Effect of Neurotropin on the reduction of COX-2 and TNF-α in human intervertebral disc cells in vitro

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ABSTRACT

INTRODUCTION

Neurotropin (NTP) is a non-protein extract from the inflamed skin of rabbits inoculated with vaccinia virus. It has been widely used clinically in Japan to treat various chronic pain conditions. A double-blind placebo-controlled cross-over study investigating its effectiveness in the treatment of acute pain syndrome (CRPS) is currently underway also in the United States. Local injection or injections into the epidural space are commonly done and achieve a good effect, especially for symptoms of intervertebral disc herniation (IDH). Although its main effect has been indicated to be activation of the descending monoaminergic pain inhibitory systems of the central pain pathway[1,2], its mechanism for local action is not clearly known. IDH is a serious condition that induces inflammatory reaction around the nerve root, leading to functional disability. It is postulated that in addition to mechanical compression of the lumbar nerve root ganglia by herniated discs, there is a chemical stimulus to the production of symptoms[3]. As a cytokine constituent of herniated nucleus pulposus tissue, recent investigations have described a central role for the proinflammatory cytokine Tumor Necrosis Factor-α (TNF-α) in the manifestations of sciatica and nerve root damage associated with IDH[3,4]. Moreover, it has been reported that prostaglandin E2 (PGE2), and the rate-limiting enzyme of the pathway, is Cyclooxygenase-2 (COX-2) in the presence of various concentrations of NTP (0, 10-7, 10-6, and 10-5 NTP). The mRNA for COX-2, TNF-α, and phospholipase A2 (PLA2) were determined by ELISA. A statistical significance between two groups was determined by Student’s t-test. Statistical significance between more than two groups was analyzed using Scheffe’s multiple comparison tests. Significance was recognized as a probability value less than 0.05.

RESULTS

The confirmation of the positive feedback loop of IL-1β

First, the cDNA bands of COX-2, TNF-α and PLA2 were recognized at each base pair by RT-PCR. And then, it is confirmed that the cells are in inflammatory conditions and in a positive feedback loop of IL-1β by real-time PCR. The effect of NTP

The mRNA expression levels of the NTP-treated cells were compared with those derived from the NTP 0 stimulated (only IL-1β stimulated) ones. The relative ratios of the COX-2 mRNA expression levels were 67.8% in 10-7 NTP (p=9.7 × 10-4), 38.0% in 10-6 NTP (p=3.9 × 10-3), and 42.9% in 10-5 NTP (p=1.3 × 10-2) of NTP. The relative ratios of the TNF-α mRNA expression levels were 62.6% in 10-7 NTP (p=5.2 × 10-4), 46.4% in 10-4 NTP (p=6.2 × 10-6), and 51.0% in 10-3 NTP (p=2.2 × 10-5) of NTP. The relative ratios of the PLA2 mRNA expression levels were 91.0% in 10-7 NTP, 100.0% in 10-6 NTP, and 130.9% in 10-5 NTP of NTP. In protein levels, ELISA was performed. The relative ratios of the COX-2 production levels were 85.8% in 10-7 NTP, 63.3% in 10-6 NTP, and 33.8% in 10-5 NTP (p=3.3 × 10-3) of NTP. The concentration level of cultured media was 88.2% in 10-5 NTP, 73.9% in 10-4 NTP, and 82.7% in 10-3 NTP of NTP. The effect of nombetone

The mRNA expression levels of nombetone-treated cells were compared with those derived from the only IL-1β stimulated ones. The relative ratios of the mRNA expression level were 180.2% (p=0.01) in COX-2, 135.5% in TNF-α, and 85.9% in PLA2. The effect of NTP and nombetone on secreted PGE2

The mean values of the PGE2 concentration levels were 73.5% in 10-5 NTP/mL, 109.4% in 10-4 NTP/mL, and 74.0% in 10-3 NTP/mL. The mean value of the PGE2 concentration level was 23.6% in 50 μg/mL of nombetone.

These results consistently showed that NTP significantly suppress the expression of COX-2 and TNF-α at mRNA levels and the concentration of COX-2 also at protein levels in a dosage-dependent manner. Nombetone was found to significantly increased COX-2 at mRNA levels, but directly suppress the concentration of PGE2 in the culture media.

DISCUSSION

The results clearly demonstrated that NTP inhibits the expression of proinflammatory cytokines (COX-2 and TNF-α) in mRNA levels and the concentration of COX-2 at protein levels in a dosage-dependent manner. These findings, to our knowledge, have not been reported by other researchers.

PLA2, COX-2, and PGE2 are all materials of the arachidonic acid cascade. PGE2, which is an end product of the arachidonic cascade produced by the action of COX-2. As for NTP, the likelihood of showing an anti-inflammatory effect was suggested by the decreasing expression of COX-2. On the other hand, the effect of NTP for PGE2 was not confirmed. It was assumed that NTP did not contribute to PG production, and the action mechanism with NSAIDs was different. Nombetone did not affect gene expression of COX-2, and it was confirmed to restrain secretion of PGE2. The substrate arachidonic acid gains access to the active site via a hydrophobic channel (COX channel) and nombetone block the biosynthesis of prostanooids as they occupy the COX channel of COX-1 and COX-2[5]. On this account, nombetone did not affect the expression level of COX-2 although it may inhibit the activity.

At present, the way of administering NTP is peroral, intravenous administration, or muscle injection. However, the basis for local injection is not yet well understood. In the present study, it is suggested that NTP has anti-inflammatory effect, so the reason why administration is effective may be due to this anti-inflammatory action.

One limitation of the present study was that we did not determine the anti-inflammatory effect of NTP in vivo. In clinical practice, however, local injections, trigger-point injections, and injections into the epidural space of NTP with local anaesthetics are generally performed, and it is known that such treatment is effective in experience, and no adverse or detrimental effects have been reported in the majority, in Japan. Different sets of experiments will be required to confirm our data and verify our working hypothesis.

CONCLUSION

These results suggest that NTP shows an analgesic effect through suppression of COX-2 and TNF-α in a focal area, and nombetone show this through suppression of PGE2 production. These results suggest that NTP could decrease pain through blocking the central pain pathway, or through increasing the focal anti-inflammatory effects.

REFERENCES