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Melatonin resists oxidative stress-induced apoptosis in nucleus pulposus cells

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ABSTRACT

Aims: Intervertebral disc degeneration (IVDD) is thought to be the major cause of low back pain (LBP), which is still in lack of effective etiological treatment. Oxidative stress has been demonstrated to participate in the impairment of nucleus pulposus cells (NPCs). As the most important neuroendocrine hormone in biological clock regulation, melatonin (MLT) is also featured by good antioxidant effect. In this study, we investigated the effect and mechanisms of melatonin on oxidative stress-induced damage in rat NPCs.

Main methods: Cytotoxicity of H_2O_2 and protecting effect of melatonin were analyzed with Cell Counting kit-8 (CCK-8). Cell apoptosis rate was detected by Annexin V-FITC/PI staining. DCFH-DA probe was used for the reactive oxygen species (ROS) detection. The mitochondrial membrane potential (MMP) changes were analyzed with JC-1 probe. Intracellular oxidation product and reductants were measured through enzymatic reactions. Extracellular matrix (ECM) and apoptosis associated proteins were analyzed with Western blot assays.

Key findings: Melatonin preserved cell viability of NPCs under oxidative stress. The apoptosis rate, ROS level and malonaldehyde (MDA) declined with melatonin. MLT/H_2O_2 group showed higher activities of GSH and SOD. The fall of MMP receded and the expression of ECM protein increased with treatment of melatonin. The mitochondrial pathway of apoptosis was inhibited by melatonin.

Significance: Melatonin alleviated the oxidative stress-induced apoptosis of NPCs. Melatonin could be a promising alternative in treatment of IVDD.

1. Introduction

Low back pain (LBP) is one of the most common complaints in orthopedic clinics and imposes a huge financial burden on society [1]. Studies have shown that more than half of LBP cases are caused by intervertebral disc degeneration (IVDD) [2]. Various factors, including oxidative stress, infection, smoking, and trauma, are involved in the initiation and progression of disc degeneration [3,4]. However, the majority of current therapies are symptomatic treatments, which inadequately cure IVDD.

The nucleus pulposus constitutes the inner part of the intervertebral disc (IVD) and helps maintain the integrity of the extracellular matrix (ECM) and the biomechanical properties of the IVD. During IVDD, ruptures of the annulus fibrosus and degeneration of the cartilage endplates, as well as inflammation, aggravate oxidative stress in nucleus pulposus cells (NPCs) by inducing more reactive oxygen species (ROS) production [5]. Oxidative stress, which accelerates cell senescence [6,7] and induces mitochondrial apoptosis in NPCs, plays a significant role in the occurrence and development of IVDD [8,9].

Melatonin (*N*-acetyl-5-methoxytraptamine) is an indolyl hormone that has extensive physiological functions; melatonin regulates circadian rhythms, blood pressure, and immune function and inhibits cancer [10–13], and studies in rodents and humans have shown that melatonin also plays an important role in protecting the tissues and cells of the nervous system, the cardiovascular system and other organs against oxidative damage [14–16]. The first-, second-, and third-generation metabolites of melatonin are all capable of detoxifying radicals [17,18] and are thus excellent protectants in ischemia-reperfusion injury and aging-related diseases [19,20].

A.T. Turgut el al. reported that IVDD was more likely to occur after pineal excision, indicating that the pineal glands are associated with vertebral diseases [21,22]. Therefore, melatonin supplementation may have beneficial effects on degenerated IVDs. Since oxidative stress has been confirmed to be involved in rat IVDD [23], in the present study,

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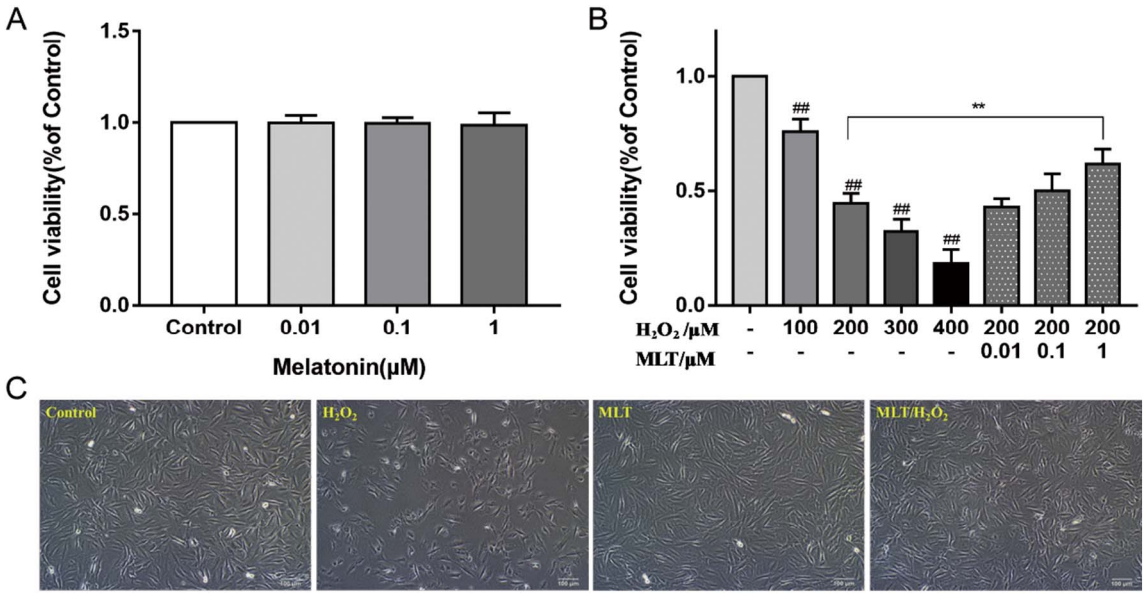

Fig. 1. Cell viability after treatment of melatonin and H_2O_2. **A**. Toxicity of melatonin on NP cells was assessed by CCK-8 assay. The values were expressed as mean ± SD (#, $P < 0.05$, ##, $P < 0.01$ vs. control; *, $P < 0.05$, **, $P < 0.01$ vs. H_2O_2 group). **B**. Toxicity of H_2O_2 on NP cells and the protective effects of melatonin against H_2O_2-induced cell injury. The values were expressed as mean ± SD (#, $P < 0.05$, ##, $P < 0.01$ vs. control; **, $P < 0.01$ vs. 200 μM H_2O_2 group). **C**. Morphological changes of NP cells after different treatments, observed with phase contrast microscope. Bar = 100 μm.

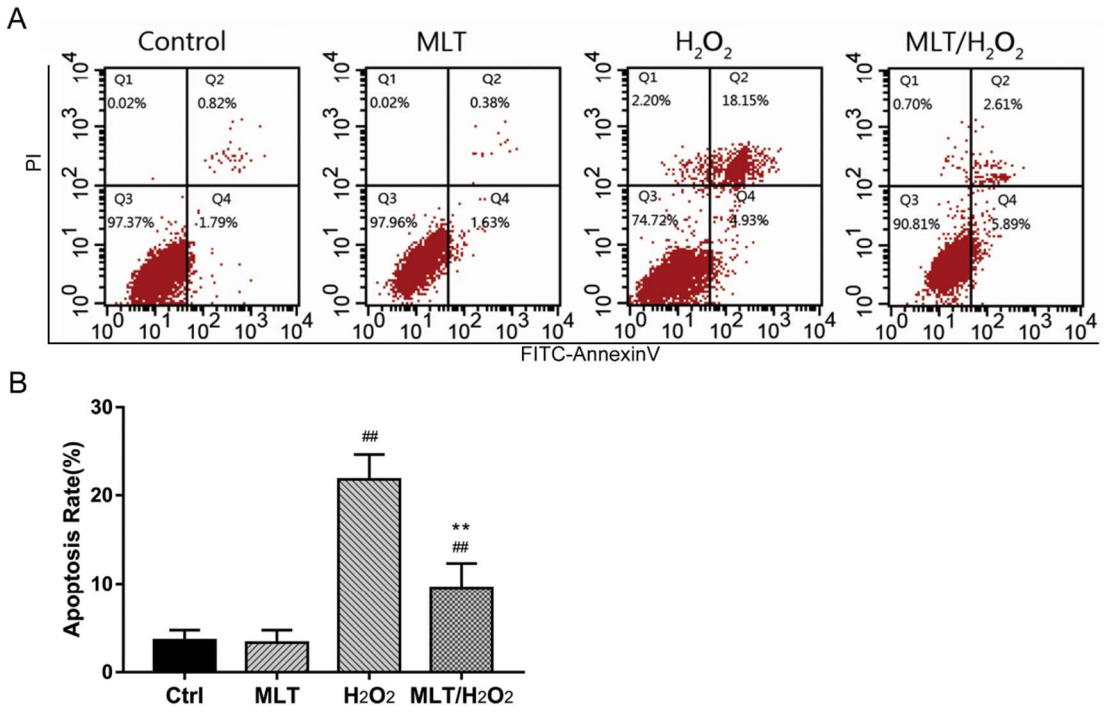

Fig. 2. Protective effects of melatonin on H_2O_2-induced apoptosis in NP cells. **A**. NP cells stained with Annexin V-FITC/PI were analyzed by flow cytometry; **B**. Histogram of the apoptotic rate in different groups, with values expressed as mean ± SD (#, $P < 0.05$, ##, $P < 0.01$ vs. control; *, $P < 0.05$, **, $P < 0.01$ vs. H_2O_2 group).

we investigated the function of melatonin in and the mechanism of its effects on the oxidative stress-induced apoptosis of rat NPCs to provide evidence for its clinical use in the treatment and prevention of IVDD.

2. Materials and methods

2.1. Isolation and culture of NPCs

All of the experiments were approved by the Animal Experimentation Committee of Huazhong University of Science and Technology. Primary rat NPCs were isolated and cultured as previously described [24,25]. Briefly, after 12-week-old Sprague-Dawley rats (250–300 g) were euthanized with an intraperitoneal injection of chloride hydrate (350–400 mg/kg), the lumbar spine was separated from their bodies. The nucleus pulposus of each disc was acquired with tweezers and minced into floccules smaller than 1 mm^3. The isolated tissues were digested with 0.25% type II collagenase (Gibco, USA) for 30 min at 37 °C. Complete culture medium (DMEM/F-12 (HyClone, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin/streptomycin (Solarbio, USA)) was used to terminate