THE EFFECTS OF EXTRACELLULAR ATP ON CULTURED FIBROBLAST-LIKE CELLS FROM HUMAN CERVICAL LIGAMENTUM FLAVUM

**Sawada T; Furukawa, K-I; Kishiya, M; Kanemaru, K; Motomura, S; Toh, S

+Department of Pharmacology, Hirosaki University School of Medicine, Hirosaki, Japan

**Department of Orthopaedic Surgery, Hirosaki University School of Medicine, Hirosaki, Japan

h03gm104@cc.hirosaki-u.ac.jp

Introduction: Ossification of the posterior longitudinal ligament of the spine (OPLL) is a common disease among Japanese and other Asian populations, characterized by ectopic bone formation in the spinal ligament. There is no effective therapy for OPLL, except for spinal operations as an established one, therefore, safe and effective drug therapy is needed. However, the mechanism promoting the ossification is not known yet. Roles of extracellular factors in this disease are thought to be important, therefore, blocking the factors may be effective as a therapy for the disease. There are important extracellular substances related to homeostasis of bone tissue. Extracellular nucleotides such as ATP have been reported to play a pivotal role in the bone remodeling via binding to receptors termed P2-purinoceptors. These receptors are subdivided into P2Y and P2X subtypes based on the mechanism of signal transduction. If there are significant differences of the expression of the receptors between OPLL and non-OPLL cells, extracellular ATP may affect the initiation and progression of OPLL. The purpose of this study is to explore roles of extracellular ATP and P2-purinoceptors in the process of OPLL.

Material and Method: Informed consent was obtained from each patient and this study was approved by the Ethics Committee of Hirosaki University School of Medicine. C3-C4 level ligamentum flavum was harvested aseptically from OPLL and non-OPLL patients during cervical operation. Harvested flavum were cultured by the explant method. Fibroblast-like cells migrated from explants were harvested and cells from the fifth passage cultures were used. SaOS-2 (Human osteosarcoma cell line) cells were cultured and used as a control cell line expressing P2-receptor subtypes. Total RNA was extracted from cells and mRNA expressions of P2-receptor subtypes were analyzed by the RT-PCR and real-time PCR methods. Cells were stimulated with ATP (P2Y1 agonist) and expressions of osteogenic markers such as alkaline phosphatase (ALP) and osteopontin were analyzed by the real-time PCR method. Glyceraldehyde 3-phosphate dehydrogenase (G3PDH) was used as an internal standard.

Result: RT-PCR demonstrated a variable spectrum of expression of P2 receptor subtypes in OPLL, non-OPLL, and SaOS-2 cells, i.e., P2Y1, 2, 4, 6, 12, P2X4 in OPLL, P2Y1, 2, 4, 6, 12, P2X1, 4, 6, 7 in SaOS-2 cell line. In OPLL group (n=11), P2Y1 expression was as much as 7 times higher than that in non-OPLL group (n=9) (p<0.05) and almost equal to that in SaOS-2 (Figure1). In OPLL group (n=5), ATP (100 μM) as a P2Y1 agonist increased the mRNA level of ALP expression in a time-dependent manner up to 4 hr and a peak appeared at 1hr (p=0.05). On the other hand, significant change was not found in non-OPLL group (n=5) (Figure2). Messenger RNA expression of osteopontin was also stimulated by ATP in OPLL group (n=4) but not in non-OPLL group (n=5) (Figure3).

Discussion: Several lines of evidence have demonstrated that extracellular ATP affects on many physiological and pathophysiological activities of human cell lines. P2 receptor subtypes are detected in osteoblast, osteoclast and osteosarcoma. ATP binding to P2Y1 stimulated expressions of some transcriptional factors being related to bone metabolism, i.e., Egr-1, c-fos and Runx2/Cbfα1. In this study, the spectrum of expressions of P2 receptor subtypes in OPLL group was similar to that in SaOS-2 group but not in non-OPLL group. Messenger RNA expressions of P2Y1 in OPLL and SaOS-2 groups were statistically higher than that in non-OPLL group. Addition of ATP evoked a higher increase in not only ALP-mRNA but also osteopontin-mRNA expressions in OPLL and SaOS-2 groups but not in non-OPLL group. Therefore, it is suggested that one of extracellular factors influencing the process of ossification, a pathophysiological action of ATP via P2Y1 is important.

Reference

52nd Annual Meeting of the Orthopaedic Research Society

Paper No: 1249