ANTI-HMGB1 NEUTRALIZATION ANTIBODY IMPROVES PAIN-RELATED BEHAVIOR INDUCED BY APPLICATION OF AUTOLOGOUS NUCLEUS PULPOSUS ON NERVE ROOT IN RATS

INTRODUCTION

Nucleus pulposus (NP) applied on the dorsal root ganglion (DRG) induces chemical inflammation of the nerve and evokes neuropathic pain. Several cytokines has been reported to play an important role to induce chemical reactions. A high mobility group box-1 (HMGB-1), originally known as an architectural nuclear protein, is identified as a late mediator of inflammation and induces neuropathic pain\(^1\). The purpose of this study was to investigate the role of HMGB1 in the pain-related behavior induced by NP application on to the DRG.

MATERIALS AND METHODS

Experiment 1: Certification of HMGB1 in the naive nucleus pulposus

The rats were placed in the prone position, and an incision was made in the middle of the proximal tail (n=3). NP was harvested from the disc of coccygeal spine by the forceps and froze in liquid nitrogen. Immunoblotting of naive NP was performed.

Experiment 2: HMGB1 involvement in neuropathic pain induced by nucleus pulposus application

Surgical procedure: Adult male Sprague-Dawley rats (200-300g, n=39) were used. The left L5/6 facet joint was removed and L5 DRG was exposed. NP was harvested from the tail was applied to the left L5 DRG.

Drug administration: 200 µg chicken anti-HMGB1 polyclonal antibody (SHINO-TEST, Kanagawa, Japan) (aHMGB1 group), or same amount of solvent (PBS) (PBS group) was administrated i.p. 12h and 36h after surgery (n=14 for each group)

Behavioral testing: To investigate the effect of HMGB1 antibody, sensitivity to non-noxious mechanical stimuli was measured using the von Frey test in the aHMGB1 group and PBS groups. The left hind paw withdrawal response to von Frey filament stimulation of the foot pads was determined on postoperative days 2, 7, 14, 21, 28, 35, 42, 49, and 56 (n=6 for each group).

Immunohistochemistry: Histological examinations of DRG with surrounding tissues were performed on postoperative days 2 (n = 5 for each group). Naive rats were used as controls (n = 5). Immunohistochemical examination was performed using rabbit anti-ATF3 antibody (Santa Cruz, Delawere, CA, USA), mouse anti-ED1 antibody (Chemicon, Temecula, CA, USA), goat anti-rat TNF-α antibody (RD System Minneapolis, MN, USA), and rabbit anti HMGB1 antibody(SHINO-TEST, Kanagawa, Japan).

Immunoblotting: Immunoblotting examinations of DRG was performed on postoperative days 2 (n = 3 for each group). Naive rats were used as controls (n = 3). The primary antibodies used for incubation were mouse anti-TNF-α (1:100; RD System), rabbit anti-HMGB-1 (1:100, SINO-TEST), and mouse anti-β actin (1:5000; Sigma).

RESULTS

Experiment 1: HMGB1 was clearly expressed on immunoblotting of NP using anti HMGB1 antibody, whereas there was no band in the lysis buffer. This result suggested that NP itself contained HMGB1.

Experiment 2: The mechanical withdrawal threshold in the aHMGB1 group was significantly increased from day 0 to day 7 compared with the PBS group (p<0.05) (Figure1). Double-labeled immunohistochemistry revealed that macrophages was immunoreactive for HMGB1 (Figure2) and TNF-α (Figure3). Immunoblotting revealed that NP contained HMGB1 and TNF-α expression was significantly decreased in the aHMGB1 group compared with the PBS group (Figure4).

DISCUSSION

HMGB1 would play an important role in developing neuropathic pain induced by application of nucleus pulposus. HMGB1 from applicated nucleus pulposus would act as a proinflammatory mediator together with pro-inflammatory cytokines which were also contained in the nucleus pulposus. HMGB1 blocking therapy might become one of the new treatment methods of neuropathic pain and provide additional clinical benefit to TNF and IL-1 blocking therapy.

REFERENCES