Molecular characterization and comparison of the effects of several opioid agonists clinically used in Japan - Using the CellKey™ and internalization assays with stable cells expressing opioid μ , δ or μ/δ dimerized receptors

Yuriko Fujii^{1,2}, Kanako Miyano¹, Kaori Oshima^{1,3}, Sei Manabe^{1,4,5}, Ryoto Someya⁶, Kazumi Yoshizawa³, Hiroshi Morimatsu⁴, Masako Iseki², Eiichi Inada², Yasuhito Uezono^{1,7}

¹Division of Cancer Pathophysiology, National Cancer Center Research Institute, Tokyo, Japan, ²Department of Anesthesiology and Pain Medicine, Juntendo University School of Medicine, Tokyo, Japan, ³Department of Pharmacology and Therapy, Faculty of Pharmaceutical Science, Tokyo University of Science, Chiba, Japan, ⁴Department of Anesthesiology and Resuscitology, Okayama University Medical School, Okayama, Japan, ⁵Department of Anesthesiology & Critical Care Medicine, National Cancer Center Hospital, Tokyo, Japan, ⁶Laboratory of Medicinal Chemistry, Kitasato University School of Pharmacy, Tokyo, Japan, ⁷Division of Supportive Care Research, Exploratory Oncology Research & Clinical Trial Center, National Cancer Center, Tokyo, Japan

Morphine(MRP), fentanyl(FEN), oxycodone(OXY) and hydromorphone(HDM) are approved as opioid analgesics in Japan, and they show analgesia mainly through activation of μ opioid receptors (μ OR) among μ , δ , and κ ORs. However, these analgesics show different side effects, possibly due to their distinct intracellular signaling. Recently, it was demonstrated that μ OR forms a heterodimer with δ OR, and its specific agonist ML335 has been reported to have less side effects and lower tolerance. However, the precise mechanism of ML335 and other opioid analgesics on OR activities are poorly clarified.

The present study thus aimed to investigate intracellular signaling induced by these opioid analgesics and compared them with those by ML335, by determining Gi/o-mediated signaling and internalization profiles using cells expressing μ OR, δ OR or dimerized μ/δ OR.

[Methods]

Halo-tagged μ OR, Halo- δ OR, and dimerized Halo- μ OR/T7-tagged δ OR stably expressing cells were used for assays. The values by activation of each OR by agonists were evaluated with CellKey™ system by measuring electronic resistance changed by opioid analgesics as Gi/o-mediated signaling. Internalization of ORs was also evaluated by counting internalized OR with fluorescence-dyed technique using a pH-sensitive HaloTag® ligand that elicits red fluorescence when entered into the endoplasmic reticulum.

[Results]

Four analgesics as well as ML335 increased the electric resistance values in μ , δ , μ/δ OR expressing cells in a concentration-dependent manner. The rates of EC₅₀ values in each OR were found; μ OR: FEN <HDM <MRP <ML335 <OXY, δ OR: HDM ≤ FEN <MRP <OXY <ML335, and μ/δ OR: FEN <HDM <MRP <ML335 <OXY. In OR internalization assay, a pronounced μ OR internalization was observed by FEN as compared with MRP, OXY, HDM or ML335. The rank order of internalized ORs by the agonists are underway.

[Conclusion]

This study revealed that ML335 activates not only μ/δ OR, but also μ OR and δ OR. Furthermore, in comparison to ML335, FEN, HDM and MRP rather have high affinity for μ/δ OR. Among four opioid analgesics, character of MRP seems to be similar with that of ML335. When completed internalization assay of μ OR, δ OR and dimerized μ/δ OR, collected data from CellKey™ and internalization assay will be analyzed and be presented at the meeting.