JNJ-26070109 [(R)4-Bromo-N-[1-(2,4-difluoro-phenyl)-ethyl]-2-(quinoxaline-5-sulfonylamino)-benzamide]: A Novel, Potent, and Selective Cholecystokinin 2 Receptor Antagonist with Good Oral Bioavailability

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ABSTRACT

JNJ-26070109 [(R)4-bromo-N-[1-(2,4-difluoro-phenyl)-ethyl]-2-(quinoxaline-5-sulfonylamino)-benzamide] is a representative of a new chemical class of competitive antagonists of cholecystokinin 2 (CCK2) receptors. In this study, the primary in vitro pharmacology of JNJ-26070109 was evaluated along with the pharmacokinetic and pharmacodynamic properties of this compound in rat and canine models of gastric acid secretion. JNJ-26070109 expressed high affinity for human (pKᵦ = 8.49 ± 0.13), rat (pKᵦ = 7.99 ± 0.08), and dog (pKᵦ = 7.70 ± 0.14) CCK2 receptors. The selectivity of JNJ-26070109 at the CCK2 receptor versus the CCK1 receptor was species-dependent, with the greatest degree of selectivity (>1200-fold) measured at the human isoforms of the CCK1 receptor (selectivity at CCK2 versus CCK1 receptors: human, ~1222-fold; rat, ~324-fold; dog ~336-fold). JNJ-26070109 behaved as a surmountable, competitive, antagonistic of human CCK2 receptors in a calcium mobilization assay (pKᵦ = 8.53 ± 0.05) and in pentagastrin-stimulated gastric acid secretion in the isolated, lumen-perfused, mouse stomach assay (pKᵦ = 8.19 ± 0.13). The pharmacokinetic profile of this compound was determined in vivo in rats and dogs. JNJ-26070109 was shown to have high oral bioavailability (%F rat = 73 ± 16; %F dog = 92 ± 12) with half lives of 1.8 ± 0.3 and 1.2 ± 0.1 h in rat and dog, respectively. The pharmacodynamic properties of this compound were investigated using two in vivo models. In conscious rat and dog chronic gastric fistula models of pentagastrin-stimulated acid secretion, JNJ-26070109 had oral EC₅₀ values of 1.5 and 0.26 μM, respectively. Overall, we have demonstrated that JNJ-26070109 is a high-affinity, selective CCK2 receptor antagonist with good pharmacokinetic properties.

Introduction

Gastrin, acting through the CCK2 receptors, regulates a number of biological functions including gastric acid secretion, anxiety, satiety, and growth of the gastrointestinal mucosa (Miyake et al., 1994; Wettstein et al., 1994; Baldwin, 1995; see Noble et al., 1999). CCK2 receptors have also been implicated in the pathophysiology of cancer (Baldwin, 1995; Takhar et al., 2004) and Barrett's esophagus (Haigh et al., 2003). The dual function of CCK2 receptors in regulating gastric acid secretion and growth of the gastrointestinal mucosa make this an attractive and novel target for the treatment of gastroesophageal reflux disease. Thus, potentially, antagonism of these receptors could reduce the total acid secretory capacity of the stomach through growth modulatory effects in combination with the inhibition of acid secretion. Alternatively, CCK2 receptor antagonists could provide an adjunct therapy to proton pump inhibitors with the aim of reducing the effects of proton pump inhibitor-associated hypergastrinemia (Scarpignato et al., 2006). Taken together, it is possible that CCK2 antagonists would offer advantages over the current standard of care for gastroesophageal reflux disease (histamine H2 receptor antagonists or proton pump inhibitors), which have clinical limitations including delayed...
There are several distinct chemical classes of CCK2 receptor antagonists that have previously been identified through drug development programs. These small molecules include benzodiazepine-based compounds \([N-[(3R)-2,3\text{-dihydro-1\text{-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl}]\text{-N}}\text{-3-(methylpheny)urea (L-365,260, 1-(2,3\text{-dihydro-1-(2'-methylphenacyl)-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)}}\text{-3-(3-methylpheny)urea (YM022), and (R)-1-[2,3\text{-dihydro-2-oxo-1-pivalovalylmethyl-2'-(2'-pyridyl)-1H-1,4-benzodiazepin-3-yl}]\text{-3-(3-methyl-phenyl)urea (YF476), peptides [4-[[1R]-2-[[2R]-3-(1H-indol-3-yl)]-2-methyl-1-oxo-2-[[tricyclo[3.3.1.13,7]dec-2-ylxyloxy]carbonyl]amino]propyl[amino]-1-phenylethylamino-4-oxobutanoic acid (PD-134,308), and indoles [5-[[2S]-2-[[6-(1-adamantylmethylcarbamoyl)]1H-indole-5-carbonyl]amino-3-phenylpropanoyl]amino]benzene-1,3-dicarboxylic acid (JB93182); for review see Herranz, 2003]. In addition, a vaccine aimed to neutralize gastrin (Gastrimmune or INSEGIA; Aptphon Corporation, Miami, FL) has also been developed, and this therapy showed promise in phase II clinical trials for gastric, colon, and pancreatic carcinoma (Billiam and Watson, 2007). However, despite the variety of CCK2 receptor-targeted ligands, no compounds are currently available for clinical use. This may be because many compounds in this class have been associated with poor or variable pharmacokinetics, both with respect to gastrointestinal absorption and blood-brain barrier penetration (Herranz, 2003). For example, the CCK2 receptor antagonist, JB95008 (5-[[2S]-2-[[5-[[cycloheptylmethylamino]carbonyl]-1H-benzimidazol-6-yl]carbonyl]amino]-3-(2-fluorophenyl)-1-oxo-propylamino]-1,3-benzenedicarboxylic acid), demonstrated efficacy in phase II trials of pancreatic cancer; however, this compound had to be administered by continuous intravenous infusion because of its poor oral bioavailability (Chau et al., 2005). For example, the CCK2 receptor antagonist, JB95008 (5-[[2S]-2-[[5-[[cycloheptylmethylamino]carbonyl]-1H-benzimidazol-6-yl]carbonyl]amino]-3-(2-fluorophenyl)-1-oxo-propylamino]-1,3-benzenedicarboxylic acid), demonstrated efficacy in phase II trials of pancreatic cancer; however, this compound had to be administered by continuous intravenous infusion because of its poor oral bioavailability (Chau et al., 2005).

Where feasible, the CCK2 receptor antagonist YF476 was considered suitable for clinical investigation.

### Materials and Methods

All procedures and experiments were performed according to the internationally accepted guidelines for the care and use of laboratory animals in research, and they were approved by the local Institutional Animal Care and Use Committee or the Beerse Institutional Ethical Committee.

#### Cell Culture

Chinese hamster ovary-K cells that had undergone stable transfection with the rat, human CCK1 receptor or the rat and canine CCK2 receptor were maintained in Dulbecco’s modified Eagle’s medium (Ham’s F-12) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 50 U mL −1 penicillin, 50 μg mL −1 streptomycin, and 0.6 μg mL −1 Geneticin for continuous selection (all materials from Invitrogen, Carlsbad, CA). For radioligand binding studies, the cells were harvested by cell scraping, and resulting pellets were immediately frozen at −80°C (approximately 50 10^6 cells/pellet).

#### Radioligand Binding Studies

Frozen pellets of Chinese hamster ovary cells, stably transfected with the CCK1 or CCK2 receptor of interest, were used. For the human CCK2 receptor assay, HEK cells that had been transfected with selective zinc finger proteins to up-regulate human CCK2 receptor expression were used (HEKZFPP system; for details see Morton et al., 2005). All cell pellets were defrosted on ice in 15 mL of assay buffer (10 mM HEPES, 130 mM NaCl, 4.7 mM KCl, 5 mM MgCl2, and 0.089 mM bacitracin, pH 7.2 at 21 ± 3°C) and then they were homogenized in a Polytron homogenizer (setting 10, 7 × 3 s; Brinkmann Instruments, Westbury, NY). The homogenate was centrifuged (800g for 5 min at 4°C), and the pellet was discarded. The supernatant was recentrifuged (26,892 g, 100 min (total volume of 150 mL). All binding assays were conducted in 96-well Multiscreen GF/B filter plates (Millipore Corporation, Billerica, MA) that were proasoked in assay buffer for 1 h. For competition studies, cell membranes (45 μL) were incubated with 60 pM 125I-Bolton Hunter-CCK-8S (50 μL) in the presence of competing ligand (15 μL) for 100 min (total volume of 150 μL). Nonspecific binding was determined by inclusion of 1 μM JB93182 (a highly selective CCK2 receptor antagonist; Harper et al., 1996). The bound radioactivity was separated by filtration using a...
Multiscreen Resist manifold (Millipore Corporation). The filters were washed three times with ice-cold phosphate-buffered saline, pH 7.5, and radioactivity retained on the filters was measured by liquid scintillation counting using a TopCount (PerkinElmer Life and Analytical Sciences, Waltham, MA). Radioligand binding studies on human gallbladder membranes were conducted as previously (Morton et al., 2002) using \[^{3}H\]H-364,718 [N-(3S)-2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl]-1H-indole-2-carboxamide] as radiolabel.

**Calcium Mobilization Measured Using FLIPR.** Cells were seeded into black-walled 384-well plates (Bioxell; BD Biosciences, San Jose, CA) at 5000 cells/well and grown for 24 h in culture medium before stimulating the receptor stimulus with 2 ng/ml of doxycyclin for 48 h. On the day of the experiment, cells were preincubated with devazepide at 100 nM for 20 min before the addition of different concentrations of JNJ-26070109 and calcium assay kit solution (BD Biosciences) containing 50 µM probenecid (Sigma-Aldrich, St. Louis, MO) for 45 min at 37°C and 15 min at room temperature. The intracellular Ca\(^{2+}\) flux was assayed using a FLIPR Tetra (Molecular Devices, Sunnyvale, CA) to simultaneously monitor fluorescence in all wells (excitation, 488 nm; emission, 540 nm). Cells were challenged with different concentrations of CCK8-S (Sigma-Aldrich) agonist peptide (delivered at a velocity of 20 ml · s\(^{-1}\)), and the fluorescence intensity was captured every 1 s for 1 min then every 6 s for 3 min after agonist addition.

**Receptor Binding at a Panel of Additional Pharmacological Targets for Selectivity Determination.** The selectivity of JNJ-26070109 was assessed in a standard panel of radioligand-binding assays for 50 additional pharmacological loci (standard selectivity screening panel from Cerep (Celle L’Evescault, France). JNJ-26070109 was tested at 1 µM. All studies were internally controlled with reference ligands.

**In Vitro, Lumen-Perfused Mouse Stomach Acid Secretion Assay.** Gastric acid secretion was measured in the mouse, isolated, lumen-perfused stomach preparation (Black and Shankley, 1985). Isolated whole stomachs were removed from fasted, young adult mice (22–26 g) of either gender, after euthanasia by asphyxiation using a rising concentration of CO\(_2\). The abdomen was opened and the stomach was cannulated via the duodenal sphincter. The oesophagus was ligated at the level of the cardiac sphincter and the stomach was excised from the abdomen. A small incision was made in the fundic region, a cannula was ligated tightly into the incision, and the contents of the stomach were flushed through with mucosal solution (118 mM NaCl, 4.8 mM KCl, 1.2 mM MgSO\(_4\), 1.3 mM CaCl\(_2\), 31.6 mM glucose) to remove any remaining food. The stomach was placed into an organ bath containing 30 ml of buffered serosal solution (118 mM NaCl, 5.9 mM KCl, 2.5 mM CaCl\(_2\), 1.2 mM MgSO\(_4\), 1.0 mM Na\(_2\)HPO\(_4\), 25 mM NaHCO\(_3\), and 10 mM d-glucose; Sigma Chemical, Poole, Dorset, UK) at 29°C and continuously gassed with O\(_2\), CO\(_2\) (95:5). A resting tension of 1 g was applied to the tissue strips, and they were then equilibrated for 1 h during which time the buffer was replaced at 30-min intervals. CCK-8S concentration-effect (E(1/2)) curves were obtained by cumulative dosing in each tissue, and once a maximal response was obtained, the preparations were washed by replacing the buffer at 10-min intervals until the response returned to baseline. Antagonists, were equilibrated for 90 min before another CCK-8S concentration-effect curve was obtained (repeat-curve design). Contractile tissue responses were measured using isometric transducers and displayed on dual channel flat-bed recorders (Kipp and Zonen, Deif, The Netherlands).

**In Vivo Pharmacokinetic Analysis of JNJ-26070109 Using Caco-2 Cells.** The absorption potential of JNJ-26070109 was evaluated using the human intestinal cell line Caco-2 (experiments conducted by Absorption Systems, Exton, PA). In summary, Caco-2 monolayers were grown to confluence on collagen-coated, microporous, polycarbonate membranes in 12-well Costar Transwell plates (Corning Life Sciences, Lowell, MA). The permeability assay buffer was Hank’s balanced salt solution containing 10 mM HEPES and 14 mM glucose at a pH of 7.0. JNJ-26070109 was added to the apical side (A-to-B) or basolateral side (B-to-A) and incubated at 37°C with 5% CO\(_2\) and 90% relative humidity. At 1 h and 2 h after addition, a 200-µl aliquot was taken from the receiver chamber and replaced with fresh assay buffer. Permeability through a cell-free (blank) membrane was studied to determine nonspecific binding and free diffusion of the compound through the device. The flux of the dye, Lucifer yellow, was also measured for each monolayer after being subjected to the test compounds to ensure no damage was inflicted to the cell monolayers during the incubation period. All samples were assayed by high-pressure liquid chromatography/tandem mass spectrometry in the electrospray-positive mode by selected reaction monitoring (ACE C18 column, 2 × 50 mm, 3- or 5-µm particle size; Phenomenex, Torrance, CA).

**In Vitro Pharmacokinetic Profiling of JNJ-26070109 in Human Hepatoctyes.** Pharmacokinetic analysis of JNJ-26070109 in human hepatocytes was conducted by Absorption Systems. In summary, viable hepatocytes from three donors (two female and one male) were purchased from XenoTech, LLC (Kansas City, KS). These were separated by centrifugation in a Percoll gradient and pooled at 1.5 × 10\(^{6}\) cells/ml in Krebs-Henseleit buffer. The experiments were initiated by the addition of 1 mM test compound stock solution in 100% dimethyl sulfoxide to 1.2 ml of cell suspension. The final test compound concentration was 5 µM. The cell suspension was immediately divided into three aliquots and incubated in a shaking water bath at 37°C. At 15, 30, 60, and 120 min, 60 µl of cell suspension were removed and mixed with 180 ml of 100% acetonitrile. The samples were then vortexed and sonicated for 5 min. After centrifugation, at 148g for 15 min, the supernatant was diluted with 2 volumes of distilled H\(_2\)O and analyzed by high-pressure liquid chromatography/tandem mass spectrometry in the electrospray-positive mode by selected reaction monitoring (ACE C18 column, 2 × 50 mm, 3- or 5-µm particle size; Phenomenex). Testosterone (20 µM) was included as an internal reference. Data are reported as the percentage remaining of triplicate samples.

Cross-species plasma protein binding was conducted by Absorption Systems using an ultracentrifugation-based method and following their standard protocols.

**In Vivo Pharmacokinetics of JNJ-26070109.** The pharmacokinetic profile of JNJ-26070109 was assessed in male Sprague-Dawley rats (230–350 g) and male beagle dogs (8–14 kg). In all studies, the intravenous formulation was prepared in 5% (v/v) N-methyl-2-pyrrolidone in 20% (v/v) hydroxypropyl-β-cyclodextrin solution. For the oral formulation, JNJ-26070109 was mixed with 1 M equivalent of arginine, followed by the addition of a solution consisting of 10% (v/v)
water in polyethylene glycol 400. The resulting mixture was stirred at room temperature until a solution was obtained (overnight). For the rat studies, 2 μmol · kg⁻¹ of JNJ-26070109 was administered by oral gavage to animals that had been fasted for 18 h. An additional group of rats were given 2 μmol · kg⁻¹ JNJ-26070109 via the tail vein. All rats were allowed water ad libitum and those fasted were refed 4 h after oral dosing. For the canine studies, 18.5 μmol · kg⁻¹ was administered orally and 5.5 μmol · kg⁻¹ was bolus-dosed via brachial vein puncture. Blood samples were collected from the tail vein in rats (250 μl) or from the saphenous vein in dogs (4–5 ml) at various time intervals (up to 26 h) and stored on melting ice for approximately 30 min before centrifugation (10 min at 3000g). Plasma was collected and frozen at −20°C pending analysis of JNJ-26070109 using high-pressure liquid chromatography/tandem mass spectrometry in the electrospray-positive mode by selected reaction monitoring (ACE C18 column, 2 × 50 mm, 3- or 5-μm particle size; Phenomenex). The lower limit of quantification was 0.03 μM in both rat and dog plasma.

Inhibition of Pentagastrin-Stimulated Gastric Acid Secretion in Anesthetized Rats: The Ghosh and Schild Model. Male Sprague-Dawley rats (230–350 g) were anesthetized with isoflurane (1–3%) mixed in room air. Gastric acid secretion was measured using the Ghosh and Schild preparation (Ghosh and Schild, 1958) with modifications. In brief, the carcot artery and jugular veins were cannulated to allow measurement of blood pressure and infusion of pentagastrin, respectively. The femoral vein was also cannulated and used for bolus administration of test compounds. The stomach was cannulated with an inflow tube positioned near the greater curvature of the stomach and an outflow cannula was inserted into the duodenum. The esophagus was ligated to prevent back flow of the mucosal solution. The stomach lumen was washed with 20 to 60 ml of a nonbuffering solution (mucosal solution: 135 mM NaCl, 1.2 mM MgSO₄, 1.3 mM CaCl₂, and 31.6 mM glucose at pH 6.6) to prevent back flow of the mucosal solution. The stomach lumen was washed with 20 to 60 ml of a nonbuffering solution (mucosal solution: 135 mM NaCl, 1.2 mM MgSO₄, 1.3 mM CaCl₂, and 31.6 mM glucose maintained at 37°C and gassed with 95% O₂). The mucosal solution was continuously perfused through the stomach at the rate of 1 ml·min⁻¹ using a Carter multichannel precision pump (Carter, Manostat, IL). The perfusate was then passed over a pH electrode system (Beckman Coulter, Fullerton, CA) to allow the pH of the effluent from the stomach to be monitored continuously using a digital data acquisition system (ADInstruments). Preliminary agonist dose-response studies demonstrated that infusion of 100 nmol·kg⁻¹·h⁻¹ pentagastrin produced ~80% of the maximal response (ED₅₀), and this concentration was continuously infused for subsequent antagonist studies. To determine the effects of JNJ-26070109, the compound was administered according to a cumulative dosing regimen (10 nmol·kg⁻¹ to 10 μmol·kg⁻¹ when a stable response was obtained to the pentagastrin infusion after ~30 min). After each dose of antagonist, blood samples were taken to determine the corresponding plasma levels. Changes in the pH of the stomach perfusate were monitored continuously.

Inhibition of Pentagastrin-Stimulated Gastric Acid Secretion in Conscious Rats: The Gastric Fistula Model. Male Sprague-Dawley rats (160–190 g), fasted for 18 h (water ad libitum), were monitored continuously. Animals were fitted with a stainless-steel gastric fistula. Analgesia was provided by subcutaneous injection of 1–3% mixed in room air. Gastric acid secretion was measured using a Carter multichannel precision pump (Carter, Manostat, IL) or from the saphenous vein in dogs (4–5 ml) at various time intervals (up to 26 h) and stored on melting ice for approximately 30 min before centrifugation (10 min at 3000g). Plasma was collected and frozen at −20°C pending analysis of JNJ-26070109 using high-pressure liquid chromatography/tandem mass spectrometry in the electrospray-positive mode by selected reaction monitoring (ACE C18 column, 2 × 50 mm, 3- or 5-μm particle size; Phenomenex). The lower limit of quantification was 0.03 μM in both rat and dog plasma.

Measurement of Gastric Acid Secretion in Conscious Gas-tric-Fistula Dogs. Female beagles (9–12 kg) instrumented with chronic gastric and duodenal fistulae were used for these studies. Experiments were performed using six dogs, allocated to treatment or vehicle, fasted for up to 18 h before experimentation. Dogs were positioned in Pavlov frames, and after washout of residual stomach content, gastric secretions were collected by gravity drainage from the gastric fistula for four 15-min periods. The volume of secretion was measured and total acid secretion was determined by titration of a 0.25-mL sample with 0.01 N NaOH to pH 7.0 using an automatic titration assembly (Metrohm Herisau). After a 45-min basal acid secretion period, doses of JNJ-26070109 (0.55, 1.8, and 5.5 μmol·kg⁻¹) were administered intraduodenally via the duodenal fistula (12.5 ml). After an additional 45 min, during which time the fistula was closed, a submaximal dose of pentagastrin was administered subcutaneously (4 μg·kg⁻¹). Gastric secretions were collected for an additional 90 min in 15-min aliquots, and the amount of acid secreted in each aliquot was determined by titration with 0.01 N NaOH to pH 7.0 using an automatic titration assembly (Metrohm Herisau). Blood samples were taken at the end of the experiment, and plasma was collected for determination of antagonist concentrations. To determine the duration of inhibition of gastric acid secretion, an additional experiment was conducted where a single dose of JNJ-26070109 (3.65 μmol·kg⁻¹) was administered 30 min after initiation of a continuous intravenous infusion of pentagastrin (39 pmol·kg⁻¹·min⁻¹). Gastric secretion aliquots were collected at 15-min intervals for the duration of the experiment to monitor the response to JNJ-26070109. Blood samples were collected throughout the experiment (0.25, 0.5, 1, 2, and 4 h), and the plasma concentration of JNJ-26070109 was determined as described above.

Data Analysis and Statistics. Values are represented as the mean ± S.E.M. (n = 3–9). Statistical significance was determined using one-way analysis of variance (p < 0.05) followed by a Dunnett’s test for multiple comparisons.

For the gastric acid secretion experiments, responses were expressed as change in pH (ΔpH) or as the total number of moles H⁺ secreted over the investigated collection periods.

For the isolated in vitro stomach assay, individual E/A curve data were fitted to the following Hill equation (eq. 1), to provide estimates of midpoint location (A₅₀), maximal asymptote (A), and Hill slope (nₕ) parameters, where [A] is the agonist concentration and E is the measured effect expressed as ΔpH.

\[
E = \frac{A}{A_{50} + [A]^{n_h}}
\]

Analysis of competitive antagonism, expressed as pKᵦ values, was performed by direct model-fitting to the Gaddum-Schild equation as described by Black et al. (1985). For in vivo experiments, ED₅₀ refers to the dose of compound that produced a half-maximal effect and EC₅₀ denotes the corresponding plasma concentration. For radiol-
gand competition-inhibition curve data and in vivo analysis of the inhibition of gastric acid secretion, concentration-response curve data were fitted to a four-parameter general logistic function (eq. 2).

\[
B = \frac{\alpha_{\text{min}} + (\alpha_{\text{max}} - \alpha_{\text{min}})}{1 + 10^{(\text{logEC}50 - \text{logX})/\text{H11005}}}
\]

Results are presented as mean values ± S.E.M. Data between treatment groups were compared using Student’s two-tailed t tests with p < 0.05 being considered statistically significant. All data were analyzed using the software package Prism, version 3.01 (GraphPad Software Inc., San Diego, CA).

Noncompartmental analysis of JNJ-26070109 pharmacokinetics was performed using WinNonlin Professional, version 4.0.1 (Pharsight, Mountain View, CA). Individual plasma concentrations and sample times for each animal were used in the analysis.

**Drugs.** JNJ-26070109 was synthesized in-house (see Woods et al., 2007 for description of synthesis), an YF476 was a generous gift from the James Black Foundation Ltd. (London, UK) (see Fig. 1 for structure of JNJ-26070109).

**Results**

**Affinity and Selectivity of JNJ-26070109 at Human, Rat, and Canine CCK Receptors.** The affinities of JNJ-26070109 (see Fig. 1 for structure) and YF476 were determined across a number of different species using both radioligand binding (Table 1) and in vitro organ bath bioassays. The affinity of JNJ-26070109 for human CCK2 receptors (pKᵢ = 8.49 ± 0.13) was approximately ~1200-fold higher than for human CCK1 receptors (pKᵢ = 5.41 ± 0.06; Fig. 2A). The CCK2/CCK1 selectivity of JNJ-26070109 was lower for canine (~336-fold; Fig. 2B) and rat (~324-fold; Fig. 2C) receptors, which was largely a consequence of lower affinity for the rat and canine CCK2 receptor (all cell-based radioligand binding studies were n = 4, conducted in triplicate). The human CCK1 receptor affinity of JNJ-26070109 was also confirmed in a binding assay prepared from a single sample of human gallbladder (pKᵢ = 5.3, conducted in sextuplet) and in an in vitro functional assay of guinea pig gallbladder, where 60-min preincubation with 300 μM JNJ-26070109 had no significant effect on CCK-8S contractile E/[A] curves (data not shown).

YF476 expressed approximately 10-fold higher affinity for human, rat, and canine CCK2 receptors (pKᵢ values = 9.73, 9.50, and 9.12, respectively) than JNJ-26070109 (Table 1). However, the selectivity of YF476 for the CCK2 receptors in all species was lower than that of JNJ-26070109 (human, ~80-fold; dog, ~20-fold; rat, ~77-fold), because of the relatively higher affinity of YF476 for the CCK1 receptors.

**Inhibition of Cholecystokinin-Stimulated Calcium Flux by JNJ-26070109 at the Human CCK2 Receptor Expressed in HEK293 Cells in the FLIPR Assay.** CCK-8S stimulated calcium flux with a pA₅₀ of 7.9 ± 0.03 (n = 3, conducted in triplicate) at the human CCK2 receptor. The response was inhibited by JNJ-26070109 in a concentration-dependent manner, and a pKᵢ value of 8.53 ± 0.05 was estimated from these data (Fig. 3). JNJ-26070109 behaved as a simple, competitive antagonist, and no change in maximal asymptote or Hill slope were observed over the concentration range evaluated and the Gaddum-Schild slope was not significantly different from unity (0.97 ± 0.03).

**Effect of JNJ-26070109 on Pentagastrin-Stimulated Acid Secretion in the Lumen-Perfused Mouse Stomach Assay In Vitro.** Pentagastrin, an established experimental surrogate for the hormone gastrin that retains the full biological activity of the hormone, produced a concentration-dependent increase in gastric acid secretion in the isolated mouse stomach assay (pA₅₀ = 8.38 ± 0.12; nH = 0.66 ± 0.05; α = 0.29 ± 0.03; ΔpH, n = 10). JNJ-26070109 (20–200 nM; n = 6–9) produced a concentration-dependent rightward shift of the pentagastrin E/[A] curve with no significant effect on basal acid secretion or the midpoint Hill slope and upper asymptote of the pentagastrin curves (Fig. 4A). Analysis of the log [A]₅₀ values obtained in the absence and presence of JNJ-26070109 gave a Gaddum-Schild plot slope estimate (1.05 ± 0.15) that was not significantly different from unity (Fig. 4B). When the data were refitted to the Gaddum-Schild equation with the slope parameter constrained to unit value, a pKᵢ value of 8.19 ± 0.13 was obtained.

**In Vitro Pharmacokinetic Analysis of JNJ-26070109 in Caco-2 Cells and Human Hepatocytes.** JNJ-26070109 showed high absorption potential in this endothelial cell permeability assay (two separate experiments were conducted). Compound flux from the apical to basolateral side (15.9 ± 0.5 cm · s⁻¹; n = 2) was nearly identical to flux measured in the reverse direction (17.3 ± 1.2 cm · s⁻¹; n = 2) indicating that the compound was not subject to active efflux mechanisms.

**Table 1**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Species</th>
<th>pKᵢ Value</th>
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<tbody>
<tr>
<td></td>
<td>JNJ-26070109</td>
<td>YF476</td>
</tr>
<tr>
<td>CCK2</td>
<td>Human</td>
<td>8.49 ± 0.13</td>
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<tr>
<td></td>
<td>Rat</td>
<td>7.99 ± 0.08</td>
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<tr>
<td></td>
<td>Canine</td>
<td>7.70 ± 0.14</td>
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<tr>
<td>CCK1</td>
<td>Human</td>
<td>5.41 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Human gallbladder</td>
<td>5.34</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>5.45 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Canine</td>
<td>5.17 ± 0.08</td>
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*These data are taken from Morton et al., 2002.
In isolated human hepatocytes, the quantity of JNJ-26070109 remaining after 15, 30, 60, and 120 min was determined to be 93, 58, 69, and 59%, respectively. These data indicate that the half-life of JNJ-26070109 in this system was longer than 2 h in this system. JNJ-26070109 was highly protein bound in human and rat plasma (human, 99.9%; rat, 99.9%). Slightly less protein binding (98.8% bound) was measured in dog plasma.

**In Vivo Pharmacokinetic Analysis of JNJ-26070109 in Rats and Dogs.** JNJ-26070109 exhibited a small volume of distribution and clearance in both rats and dogs (see Table 2 for calculated and measured pharmacokinetic values and Fig. 5 for a graphical representation of these data). The half-life for JNJ-26070109 was similar between rats ($t_{1/2} = 1.8 \pm 0.3$ h; $n = 3$) and dogs ($t_{1/2} = 1.2 \pm 0.1$ h; $n = 3$). Oral administration of JNJ-26070109 showed the compound was well absorbed with a $T_{max}$ of 1.3 and 3.3 h in rat and dog, respectively. JNJ-26070109 was found to have a high oral bioavailability and there was no significant difference between rat and dog (73 and 92%, respectively).

**Analysis of the Inhibition of Gastric Acid Secretion by JNJ-26070109 in the Anesthetized Rat: Ghosh and Schild Model.** Intravenous pentagastrin infusion produced a stable baseline of acid secretion for 4 to 6 h. The assay was validated by including omeprazole and YF476, which both inhibited pentagastrin-stimulated acid secretion with pED$_{50}$.
values of 3.7 ± 0.9 (22 mmol · kg⁻¹) and 7.1 ± 0.1 (80 mmol · kg⁻¹), respectively (n = 3). Intravenous bolus administration of JNJ-26070109 produced a dose-dependent inhibition of gastric acid secretion with a pED₅₀ value of 6.57 ± 0.07 (270 nmol · kg⁻¹; Fig. 6A; n = 6). The corresponding plasma concentration-response curve was obtained from analysis of samples taken at the plateau of each response to JNJ-26070109, and from these data the concentration of drug in the plasma that produced a half-maximal effect was calculated to be ≈1 μM (pEC₅₀ = 6.0 ± 0.1; Fig. 6B).

Analysis of the Inhibition of Gastric Acid Secretion by JNJ-26070109 in the Conscious Rat: Gastric Fistula Model. Subcutaneous administration of pentagastrin (30 nmol · kg⁻¹) increased acid secretion (Fig. 7). The increase in acid secretion was maximal 1 h after administration. Oral administration of JNJ-26070109 produced a dose-dependent inhibition of pentagastrin-stimulated acid secretion with an ED₅₀ value ≈3 μmol · kg⁻¹ and corresponding EC₅₀ value ≈1.5 μM (pEC₅₀ = 5.82 ± 0.24; Fig. 7, A and C; n = 6). There was no significant effect of 30 μmol · kg⁻¹ JNJ-26070109 after oral administration on the secretory response to a near-maximal dose (30 μmol · kg⁻¹) of histamine (data not shown).

Analysis of the Inhibition of Gastric Acid Secretion by JNJ-26070109 in the Conscious Dog: Gastric Fistula Model. JNJ-26070109 inhibited pentagastrin-stimulated acid secretion in a dose-dependent manner (Fig. 7, B and D;...
CCK2 receptor antagonists could provide therapy for a number of different illnesses, including cancer, anxiety, and disorders of gastric acid secretion. Indeed, promising clinical data have been presented for some CCK2 receptor antagonists, including single-dose administration of YF476 in the regulation of gastric acid secretion (Boye et al., 2000a) and continuous infusion of JB95008 in the treatment of pancreatic cancer (Chau et al., 2006). However, despite the discovery and development of a number of CCK2 receptor ligands, there are no commercially available compounds for use in the clinic. Although not clearly established, it would seem that many compounds in this class have been discontinued because of a number of reasons including loss of efficacy upon repeat dosing (Boye et al., 2000b) and poor and variable pharmacokinetics (Herranz, 2003). Therefore, a medicinal chemistry program was initiated with the goal of discovering novel chemical classes of antagonist with optimal biopharmaceutical properties, most notably high oral bioavailability and a robust relationship between plasma concentration and pharmacological effect (Allison et al., 2006; Woods et al., 2007; Rosen et al., 2008). This program resulted in the identification of JNJ-26070109. The in vitro pharmacology of this compound along with in vivo efficacy data are presented here.

The human CCK2 receptor binding affinity was determined in a system in which the level of CCK2 receptor expression in a human cell line was up-regulated by engineered transcription factors (see Morton et al., 2005). This expression system allows the CCK2 receptor to be investigated in its wild-type cellular context and, in the absence of an assay of human tissue, provides a receptor-expression system for the investigation of CCK receptor ligands. In this assay, JNJ-26070109 expressed a high affinity at the human CCK2 receptor (pKᵦ = 8.49 ± 0.13). JNJ-26070109 also expressed a high affinity at the cloned rat and dog CCK2 receptors; however, the affinity values estimated at these isoforms were lower than at the human CCK2 receptor (~3-fold lower at rat and ~6-fold lower at dog CCK2 receptor).

Species differences in the affinity of CCK receptor ligands have been previously documented. For example, a single amino acid substitution in the CCK2 receptor has been shown to account for the reverse selectivity of the nonpeptide antagonists L-365,260 and L-364,718 between dog and human CCK2 receptors (Beinborn et al., 1993). The differences in affinity observed in our study did not result in the reversal of selectivity for JNJ-26070109 in any species investigated. Indeed, JNJ-26070109 was shown to be highly selective for the CCK2 receptor across all species with the greatest fold-selectivity (~1200) observed at the human isoforms of the CCK receptors. Therefore, JNJ-26070109 represents one of the most selective CCK2 receptor antagonists identified to date.

The functional effects of JNJ-26070109 were evaluated in vitro, in both a cell-based calcium flux assay and an isolated, lumen-perfused mouse stomach assay. In both of these studies, JNJ-26070109 behaved as a surmountable, competitive antagonist with an equilibrium dissociation constants estimate (pKᵦ ≈ 8.2 for the mouse and pKᵦ ≈ 8.5 for the human) consistent with the pKᵢ value generated for the human CCK2 receptor in the radioligand binding studies.

Gastric acid secretion assays were also used to assess the pharmacodynamic properties of this compound in vivo. In the rat, two models were used such that the activity of the compound could be assessed after intravenous and oral dosing. These studies demonstrated that JNJ-26070109 inhibited pentagastrin-stimulated gastric acid secretion with a similar potency for both routes of administration. This is consistent with the high oral bioavailability estimated for this compound in the pharmacokinetic studies. Other CCK2 receptor antagonists have also been evaluated in this model. For example, YF476 was shown to inhibit pentagastrin-stimulated gastric acid secretion in anesthetized rats with an ED₅₀ value of ~0.01 μmol/kg for bolus intravenous administration (Takinami et al., 1997). This value is consistent with the 37-fold higher affinity of YF476 for the rat CCK2 receptor compared with JNJ-26070109 (pKᵢ values 9.55 and 7.89, respectively).

Pentagastrin-stimulated gastric acid secretion was also measured in conscious dogs after intraduodenal administration of JNJ-26070109. In these studies, JNJ-26070109 dose-dependently inhibited pentagastrin-stimulated acid secretion. Despite the ~3-fold lower affinity JNJ-26070109 expressed for the canine CCK2 receptor compared with the rat, the plasma concentration required to cause a half-maximal inhibition was ~3-fold lower in the dog than in the rat. The reason for this is not clear but could be related to differ-
ences in plasma protein binding or in the distribution of the compound to the gastric mucosa between the species. In the canine model, the kinetic nature of this response was investigated further and the data obtained demonstrated that the time-dependent level of inhibition was paralleled by the plasma concentration profile.

The pharmacokinetic properties of JNJ-26070109 were evaluated in human in vitro assays and in vivo using both rats and dogs. These studies revealed that this compound was not subject to active transport in human Caco-2 cells and expressed a half-life longer than 2 h in isolated human hepatocytes. In vivo, JNJ-26070109 had high oral bioavailability in rats and dogs (%F = 70–92%) and seemed to have a moderate clearance and volume of distribution.

Overall, JNJ-26070109 behaved as a potent and selective CCK2 receptor antagonist and displayed a pharmacokinetic-pharmacodynamic profile that makes it suitable for further evaluation in repeat-dose, preclinical disease models and the clinic.

Authorship Contributions

Participated in research design: Morton, Barrett, Freedman, Li, Rizzolio, Prendergast, Wu, Moreno, Pyati, Figueroa, Cagnon, Lagaud, Ver Donck, Ghoos, and Shankley.

Conducted experiments: Morton, Barrett, Freedman, Li, Rizzolio, Prendergast, Wu, Moreno, Pyati, Figueroa, Cagnon, Lagaud, Ver Donck, and Ghoos.

Contributed new reagents or analytic tools: Allison and Rabinowitz.

Performed data analysis: Morton, Barrett, Freedman, Li, Rizzolio, Prendergast, Wu, Moreno, Pyati, Figueroa, Cagnon, Lagaud, Ver Donck, Ghoos, and Shankley.

Wrote or contributed to the writing of the manuscript: Morton, Barrett, Moreno, and Shankley.

References


