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Taurine protects against knee osteoarthritis development in experimental rat models

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ABSTRACT

Background: Osteoarthritis (OA) is one of the complex diseases that affect a large population of the world. The aim of the current study was to explore the roles of taurine in OA rat models, and discover the related mechanisms.
Methods: OA rat models were established by anterior cruciate ligament transection (ACLT) plus medial meniscus resection (MMx) surgery on the right knees. Secondary mechanical allodynia, weight-bearing alterations and knee joint width were evaluated before surgery and every two weeks after surgery. At 14 weeks, histopathological analysis was conducted on the knee joint cartilage. Protein amount of MMP-3 and CHOP was evaluated by western blot.
Results: Taurine injection after surgery significantly relieved the symptoms of OA in rat models in a dose-dependent and time-dependent manner, as shown by alleviation of secondary mechanical allodynia, decrease in hind limb weight-bearing alterations, and inhibited knee swelling. Moreover, histopathological analysis showed that taurine inhibited matrix loss and cartilage degeneration in a dose-dependent manner. Taurine administration strikingly suppressed the expression of matrix metalloproteinase-3 (MMP-3) and CHOP.
Conclusion: These results indicated that taurine administration exhibited protective effects by inhibiting MMP-3 and CHOP expression, and subsequently alleviated the OA symptoms in experimental rat models.

1. Introduction

Osteoarthritis (OA) – caused by aging, obesity, trauma, joint instability, overuse or other abnormal loading conditions – is a complex disease that afflicts millions of people worldwide [1–3]. It is mainly characterized by progressive articular cartilage (AC) degradation and chondrocyte death, and may lead to severe disability [4,5]. Chondrocytes, the only cells in AC, are responsible for maintaining healthy cartilage and joint mobility in humans and animals, and are considered as target cells for OA therapy. OA animal models have been used for understanding the generation and progression of OA, and the efficacy of anti-OA drugs [6,7]. Previous studies have reported that anterior cruciate ligament transection (ACLT) plus medial meniscus resection (MMx) surgery can rapidly induce OA disease in animal models, and the manifestations are relatively stable [2,8].

Taurine (2-aminoethane sulfonic acid), a well-documented semi-essential amino acid, is one of the richest free amino acids in most mammalian tissues. Taurine is known to perform various functions in both human and animal physiology, including antioxidant, anti-infection, anti-inflammation, osmoregulation, regulation of blood pressure, neuromodulation, maintenance of cardiac function, regulation of neuroendocrine activity, and immune regulation [9–15]. Moreover, it is also reported that the above func-

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tions of taurine are not tissue-specific, and the anti-apoptosis and anti-oxidation effects are important for cytoprotective activities of taurine [16]. However, few studies regarding the anti-OA effects of taurine can be found.

The purpose of the current study was to illustrate the possible contribution and underlying mechanisms of taurine to the pathogenesis of OA in experimental rat models. Therefore, OA rat models were established through ACLT plus MMx surgery. The OA rats were treated with different doses of taurine through tail vein injection for 14 weeks after surgery. The results of serial experiments showed that taurine administration significantly prevented exacerbation of OA symptoms in the ACLT plus MMx surgery-treated rats, as shown by alleviation of secondary mechanical allodynia, decrease in hind limb weight-bearing alterations, and inhibited knee swelling. In addition, histopathological analysis showed that taurine treatment inhibited matrix loss and cartilage degeneration in a dose-dependent manner. Moreover, western blot analysis indicated that taurine strikingly down-regulated the expression of matrix metalloproteinase-3 (MMP-3) and C/EBP Homologous Protein (CHOP) protein in OA rat cartilage.

2. Methods and materials

2.1. Osteoarthritis rat models

The osteoarthritis rat models were established as described previously [2]. The use of animals was reviewed and approved by the Institutional Ethical Review Board of our hospital. Twelve-week-old male Wistar rats (weight 330–350 g) were maintained on a 12-hour light/dark circadian cycle, at 22–24 °C and a humidity of 55%. The animals were provided standard rat diet and water ad libitum. OA was induced by ACLT plus MMx surgery to the rats' right knees, while the left knees were untreated. In brief, the rats were anesthetized with halothane. The right knee joints were then shaved and disinfected, and subsequently exposed by medial parapatellar arthrotomy followed by ACLT and MMx. In the sham group, the ACL was just exposed through a small medial parapatellar incision, then the joint was washed with saline and the incision was sutured. The rats were given supplemental heat and were closely monitored until fully recovered from the anesthesia. The rats were also monitored daily for pain, infection and other complications of surgery.

2.2. Experimental design and taurine administration

The ACLT plus MMx surgery was conducted at week 0. The animals were treated with 0.5× taurine (100 mg/kg), 1× taurine (200 mg/kg), or 2× taurine (400 mg/kg) by tail vein injection following surgery. Secondary mechanical allodynia was assessed with Von Frey Filaments (North Coast Medical, Inc. Morgan Hill, CA, USA) before and every two weeks after surgery. A weight-bearing distribution test was performed via an incapacitance tester (Singa Technology Corporation, Taipei, China) before and every two weeks after surgery. Additionally, the knee joint width was measured bilaterally via calipers (AA847R, Aesculap, Eindhoven, Netherlands) before and every two weeks after surgery. Rats were sacrificed at 14 weeks after surgery and the knee tissues were harvested for further analyses.

2.3. Incapacitance test (weight-bearing test)

The change in postural equilibrium, which is an index of joint discomfort, was measured as previously described [17]. Animals were positioned standing on their hind paws in an inclined plexiglass chamber, and the body weight applied by each hind limb was independently calculated by the incapacitance tester. Five readings were taken for each animal, and the mean value was calculated. Data were expressed as the weight-bearing difference between the contralateral and ipsilateral hind limbs.

2.4. Secondary mechanical allodynia

Allodynia was evaluated according to a previously reported method [18]. Von Frey Filaments were applied to determine the 50% paw withdrawal threshold through Chaplan's up–down method. In brief, each Von Frey Filament was tested on the plantar surface of each hind paw for five seconds. Paw lifting movements indicated a positive response, and the next weaker filament was then applied. The absence of paw withdrawal responses indicated negative feedback, and the next stronger filament was applied. Von Frey Filaments were used for each hind paw at an interval of three minutes for five trials.

2.5. Histopathological analysis of rat knee joints

The harvested knee joints were fixed in four percent paraformaldehyde for 72 h at 4 °C. After decalcification, the tissues were dehydrated in series of ethanol, cleared with xylene and embedded in paraffin. Serial sections of five microns were obtained using microtome, and stained with hematoxylin/eosin (H&E). The morphological changes were scored using the modified Osteoarthritis Research Society International scoring system. The scoring was repeated independently three times through blinded experiments.

2.6. Western blot analysis

Antibodies for MMP-3 and CHOP were purchased from Santa Cruz Biotechnology Dallas, TX, USA. Cartilage protein extraction was performed as previously described [19]. The soluble supernatants of the extracts were collected and assayed by a bicinchoninic acid assay reagent kit (Pierce, Rockford, IL, USA). Equivalent amounts of protein were separated by sodium dodecyl

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