INTRODUCTION: Chronic low back pain is a debilitating disease with increased sensitivity to noxious stimuli because of altered neuropathic pain processes. A recent study indicated that a significant degradation of facet joint cartilage occurs at its location close to the facet joints. The purpose of study was to determine if NTP reduces pain and to reveal the molecular mechanism in the DRG in the rat thrombin-induced arthritis model.

METHODS: Rat Thrombin-induced Facet Joint Arthritis Model. Twenty-four female Sprague-Dawley rats, weighing 300-550g, were used with IACUC approval. To induce facet joint inflammation, the right L4/5 level was carefully exposed without damaging the capsule through a posterior approach. Bovine thrombin (20U/2µL) was slowly injected into the facet joint space using a 5µL syringe with a 33G needle. From the day after surgery, animals received daily injections (1ml/100g BW, sq.) of saline (12 rats; Control) or NTP (12 rats; 1ml/100g BW, 20 NTP Units/ml, Nippon Zoki Pharm., Japan) until sacrifice. All animals were euthanized at 28 days and DRGs were isolated (8 DRGs per rat, 4 rats per group, 8 rats total for molecular analysis).

von Frey test: Tactile allodynia was assessed using the von Frey test preoperatively and at days 6, 9, 12, 16, 20 and 27 after surgery. Fifty percent paw withdrawal thresholds were determined using a modification of the up-down method as previously published.

Gene Expression Analyses: Gene expressions of cytokine (TNF-α), pain molecules [prostaglandin-endoperoxide synthase 2 (PTGS2) and nerve growth factor (NGF)] were assessed by QPCR. DRGs were stored in RNAalter and total RNA was extracted using the RNaseasy kit (Qiagen, CA). RNA was reverse-transcribed to cDNA and amplified using specific primers (SA Bioscience, MA). Standards were used prepared by cloning PCR products into pdrive vectors using the PCR cloning kit (Qiagen). 18s rRNA was used as internal control.

Statistical analyses: Two-repeated or one-way ANOVA with Fisher LSD post hoc test.

RESULTS: von Frey test: After the injection of thrombin, a significant decrease of withdrawal thresholds in the right paw of the rats was achieved on day 14 (p<0.05) (Fig. 1). A two-way repeated ANOVA of thresholds showed a significant difference between the saline and the NTP groups during the course of study. Thresholds in the NTP group were significantly higher than those of the saline control on days 12, 16 and 20 (p<0.05, Fig. 2A). Gene Expression: TNF-α expression at L2-L5 in the NTP group was significantly lower than that in the saline group (p<0.001). The expression peaked at L3 and L4 and was generally higher in the right DRG; however, a similar trend was observed on the left side. Similar trends showing the distribution of a high gene expression at L3/4 and the suppressive effect of NTP were observed for PTGS2 (p<0.001, Fig. 2B) and NGF (p<0.001, Fig. 2C).

DISCUSSION: NTP has been used clinically for back pain and neurotropic pain and is now being studied in two National Institute of Nursing Research pain treatment trials (fibromyalgia and chronic neuropathic pain). The mechanism of action for back pain is considered to be due to the activation of a descending pain inhibitory system in the brain. Recently, the direct effects of NTP on matrix synthesis and cytokine and COX-2 and TNFα gene expression have also been reported; these may suggest the presence of a multiple pathway for the mechanism of action of this analgesic agent. Our study indicated that NTP suppresses TNF-α, PTGS and NGF gene expression in L2-L5 DRGs in the rat thrombin-induced facet joint arthritis model. This suggests that the significant suppression of tactile allodynia may be due to this suppressive effect of NTP.