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Ghrelin improves vascular autophagy in rats with vascular calcification

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ABSTRACT

Backgrounds: This study aimed to investigate whether ghrelin ameliorated vascular calcification (VC) through improving autophagy.
Methods: VC model was induced by nicotine plus vitamin D_3 in rats and β-glycerophosphate in vascular smooth muscle cell (VSMC). Calcium deposition was detected by von Kossa staining or alizarin red S staining. ALP activity was also detected. Western blot was used to assess the protein expression.
Results: Ghrelin treatment attenuated the elevation of calcium deposition and ALP activity in VC model both in vivo and in vitro. Interesting, the protein levels of autophagy markers, LC3 and beclin1 were significantly upregulated by ghrelin in VC model. An autophagy inhibitor, 3-methyladenine blocks the ameliorative effect of ghrelin on VC. Furthermore, protein expressions of phosphate-AMPK were increased by ghrelin treatment both in calcified aorta and VSMC. The effect of ghrelin on autophagy induction and VC attenuation was prevented by AMPK inhibitor, compound C.
Conclusions: Our results suggested that ghrelin improved autophagy through AMPK activation, which was resulted in VC amelioration. These data maybe throw light on prevention and therapy of VC.

1. Introduction

Autophagy is a dynamic and refinedly controlled process of self-digestion. It demonstrates primary to sustain cellular homeostasis through the removal of impaired protein and organelles, as well as affording a survival mechanism for cells suffering stress [1]. Autophagy is commonly considered to exist basically in three different forms, including microautophagy, chaperone-mediated autophagy and macroautophagy [2,3]. The more common term autophagy is usually referred to macroautophagy that has been comprehensively focused in most investigations. There are accumulating literatures attempting to investigate how normal and abnormal autophagy mediates vascular pathophysiology, such as vascular calcification (VC) [4].

VC underlies massive cardiovascular morbidity and mortality, via deleterious mechanical effects on vascular compliance and vasomotion [5]. It may appear in the tunica intima and tunica media. Intimal calcification is usually observed in advanced atherosclerotic lesions, whereas medial calcification is independent of atherosclerosis but has a firm correlation with aging, metabolic syndrome, chronic renal failure and hypertension [6,7]. Growing evidence has supported that vascular smooth muscle cells (VSMCs) transformation from contractile phenotype to osteogenic phenotype promote process of VC [8,9]. Recently, emerging articles have reported that autophagy is promoted in calcified vascular both in in vitro calcification model of VSMCs, and in vivo VC model of rats. Moreover, the promotion of autophagy can ameliorate pathologies of VC both in vivo and in vitro [10–12]. However, regulated mechanism of autophagy during progression of VC still remains unclear.

Ghrelin is a 28 amino acid peptide that firstly has been separated from stomach by Kojima in 1999, and functions as a natural endogenous ligand for growth hormone secretagogue receptor (GHSR) [13]. Beside of stomach, ghrelin are also produced by other organs such as heart, lung and kidney [14]. Ghrelin has extensively crucial physiological effects, including stimulating growth hormone (GH) secretion from the pituitary, promoting food intake, and maintaining energy homeostasis [15,16]. Moreover, accumulating investigations have demonstrated beneficial effect of ghrelin on cardiovascular system, including enhancing myocardial contractility, reducing mean arterial pressure, vasodilatation, ameliorating heart failure and ventricular remodeling, as well as protecting myocardium against ischemia/reperfusion injury [17,18]. These findings highlight the ghrelin system as a potential candidate for cardiovascular drug discovery [19]. mRNA expression and contents of ghrelin in vascular was decreased during VC, whereas exogenous supplementation of ghrelin significantly ameliorates pathology of VC [20,21]. Another article further demonstrates that ghrelin prevents osteoblastic transformation and mineralization of VSMCs mediated by GHSR/ERK signaling pathway [22]. These results reveal the ameliorative of ghrelin on VC. However, detailed mechanism of ghrelin improving pathology of VC still remains unclear.

Recently, cardioprotection of ghrelin due to promotion of autophagy has been verified by numbers of articles. Ghrelin protects

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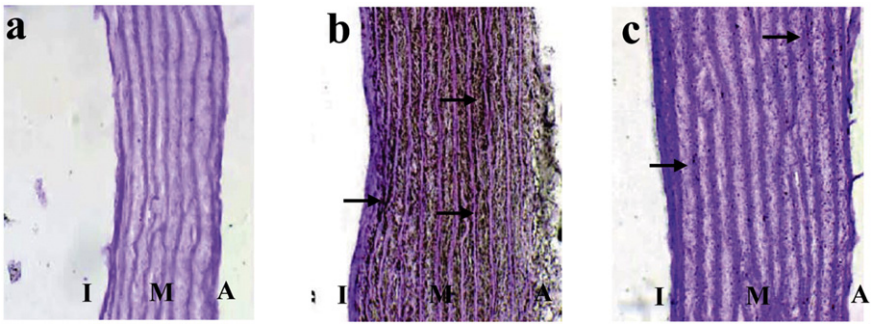

Fig. 1. Representative image of von Kossa staining for aortic calcification, showing a positive staining of black/brown areas (arrow shows) among elastic fibers of aortic media in calcified aorta (original magnification ×100). a, control group; b, VC group; c, VC plus ghrelin group. (n = 8 in each group) A, adventitia; M, media; I, intima.

myocardiocytes against hypoxia induced injury by inducing protective autophagy in an AMPK-dependent manner [23]. Ghrelin also protects against cardiac dysfunction in type 2 diabetic mice by enhancing autophagy via AMPK/ERK1/2 signaling pathways [24]. Otherwise, the inducing effect of ghrelin on autophagy has been determined in other tissues and cells, such as skeletal muscles [25,26] and liver [27,28]. Given that promotion of autophagy can ameliorate pathology of VC, we hypothesize that ghrelin enhance autophagy in calcified vessel which is associated with the ameliorative effect of ghrelin on VC. Here, a rats model of VC induced by Vitamin D_3 ($VitD_3$) and nicotine and an in vitro model of rats VSMCs calcification induced by β-glycerophosphate are used to identify the effect of ghrelin on autophagy in VC scenario.

2. Materials and methods

2.1. In vivo model of VC in rats

Three-month-old male Sprague–Dawley (SD) rats were from Vital River (Beijing, China). Rats were housed under standard conditions (room temperature 20 ± 8 °C, humidity 60 ± 10%, lights from 6:00 to 18:00) and were given standard rodent chow and water freely. All animal procedures were complied with the Animal Management Rule of the Ministry of Health, People's Republic of China (documentation No. 55, 2001) and the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and approved by the Animal Care Committee of Guangdong Medical University.

Rats were randomly divided into three groups (n = 8 in each group): control group, VC group and VC plus ghrelin group. The rat model of VC was established according Niederhoffer N et al. describing [29]. Briefly, rats in VC group and VC plus ghrelin group were treated with Vitamin D_3 (intramuscularly, 300,000 IU/kg, from Sigma, St. Louis, United States) and nicotine (intragastrically, 25 mg/kg in olive oil, from Sigma, St. Louis, United States), and nicotine was given again after 12 h. The rats in control group were treated with solvent. The rats in VC plus ghrelin were injected intraperitoneally with ghrelin (100 nmol/kg, twice per day, from Phoenix Pharmaceutical, Belmont, United States), whereas rats in control group and VC group injected intraperitoneally with saline. Four weeks later, the rats were anesthetized with 50 mg/kg pentobarbital sodium. Blood samples were collected and the thoracoabdominal aorta was removed into ice-cold phosphate buffered saline (PBS).

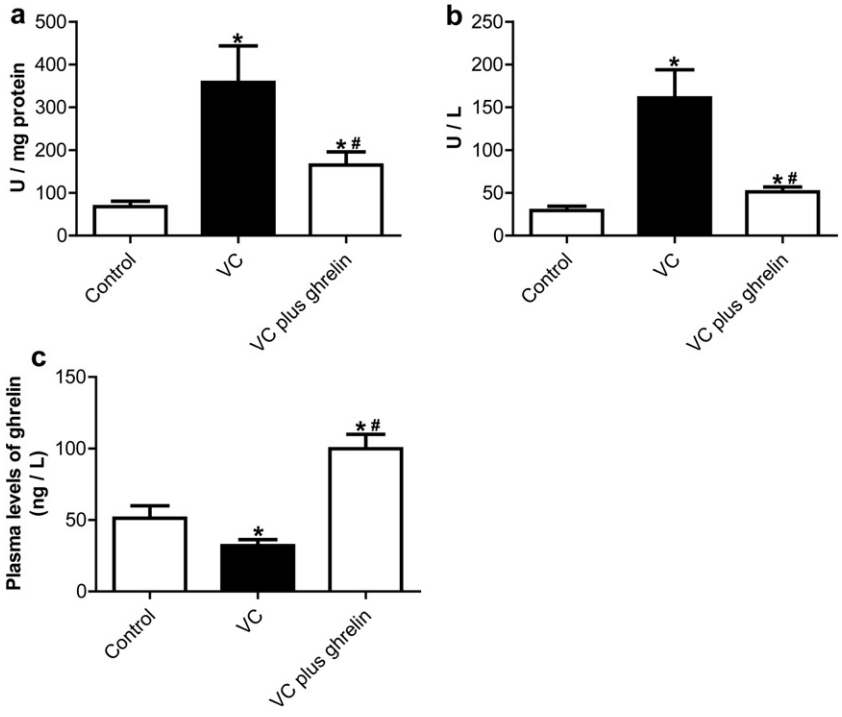

Fig. 2. Activity of ALP in aortic tissues (a) and plasma (b), and plasma levels of ghrelin (c). * P < 0.05 vs. control group; # P < 0.05 vs. VC group. (n = 8 in each group).