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Curcumin improves neural function after spinal cord injury by the joint inhibition of the intracellular and extracellular components of glial scar

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ABSTRACT

Background: Spinal cord injury (SCI) is characterized by a high rate of disability and imposes a heavy burden on society and patients. SCI can activate glial cells and lead to swelling, hyperplasty, and reactive gliosis, which can severely reduce the space for nerve growth. Glial cells can secrete a large amount of extracellular inhibitory components, thus altering the microenvironment of axon growth. Both these factors seriously impede nerve regeneration. In the present study, we investigate whether curcumin (cur), a phytochemical compound with potent anti-inflammatory effect, plays a role in the repair of SCI.

Materials and methods: We established a rat model of SCI and treated the animals with different concentrations of cur. Using behavioral assessment, immunohistochemistry, real-time polymerase chain reaction, Western blotting, and enzyme-linked immunosorbent assay, we detected the intracellular and extracellular components of glial scar and related cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, nuclear factor (NF)-κb, transforming growth factor (TGF)-β_1, TGF-β_2, and sex determining region Y-box (SOX)-9.

Results: We found that cur inhibited the expression of proinflammatory cytokines, such as TNF-α, IL-1β, and NF-κb; reduced the expression of the intracellular components glial fibrillary acidic protein through anti-inflammation; and suppressed the reactive gliosis. Also, cur inhibited the generation of TGF-β_1, TGF-β_2, and SOX-9; decreased the deposition of chondroitin sulfate proteoglycan by inhibiting the transforming growth factors and transcription factor; and improved the microenvironment for nerve growth. Through the joint inhibition of the intracellular and extracellular components of glial scar, cur significantly reduced glial scar volume and improved the Basso, Beattie, and Bresnahan locomotor rating and axon growth.

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Conclusions: Our data support a role for curcumin in promoting neural function recovery after SCI by the joint inhibition of the intracellular and extracellular components of glial scar, providing an important strategy for treating SCI.

1. Introduction

Glial scar, which is caused by activated astrocyte after spinal cord injury (SCI), is a significant obstacle to nerve regeneration [1]. The inhibiting effect of glial scar is manifested in two ways. First, the swelling, hyperplasia, and hypertrophy of glial cells lead to reactive gliosis, which is mainly characterized by the increased expression of the cytoskeletal intermediate filament (IF) proteins such as glial fibrillary acidic protein (GFAP). These act as a physical barrier preventing newly generated axons from continuing growth. Second, glial cells secrete various extracellular inhibitory substances, such as chondroitin sulfate proteoglycan (CSPG), change the microenvironment of nerve growth, and act as a chemical barrier inhibiting nerve regeneration [2].

The expression of the cytoskeletal protein GFAP is closely related to the inflammatory response [3]. Microglia and macrophages release inflammatory cytokines involved in the initial reaction, such as tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β) [4]. These inflammatory cytokines can activate astrocytes, which then secrete more inflammatory cytokines. An appropriate inflammatory response can protect the body, but sustained excessive inflammatory response is an important cause for secondary SCI. It is essential to control the inflammatory response so as to inhibit glial scar formation [5]. The transforming growth factor (TGF)-β family regulates a wide range of biological responses, such as cell growth and differentiation, particularly the deposition of extracellular matrix (ECM) components [6]. TGF-β can affect the astrocytes and lead to the secretion of the inhibitory ECM CSPG. The axons will stop extending downward when they reach the CSPG-rich regions [7]. Hence, controlling the ECM secretion is important in suppressing glial scar formation [8]. Despite the large number of studies focusing on glial scars, there is still a lack of effective means for suppressing glial scar formation.

Curcumin (cur) is isolated from the dried stalks of the perennial herb turmeric in the Curcuma genus of the Zingiberaceae family and has long been widely used as a spice and food coloring [9]. Because cur has various functions, such as powerful anti-inflammation, anticancer, antifibrosis, and anti-oxidation effects, it has been studied in various disease models [10–12]. In the central nervous system, cur has neuroprotective effects and can significantly improve the recovery of neurologic function after neurodegenerative diseases and injury [13,14]. Despite many studies focusing on the relationship between cur and glial scar, the specific mechanism of action requires further study.

Previous research into the inhibition of glial scar formation mainly focused either on suppressing glial cell proliferation [15] or on decreasing the secretion of ECM [16] and rarely combined the mentioned two aspects to achieve regulation. Therefore, we wished to identify an effective method that can reduce the expression of both intracellular and extracellular components to simultaneously suppress physical and chemical barriers, thus providing more effective inhibition of glial scar formation. This study used cur for the treatment of SCI rats and detected the effects and possible mechanisms of the subsequent joint inhibition of GFAP and CSPG.

2. Methods

2.1. Establishment of the SCI model

Female Sprague–Dawley rats weighing 180–220 g were selected, and modeling was initiated after a week of adaptation. The rats were anesthetized with an intraperitoneal injection of 5% chloral hydrate and fixed in the prone position. The laminae of the thoracic vertebrae T8-10 were exposed, and the spinal cord was dissected by blunt dissection. An aneurysm clip with a fixed force of 50 g was used to clip the T9 spinal cord for 60 s. The rats immediately showed tail wagging reflex, hind limb and torso retraction and flutter, and subsequent paralysis of the hind limbs, suggesting successful modeling [17]. The muscle and skin were sutured, and the rats received three consecutive days of intramuscular injection of 40,000 U gentamicin once per day. The experimental groups were as follows: 1, the sham group; 2, the simple injury group; 3–5, the cur-treatment groups; and 6, the methylprednisolone (MP) treatment group. The spinal cord in group 1 was exposed but not clipped, and the rats received normal saline after surgery. Group 2 received dimethyl sulfoxide after surgery. Groups 3–5 were administered 300, 100, and 30 mg/kg of cur, respectively, and group 6 was administered 30 mg/kg of MP. With the operation completed, the first dose of cur or MP was given to the rat immediately. All drugs were administered via an intraperitoneal injection once per day for 7 d. All animal experiments and care were approved by the Third Military Medical University Committee on Ethics in the Care and Use of Laboratory Animals.

2.2. Behavioral test using the Basso, Beattie, and Bresnahan locomotor rating scale

We randomly selected eight rats from each group at 1 d and 1, 2, 4, 6, and 8 wk after surgery. The rats were placed on a circular platform with a diameter of 2 m and allowed to crawl. The rats were observed continuously for 4 min, and the Basso, Beattie, and Bresnahan (BBB) scoring method was used to evaluate rat hind limb motor function recovery. A score in the range of 0–21 points was assigned based on conditions such as joint activities, coordinated movement of fore and hind limbs, and trunk and tail positions [18]. All observations were