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Tanshinone IIA represses inflammatory response and reduces radiculopathic pain by inhibiting IRAK-1 and NF-κB/p38/JNK signaling

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ABSTRACT

Intervertebral disc (IVD) disease, a most common cause of disc failure and low back pain, is characterized by age-related changes in the adult disc. In this study we aimed to investigate the potential of Tanshinone IIA (TSA) for the treatment of IVD disease, through exploring its anti-inflammatory and anti-catabolic activities in both in vitro IVD cell culture and in vivo animal models. After the inflammatory response was induced in IVD cells by IL-1β, the activity and expression of inflammatory mediators, and potentially involved pathways were investigated in the presence or absence of TSA. The p38-MAPK inhibitor, SB239063, was also used to investigate the involvement of the MAPK signaling pathway in the observed effects. Meanwhile, the analgesic properties of TSA were analyzed by the von Frey filament test in Sprague–Dawley rats. Our results indicated that TSA significantly inhibited the expression of pro-inflammatory mediators and matrix metalloproteinases in vitro, as well as radiculopathic pain in vivo, probably by modulation of the activity of interleukin-1 receptor-associated kinase 1 (IRAK-1) and its downstream effectors p38, JNK and NF-κB. Our current study strongly demonstrates the potential of TSA for the treatment of inflammation and followed pain in degenerative disc disease.

1. Introduction

Intervertebral disc (IVD) degeneration is an inevitable consequence of aging, which starts in the late second or early third decade [1]. Although usually asymptomatic, specific histological, biochemical and functional changes have been discerned during IVD degeneration, which are consistently associated with pain generation. IVD degeneration is a most common cause of lower back pain in adults. It occurs as a result of the imbalance between anabolic and catabolic processes, leading to upregulated generation of matrix metalloproteinases (MMPs), loss of collagen and proteoglycan and concomitant reduction in water content [2–4]. These degenerative processes lead to mechanical malfunction and redistributed stress along the tissue, increasing the risk of load-induced structural failure in the annulus fibrosus (AF), e.g., clefts and tears or fissures. Pain sensation can be then triggered by the leakage of nucleus pulposus (NP) materials through the AF and subsequent irritation of spinal nerves such as in radiculopathic pain or nerve infiltration into the compromised disc in nociceptive pain.

Degenerated IVD can be painful even in the absence of a disc prolapse, especially when a large amount of pro-inflammatory mediators are secreted. The interleukin-1 (IL-1) family cytokines irritate nerve endings in the AF [5], as well as stimulate the production of matrix-degrading enzymes which further deteriorate the IVD degeneration [6–8]. Normally, healthy IVD is seen as an immune-privileged organ which is excluded from the development of immunologic tolerance and is not exposed to systemic circulation [9], whereas diseased human discs are heavily invaded by blood vessels and nociceptive nerve fibers [10]. The immune system will recognize it as a foreign body once NP extrudes from the IVD to the systemic circulation, resulting in autoimmune reactions which may further accelerate the damage of NP tissue inside the disc [11]. On the other hand, some immune cells, e.g., macrophages, may benefit the closing of the IVD wound and immune privilege maintenance at early stages of the degeneration, as well as the absorption of herniated NP [9,12,13].

Traditional treatment of degenerative disc disease is pretty limited. Conservative treatment such as physical therapy often fails and leaves patients with disc herniation or painful degenerative disc disease subject to surgical interventions. Therefore, less invasive and more targeted strategies are being developed over the years. NP replacement, e.g., by the injection of biocompatible hydrogels with or without cells, have been proposed to restore normal disc height and load distribution, and limit degenerative changes in adjacent discs [5]. Recently, anti-inflammatory and anti-catabolic substances that target the metabolism and inflammatory signaling within the IVD have exhibited interesting and promising treatment potentials [14,15].

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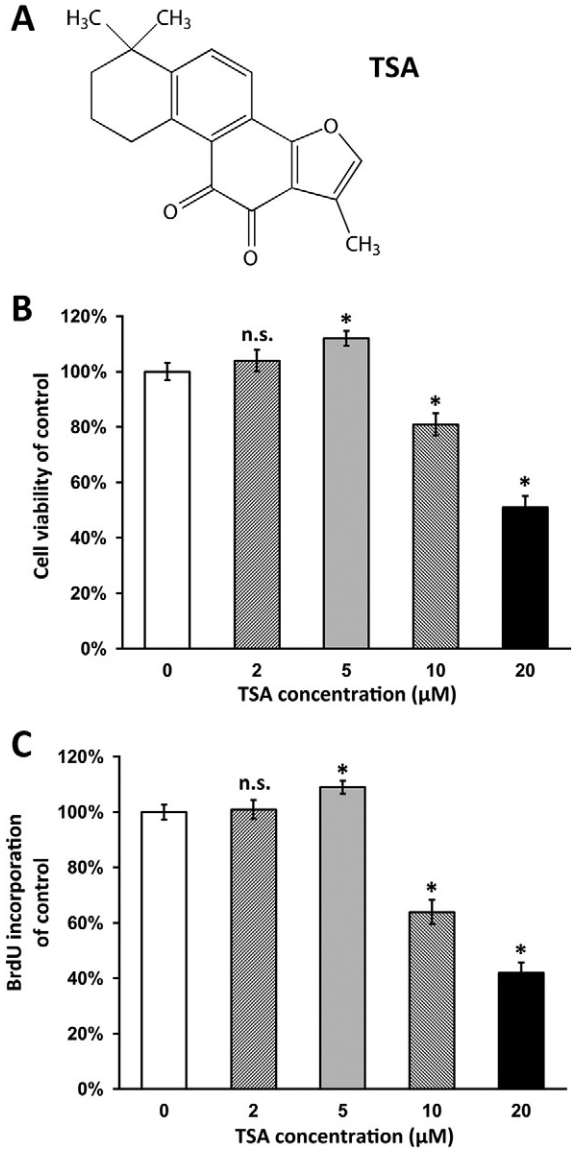

Fig. 1. Effects of TSA on the viability and proliferation of human IVD cells in vitro. (A) Chemical structure of TSA. (B) Relative cell viability and (C) proliferation of the IVD cells to the control (0 μM) following TSA treatment, measured by MTT assay (B) and BrdU incorporation assay (C) respectively. Data were presented by the mean ± SEM. n.s.: not statistically significant vs control. *$p < 0.05$ vs control.

Of clinical interest, Tanshinone IIA (TSA, Fig. 1A), also known as Dan Shen ketone, has sparked our interest for the treatment of IVD degeneration due to its potent anti-inflammatory effects. TSA is an important lipophilic diterpene extracted from a traditional herbal medicine *Salvia miltiorrhiza* Bunge. It is widely used for the treatment of many different diseases, such as cardiovascular [16,17], cerebrovascular [18,19] and postmenopausal syndromes [20]. TSA has been demonstrated to exhibit potent anti-inflammatory properties in different cell types, such as RAW 264.7 cells [21], BV-2 cells [22], and osteoclast progenitor cells [23]. Meanwhile, its beneficial anti-inflammatory effects are also observed in different animal models [24–26]. Moreover, previous studies demonstrate that TSA exert a series of biochemical effects through its antioxidative [27] and anti-apoptotic [28] properties in addition to it potent anti-inflammatory effect. However, its effect on IVD degeneration is not yet clear.

In this study, we aimed to investigate the effect of TSA on IVD degeneration. The effect of TSA on the IL-1β-induced inflammatory responses was firstly examined in IVD cells in vitro. We found that TSA significantly reduced the inflammatory response stimulated by IL-1β, evidenced by suppressed MMP activity and down-regulated mRNA and protein expressions. Its anti-inflammatory effects were also observed in its inhibition of the generation of various cytokines. Further mechanistic study revealed that this anti-inflammatory effect of TSA was mediated through the NF-κB/p38/JNK signaling pathway. Moreover, we analyzed the analgesic properties of TSA using the von Frey filament test in Sprague–Dawley rats, and found that it significantly inhibited radiculopathic pain in vivo. In short, our study strongly demonstrates the potential of TSA for the treatment of inflammation and pain in degenerative disc disease.

2. Materials and methods

2.1. Human IVD cell culture preparation

The study was approved by ethic committees of Shandong Provincial Hospital, Shandong University. Human NP tissue was removed from patients undergoing spinal surgery for degenerative disc disease or disc herniation after informed consent was granted. Tissue was enzymatically digested using a mixture of 0.2% collagenase NB4 (Serva, Germany) and 0.3% dispase II (Roche, Switzerland) for 4–8 h at 37 °C and isolated primary cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Sigma, USA) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin (50 units/mL), streptomycin (50 μg/mL) and ampicillin (125 ng/mL, Gibco, USA). Cells were subcultured up to passage 3 using 0.5% trypsin (Gibco). Cells of passage 1–3 that were cultured adherently were used for further experiments.

2.2. TSA preparation

TSA (Sigma) was dissolved in DMSO and then diluted in saline to a final concentration of 1.0 mM as a stock solution. In the TSA treated groups, TSA stock solution was diluted to a desired concentration with DMSO before using. The control group received a corresponding volume of vehicle DMSO injection.

2.3. MTT assay

For the measurement of metabolic activity, 3-(4,5-dimethyl-2-thiazoyl)-2, 5-diphenyltetrazolium bromide (MTT) was used according to the manufacturer's instructions (Roche, USA). Cells were incubated with 0.5 mg/mL MTT at 37 °C, and incubated with lysis buffer overnight in an incubator. The optical density of solubilized formazan was measured at 570 nm on a plate reader (BioRad, USA).

2.4. BrdU incorporation assay

Proliferation of IVD cells was assessed by bromodeoxyuridine (BrdU) incorporation assay using the Proliferation Assay Kit (Millipore, USA). In brief, cells were seeded in 96-well plates (5000 cells/well) and treated with different concentrations of TSA for 48 h. BrdU was then added 2 h earlier before examination. The assay was performed according to the manufacturer's instructions.

2.5. Enzyme-linked immunosorbent assay (ELISA)

To determine activities of MMP-1 and MMP-13 in IVD cell culture, culture supernatant was collected and assayed using MMP-1 and MMP-13 Biotrak ELISA kits (Amersham, USA) according to the instructions, and expressed as $\delta Absorbance_{405}/h^2 \times 1000$.

To detect secreted proteins, IVD cells in passages 1–3 were seeded in 6-well plates (3×10^5 cells/well), serum starved for 2 h, and then exposed to 5 ng/mL IL-1β for 2 h before treatment with 5 μM TSA. Cell culture medium was collected 18 h later and the level of IL-6 protein expression was analyzed by ELISA according to the producer's protocol (Human IL-6 ELISA set, BD Biosciences, USA). Briefly, 96-well plates