Effect of Intra-articular 4-([S]-Amino-5-([4-[4-[2,4-dichloro-3-(2,4-dimethyl-8-quinolyloxymethyl)phenylsulfonamido]-tetrahydro-2H-4-pyranyl(carbonyl)] piperazino)-5-oxopentyl][(trimethyl)ammonium chloride hydrochloride (MEN16132), a Kinin B₂ Receptor Antagonist, on Nociceptive Response in Monosodium Iodoacetate-Induced Experimental Osteoarthritis in Rats

Cecilia Cialdai, Sandro Giuliani, Claudio Valenti, Manuela Tramontana, and Carlo Alberto Maggi
Pharmacology Department, Menarini Ricerche S.p.A., Florence, Italy
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ABSTRACT

The present study was designed to investigate the role of bradykinin (BK) in the knee joint osteoarthritis induced by intra-articular (i.ar.) administration of monosodium iodoacetate (MIA) in the rat, and to determine the efficacy of the kinin B₂ receptor antagonists, 4-([S]-amino-5-([4-[4-[2,4-dichloro-3-(2,4-dimethyl-8-quinolyloxymethyl)phenylsulfonamido]-tetrahydro-2H-4-pyranyl(carbonyl)] piperazino)-5-oxopentyl][(trimethyl)ammonium chloride hydrochloride (MEN16132) and icatibant, in reducing pain. Rats received MIA (1 mg/25 μl i.ar.) in the right knee. MEN16132, icatibant (1, 3, and 10 μg/25 μl i.ar.), or saline were administered 7 days after MIA treatment, and their antinociceptive effect was observed for 2 weeks. MEN16132 induced a marked and sustained reduction of incapacitation produced by MIA, approximately 56% inhibition of pain at 3 μg/knee. MEN16132 analgesia was more potent and longer lasting, up to 10 days, than icatibant. MEN16132 (3 μg/knee), at different time points from MIA treatment in separate groups of animals, produced comparable maximal antinociceptive effects, whereas the pain response induced by MIA was unaffected if MEN16132 (10 μg/knee) was administered in the contralateral knee. Indomethacin at high doses (100–625 μg/knee) inhibited by approximately 40% but with a short duration the MIA-induced pain. MIA treatment produced a significant increase of BK and prostaglandin E₂ (PGE₂) metabolite levels in synovial fluid up to 21 days, and PGE₂ metabolite levels were reduced almost to basal values by MEN16132. In conclusion the potent and long-lasting analgesic effect of MEN16132 in MIA-induced osteoarthritis indicates an important role for BK in osteoarthritic pain, and suggests that MEN16132 can be a candidate for the treatment of this chronic disease.

Osteoarthritis (OA) is a degenerative joint disease that affects most of the elderly population causing chronic pain and joint disability. In particular, the OA of the knee is characterized by histological changes of the joint involving degeneration of articular cartilage with fibrillation and erosion, synovitis, remodeling of subchondral bone with osteophytes formation at the joint margins, and sclerosis of subchondral bone (Goldring and Goldring, 2007). The pathophysiology behind these modifications is complex and involves a combination of mechanical, cellular, and biochemical processes that are not yet completely understood. Inflammation contributes to increase structural degeneration by releasing catabolic and proinflammatory mediators including cytokines, nitric oxide, and matrix metalloproteinases that may activate chondrocytes and synovial fibroblasts leading to cartilage destruction (Goldberg et al., 1982; Yasuda, 2006; Sutton et al., 2009). No drug modifying OA progression is currently available, and treatment options are focused on symptomatic relief of pain and inflammation to improve the joint function. Nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and opiates are widely used, but no drug modifying OA progression is currently available.
none of them provide complete relief and side effects are often associated with therapeutic treatments (Hinton et al., 2002).

Pain in osteoarthritic rats is related to the rich sensory innervation of the knee joints. Small-diameter sensory afferent nerve fibers, including unmyelinated C fibers and thin myelinated Aδ fibers, have been identified in the knee capsule, ligaments, menisci, periosteum, synovial layer, and subchondral bone, and cartilage is devoid of nervous endings (Marinozzi et al., 1991; McDougall, 2006). The terminals of such fibers may be directly activated and sensitized to mechanical stimuli by inflammatory mediators such as bradykinin, neuropeptides, eicosanoids, and ion-channel ligands (Neugebauer et al., 1989; Schaible et al., 2002; Pawlak et al., 2008). Bradykinin (BK), produced during tissue injury and inflammation by the action of plasma and tissue kallikreins on kininogens, is an important mediator of inflammatory pain. By stimulating the constitutively expressed kinin B₂ receptors, it mediates many processes such as vasodilation, plasma extravasation (Green et al., 1994; Lo et al., 1999), inflammatory cell recruitment, and activation and sensitization of nociceptive afferent nerve terminals (Kanaka et al., 1985; Dray and Perkins, 1988, 1993; Steranka et al., 1988; Dray, 1997). A pathogenetic role for bradykinin in osteoarthritis has been hypothesized (Meini and Maggi, 2008), and there is evidence that, when injected in the rat knee joint, BK causes a progressive incapacitation that was inhibited by the selective B₂ receptor antagonist icatibant (Tonussi and Ferreira, 1997). Icatibant, in a model of adjuvant-induced arthritis in the rat, showed anti-inflammatory effects on knee joint swelling and reduction of tissue kallikrein levels in synovial tissue (Sharma and Wirth, 1996).

The presence of BK has been found also in the synovial fluid of patients affected by rheumatoid arthritis, and the B₂ receptors have been demonstrated on synovial lining cells, fibroblasts, and endothelial cells of blood vessels from patients affected by OA (Bond et al., 1997; Cassim et al., 1997). Moreover, BK involvement in OA pain, through B₂ receptors, has been shown in patients with symptomatic knee osteoarthritis in which intra-articular injection of the B₂ receptor antagonist icatibant reduced pain intensity (Sorbera et al., 2006; Song et al., 2009).

Despite a considerable evidence of BK involvement in nociceptive activation, pain generation, and transmission, the role of this mediator has not yet been investigated in the monosodium iodoacetate (MIA)-induced experimental model of osteoarthritis. MIA is a metabolic inhibitor of glycolysis in cartilage, synovial chondrocytes that causes cartilage degradation and loss followed by subchondral bone alterations and induction of synovial inflammation (Guzman et al., 2003; and pain (Ponomis et al., 2005; Ivanavicius et al., 2007) resembling the human pathology.

Different analgesic and anti-inflammatory drugs, including naproxen, rofecoxib, celecoxib, and acetaminophen, have already been tested after oral administration against MIA-induced incapacitation. Among these compounds the most potent was naproxen, which has shown an acute antinociceptive activity, at day 14 from MIA treatment, that lasted for a few hours only (Bove et al., 2003; Ivanavicius et al., 2007).

The aim of this study was to investigate the efficacy of the novel potent and selective nonpeptide kinin B₂ receptor antagonist MEN16132 (Cucchi et al., 2005; Valenti et al., 2005) in reducing MIA-induced osteoarthritic pain in the knee joint compared with the peptide B₂ receptor antagonist icatibant.

Materials and Methods

Experiments were carried out in accordance with the principles and guidelines of the European Union, the Italian Government regulations, and the local ethics committee.

Male Wistar rats (Harlan Laboratories, Udine, Italy) weighing 200–250 g were used. Animals were housed under a 12-h dark/light cycle with free access to a standard pellet diet and to water ad libitum.

Induction of Osteoarthritis. Osteoarthritis was induced by intra-articular (i.ar.) injection of monosodium iodoacetate solution in the knee joint. Animals were anesthetized with pentobarbital (40 mg/kg, 3 ml/kg i.p.) and received a single injection of MIA (0.3, 1, and 3 mg in 25 μl of 0.9% saline) into the joint space of the right knee through the infrapatellar ligament after shaving the skin and by a gentle flexion of the knee. The left knee received an equal volume of 0.9% sterile saline. MIA and drug solutions were prepared under sterile conditions and injected by use of a 50-μl Hamilton microliter syringe with a 6-mm, 27-gauge needle that was inserted into the joint for approximately 2 to 3 mm. The dose of MIA, producing a prolonged, altered hind limb weight distribution to test the effect of kinin B₂ receptor antagonists, was selected on the basis of the dose–response experiment.

Evaluation of Pain-Related Behavior. Pain associated with OA was characterized by changes in weight distribution on each hind paw. A hind limb weight-bearing apparatus (incapacitance tester; Linton Instrumentation, Norfolk, UK) was used to assess the difference in the distribution of weight between the right (osteoarthritic) and the left (contralateral control) hind limb. Animals were placed into a Plexiglas chamber with each hind paw on the separate force plate, and they were allowed to become accustomed to the apparatus. When stationary, the force exerted on the plate by each hind paw was recorded over a period of 5 s and expressed in grams. A total of four readings were taken for each rat at each time point, and the mean was used for calculation. The study was performed in a no blinded fashion.

Antinociceptive Activity of the B₂ Receptor Antagonists. The relationship between increasing concentrations of MIA (0.3, 1, and 3 mg/25 μl i.ar.) and development and duration of osteoarthritis pain was evaluated at various time points after MIA injection. The antinociceptive effect of the B₂ receptor antagonists MEN16132 and icatibant was assessed in rats treated with 1 mg/25 μl i.ar. MIA.

MEN16132 or icatibant (1, 3, and 10 μg/25 μl in 0.9% saline) were injected in the right knee on day 7 after MIA treatment when the nociception reached a plateau, while the left knee received saline, and their effect on pain-related behavior tested for 2 weeks at selected time points (days 1, 3, 7, 10, and 14) from their administration. At the end of this period, in the group receiving MEN16132 (3 μg/25 μl i.ar.), the dose was repeated and the effect on weight bearing was observed for another 14 days at the same time points as above to determine the reproducibility of the MEN16132 antinociceptive effect.

The antinociceptive activity of MEN16132 was also checked after its administration at different time points of the experimental OA development. MEN16132 (3 μg/25 μl i.ar.) was injected in the right knees of different groups of animals at days 3, 7, 14, and 21 after MIA (1 mg/25 μl i.ar.) treatment. The effect on pain behavior was evaluated, with use of the incapacitance tester, 1 and 3 days after its administration, corresponding to MEN16132 maximal analgesic activity.

MEN16132 in the Contralateral Normal Knee of MIA-Treated Rats. MIA (1 mg/25 μl) was injected in the right knee to induce osteoarthritis, and the nociceptive response was measured with the incapacitance tester. Seven days later, MEN16132 (10
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**Antinociceptive Activity of Indomethacin.** Indomethacin was administered intra-articularly in the right knee 7 days after MIA (1 mg/25 μl) treatment, and its antinociceptive activity was observed for 2 weeks with the same protocol used for the B2 receptor antagonists.

**MEN16132 on the Knee Edema Induced by MIA.** MIA (1 mg/25 μl i.ar.) or saline were administered in the right knee according to the experimental protocol described above. MEN16132 (3 μg/knee) was administered 3 days after MIA treatment and its anti-inflammatory effect, as inhibition of the knee swelling, was measured with a calipers 1 day later.

**Bradykinin and PGE2 Metabolite Levels in the Synovial Fluid.** Collection of synovial fluid was performed on days 3, 7, 10, 14, and 21 after MIA treatment (1 mg/25 μl i.ar.) in rats anesthetized with urethane (1.2 g/kg i.p.) by intra-articular lavage of synovial cavity. After skin shaving, a 27-gauge needle was inserted into the joint capsule for approximately 2 mm from the surface and connected, through a catheter, to an infusion pump (Ismatec, Glattbrugg, Switzerland). The joint capsule was first infused with 100 μl of 0.9% saline to dilate the capsule wall and to induce a slight increase of internal pressure. This was done to allow the correct insertion of a second 27-gauge needle, next to the first one, and to make the synovial fluid collection easier. Then, the infusion proceeded until 500 μl was taken. Synovial fluid was centrifuged at 10,000g for 5 min at 4°C and stored at -80°C until analysis. Bradykinin levels were measured in the synovial lavage fluid through an enzyme-linked immunosorbent assay. Bradykinin standards (0.008–2.5 μg/ml) (PolyPeptide Group, Strasbourg, France) or samples were added to MaxiSorp immunoplate (Nalge Nunc International, Rochester, NY) and incubated overnight at 4°C. The following incubations with goat anti-bradykinin antibody 1:500 in PBS-T (Santa Cruz Biotechnology, Santa Cruz, CA), biotinylated donkey anti-goat IgG 1:1000 in PBS-T (Santa Cruz Biotechnology), streptavidin-conjugated horseradish peroxidase 1:1000 in PBS-T, o-phenylenediamine peroxidase substrate (Sigma Fast OPD; Sigma-Aldrich, St. Louis, MO) were performed at room temperature, and the absorbance read at 490 nm against a reference wavelength of 620 nm. Bradykinin levels were expressed as the total amount (nanograms per milliliter) in the synovial fluid. Because prostaglandin E2 (PGE2) produced in synovial fluid is rapidly converted in vivo to its more stable metabolites, an estimate of the amount released was obtained through the determination of a PGE2 metabolite (PGE2) assay (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s instruction. PGE2 levels were expressed as the total amount in the synovial fluid (picograms per milliliter).

**Drugs and Chemicals.** MEN16132 (Cucci et al., 2005) and icatibant or HOE140 or D-Arg-[Hyp3, Thi5, D-Tic7, Oic8]bradykinin (Hock et al., 1991), were synthesized at the Chemistry Departments of Menarini Ricerche, Florence and Pomezia (Italy). Indomethacin (Liometrecon, 50 mg/2 ml) was purchased from Promedica S.r.l. (Parma, Italy). Monosodium iodoacetate, sodium pentobarbital, and urethane were purchased from Sigma-Aldrich.

**Statistical Analysis.** Data related to pain behavior were expressed as the difference in weight distribution (g) between the left and the right hind limb (Δ g left–right) and then calculated, after the administration of compounds, as percentage of inhibition of the respective basal value. Each point represents the mean ± S.E.M. of the given number of animals. Statistical comparison among groups was performed with one-way analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison post test. Data related to BK and PGE2 levels were expressed as total amount of the mediators in the synovial fluid, and statistical comparison among groups was performed with ANOVA followed by Student’s t test for unpaired data. The differences among groups were considered significant at the level of P < 0.05.

**Results**

**Effect of Intra-articular MIA Administration on Hind Limb Weight Bearing.** Intra-articular injection of MIA (0.3, 1, and 3 mg/25 μl) into the right knee induced a dose-dependent joint incapacitation resulting in alteration of hind limb weight bearing (Fig. 1). Pain was characterized by a biphasic response with an initial acute and marked reduction of weight bearing on the right limb in the first week. Such discomfort was maximal on day 3 after injection, followed, after day 7, by an almost constant and long-lasting pain response that did not resolve in the course of the 6-week experimental period. Alterations in hind limb weight bearing were more prominent after 1 and 3 mg/25 μl MIA and mild at the dose of 0.3 mg/25 μl, whereas saline was without effect. The response induced by MIA at 1 and 3 mg/25 μl was maximal at 3 days from administration and similar in terms of intensity (weight distribution left–right = 65 ± 9 g and 70 ± 4 g, respectively, n = 5) and duration (weight distribution on day 21 left-right = 48 ± 7 g and 53 ± 4 g, respectively, n = 5). The dose of 1 mg/25 μl was chosen for further experiments to give an almost maximal stable and prolonged pain response.

**Effect of Intra-articular MEN16132 and Icatibant Administration on MIA-Induced Changes in Hind Limb Weight Bearing.** The kinin B2 receptor antagonists MEN16132, icatibant, or saline were injected into the right knee on day 7 after MIA treatment (1 mg/25 μl i.ar.). MEN16132 caused a greater and prolonged dose-dependent (1, 3, and 10 μg/25 μl i.ar.) inhibition of MIA-induced pain response compared with icatibant (Fig. 2). MEN16132 showed an antinociceptive effect, statistically different from the saline-treated group, starting from 1 day after its administration and lasting for the whole experimental period, whereas icatibant was active only on day 3 after its administration and the effect, at all the doses used, faded rapidly. In detail, 24 h after administration, MEN16132 (3-10 μg/25 μl i.ar.) reduced the basal MIA-induced alteration of hind
limb weight distribution (basal value) by approximately 40% ($n = 8–9$) compared with the same doses of icatibant ($16 \pm 11\%$ and $6 \pm 11\%$ inhibition at 3 and 10 $\mu g/25 l$ i.a.r., respectively; $n = 8–10$). The maximum effect on pain response was produced by both antagonists on day 3 after administration. At 3 and 10 $\mu g/25 l$ i.a.r., MEN16132 gave $56 \pm 5\%$ and $59 \pm 9\%$ inhibition, respectively ($n = 8–9$), and icatibant gave $40 \pm 4\%$ and $47 \pm 6\%$ inhibition, respectively ($n = 8–10$). The antinociceptive effect of MEN16132 reached a plateau on day 7, showing approximately 50% inhibition at both 3 and 10 $\mu g/25 l$ i.a.r.; the effect of icatibant, at both doses, faded to 25 to 30% inhibition during the same observation period (Fig. 2). The prolonged ability of MEN16132 to reduce pain and the consequent imbalance of the hind limb weight distribution lasted for 2 weeks and was still present on day 14 from its administration, when a $32 \pm 6\%$ and $42 \pm 6\%$ inhibition ($n = 8–9$) was measured at 3 and 10 $\mu g/25 l$ i.a.r., respectively, compared with $15 \pm 10\%$ and $12 \pm 11\%$ inhibition ($n = 8–10$) for icatibant. Both drugs given at the dose of 1 $\mu g/25 l$ i.a.r. have shown only a mild effect on pain response, which was not statistically different from the control saline-treated group. Moreover, to check the reproducibility of MEN16132 ($3 \mu g/25 l$ i.a.r.) activity on MIA-induced pain response, a second administration was performed 14 days after the first injection. The maximum inhibition of basal MIA-induced changes in hind limb weight distribution, measured 3 days after each MEN16132 treatment, was comparable ($56 \pm 5\%$ and $54 \pm 5\%$ inhibition, respectively, $n = 8$) (data not shown).

**Effect of MEN16132 at Various Stages of MIA Treatment.** MEN16132 (3 $\mu g/25 l$ i.a.r.) was administered at different time points (3, 7, 14, and 21 days) from MIA treatment to evaluate the role of $B_2$ receptors role at various stages of the disease, as checked by variations in the analgesic effect of the drug. The antinociceptive activity, at 3 days after the compound administration, corresponding to the maximal response, was the same at each time point and within 56 to 59% inhibition of the basal value (Fig. 3). The responses at the first day from MEN16132 administration were close to the maximal effect obtained at all the time points, with the exception of at day 3 from MIA treatment when the antinociceptive effect was significantly smaller than the maximum.

**Effect of MEN16132 in the Contralateral Knee of MIA-Treated Rats.** To evaluate whether systemic effects could participate in the observed analgesic response of MEN16132, the drug was administered (10 $\mu g/25 l$) in the contralateral knee. It did not change the alterations of weight distribution on hind limbs induced by MIA (1 mg/25 l) injection in the right (osteoarthritic) knee compared with the control group (Fig. 4).

**Antinociceptive Activity of Indomethacin in MIA-Treated Rats.** The unselective cyclooxygenase (COX) inhibitor indomethacin, administered intra-articularly (100–625
Fig. 3. Antinociceptive activity of MEN16132 (3 µg/25 µl i.ar.) at various stages of MIA (1 mg/25 µl i.ar.)-induced osteoarthritis. MEN16132 was administered on days 3, 7, 14, and 21 from MIA treatment in different groups of animals. The pain behavior was evaluated 1 (●) and 3 (●) days later at each time point. Data are the percentage inhibition of the basal value, and it is the mean ± S.E.M. of eight experiments. *P < 0.05 significantly different from the corresponding day 3 measurement (ANOVA followed by Student's t test).

Fig. 4. Antinociceptive activity of MEN16132 (10 µg/25 µl) administered in the control left knee joint 7 days after MIA (1 mg/25 µl) injection in the right knee. Pain behavior was measured as difference in weight (g) distribution on hind limbs (left-right). Each value is the mean ± S.E.M. of eight experiments.

µg/25 µl) 7 days after MIA treatment, produced comparable analgesia at both doses with a maximal effect of 42 ± 7% inhibition of pain at day 1 (Fig. 5). During the 2 weeks of the experiment, the pain behavior was reduced without reaching a statistically significant effect.

Effect of MEN16132 on the MIA-Induced Knee Edema. Intra-articular administration of MIA (1 mg/25 µl) in the right knee produced, 3 days later, a small but significant knee swelling (+12 ± 2%, n = 8) compared with the control group. MEN16132 (3 µg/25 µl i.ar.) was administered at this time point, and its effect was checked 24 h later. As shown in Fig. 6 MEN16132 completely inhibited the edema induced by MIA.

Bradykinin and PGEM Levels in Synovial Fluid. Measurement of BK levels in synovial fluid showed a peak at day 3 after MIA treatment. Thereafter, the amount of BK in synovial fluid of MIA-treated animals was relatively constant up to 21 days from MIA treatment and significantly higher than in the respective saline-treated group (Fig. 7a).

PGEM production in synovial fluid showed a similar time course compared with bradykinin with maximal levels on day 3 after MIA treatment (Fig. 7b). The PGEM increase was almost reduced to basal value by MEN16132 intra-articular administration (10 µg/25 µl, 7 days after MIA) at all time points considered.

Discussion

OA is widely diffused among the elderly population, and alleviation of pain is one of the most important issues for the therapeutic treatment of the disease. Current pharmacological treatments do not provide complete pain relief, and side effects are often associated with the therapies. NSAIDs are limited in their use by systemic, gastrointestinal, and cardiovascular side effects; corticosteroids have a short duration of action with serious adverse effects; and intra-articular hyaluronans have a slow onset of action and could cause damage at the site of action with effusion, erythema, and pain reactions (Hinton et al., 2002; Bellamy et al., 2005; Barron and Rubin, 2007).

In this study we have investigated the role of BK and the potential analgesic effect of the kinin B2 receptor antagonist MEN16132 in osteoarthritic pain caused by intra-articular administration of MIA in the rat knee joint. Present findings demonstrate a potent and long-lasting antinociceptive activity of the kinin B2 receptor antagonists, in particular, MEN16132, in this model of osteoarthritic pain, indicating a role for BK, which increased in the synovial fluid after MIA administration. The maximal BK release, measured in synovial fluid at the beginning of the pathology development, is probably ascribable to the inflammatory reaction that occurs in the synovium in response to MIA administration and chondrocyte degeneration. In the MIA-induced experimental OA, the inflammatory reaction described seems to resolve in the first week (Bove et al., 2003), but BK generation occurs at least until 3 weeks after MIA administration, and its effect may be very prolonged. Therefore, we can assume that BK could be a marker not only of early inflammation, but also of the progression of damage in knee joint tissues. Confirmatory results for the role of the B2 receptors at various stages of the disease progression have been observed when MEN16132 has been administered in separate groups at different time points from MIA treatment. In this case, MEN16132 maintains a similar profile of analgesia throughout the experiment for 3 weeks.

BK-induced joint pain could be mainly ascribable to a direct activation and sensitization of sensory nerve fibers that innervate the synovium and subchondral bone (Pawlak et al., 2008), and may be enhanced by the presence of other inflammatory mediators such as PGE2, which has been shown to sensitize articular sensory nerve fibers. The combined effect of BK and PGE2 may lead to an exacerbation of OA pain because of the greater nociceptive effect exerted by BK on sensory fibers in the presence of PGE2 (Schaible and Schmidt, 1988; Birrell et al., 1993). Moreover, BK is responsible for the increased PGE2 production measured in synovial fluid after MIA treatment, as demonstrated by the reduction of PGE2 levels after MEN16132 intra-articular administration. The increased amount of PGE2 produced in this model has a time course similar to BK, showing a strong relationship between BK and the PGE2 production that is mediated by B2 receptor activation. Further support to the anti-inflammatory effect of MEN16132 is brought by the activity on the
joint swelling induced by MIA within a few days after administration. In fact, although, in this case, the edema is of a small amplitude for a reliable study, this inflammatory response is effectively blocked by MEN16132.

In the present study we have also demonstrated that, compared with icatibant, MEN16132 is more potent and longer lasting in reducing MIA-induced pain response. MEN16132 shows affinity at the B₂ receptor in the subnanomolar range and an antagonist potency, evaluated in BK-induced contractility of rat uterine and rat urinary bladder smooth muscle preparations, that is approximately 10-fold greater than the potency of icatibant (Meini et al., 2009). Our results indicate that locally active doses of MEN16132 are in the microgram range in agreement with the high in vitro affinity.

We can hypothesize that differences in the duration of action between the two compounds are related to the better local metabolic and kinetic profile of the nonpeptide MEN16132 versus the peptide antagonist icatibant and/or to the stronger binding at the kinin B₂ receptor (Tramontana et al., 2001). In this regard, we have recently demonstrated, in reversibility experiments in rat isolated uterine strips (Meini et al., 2009), that MEN16132 induced a significant slower recovery than icatibant in the control BK-induced contractile response, after repeated washing. The metabolic stability of MEN16132, in addition to the stable binding at the B₂ receptors, could protract its presence in the site of action resulting in a prolonged and significant analgesic effect that lasted up to 2 weeks at the doses of 3 and 10 μg. Moreover, MEN16132 structure is characterized by the presence of a quaternary alkyl ammonium group, which may allow high concentrations of the compound into the inflamed joint, resulting in a reduced systemic diffusion and consequently the need of a lower effective dose. In fact, it has been demonstrated that compounds with a quaternary ammonium moiety, interacting with the anionic sites of cartilage proteoglycans, can...
concentrate in the inflamed tissue, decreasing their effective doses and systemic side effects (Yadav et al., 2008).

The current treatment of knee OA is based mainly on nonsteroidal anti-inflammatory drugs, either the selective or nonselective COX inhibitors. Among these the COX-2 inhibitors are mainly used. In previous studies on experimental osteoarthritis induced by MIA (Bove et al., 2003; Ivanavicius et al., 2007), the acute pain response, evaluated at day 14 time point, seemed to be reduced by single oral administration of NSAIDs. In another study it was shown that, when the pathology progresses, a single oral administration of indomethacin or celecoxib 21 days after MIA treatment did not have any effect on MIA-induced alteration in hind limb weight bearing assessed 1 h after drug treatment. Only repeated oral administrations of celecoxib, twice daily for 10 days, significantly reduced MIA-induced pain response (Pomoni et al., 2005). It is clear that the experimental conditions can affect the response observed. For this reason we have studied the effect of indomethacin in the same conditions as the B2 receptor antagonists, i.e., after intra-articular administration. If compared with MEN16132, the effect of indomethacin faded rapidly, within 1 day, without reaching the level of the antinociceptive effect observed with the selective B2 receptor antagonist even at a 30-fold higher dose. Therefore, compared with indomethacin, MEN16132 possesses a favorable profile of activity when administered intra-articularly. Although prostaglandins are released in the knee joint inflammatory processes, and BK is known to activate prostaglandin production from various cell types, the antinociceptive response observed with MEN16132 clearly exceeds that small, brief response observed with indomethacin. Therefore, despite the fact that the B2 receptor antagonist induces a significant decrease in PGEM levels, our results suggest that the pain response, locally activated by the stimulation by B2 receptors, largely occurs via mechanisms independent of the prostaglandins and other COX product formation in this model of OA.

The antinociceptive activity of the B2 receptor antagonists is characterized by a maximal effect at 1 to 3 days from administration that could suggest some delay in reaching the B2 receptors in the knee joint and/or the involvement of central effects due to central nervous system penetration after systemic absorption. We actually have no evidence that MEN16132 is able to reach the central nervous system because of its positive charge, which hampers this possibility. To verify possible systemic effect we have administered MEN16132 in the contralateral normal knee of MIA-treated rats. In this condition no analgesic effect was observed, suggesting that MEN16132 produces its effect mainly by direct blockade of the B2 receptors present in the affected knee joint.

As a whole, these results clearly demonstrate the efficacy of the kinin B2 receptor antagonist in experimental osteoarthritic pain. These findings suggest the potential therapeutic application of MEN16132, with a new mechanism of action in comparison with the current treatment with NSAIDs, corticosteroids, and hyaluronans, and hopefully with a safer profile. It is noteworthy that OA is a chronic disease that requires prolonged drug treatments, and the medical need is for safer drugs than those used at present for this very common pathology. Further advantages, in terms of safety, may be obtained with intra-articular drug application that allows the reduction of possible systemic side effects. In particular, MEN16132, because of its potency, long duration of action, and the reproducibility of its effect, allows delayed repeated intra-articular administration. Recently Song et al. (2009) demonstrated, in a clinical trial with patients affected by knee OA, long-lasting analgesic effect of icatibant after its intra-articular administration either at rest or during exercise, supporting the experimental finding that inhibition of the B2 receptor pathway could have important therapeutic applications.

We can conclude that the kinin B2 receptor antagonist MEN16132 has a potent and long-lasting antinociceptive effect when given locally in the knee joints in the model of MIA-induced osteoarthritis in rats. Beside pain, periods of inflammation occur in OA and because BK is a mediator of inflammation, the anti-inflammatory properties of MEN16132 could represent an additional effect to counteract the overall outcome and symptoms of OA.

References


Address correspondence to: Dr. Sandro Giuliani, Pharmacology Department, Menarini Ricerche S.p.A., via Rismondo 12A, I-50131, Florence, Italy. E-mail: sgiuliani@menarini-ricerche.it