Analgesic Effects of Intra-arterial Infusion of Imipenem/Cilastatin Sodium in a Rat Model of Knee Osteoarthritis.

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INTRODUCTION: Knee osteoarthritis (KOA) is a prevalent disease, especially among the elderly, presenting challenges globally [1][2]. Knee pain, a primary symptom, can drastically affect physical activity and quality of life, emphasizing the need for effective pain management [3][4]. Okuno et al. [3][5] introduced the idea that neovessels associated with nerves might be pain sources in musculoskeletal disorders. They advocated for Transcatheter Arterial Micro-Embolization (TAME) using imipenem/cilastatin sodium (IPM/CS) as an effective pain relief method for KOA. IPM/CS is known as an antibiotic, and acts as an embolic agent on small blood vessels. Despite TAME's clinical success, its mechanisms remain elusive. Its analgesic effects have not been demonstrated in animal models. The aim of this study was to investigate the analgesic effects of intraarterial embolization with IPM/CS in KOA rat model, using pain behavior assessments and in vivo whole-cell patch-clamp techniques.

METHODS: All experimental procedures involving the use of animals were approved by the Ethics Committee on Animal Experiments, Wakayama Medical University, and were in accordance with the UK Animals (Scientific Procedures) Act, 1986, and associated guideline.

1. Induction of KOA: Male Sprague–Dawley rats (3 weeks old, 70-110g) were given a single intra-articular (i.a) injection of 3mg of Sodium monoiodoacetate (MIA) in one knee to induce unilateral KOA, under 1–3% isoflurane anesthesia. Another group of rats received a sham injection of saline alone (Sham group). After three weeks, histopathological changes in their knees were assessed using the OARSI scoring system [6].

2. Intra-vessel infusion of IPM/CS: The femoral neurovascular bundle was exposed, and the femoral sheath was opened with a microscope under 1–3% isoflurane anesthesia. For drug administration into the femoral artery/vein, a 30-gauge needle was directly punctured. A suspension of 0.5 g IPM/CS in 5 mL of 0.9% saline was prepared and 0.2 ml of the solution injected into either the femoral artery or vein. For histological verification of embolic material presence, we compared the rats with intra-femoral artery/vein administration of IPM/CS to those given saline in the KOA model. Both groups were sacrificed immediately post-administration and subsequently evaluated using hematoxylin and eosin (HE) staining.

3. Pressure Application Measurement (PAM): Mechanical pain threshold in rodents was measured with PAM device (Ugo Basile) [7] . The device is designed to apply a gradually increasing squeeze pressure directly across the knee joint of rats until the animal gives an indication of pain or discomfort. The peak gram force (gf) which applied immediately prior to limb withdrawal was recorded, and this value was designated the limb withdrawal threshold (LWT). Data were collected from the sham group, KOA group, and KOA groups with intra-arterial and intra-venous infusions.

4. In vivo patch-clamp recordings: The methods used for the in vivo patch-clamp recordings were as described previously [8][9][10][11][12]. The lumbar spinal cord was exposed from L3 to L5 following thoraco-lumbar laminectomy from Th11 to L2. The dura was cut and a recording electrode was advanced into the SG from the surface of the spinal cord. The surface of the spinal cord was irrigated with Krebs solution (10 -15 ml/min) and equilibrated with a 95% O2,5%CO2 gas mixture through glass pipettes. The electrode was advanced at an angle of 30–45° into the SG through a window in the pia-arachnoid membrane using a micromanipulator. A tight seal (resistance of at least 10 GΩ) was then formed with neurons at a depth of 30–150 μm. After forming the seal, the membrane patch was ruptured by a brief period of more negative pressure, thus resulting in a whole cell configuration for excitatory postsynaptic current (EPSC) recordings. The data were recorded in the L4 medullary segment level where pain information from the knee joint is considered to be input. Data were collected from the sham group, KOA group, the KOA group with intra-arterial infusion of saline, and the KOA group with intra-arterial infusion of IPM/CS. We then analyzed the spontaneous EPSCs for each group.

RESULTS:

1 Histopathologic finding: In KOA group, weak safranin-O staining in cartilage matrix was observed, indicating differences in OARSI scores between KOA and sham groups. Upon intra-arterial infusion of IPM/CS to the KOA group, IPM/CS particles were seen inside synovial tissue arteries (Fig 1).

2 Pressure application measurement (PAM): Mechanical threshold tests after KOA induction showed a significant decrease in average ipsilateral LWT in the MIA group over 21 days compared to contralateral joints. After intra-arterial infusion in KOA group, the ratio of right LWT to left one significantly improved in IPM/CS group than saline group after infusion ($P < 0.05$) (Fig 2 a). After intra-venous infusion, both in IPM/CS and saline groups, the ratio of right LWT to left one did not improve (Fig 2 b).

3 *In vivo* patch-clamp recording: The average frequency and amplitude of spontaneous EPSCs in sham group were 3.2±0.6 Hz and 6.7±0.9 pA (n=9). The average frequency and amplitude of spontaneous EPSCs in MIA group were 10.1±1.4 Hz and 5.9±0.3 pA (n=7). The average frequency and amplitude of spontaneous EPSCs in intra-arterial infusion of saline group were 16.5±3.9 Hz and 7.9±1.1 pA (n=5). The average frequency and amplitude of spontaneous EPSCs in intra-arterial infusion of IPM/CS group were 3.5±0.7 Hz and 6.5±1.5 pA (n=8). The average frequency of spontaneous EPSCs in intra-arterial infusion of IPM/CS on KOA was significantly lower than that of KOA without intra-arterial infusion rats and intra-arterial infusion of saline on KOA group ($p<0.05$) (Fig 3). There was not significant difference in the average amplitude of spontaneous EPSCs among the groups.

DISCUSSION: There are few basic reports using animal models for TAME. Taguchi et al [13] reported that transcatheter arterial embolization using IPM/CS decreased the number of blood vessels and inflammatory changes in the frozen shoulder, and increased the moving distance and speed of in rat after 1 week. In this study, following the administration of IPM/CS, the pain threshold in the knee osteoarthritis rat model increased, and there was a reduction in the frequency of spontaneous EPSCs. Histopathologically, similar to the findings reported by Taguchi et al., IPM/CS particles were observed inside the arteries within the synovial tissue of the KOA group that received intra-femoral arterial IPM/CS infusion. The intra-arterial infusion of IPM/CS potentially reduces the number of vascular endothelial cells and infiltrating inflammatory cells (and their associated chemical mediators). It is anticipated that further investigation into the pathological changes occurring peripherally is necessary, taking into account factors such as inflammation and chemical mediators.

SIGNIFICANCE/CLINICAL RELEVANCE: Analgesic effect of intra-arterial infusion of IPM/CS to knee osteoarthritis model rats was shown. This approach could be a potential pain relief solution for patients with persistent knee osteoarthritis pain.

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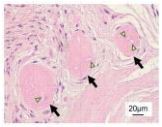

Fig 1. IPM/CS particles inside arteries

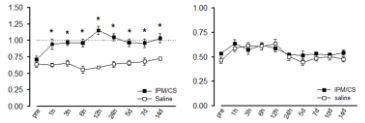

Fig 2. LWT (a) intra-arterial infusion (b) intra-venous infusion

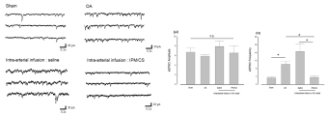
Fig 3. Spontaneous EPSCs and Summary