JOURNAL OF FOOD AND DRUG ANALYSIS XXX (2017) 1–9

Available online at www.sciencedirect.com

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journal homepage: www.jfda-online.com

Original Article

Sialoglycoprotein isolated from eggs of *Carassius auratus* promotes fracture healing in osteoporotic mice

Fei Wang, Lihua Han, Xiaohong Wang, Yuanyuan Li, Yujie Zhu, Jingfeng Wang, Changhu Xue*

College of Food Science and Engineering, Ocean University of China, Qingdao, Shandong Province, China

ARTICLE INFO

Article history:
Received 9 January 2017
Received in revised form
22 July 2017
Accepted 24 July 2017
Available online xxx

Keywords:
Carassius auratus eggs
Sialoglycoprotein
OVX
Fracture healing
Endochondral ossification

ABSTRACT

In this study, open tibial fracture surgery was performed on mice with ovariectomy induced osteoporosis to investigate the effect of a treatment with sialoglycoprotein isolated from *Carassius auratus* eggs (*Ca*-SGP) on fracture healing. Dynamic histological analysis showed that *Ca*-SGP promoted the generation of cartilage callus on day 5 post-surgery, then facilitated the transformation of the cartilage callus to bony callus on days 11 and 24 post-surgery, and enhanced the remodeling of bony callus on 35 day post-surgery. Moreover, *Ca*-SGP significantly decreased the secretion of TNF-α and IL-1β in serum on day 5 post-surgery, thus inhibiting the negative spread of the inflammatory reaction. On day 11 post-surgery, *Ca*-SGP clearly decreased the serum level and the mRNA expression of Aggrecan but also increased the secretion and the expression of VEGF and MMP13, thus promoting the degradation of the cartilage matrix and vascular invasion. On day 24 post-surgery, *Ca*-SGP remarkably increased the mRNA expression of osteogenesis markers Col1a and OCN, and increased callus BV/TV and Tb.N, this facilitating the formation of woven bone. On day 35 post-surgery, *Ca*-SGP enhanced the transformation of woven bone into lamellar bone and improved the callus biomechanical property. In conclusion, *Ca*-SGP promoted fracture healing in osteoporotic mice by accelerating endochondral ossification.

1. Introduction

Osteoporosis affects hundreds of millions of people worldwide, and is characterized by low bone density and strength, high bone fragility with consequent susceptibility to fracture [1,2]. Osteoporosis and osteoporosis-related fractures are currently the major causes of morbidity and mortality among the elderly [3]. Numerous studies have reported that

Abbreviations: Ca-SGP, sialoglycoprotein isolated from eggs of *Carassius auratus*; ALN, alendronate sodium; IL-1β, interleukin 1β; VEGF, vascular endothelial growth factor; MMP13, matrix metallopeptidase 13; Col10a, collagen type X; Col1a, collagen type I; OCN, osteocalcin.

* *Corresponding author*. Fax: +86 0532 82032468.
E-mail address: jfwang@ouc.edu.cn (J. Wang).

https://doi.org/10.1016/j.jfda.2017.07.017

Please cite this article in press as: Wang F, et al., Sialoglycoprotein isolated from eggs of *Carassius auratus* promotes fracture healing in osteoporotic mice, Journal of Food and Drug Analysis (2017), https://doi.org/10.1016/j.jfda.2017.07.017

osteoporosis could result in not only delayed fracture healing but also in disturbed callus formation with decreased bone mineral density, and volume, and altered biomechanical properties [4–6]. Ovarian hormone deficiency is a key risk factor for postmenopausal osteoporosis. Estrogen has been reported to enhance fracture healing in mice thanks to its effect on the regulation of skeletal growth, development and maintenance, as well as on the cartilage homeostasis, growth, and maturation [7,8].

Currently, some anti-resorptive drugs such as bisphosphonates, estrogen, selective estrogen receptor modulators and calcitonin, have been used to promote callus formation during fracture healing [9,10]. However, these existing therapeutic options are limited not only due to their inherent adverse effects, but also because of the delay that they imply in bone remodeling at the late healing stage. Thus, the identification of novel alternatives for the treatment of osteoporotic fracture is urgently needed. Sialoglycoproteins (SGPs) belong to the family of highly acidic glycoproteins and can be found in the eggs of many vertebrates [11]. Fish eggs are one of the major byproducts of the fish-processing industry and can be easily retrieved. In our previous work, sialoglycoprotein was successfully isolated from the eggs of *Carassius auratus* (*Ca*-SGP), and its anti-osteoporotic effect was demonstrated using ovariectomized (OVX) rats and the senescence-accelerated mouse strain P6 *in vivo* [12–14].

Fracture healing involves a well-characterized cascade of events including three major phases: the reactive phase, the reparative phase, and the remodeling phase. Endochondral ossification is identified as an indispensable process for reparative phase in fracture healing [15,16]. It begins with mesenchymal stem cell condensation, followed by chondrocyte proliferation, differentiation, maturation, and apoptosis, as well as vasculature invasion of the fracture site. In this study, in order to explore the value of *Ca*-SGP use on bone metabolism, open tibial fracture surgery was performed on mice with OVX-induced osteoporosis to investigate the effect of a *Ca*-SGP treatment on fracture healing.

2. Materials and methods

2.1. Materials and reagents

Fresh female *C. auratus* were purchased at a seafood market in Qingdao, China. The ovaries were quickly taken out under an ice bath. After removing the egg envelope, the fish eggs were extracted and stored at −80 °C until use.

Moloney murine leukemia virus reverse transcriptase (M-MLV), dNTPs, random primer, and PageRuler prestained protein ladder were procured from TaKaRa Bio Inc. (Otsu, Shiga, Japan). FastStart Universal SYBR Green Master (Rox) was purchased from Roche (Roche Applied Science, Mannheim, Germany). The primers of genes including Aggrecan, collagen type X(Col10a), vascular endothelial growth factor (VEGF), matrix metallopeptidase 13 (MMP13), collagen type I(Col1a) and osteocalcin (OCN) were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China).

2.2. Preparation of Ca-SGP

Ca-SGP was prepared as previously reported [14]. Its purity was 94.76%, and its molecular weight 195.35 kDa. *Ca*-SGP contained 62.81% hexose, 14.33% protein, and 19.72% N-acetylneuraminic acid. Monosaccharide composition analysis showed the presence of mannose, glucosamine, galactosamine, and galactose with a molar ratio of 1:2.98:2.65:5.09.

2.3. Animals and experimental design

This study was approved by the Ethics Committee of Experimental Animal Care at the Ocean University of China (certificate no. SYXK20120014). Nine-week-old female C57BL/6J mice (20 ± 2.0 g) were purchased from the Vital River Laboratory Animal Center (Beijing, China; license ID SCXK2012-0001). The animals were housed four per cage at 23 ± 1 °C with a 12 h light/12 h dark cycle and they were given food and water ad libitum. After 7 days of acclimatization, the animals were sham-operated (control, n = 54) or subjected to bilateral ovariectomy (n = 150) and then left untreated for 10 weeks to allow bone loss. The femurs of six mice were collected in each group to demonstrate the successful establishment of the osteoporotic model using histological analysis. Then the OVX animals were randomly allocated to one of the three following groups (n = 48 per group): a model group (treated with physiological saline), an OVX + ALN group (treated with alendronate sodium as a positive control; 1 mg/kg body weight) and an OVX + *Ca*-SGP group (treated with *Ca*-SGP; 500 mg/kg body weight). The control group was also treated with physiological saline. In each group, animals were given intragastric administration of either physiological saline or their respective drugs (1 mL/100 g body weight) once a day. After 7 days of treatment, all the animals were subjected to an open fracture operation according to the method described in Ref. [17], with slight modifications. Briefly, a transverse osteotomy was made at the proximal third of the right tibia diaphysis under general anesthesia and sterile conditions. Then, the patella was laterally deflected and a hole was drilled through the intercondylar eminence of the tibia. Subsequently, the fracture site was connected by a 0.45-mm Kirschner wire inserted through the hole across the fracture ends. The locations of the fracture and the inserted Kirschner wire were monitored by a HY-450DR X-ray system (KangDa, Shanghai, China) after operation. Drug administration was continued after fracture surgery.

Serum was dynamically collected on days 3, 5 and 11 post-surgery for the determination of biochemical indicators (n = 8 per group). Tibiae callus (n = 6 per group) collected days 5, 11, 24 and 35 post-surgery were used for histological analysis. Similarly, six tibial calluses per group were collected for a micro-CT analysis on day 24 post-surgery, for a biomechanical property testing on day 35 post-surgery, and for the measurement of mRNA expression on days 11 and 24 post-surgery.

2.4. Determination of serum biochemical indicators

The biochemical indicators including TNF-α, interleukin 1β (IL-1β), VEGF, Aggrecan and MMP13 in serum were measured using commercial ELISA kits (R&D Systems, Minneapolis, MN, USA).

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