Pharmacological Profile of JNJ-27141491 [(S)-3-[3,4-Difluorophenyl]-propyl]-5-isoxazol-5-yl-2-thioxo-2,3-dihydro-1H-imidazole-4-carboxylic Acid Methyl Ester, as a Noncompetitive and Orally Active Antagonist of the Human Chemokine Receptor CCR2

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ABSTRACT

The interaction between CC chemokine receptor 2 (CCR2) with monocyte chemoattractant proteins, such as MCP-1, regulates the activation and recruitment of inflammatory leukocytes. In this study, we characterized (S)-3-[3,4-difluorophenyl]-propyl]-5-isoxazol-5-yl-2-thioxo-2,3-dihydro-1H-imidazole-4-carboxylic acid methyl ester (JNJ-27141491) as a noncompetitive and orally active functional antagonist of human (h)CCR2. JNJ-27141491 strongly suppressed hCCR2-mediated in vitro functions, such as MCP-1-induced guanosine 5′-[3-35S]triphosphate binding; MCP-1, -3, and -4-induced Ca2+ mobilization; and leukocyte chemotaxis toward MCP-1 (IC50 = 7–97 nM), whereas it had little or no effect on the function of other chemokine receptors tested. The inhibition of CCR2 function was both insurmountable and reversible, consistent with a noncompetitive mode of action. JNJ-27141491 blocked the binding of 125I-MCP-1 to human monocytes (IC50 = 0.4 µM), but it failed to affect MCP-1 binding to mouse, rat, and dog cells (IC50 > 10 µM). Therefore, transgenic mice, in which the mouse (m)CCR2 gene was replaced by the human counterpart, were generated for in vivo testing. In these mice, oral administration of JNJ-27141491 dose-dependently [5–40 mg/kg q.d. (once daily) or b.i.d.] inhibited monocyte and neutrophil recruitment to the alveolar space 48 h after intratracheal mMCP-1/lipopolysaccharide instillation. Furthermore, treatment with JNJ-27141491 (20 mg/kg q.d.) significantly delayed the onset and temporarily reduced neurological signs in an experimental autoimmune encephalomyelitis model of multiple sclerosis. Taken together, these results identify JNJ-27141491 as a noncompetitive, functional antagonist of hCCR2, capable of exerting oral anti-inflammatory activity in transgenic hCCR2-expressing mice.

CCR2 is a G protein-coupled chemokine receptor, expressed on monocytes, macrophages, basophils, dendritic cells, natural killer cells, and activated T lymphocytes, that mediates the activation and movement of responsive leukocytes along a chemotactic gradient. CCR2 has two isoforms (CCR2A and CCR2B) that are generated by alternative splicing and differ only in their carboxyl-terminal tail. Leukocytes predominantly express the longer CCR2B variant (Tanaka et al., 2002). CCR2B (henceforth called CCR2) is activated by several members of the CC-chemokine subfamily, consisting of monocyte chemoattractant protein-1 (MCP-1/CCL2), MCP-2/CCL8, MCP-3/CCL7, MCP-4/CCL13, and MCP-5/CCL16. MCP-1 exclusively interacts with CCR2 and is therefore recognized as the prime CCR2 agonist. Engagement of CCR2 inhibits adenylyl cyclase, promotes intracellular calcium mobilization, stimulates mitogen-activated protein kinase and phosphatidylinositol 3-kinase pathways, and promotes chemotaxis.

ABBREVIATIONS: CCR, CC chemokine receptor; MCP, monocyte chemoattractant protein; MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; GTPγS, guanosine 5′-O-(3-thiotriphosphate); h, human; CHO, Chinese hamster ovary; JNJ-27141491, (S)-3-[3,4-difluorophenyl]-propyl]-5-isoxazol-5-yl-2-thioxo-2,3-dihydro-1H-imidazole-4-carboxylic acid methyl ester; m, mouse; PBMC, peripheral blood mononuclear cells; DMSO, dimethyl sulfoxide; rAM, resident alveolar macrophages; PBS, phosphate-buffered saline; LPS, lipopolysaccharide; BAL, bronchoalveolar lavage; FSC, forward scatter; FL, fluorescence channel; MOG, myelin oligodendrocyte glycoprotein; MIP, macrophage inflammatory protein; IL, interleukin; q.d., quaque die, once daily; CNS, central nervous system.
motaxis (Jiménez-Sainz et al., 2003). Recent evidence suggests that CCR2 is critical for monocyte egress from the bone marrow (Tsou et al., 2007), although CCR2 has mostly been implicated in the pathophysiology of a wide range of both acute and chronic inflammatory conditions characterized by mononuclear cell infiltration, such as rheumatoid arthritis, multiple sclerosis (MS), atherosclerosis, uveitis, asthma, chronic obstructive pulmonary disease, psoriasis, diabetes, inflammatory bowel disease, and transplant rejection (Dawson et al., 2003; Belvisi et al., 2004; Feria and Díaz-Gonzalez, 2006). Evidence for CCR2 involvement in these diseases comes from clinical studies showing elevated expression of MCP-1 correlating with leukocyte infiltration (Ellingsen et al., 2001; Mahad and Ransohoff, 2003; Rose et al., 2003; Schober and Zernecke, 2007). Moreover, genetic deletion of either CCR2 or MCP-1 showed a selective defect in migration of macrophages to sites of inflammation (Kurihara et al., 1997; Lu et al., 1998) and protection in inflammatory disease models such as experimental autoimmune encephalomyelitis (EAE) (Fife et al., 2000; Izikson et al., 2000; Huang et al., 2001) and atherosclerosis (Boring et al., 1998; Gosling et al., 1999). Furthermore, a reduction of disease in models of arthritis, MS, and asthma was demonstrated after treatment with anti-MCP-1 antibodies (Ogata et al., 1997; Kennedy et al., 1998), an anti-CCR2 antibody (Mellado et al., 2008), or low-molecular weight CCR2 antagonists (Brodmerkel et al., 2005; Higgins et al., 2007; Xia et al., 2007). These observations led to the hypothesis that CCR2 could represent a potentially attractive disease target.

By functional screening in a guanosine 5′-O-(γ-thio)triphosphate ([35S]GTPyS) binding assay using hCCR2-expressing CHO cells, we hit upon a series of thioimidazole derivatives with micromolar CCR2 antagonistic potency. Subsequent targeted chemical synthesis led to compound JNJ-27141491 (Fig. 1) (Van Lommen et al., 2005; Doyon et al., 2008). The present study characterizes this compound as a potent, selective, and noncompetitive antagonist of the hCCR2. Oral activity is demonstrated in transgenic mCCR2 knockout/hCCR2 knockin mice, using a model measuring monocyte and neutrophil recruitment to the alveolar space after i.t. MCP-1/LPS instillation and in the mouse EAE model of multiple sclerosis.

**Materials and Methods**

**Animals.** C57BL/6 mice and Lewis rats were obtained from Charles River (Sulzdorf, Germany). Beagle dogs were obtained from CEDS (Mézières, France). mCCR2 knockout/hCCR2 knockin mice were generated as described in the supplemental data, in collaboration with Lexicon Pharmaceuticals Inc. (The Woodlands, TX), using targeted 129Sv/Evbrd (LEX1) embryonic stem cell clones injected into C57BL/6 mice (Supplemental Fig. A). Expression of the hCCR2B transcript was confirmed by quantitative reverse transcription-polymerase chain reaction performed on spleen, kidney, thymus, and lung total RNA from heterozygous and homozygous hCCR2B knockin mice (Supplemental Fig. B). Backcrossing into C57BL/6 genetic background continued to the eighth generation. Tranagenic mice were maintained in a specific-pathogen-free facility. Other mice strains, rats, and dogs were housed in facilities that meet all Belgian and European requirements for animal care. Experimental procedures were carried out in accordance with the European Communities Council Directives (86/609/EEC) and were approved by the institute’s animal care and use committee.

**Cells.** Human CCR2B-transfected CHO (hCCR2-CHO) cells (purified from Euroscreen, Brussels, Belgium) were cultured in Dulbecco’s modified Eagle’s medium-NutF12 medium; human monocytic THP-1 cells [TIB-202; American Type Culture Collection (Manassas, VA)] were cultured in RPMI 1640 medium; and murine monocytic WEHI-274 cells (CRL-1679; American Type Culture Collection) were cultured in Dulbecco’s modified Eagle’s medium supplemented with 4.5 g/l glucose, 50 μM 2-mercaptoethanol, and 1% sodium pyruvate. To all media, 10% fetal bovine serum, 1% l-glutamine, and 1% penicillin/streptomycin were added (all obtained from Invitrogen, Carlsbad, CA). Peripheral blood mononuclear cells (PBMC) from heparinized blood were isolated using Ficol-Paque gradient centrifugation (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Human monocytes were further separated using the Dynal monocyte negative isolation kit (Invitrogen).

**Chemicals.** JNJ-27141491 was synthesized at the Department of Medicinal Chemistry at Johnson & Johnson Pharmaceutical Research and Development (Beershe, Belgium) according to the methods described by Doyon et al. (2008). Reference compounds were synthesized as described previously: INCB3344 (Brodmerkel et al., 2005), compound 7a (Xia et al., 2007), and UCB-102405 (Dasse et al., 2007). For in vitro experiments, compounds were dissolved at 5 mM in dimethyl sulfoxide (DMSO) and appropriately diluted in the indicated buffers, with a final DMSO concentration of 1% in [35S]GTPyS, calcium, and binding assays and 0.2% in chemotaxis assays. For in vivo experiments, JNJ-27141491 was suspended in a vehicle solution consisting of DMSO/Cremophor 0.9% NaCl saline (1:1:8) (Sigma-Aldrich, St. Louis, MO) and orally administered to mice once or twice daily in a volume of 100 μl/g of body weight.

**[35S]GTPyS Binding Assay.** To prepare a stock of membranes, hCCR2-CHO cells were centrifuged (10 min at 16,000 rpm), and pellets were resuspended in cell homogenization buffer A (15 mM MgCl2, 0.3 mM EDTA, and 1 mM EGTA). After homogenization, membranes were pelleted (25 min at 22,000 rpm at 4°C) and again resuspended in buffer A, homogenized, and centrifuged (25 min at 22,000 rpm). Finally, the membrane pellet was resuspended in buffer B (7.5 mM Tris-HCl, pH 7.5, 12.5 mM MgCl2, 0.3 mM EDTA, 1 mM EGTA, and 250 mM sucrose), homogenized, aliquoted at 1700 μg/ml, and stored at −70°C until analysis. Membranes were diluted in incubation buffer (20 mM HEPES, 100 mM NaCl, 10 μM GDP, and 1 mM MgCl2, pH 7.4) supplemented with 14.3 μg/ml saponin and preincubated with compound for 30 min at 30°C in 96-well plates (10 μg protein/well). Then, MCP-1 (100 nM) was added for 30 min. Finally, the mixture was incubated for another 30 min with [35S]GTPyS (~1100 Ci/mmol, 2.5 nM; GE Healthcare) before membranes were harvested on GF/B filter plates (PerkinElmer Life and Analytical Sciences, Waltham, MA) using a Packard Filtermate harvester (Packard, Waltham, MA). The plates were counted in a TopCount NXT (Packard) after loading with MicroScint 0 (Packard).

**Calcium Mobilization Assays.** Cells were fluorescently labeled with Fluo-3 acetoxyethyl ester (Invitrogen) as described in Van Lommen et al. (2005) and preincubated with compound for 20 min before adding recombinant human MCP-1, MCP-2, MCP-3, MCP-4, or mouse (m)MCP-1/βE (R&D Systems, Minneapolis, MN). Cells were preincubated for 20 min in 1% DMSO when compound and agonist were added simultaneously and when compound was added alone. Changes in intracellular free [Ca2+] were measured using the fluorescent imaging plate reader (Molecular Devices, Sunnyvale, CA) or FDSS6000 screening system (Hamamatsu, Bridgewater, NJ). Functional testing of chemokine receptor selectivity was performed.
Identification of JNJ-27141491, a Novel CCR2 Antagonist

JNJ-27141491 Inhibits hCCR2 Activation in Vitro. Nanomolar concentrations of JNJ-27141491 inhibited MCP-1-induced \(^{35}\)S binding to hCCR2-CHO cell membranes (IC\(_{50} = 38 \pm 9 \text{ nM; Fig. 2A}) and reduced MCP-1-induced Ca\(^{2+}\) mobilization in hCCR2-CHO cells (IC\(_{50} = 13 \pm 1 \text{ nM}) in THP-1 cells (IC\(_{50} = 13 \pm 2 \text{ nM}) and in human blood monocytes (IC\(_{50} = 43 \pm 4 \text{ nM}) (Fig. 2, B–D). For comparison, in the MCP-1-induced Ca\(^{2+}\) mobilization assay in THP-1 cells, the known experimental CCR2 antagonists INCB3344 (Brommerkel et al., 2005), compound 7a (Xia et al., 2007), and UCB-102405 (Higgins et al., 2007) suppressed the reaction, with IC\(_{50}\) values of 13, 13, and 0.8 nM, respectively.

JNJ-27141491 also inhibited chemotaxis of human PBMC toward MCP-1, with an IC\(_{50}\) value of 97 \pm 16 nM (Fig. 2E). To assess the antagonistic effect of JNJ-27141491 on other CCR2 agonists, its activity was determined on Ca\(^{2+}\) mobilization in hCCR2-CHO cells stimulated by MCP-3 and MCP-4 and in THP-1 cells stimulated with mouse MCP-1. As shown in Fig. 2, B and C, JNJ-27141491 reduced Ca\(^{2+}\) influx with comparable potency against all agonists (IC\(_{50}\) = 7 \pm 1, 7 \pm 2, and 20 \pm 4 nM, respectively). Incubation of JNJ-27141491 alone (1 \muM) with THP-1 cells or human monocytes did not induce Ca\(^{2+}\) mobilization, indicating that the compound does not exert intrinsic agonist activity.

JNJ-27141491 Competes with the Binding of MCP-1 to hCCR2. In competition binding experiments, JNJ-27141491 blocked the binding of \(^{125}\)I-MCP-1 to hCCR2-CHO cells, THP-1 cells, and human PBMC, with IC\(_{50}\) values of 95 \pm 9 nM, 390 \pm 110 nM, and 380 \pm 80 \muM, respectively (Fig. 2F).

JNJ-27141491 Acts as a Noncompetitive Antagonist of the CCR2 Receptor. To examine the marked potency difference between the compound's functional and binding antagonism of CCR2, we performed Schild analysis in MCP-1-induced Ca\(^{2+}\) mobilization experiments in THP-1 cells. The
MCP-1 dose-response curves (Fig. 3), measured after 20-min preincubation with 0, 3, 9, or 15 nM JNJ-27141491, indicate that JNJ-27141491 acts as an insurmountable antagonist of CCR2. Indeed, the maximal response (E\text{max}) to MCP-1 in the presence of 3, 9, or 15 nM JNJ-27141491 was reduced to 98, 81, and 57%, respectively, of the E\text{max} measured in the absence of compound, whereas MCP-1 potency was only slightly affected (EC\text{50} values of 4, 8, 14, and 14 nM, respectively). The decline was near complete after preincubation with 50 nM JNJ-27141491 (data not shown). To find out whether an insurmountable antagonist is competitive, it is essential for the receptors to be exposed to the antagonist and agonist simultaneously. A decrease of the maximal agonist-evoked response is only to be expected in noncompetitive antagonism (Vauquelin et al., 2002). As shown in Fig. 4, the E\text{max} values of the MCP-1 dose-response curves were reduced to 99, 92, 73, and 44% after coincubation with 0.5, 1, 3, or 10 mM JNJ-27141491 (albeit >100-fold higher than in the preincubation experiments), whereas MCP-1 potency was only slightly affected; EC\text{50} values were 6, 8, 12, and 23 nM, respectively (4 nM without compound). Furthermore, the observation that the effect of JNJ-27141491 was readily lost after cell washings supported the reversible character of CCR2 antagonism (data not shown). In addition, IC\text{50} values

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**Fig. 2.** In vitro inhibitory activity of JNJ-27141491 on MCP-1-induced [35S]GTP\gammaS binding using hCCR2-CHO cell membranes (A); and MCP-1, MCP-3, MCP-4, or mMCP-1-induced Ca\textsuperscript{2+} mobilization in hCCR2-CHO cells (B), THP-1 cells (C), and human monocytes (D); and chemotaxis of human PBMC toward MCP-1 (E). Data shown are mean ± S.E.M. inhibition of two to six independent experiments carried out in duplicate or triplicate. Cells were stimulated with a MCP-1, -3, -4, or mMCP-1 concentration that caused 80% of the maximal agonist-induced response (EC\text{80}). MCP-2 induced only weak [Ca\textsuperscript{2+}] increases in hCCR2-CHO cells, precluding inhibition testing with compound. Effect of JNJ-27141491 on 125I-MCP-1 receptor binding to hCCR2-CHO cells, THP-1 cells, or human PBMC is shown as mean ± S.E.M. percentage of 125I-MCP-1 bound of two to three independent experiments carried out in duplicate (F).
Identification of JNJ-27141491, a Novel CCR2 Antagonist

Effect of JNJ-27141491 on other chemokine-chemokine receptor functional interactions

Results from experiments carried out by Euroscreen using their aequorin-based calcium mobilization method for testing functional G protein-coupled receptor activity (AequoScreen, www.euroscreen.com). Results are represented as IC50 values of duplicate determinations.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Chemokine</th>
<th>IC50 (μM)</th>
</tr>
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<tbody>
<tr>
<td>hCCR1</td>
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</tr>
<tr>
<td>hCCR2</td>
<td>MCP-1</td>
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</tr>
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<td>hCCR2</td>
<td>MCP-3</td>
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<tr>
<td>hCCR3</td>
<td>Eotaxin</td>
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</tr>
<tr>
<td>hCCR4</td>
<td>Macrophage-derived chemokine</td>
<td>&gt;5</td>
</tr>
<tr>
<td>hCCR5</td>
<td>Regulated on activation normal T cell expressed and secreted</td>
<td>~5</td>
</tr>
<tr>
<td>hCCR6</td>
<td>MIP-3α</td>
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<tr>
<td>hCXCR3</td>
<td>Interferon-inducible T cell α chemokine</td>
<td>&gt;5</td>
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**CCR2 Antagonism Is Restricted to the Human CCR2.** To determine the species selectivity of JNJ-27141491, [125I]MCP-1 competition binding assays were performed using human, dog, and rat PBMC and murine WEHI cells. As shown in Fig. 5, JNJ-27141491 inhibited the binding of [125I]MCP-1 to human PBMC (IC50 = 0.38 μM), but it produced no blocking effect on binding of MCP-1 to murine WEHI cells or rat or dog PBMC when tested up to 10 μM. These data indicate that the antagonism of JNJ-27141491 is restricted to hCCR2. Therefore, in vivo studies were done in transgenic mice in which the mCCR2 gene was knocked out and replaced by the human orthologous gene (Supplemental Data).

**Pharmacokinetics in Transgenic hCCR2 Knockin Mice.** Oral exposure of JNJ-27141491 in the hCCR2 knockin mice was determined at different time points after the mice were orally administered with 20 mg/kg compound. The maximum plasma concentration (Cmax = 2.5 μM; n = 3) was already observed at 30 min after dose (Tmax), indicating a fast absorption of the compound from the intestinal track. The t1/2 and area under the curve (AUC) values were, respectively, 2 h and 1240 ng·h/ml. At 8 h after dose, the plasma concentration was 0.04 μM (n = 3), whereas after 24 h all individual levels were below the limit of quantification.

**Fig. 5.** Effect of JNJ-27141491 on binding of [125I]MCP-1 to human and dog PBMC and [125I]mMCP-1 to WEHI cells and rat PBMC. Results are expressed as mean ± S.E.M. percentage of bound [125I]-MCP-1 of duplicate determinations from two to three independent experiments.
Based on these pharmacokinetic data, mice were treated q.d. and/or b.i.d. in further in vivo experiments. In addition, oral exposure in transgenic mice was comparable with data obtained in wild-type C57BL/6 mice (data not shown).

**JNJ-27141491 Inhibits Recruitment of Monocytes and Neutrophils into the Alveolar Air Space of Transgenic hCCR2 Knockin Mice.** The in vivo activity of JNJ-27141491 was tested in a mouse model that permits the identification and quantification of rAM and recruited monocytes and neutrophils in the lungs after i.t. instillation of PBS, 10 ng of LPS, or 20 μg of mMCP-1 in either absence or presence of LPS (Maus et al., 2002a). No cell influx was observed after i.t. instillation of PBS; only resident rAM were detected in the BAL fluid. Bronchoalveolar deposition of mMCP-1 alone provoked a monocyte influx, which peaked at 48 h, and LPS alone provoked sole neutrophil influx that peaked at 12 h (data not shown). Upon alveolar cochallenge with mMCP-1 and a low dose of LPS, the magnitude of the neutrophil influx (early phase) and monocyte influx (later phase) into the alveolar compartment was strongly increased compared with the respective monostimuli. Using flow cytometry, recruited monocytes and neutrophils could easily be discriminated from rAM in the BAL fluid, because the rAM strongly accumulated the red fluorescent dye PKH26. The gating of cell populations based on FL1 or FSC versus FL2 (PKH26) properties as described by Maus et al. (2001) was confirmed by positive staining of monocytes for CD14 and neutrophils for Ly6G/6C and cytospin analysis (data not shown). Figure 6 shows representative dot plots of BAL cells from a vehicle-treated (Fig. 6A) and a JNJ-27141491-treated mouse (20 mg/kg b.i.d.) (Fig. 6B), 48 h after mMCP-1/LPS instillation. The BAL fluid of vehicle-treated mice contained 792 ± 47 × 10^3 cells (mean ± S.E.M.; n = 16), consisting of 23 ± 5% monocytes, 21 ± 7% neutrophils, and 56 ± 11% rAM (mean ± S.D.), whereas the total number was significantly reduced to 636 ± 34 × 10^3 (n = 5) after b.i.d. JNJ-27141491 treatment (20 mg/kg) (p < 0.05). This reduction was solely due to reduced numbers of recruited monocytes and neutrophils, making up, respectively, 7 ± 1 and 14 ± 4% of the BAL cells (both p < 0.05), with no significant effects on the total number of rAM. Figure 7 demonstrates that the effect of JNJ-27141491 treatment on the number of infiltrated monocytes and neutrophils was dose-dependent. Once-daily oral treatment with 40, 20, 10, or 5 mg/kg inhibited the monocyte influx by 77, 57, 49, and 27%, respectively, compared with vehicle treatment, whereas this value was 74 and 22% after twice-daily oral treatment with 20 or 5 mg/kg (n = 5–16 mice/group). The neutrophil influx was also reduced; neutrophil numbers were decreased, with 56, 45, 20, and 8% after 40, 20, 10, or 5 mg/kg q.d. treatments and with 45 and 20% after 20 and 5 mg/kg b.i.d. treatments (Fig. 7). The amount of rAM did not significantly differ in any of the tested groups (data not shown). As expected, treatment of wild-type C57BL/6 mice with 20 mg/kg JNJ-27141491 (b.i.d.) had no inhibitory effect on mMCP-1/LPS-induced monocyte or on neutrophil pulmonary influx (data not shown).

**JNJ-27141491 Delays EAE in Transgenic CCR2 Mice.** Figure 8 shows a representative MOG35-55-induced EAE experiment in hCCR2 knockin mice. Mice in one group (n = 8) were orally treated with JNJ-27141491 (20 mg/kg) once daily from day 0 until day 16, whereas the control animals (n = 8) received vehicle at the same time points. Figure 8A shows that oral treatment with JNJ-27141491 significantly (p < 0.05) reduced the clinical disease scores on day 7 (from 0.7 ± 0.2 to 0.1 ± 0.1), day 8 (from 0.8 ± 0.2 to 0.3 ± 0.2), day 9 (from 1.3 ± 0.3 to 0.4 ± 0.2), and day 10 (from 1.3 ± 0.3 to 0.6 ± 0.2). Furthermore, JNJ-27141491 treatment significantly delayed the start of clinical signs of disease (mean day of onset 9.6 ± 0.7 in the compound-treated group versus 7.5 ± 0.7 in the vehicle-treated group; p < 0.05), with lower disease grades (Fig. 8B). However, compound treatment failed to significantly attenuate clinical disease day 11 onward, resulting in a mean clinical score of 1.9 ± 0.3 in the treated group versus 2.3 ± 0.3 in the control group on day 16.

**Discussion**

Numerous studies support the important role of the interaction between MCP-1 and CCR2 in the pathophysiology of a wide range of inflammatory conditions, which has put forward CCR2 as a potential therapeutic target. In this study, we characterized JNJ-27141491 as a potent, selective, and noncompetitive antagonist of hCCR2 without intrinsic agonist activity, as determined by its inability to induce Ca2+ mobilization. JNJ-27141491 inhibited hCCR2 function in vitro, with IC50 values of 7 to 97 nM as measured in [35S]GTPγS binding assays, Ca2+ mobilization assays, and chemotaxis assays using either hCCR2-CHO cells, THP-1 cells, or human PBMC. The compound showed high selectivity against nine other chemokine receptors tested (5 μM),
with minimal functional activity on the MIP1-α/CCR1 interaction (IC_{50} = 0.6 μM). Markedly higher compound concentrations were required for inhibiting ^{125}I-MCP-1 binding to hCCR2 (IC_{50} = 0.38–0.40 μM). These findings are reminiscent of the noncompetitive CCR1/CCR3 antagonist UCB-35625 and CXCR1 antagonist repertaxin. Both compounds inhibit chemokine receptor function, with low effect on binding (Sabroe et al., 2000; Bertini et al., 2004). Our results showing that JNJ-27141491 behaves as a reversible inhibitor of hCCR2 and as an insurmountable antagonist of hCCR2 in Schild analyses in both pre- and coincubation settings (Vauquelin et al., 2002) suggest that JNJ-27141491 is a noncompetitive CCR2 antagonist. Insurmountable antagonists exhibiting adequate receptor selectivity may have great potential as therapeutic drugs, because they exert their blocking action irrespective of the concentration of the natural agonist. In addition, in terms of potency, our compound proved to be comparable with that of other reported CCR2 antagonists such as INCB3344 (Brodmerkel et al., 2005), compound 7a (Xia et al., 2007), and UCB-102405 (Higgins et al., 2007), which suppressed MCP-1-induced Ca^{2+} mobilization in THP-1 cells, with IC_{50} values of 13, 13, and 0.8 nM, respectively. Despite good activity toward hCCR2, the compound had no effect on the binding of ^{125}I-MCP-1 to murine, rat, or dog leukocytes (IC_{50} > 10 μM). In vivo experiments were therefore carried out using transgenic mice in which the mCCR2 gene was replaced by the human counterpart. Pharmacokinetic analysis after oral dosing with 20 mg/kg showed good absorption and bioavailability, with no differences compared with wild-type C57BL/6 mice.

In these transgenic animals, once- or twice-daily oral treatment with JNJ-27141491 dose-dependently (5–40 mg/kg) inhibited the recruitment of monocytes and neutrophils into the alveolar space, 48 h after i.t. mMCP-1/LPS instillation. In this pharmacodynamic model, mice were cochallenged with mMCP-1 and a low dose of LPS, because this increased the magnitude of the neutrophil influx (early phase) and the monocyte influx (later phase) largely, compared with the respective monostimuli. Although both mMCP-1 and hMCP-1 induced neutrophil and monocyte recruitment in the presence of LPS in the transgenic CCR2 mice, we choose mMCP-1 for testing JNJ-27141491 at different doses, because it was considered more compatible with the murine system. The mechanism underlying the synergism of MCP-1 and LPS in eliciting inflammatory lung events could be explained in different ways. Alveolar deposited or locally liberated MCP-1 might directly pass the epi-/endothelial barrier to bind to and chemoattract circulating CCR2-expressing monocytes from the vascular to the alveolar compartment. Alternatively, rAM located at the air-tissue interface of the lung are strategically positioned to respond to the local appearance of microbial or inflammatory agents. Because rAM constitutively express CCR2, they might be directly involved in the MCP-1-driven monocyte recruitment process. In vitro...
studies have shown that alveolar macrophage-derived products such as tumor necrosis factor-α can activate alveolar epithelial cells to release chemokines such as IL-8 and MCP-1, which may then promote both alveolar neutrophil and monocyte recruitment. Furthermore, alveolar-recruited and activated neutrophils are well known to be capable of releasing MCP-1 in the alveolar air space, implying a potential role of corecruited neutrophils in the alveolar monocyte traffic (Maus et al., 2002b). Using anti-CCR2 antibodies and CCR2−/− mice, Maus et al. (2002a) have demonstrated that CCR2 is necessary not only for the monocyte influx after i.t. mMCP-1/LPS instillation but also for the earlier accumulation of neutrophils. Blocking CCR2 with a small molecule CCR2 antagonist confirmed these findings, because JNJ-27141491 also inhibited both the influx of monocytes and neutrophils in this model.

The efficacy of JNJ-27141491 was also tested in an animal model of chronic inflammation. EAE, the animal model that closely resembles MS (Owens and Sriram, 1995), can be induced by immunization with central nervous system (CNS)-specific antigens, such as MOG35-55 peptide, and it is characterized by leukocyte infiltration, demyelination, and paralysis. A clear role for MCP-1 and CCR2 in monocyte trafficking to the CNS and in the pathogenesis of EAE was established by studies of Fife et al. (2000), Izikzon et al. (2000), and Huang et al. (2001). When EAE was induced in the hCCR2 knockin mice, daily oral treatment with 20 mg/kg JNJ-27141491 significantly delayed the day of onset of neurological signs and temporarily reduced the clinical scores. However, from day 11 after immunization, all mice showed signs of EAE, with no significant difference in clinical grade in control and compound-treated groups. These data might be in agreement with a study of Gaupp et al. (2003), demonstrating that mice with CCR2 deletions remain susceptible to EAE. In contrast to the studies of Fife et al. (2000) and Izikzon et al. (2000), these CCR2−/− mice showed a modified EAE disease course, but CNS lesions in which the process of demyelination was not affected. The authors suggested that the lack of CCR2 was compensated by an altered chemokine/chemokine receptor environment and CNS infiltrate (more neutrophils).

Because of the redundancy of the chemokine system, it might be naïve to think that the blockade of a single chemokine can abrogate the recruitment of leukocytes to inflammatory foci in human pathology (Sørensen et al., 2004). In accordance with this, phase II clinical trials in rheumatoid arthritis patients, testing systemic inhibition of CCR2 using MLN1202, a humanized anti-CCR2 monoclonal antibody (Vergunst et al., 2006) and MK-0812, a small molecule CCR2 antagonist (Braddock, 2007) showed—despite good safety—disappointing efficacy.

In conclusion, we have characterized JNJ-27141491 as a potent, noncompetitive antagonist of hCCR2, with high selectivity among other chemokine receptors. This compound showed good oral bioavailability and dose-dependent efficacy on inhibition of monocyte and neutrophil recruitment to the lungs in a pharmacodynamic model using hCCR2-expressing mice. Furthermore, oral administration of JNJ-27141491 (20 mg/kg) could significantly delay and temporarily reduce neurological signs in EAE. However, no disease-improving effects on the longer term could be demonstrated. Accepting the predictability of the EAE model, the use of JNJ-27141491 in MS patients is probably going to be unsuccessful.

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References

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