THE ROLE OF LEUKOCYTES IN NEUROPATHIC PAIN INDUCED BY THE NUCLEUS PULPOSUS IN THE RAT


Introduction

The precise mechanism of painful radiculopathy secondary to lumbar disc herniation remains unknown. We examined here, the role of leukocytes in the pain mechanism induced by the nucleus pulposus. Some studies have assessed histologically verified inflammatory cells such as macrophages, lymphocytes and neutrophils in herniated lumbar disc tissues. However, little is known regarding the relationships between clinical symptoms including radicular pain and the presence of inflammatory cells. We demonstrated that autologous nucleus pulposus, which is relocated on the lumbar nerve root in the rat, produces time dependent and reversible mechanical hyperalgesia, 1 which is thought to be a pain-related behavior in peripheral neuropathic pain models. The purpose of this study was to determine whether or not leukocytes are correlated to mechanical hyperalgesia induced by the nucleus pulposus and to further characterize the role of leukocytes in radicular pain due to lumbar disc herniation.

Materials and Methods

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee at our institution. In total, seventy-six male Sprague Dawley rats, each weighing 250 grams, were used for this study. All surgical procedures were performed with deep sodium pentobarbital anesthesia (50 mg/kg, i.p.). Nitrogen mustard (NM) was used to develop and evaluate leukocytopenia in the rats. Circulating leukocytes were counted in normal rats and NM-treated rats 3, 7 and 14 days after administration of NM (0.5, 1.0 or 1.5 mg/kg i.v., each n=6). Forty-eight rats were divided into 6 experimental groups (each n=8). In the S+L group, saline was intravenously administered, the tail was amputated and then the left L4 and 5 nerve roots were exposed after partial laminectomy. The 1.0NM+L group was treated the same as the S+L group but received an intravenous injection of 1.0 mg/kg NM. In the S+NP group, after administration of saline and amputation of the tail, the nucleus pulposus obtained from the amputated tail was relocated on the exposed nerve roots. The 0.5NM+NP, 1.0NM+NP and 1.5NM+NP groups were treated the same as the S+NP group but received an intravenous injection of 0.5, 1.0 and 1.5 mg/kg NM, respectively. Rats, in which the tail only was amputated, served as the control group (n=4). Motor function and reflex responses to noxious mechanical and thermal stimuli to both hind paws were measured in all rats preoperatively and by 14 days postoperatively (PO). The percentage difference between both hind paw responses to noxious stimuli was computed so that negative percentages reflected hyperalgesia, and positive percentages represented hypoalgesia. All rats, which the nucleus pulposus obtained from the tail was relocated on the nerve roots, were killed at 2 weeks PO. The specimens on the treated nerve roots were fixed routinely in a buffered formalin solution and embedded in paraffin, 5 μm-thick sections were subsequently stained with hematoxylin-eosin. According to the number of inflammatory cells and the formation of granulation tissue, the specimens were examined microscopically and classified by 2 independent examiners in a blinded manner. Data obtained from these measurements were analyzed by ANOVA and Student’s t-test for statistical analysis. A p value < 0.05 was considered significant.

Results

Leukocytopenia was observed in rats administered 1.5 mg/kg NM, but not in rats that received 0.5 mg/kg NM. Administration of 1.0 mg/kg NM resulted in leukocytopenia only 3 days after injection (Figure 1). No rats in any groups exhibited motor paresis or stress reactions before or after surgery. Rats in the control, S+L and 1.0NM+L groups showed normal responses to noxious stimuli over time. However, rats in the 0.5NM and S+NP groups exhibited evidence of mechanical hyperalgesia from 3 days to 14 days PO (p < 0.05). On the other hand, rats in the 1.5NM+NP group failed to show hypersensitivity to mechanical noxious stimuli. Rats in the 1.0NM+NP group showed evidence of mechanical hyperalgesia only at 14 days PO (Figure 2). There were no significant differences in sensitivities to thermal noxious stimuli among the groups. Histological examination revealed the formation of granulation tissues accompanied by numerous inflammatory cells in the 0.5NM+NP and S+NP groups. However, fewer inflammatory reactions and reduced granulation tissue were observed in the 1.5NM+NP group than in the 0.5NM+NP and S+NP groups.

Discussion

Some studies have reported that the nucleus pulposus itself on the nerve root produces delayed nerve conduction velocities, intraneurual edema and reduced blood flow in the nerve root and/or the dorsal root ganglion, and may produce radicular pain. However, we demonstrated here, that relocation of the autologous nucleus pulposus on the nerve root did not result in mechanical hyperalgesia in rats with leukocytopenia, which showed fewer inflammatory reactions around the nerve root. Therefore, neuropathic pain produced by the nucleus pulposus placed on the nerve root may be related to leukocytes induced by relocation of the nucleus pulposus rather than the nucleus pulposus itself. The control in the presence of leukocytes may lead to some therapeutic interventions, that may attenuate painful radiculopathy secondary to lumbar disc herniation.

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