

Antinociceptive Effects of AS1892802, a Novel and Selective Rho Kinase Inhibitor, in Both Inflammatory and non-Inflammatory Rat Models: a New Action Mechanism of an Analgesic for Osteoarthritis Pain

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INTRODUCTION:

Osteoarthritis (OA) is a disease in which the joint cartilage is chronically abraded or defected and the form of the joint changes. The hallmark symptom of OA is pain, which is described as deep and aching, worsening with joint use and improving with rest. According to a study of hip and/ or knee OA patients, 26% of them suffer chronic pain, which they described as "quite severe" (1). Treatment is generally based on symptomatic relief of pain and inflammation related with OA to improve joint function. Non-steroidal anti-inflammatory drugs (NSAIDs) and acetaminophen are most widely used drugs and cyclooxygenase 2 (Cox2) inhibitors are as well. However, these agents are ineffective in patients with "severe pain" and have insufficient efficacy. In addition, gastrointestinal side effects and cardiovascular risk are the greatest concerns with these drugs use. Therefore potent effective analgesics with safe are needed for OA patients with pain.

Rho kinase (ROCK) is a serine/threonine kinase that acts downstream of Rho, and is suggested to be involved in various physiological functions such as cell motility, cytoskeleton control, vasoconstriction and inflammation. Some reports described the analgesic effect of ROCK inhibitors (2, 3), however, there is no report showing efficacy of administration after onset of symptom on pain or in an in vivo chronic inflammatory model or osteoarthritis model.

Here we report that administration of a new ROCK inhibitor reduces pain that develops with complete Freund's adjuvant (CFA)-induced inflammation or with monoiodoacetate (MIA)-induced non-inflammatory OA and present usefulness of ROCK inhibitor for the treatment of OA pain.

METHODS:

ROCK enzyme activities were determined using ELISA assay. Assays for determining inhibitory activities of compounds were performed with purified human ROCK I (Carna Biosciences) or rat ROCK II (Upstate), MYTP (Upstate) as a substrate of ROCKs, and anti-phosphoserine/threonine antibody, and were quantified by absorbance at 450 nM after developing with coloring agents. Data for effects of AS1892802 on other kinases were obtained from Carna Biosciences. Experimental OA was induced by an intra-articular injection of MIA (1mg, Sigma) into right hind knee of male SD rats. Hindlimb weight bearing was determined using an incapitance tester (Linton Instrumentation, Norfolk, UK). Three weeks after injection of MIA analgesic effects of compounds on the weight bearing were performed. Hyperalgesia with chronic inflammatory was induced by an intraplantar injection of CFA (Difco) in Lewis female rats. The pain threshold was measured at non-injected paw using an analgesy meter (Ugo Basile) and the change of pain threshold was calculated. Prostaglandin E2 (PGE2) contents was determined with a commercial kit (Cayman) after extracting from the paw. AS1892802 which was newly synthesized in Astellas Pharma Inc., diclofenac (Cayman Chemical Co.), fasudil (Sigma) or tramadol (Sigma) was orally administered. Naloxone (Sigma) was administered subcutaneously 10 min before AS1892802.

Data from the experiments were analyzed by analysis of variance (ANOVA) and Dunnett's t-test. Probability values less than 0.05 were considered statistically significant. The experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee of our institute.

RESULTS:

Effects of AS1892802 on inhibition activities of ROCKs were examined. The IC50 values against human ROCK I and rat ROCK II were

122 nM and 76nM, respectively. The compound was highly selective for ROCK without any kinase inhibition activities against other 110 kinases.

A weight bearing deficit, an approximate 40g, was detected in MIA-injected rats at day 21. Diclofenac (3-30mg/kg) which is one of representative NSAIDs had no effects on the weight balance. However, AS1892802 (0.1-1mg/kg) dose-dependently reversed the weight bearing deficit with ED50 value is 0.15 mg/kg. Similarly, tramadol, which is used for treatment of OA pain, significantly reduced alterations in the weight bearing (ED50: 12 mg/kg). The effects of the drug were less than those of AS1892802. Intra-articular injection of AS1892802 (3ug/site) also significantly attenuated the weight bearing deficit

In CFA-induced chronic inflammatory model, hypersensitivity for mechanical stimuli was observed 18-24 days after injection of CFA. AS1892802 (0.1-1 mg/kg) dose-dependently reduced the sensitivity with the ED50 value of 0.29 mg/kg and the efficacy is more potent than those of clinical effective dose of diclofenac. The analgesic effect of AS1892802 was not affected by the pre-treatment of naloxone. In addition, PGE2 contents in the inflammatory paw were not changed by the treatment of AS1892802.

Fasudil, a launched ROCK inhibitor in Japan for the treatment of cerebral ischaemia, also dose-dependently reduced the weight bearing deficit of MIA model and hypersensitivity for mechanical stimuli of CFA-induced chronic inflammatory model and ED50 values were 3.5 mg/kg and 4.8 mg/kg, respectively.

DISCUSSION:

Analgesic effects of AS1892802, a novel ROCK inhibitor, on chronic pain of both MIA-induced OA rats and CFA-induced inflammatory rats are more potent than commercially available diclofenac and tramadol. As antinociceptive effects of fasudil are also shown in both the chronic pain models, ROCK inhibitors will provide a new useful treatment against OA pain. Especially, at the time point of MIA rats, diclofenac had no effects on the weight balance. Therefore, ROCK inhibitors could have beneficial effects on the NSAIDs-resistant and severe pain. Although action mechanisms of ROCK inhibitor for pain are little understandings, our experiment showed that actions of AS1892802 are not related with opiate or PGE2 pathways. Peripheral pain inhibition pathway might be involved in the analgesic effects of AS1892802. In addition to treatment of severe pain AS1892802 could be expected to have additive effects of NSAIDs. In our preliminary data, repeated administrations of AS1892802 showed long lasting analgesic effects in both MIA and CFA models. Since oral administration of higher doses or local knee-injection of 3 ug/site of the compound treatment for 3 weeks significantly decreased progression of cartilage destruction of the knee in the MIA model, beneficial effects of the compound on cartilage may induce indirect pain relief. As for side effects of AS1892802, single administration of AS1892802 (10 mg/kg) did not induced gastric ulcer which was different from those of indomethacin (personal communication).

These results suggest that AS1892802 should be a potent analgesic compared with NSAIDs and tramadol without severe side effects for the treatment of OA pain.

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