffered saline (PBS, pH 7.4) for 30 min at 37 °C. The reaction was then stopped via overnight dialysis against PBS to remove free HCLO. A control incubation was performed in native RSA dissolved in PBS alone. To remove contaminated endotoxin, all samples were passed through a Detoxi-Gel column. Endotoxin levels in AOPPs-RSA and unmodified RSA were then measured using a Limulus Amoebocyte Lysate kit and were found to be below 0.05 ng/mg protein. AOPPs content was determined in the samples as described previously (Witko-Sarsat et al., 1998). Briefly, 200 μl of sample or chloramine-T was placed in a 96-well plate, followed by 20 μl of acetic acid. The absorbance at 340 nm was obtained immediately in the microplate reader. AOPPs contents in the AOPPs-RSA and unmodified RSA were 54.10 ± 3.0 μmol/g protein and 0.20 ± 0.05 μmol/g protein, respectively.

2.2. Animals and treatments

Seventy 18-month-old male SD rats (body weight 652 ± 37 g) were randomized into five groups (n = 14 in each group) matched by body weight and received one of the following treatments: group 1, intravenous (iv) injection of vehicle (PBS, pH 7.4); group 2, iv injection of native RSA at 50 mg/kg d; group 3, iv injection of AOPPs-RSA at 50 mg/kg d; group 4, iv injection of AOPPs-RSA at 50 mg/kg d and apocynin (a nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor, Sigma, USA) at 100 mg/kg d in drinking water changed daily to quantify the volume of water intake; and group 5, apocynin at 100 mg/kg d in drinking water. The above dosage of treatment was given every day. After either 8 or 16 weeks, the animals were anesthetized with isoflurane (n = 7 for each time point), blood samples were collected via abdominal aorta puncture, and plasma was then separated and stored in −80 °C. The tibias, the femurs and the fourth lumbar vertebras (L4) were excised, covered with normal saline (NS) gauze and stored at −20 °C. All animal procedures were in accordance with guidelines set by the Animal Experiment Committee of Southern Medical University.

2.3. Assay of plasma AOPPs levels and bone turnover markers

The method of determining AOPPs levels in plasma was the same as the method used for AOPPs content determination in the AOPPs-RSA compound. Preceding plasma AOPPs measurement, total serum albumin levels were quantified using an ELISA kit (Beyotime, China). The concentrations of two specific markers of bone turnover, bone-specific alkaline phosphatase (BALP) and tartrate-resistant acid phosphatase 5b (TRACP 5b), were also quantified using ELISA kits (CUSABIO, China).

2.4. BMD measurement of femurs and L4 vertebral bodies

BMD measurements of total femurs and L4 vertebral bodies were performed as previously described (Lei et al., 2009). In brief, after thawing at room temperature for 30 min, left femurs and L4 vertebras with surrounding soft tissues removed were immersed in 0.9% NS. Subsequently, total BMD (t-BMD) of each sample was measured using a dual energy X-ray absorptiometer (NORLAND XR-46, USA) running the Small-object program.

2.5. Micro-CT analysis of left tibias and L4 vertebral bodies

The trabecular microstructure of left tibias and L4 vertebral bodies were analyzed using a micro-CT system (μCT80, SCANCO MEDICAL, Switzerland) at resolutions of 12 μm and 15 μm, respectively, with a tube voltage of 50 kV and a tube current of 0.1 mA. The volume of interest (VOI) was defined as 180 slices approximately 2.0 mm from the growth plate of the proximal tibia. As for the vertebral body, the trabecular bone region was outlined for each micro-CT slice, excluding both the cranial and caudal endplate regions. Within these regions, the trabecular bone was separated from the cortical bone using boundaries defined by the endocortical bone surfaces (Bouxsein et al., 2010). 3D images were obtained for visualization. Bone morphometric parameters, including bone volume over total volume (BV/TV), trabecular number (Tb.N), trabecular separation (Tb.Sp), trabecular thickness (Tb.Th), the degree of anisotropy (DA) and the structure model index (SMI), were obtained via analysis of the VOI. The operator conducting the scan analysis was blinded to the treatments associated with the specimens.

2.6. Three-point bending test of right femurs

Prior to biomechanical examination, the right femurs were slowly thawed for 3 h at room temperature. A distance between the intermalleolar region and the inter-condylar region was measured to determine the midpoint of the diaphysis. The femur was then placed in a material testing machine (Instron ElectroPuls, E1000, USA) on two supports separated by a distance of 20 mm, and load was applied to the midpoint of the diaphysis. Thus, a three-point bending test was created. The biomechanical quality of the right femoral diaphysis was determined at a loading speed of 2 mm/min. When the central loading point was displaced, the load and displacement were recorded until the specimen was broken. Based on the load–deformation curve, the maximum load (ultimate strength), the maximum displacement, the stiffness (slope of the linear part of the curve representing elastic deformation), and energy absorption (area under the curve) were obtained.

2.7. Statistical analysis

The data are expressed as the mean ± SD. All statistical analyses were performed using Statistical Packages for Social Sciences v13.0 software (SPSS, Chicago, IL). One-way analysis of variance (ANOVA) was used to test for differences among groups at the same time points. Post-hoc analysis of the LSD was performed on multiple comparisons between groups if one-way ANOVA was found to be statistically significant. Differences of the variables between two time points were

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