The Effect of Asiaro-Erythropoietin (A-EPO) on Pain-Related Behavior and the Expression of Phosphorylated-P38 MAP Kinase (P-P38) and Tumor Necrosis Factor-Alpha (TNF) Induced by Application of Autologous Nucleus Pulposus (NP) on Nerve Root in Rat.

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Introduction: Erythropoietin (EPO), a hematopoietic growth factor, has broad tissue protective effect in organs not involved in hematopoiesis. Recently, it has been demonstrated that EPO has wide-spread neuroprotective effect in variety models of central and peripheral nerve injuries. However, EPO is a hematopoietic growth factor, therefore, EPO can cause significant side effect such as thicker blood and promotion of blood clotting. Asiaro-erythropoietin (A-EPO) is non-erythropoietic derivative erythropoietin but retains extrahematopoietic effects of EPO. Neuroprotective effect of A-EPO was also investigated various animal models. However, neuroprotective effect of A-EPO against chemical neuronal damages caused by an application of nucleus pulposus (NP) has not been demonstrated.

Tumor necrosis factor-alpha (TNF), one of the proinflammatory cytokines, is a major key factor and trigger in the pathogenesis of NP-induced inflammation and apoptosis. Several reports demonstrated that the inhibitors of TNF inhibit the reduction of nerve conduction velocity, morphologic changes, and spontaneous pain-related behavior induced by NP application. Furthermore, p38 Mitogen-activated protein kinase (MAPK), a class of mitogen-activated protein kinases, is phosphorylated by stress signals such as inflammatory cytokines, heat shock, ultraviolet irradiation, osmotic shock, and ischemia. Spinal cord injury and peripheral nerve injury cause the activation of p38 in dorsal root ganglia (DRG) neuron and spinal cord microglia. The activation of p38 MAPK in DRG neurons implicate exacerbation of pain condition. However, an effect of A-EPO against p-p38 and TNF is not investigated. The purposes of this study were to investigate effects of A-EPO on pain-related behavior induced by NP application on nerve root, and on the expression of phosphorylated-p38 (p-p38) and TNF.

Materials and Methods: Surgical procedure: Adult female Sprague-Dawley rats (Japan SLC, Shizuoka, Japan) were used (n=94). The left L5 nerve root and dorsal root ganglion (DRG) were exposed by L5-L6 facetectomy on the left side. Autologous NP was harvested from the tail and applied to the DRG (NP group). The animals in the NP group were divided into four groups. NP + non-treatment group; no administration, NP + A-EPO group; treated with A-EPO (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) (2680IU/kg), NP + EPO group; treated with EPO (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) (2680IU/kg) and NP + vehicle group; treated with vehicle (n=20 in vehicle group, n=21 in other groups.) (Table 1). The substances were administrated 1 day before surgery and daily for 2 weeks subcutaneously. The sham operated animals were exposed the L5 nerve root and NP was not applied to the DRG (sham group).

Table 1: Experimental groups

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Behavioral test: Withdrawal thresholds were determined by the von-Frey test for mechanical allodynia at 1, 7, 14, 21, and 28 days after surgery. Data were analyzed by Dunnett t-test (significant level; p<0.05).

Results: Behavioral testing: The thresholds were decreased in the NP + non-treatment and the NP + vehicle groups for 28 days. There were significant differences of the thresholds between the two groups compared with the sham group (p<0.05). In the NP + A-EPO group, the thresholds were significantly improved compared with the NP + non-treatment and the NP + vehicle groups at day 28 (p<0.05). In the NP + EPO group, the thresholds were significantly improved compared with the NP + non-treatment and NP + vehicle groups at day 21 and 28 (p<0.05) (Fig. 1).

Immunohistochemistry of p-p38 and TNF: In the NP + non-treatment and the sham groups, p-p38 and TNF positive cells were observed and co-localized with NeuN in the DRG. In the NP + non-treatment group, p-p38 and TNF positive cells were increased, whereas a few p-p38 positive cells were observed in the sham group.

Immunoblotting for p-p38 and TNF: The expression of p-p38 in the NP + A-EPO group was significantly lower compared with NP + vehicle group (p<0.05). The expression of TNF in the NP + A-EPO and NP + EPO groups were significantly lower compared with NP + vehicle group (p<0.05) (Fig 2).

Discussion: The results of this study suggested that A-EPO has the effects on improvement of pain-related behavior and reducing the expression of p-p38 and TNF at day 1. In the contrast, EPO also has the effects on improvement of pain-related behavior and reducing the expression of TNF, but not the expression of p-p38. Although A-EPO and EPO both participate with the inhibition of TNF expression, uniquely A-EPO might be related to the inhibitory action of phosphorylation of p38 MAP kinase in DRG in the early stage of NP application.

Primary antibodies used included mouse anti-rat TNF-α monoclonal antibody (1:200; R&D Systems, Minneapolis, MN, USA), rabbit anti-rat phospho-p38 MAPK antibody (1:200; Cell Signaling Technology, Boston, MA, USA), rabbit anti-rat p38 MAPK antibody (1:200; Cell Signaling Technology, Boston, MA, USA), and mouse anti-β-actin monoclonal antibody (1:5000; Sigma, Saint Louis, MO, USA). Positive bands of immunoblots were analyzed as the ratio compared to internal control β-actin using a computer-assisted imaging analysis system (Image J version 1.33u; National Institute of Mental Health, Bethesda, MD, USA).

Data were analyzed by un-paired t-test and Dunnett t-test (significant level; p<0.05).

Figure 1: Changes in the mechanical withdrawal threshold of the foot pad in rats. Data are means ± SD (n=10 for each group). *p<0.05, compared with the NP + non-treatment group. †p<0.05, compared with the NP + vehicle group.

Figure 2: Expression of p-p38 and TNF in left L5 DRGs, determined by Immunoblotting. Positive bands of p-p38 were analyzed by ratio against p38. Positive bands were analyzed by ratio against internal control β-actin. Data are means ± SD (n=5 for each group). *p<0.05, compared with the NP + vehicle group.