ABT-963 [2-(3,4-Difluoro-phenyl)-4-(3-hydroxy-3-methyl-butoxy)-5-(4-methanesulfonyl-phenyl)-2H-pyridazin-3-one], A Highly Potent and Selective Disubstituted Pyridazinone Cyclooxygenase-2 Inhibitor


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ABSTRACT

Nonsteroidal anti-inflammatory drugs (NSAIDs) are efficacious for the treatment of pain associated with inflammatory disease. Clinical experience with marketed selective cyclooxygenase-2 (COX-2) inhibitors (celecoxib, rofecoxib, and valdecoxib) has confirmed the utility of these agents in the treatment of inflammatory pain with an improved gastrointestinal safety profile relative to NSAID comparators. These COX-2 inhibitors belong to the same structural class. Each contains a core heterocyclic ring with two appropriately substituted phenyl rings appended to adjacent atoms. Here, we report the identification of vicinally disubstituted pyridazinones as potent and selective COX-2 inhibitors. The lead compound in the series, ABT-963 [2-(3,4-difluoro-phenyl)-4-(3-hydroxy-3-methyl-butoxy)-5-(4-methanesulfonyl-phenyl)-2H-pyridazin-3-one], has excellent selectivity (ratio of 276, COX-2/COX-1) in human whole blood, improved aqueous solubility compared with celecoxib and rofecoxib, high oral anti-inflammatory potency in vivo, and gastric safety in the animal studies. After oral administration, ABT-963 reduced prostaglandin E2 production in the rat carrageenan air pouch model (ED50 of 0.4 mg/kg) and reduced the edema in the carrageenan induced paw edema model with an ED50 of 1.9 mg/kg. ABT-963 dose dependently reduced nociception in the carrageenan hyperalgesia model (ED50 of 3.1 mg/kg). After 14 days of dosing in the adjuvant arthritis model, ABT-963 had an ED50 of 1.0 mg/kg in reducing the swelling of the hind paws. Magnetic resonance imaging examination of the diseased paws in the adjuvant model showed that ABT-963 significantly reduced bone loss and soft tissue destruction. ABT-963 is a highly selective COX-2 inhibitor that may have utility in the treatment of the pain and inflammation associated with arthritis.
COX-2 Inhibitors

Pierce Chemical (Rockford IL). HPLC columns were purchased from Regis Chemical Co. (Morton Grove, IL). ELISA reagents were purchased from Cayman Chemical (Ann Arbor, MI) or from Applied Biosystems (Foster City, CA). Radioimmunoassay kits were obtained from Applied Biosystems. Calcium ionophore A23187 was purchased from Sigma-Aldrich (St. Louis, MO).

Human Whole Blood Eicosanoid Formation. Whole blood eicosanoid formation was measured essentially as described by Patrignani et al. (1994). Briefly, whole blood was collected in tubes either with or without heparin. For prostaglandin H synthetase-1 activity, nonheparinized whole blood was used, and 1-ml aliquots were placed in glass tubes and allowed to clot at 37°C for 1 h. In some assays, COX-1 activity was assessed by stimulating whole blood with 10 nM A23187. The clotted blood was then spun at 2000g, and the serum was collected and frozen until TXB2 was determined. For prostaglandin H synthetase-2 activity, 1-ml aliquots containing 10 IU of heparin were incubated with LPS for 24 h. Plasma was separated by centrifugation at 2000g for 10 min, and the plasma was frozen until PGE2 levels were determined by ELISA.

WISH Cell Assay. The WISH assay was conducted as described in Hulikower et al. (1997). Briefly, WISH cells were treated with IL-1β to induce and activate COX-2 for 18 h. Compounds were added just prior to IL-1β. Culture supernatants were collected and assayed for PGE2 levels.

Platelet Assay. Washed human platelets were obtained from donors as described by Baenziger and Majerus (1974). Platelets were stimulated with 8.8 μM calcium ionophore A23187 for 10 min at 37°C. Platelets were then pelleted and supernatants assayed for TXB2.

Determination of Prostaglandins. TXB2 and PGE2 levels in plasma were determined after the samples were mixed with 4 volumes of ice-cold MeOH, and then centrifuged at 2000g for 10 min. The immunosassays were conducted on the supernatants, as recommended by the manufacturer (Cayman Chemical). The ELISAs were conducted in 96-well microtiter plates (NUNC A/S Roskilde, Denmark), and optical density was measured using a microplate reader (Vmax, Molecular Devices, Menlo Park, CA).

For conditioned media samples (as from WISH assay) and in isolated enzyme incubations, the supernatant was analyzed directly without any further treatments, except appropriate dilutions in ELISA buffer.

Determination of Drug Plasma Concentrations. ABT-963 was recovered from the plasma using liquid-liquid extraction with an ethyl acetate/hexane mixture. In a 15-ml glass tube containing 0.1 ml of internal standard and 6 ml of ethyl acetate/hexane (1:1) was added a 0.2- to 0.5-ml plasma aliquot (sample or spiked standard). The samples were vortexed vigorously for 60 s followed by centrifugation at 2800g for 10 min (4°C). The upper organic layer was transferred to a 15-ml glass centrifuge tube and evaporated to dryness with a gentle stream of dry nitrogen over low heat (~35°C). The samples were reconstituted in acetonitrile/water (1:1) by the addition of 0.1 ml of acetonitrile and vortexed for 15 s followed by the addition of 0.1 ml of water and again vortexed 15 s. Samples were transferred to autosampler vials with glass microinserts for HPLC analysis. Spiked plasma standards were assayed interspersed evenly with the samples.

ABT-963 and an internal standard were separated from each other and any coextracted contaminants by HPLC using a 5 cm x 3.0-mm Kromasil C18 column (Thermo Hypersil, Keystone Scientific Operations, Bellefonte, PA) with an acetonitrile: 1% acetic acid mobile phase [65:35 (v/v)] at a flow rate of 0.7 ml/min with multiple reaction monitoring detection of the 25- to 50-μl injection on a Sciex API III+ mass spectrometer. The assay method was linear (correlation coefficient >0.99) over the concentration range 0.2 to 100 ng/ml with a mean percent standard deviation <15% for the analysis of triplicate standards at eight separate concentrations and an estimated limit of quantitation of ~0.3 ng/ml.

Solubility. Solubility was determined by shaking 1 mg of the experimental compounds in 1 ml of 50 mM NaH2PO4 (pH 7.4) in a

Materials and Methods

Bicinchoninic acid protein assay reagents were purchased from Pierce Chemical (Rockford IL). HPLC columns were purchased from DuP-697. A-241611, A-282904, and ABT-963. The lead compound in the series, ABT-963 has improved potency compared to the currently marketed compounds, and selectivity in human whole blood, enhanced aqueous solubility, and in vitro and in vivo. These compounds, containing a central pyridazinone ring, in general show improved potency and selectivity compared with previously published compounds. Thus far, a number of published agents have the tricyclic general structure first described for DuP-697 (Fig. 1) (Gans et al., 1990). The currently marketed agents have the tricyclic general structure first described for DuP-697 (Fig. 1) (Gans et al., 1990). The currently marketed COX-2 inhibitors that have high selectivity and potency both COX-1 and COX-2. Therefore, a new distinct chemical series may offer the opportunity for improved selectivity in vitro, pharmacological superiority and enhanced safety. Our laboratory has found selective compounds. Thus far, a number of published agents have the tricyclic general structure first described for DuP-697 (Fig. 1) (Gans et al., 1990). The currently marketed agents have the tricyclic general structure first described for DuP-697 (Fig. 1) (Gans et al., 1990). The currently marketed COX-2 inhibitors that have high selectivity and potency both. Thus far, a number of published agents have the tricyclic general structure first described for DuP-697 (Fig. 1) (Gans et al., 1990). The currently marketed agents have the tricyclic general structure first described for DuP-697 (Fig. 1) (Gans et al., 1990). The currently marketed COX-2 inhibitors that have high selectivity and potency both.

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Bicinchoninic acid protein assay reagents were purchased from Pierce Chemical (Rockford IL). HPLC columns were purchased from

Fig. 1. Structures of the inhibitors used in this study: DuP-697, A-241611, A-282904, and ABT-963.
2-mL glass vial for 72 h at room temperature. The vial was then centrifuged at 6800g for 20 min. A 50-µL aliquot of the supernatant (diluted with 450 µL of mobile phase) and a 10 µg/mL standard (in mobile phase) were then analyzed for drug by reverse phase HPLC. HPLC analytical conditions: mobile phase of 55:45 (CH3CN/0.1% trifluoroacetic acid) at a 1.0 mL/min flow with a Luna C18 (100 x 4.6 mm) and UV detection at 210 nm.

**Compound Dosing.** Compounds were suspended/dissolved in 0.2% hydroxypropylmethyl cellulose (Abbott Laboratories, Abbott Park, IL) and dosed in a volume of 5 mL/kg orally by the use of an oral gavage needle.

**Carrageenan-Induced Air Pouch Prostaglandin Biosynthesis Model.** Air pouches were formed in the backs of male Sprague-Dawley rats by injecting 20 mL of sterile air on day 0 after Segwick et al. (1983). Three days later, the pouch was reinflated with 10 mL of sterile air. On day 7, 1 mL of saline containing 0.2% carrageenan was injected into the pouch to induce the inflammatory reaction, which was characterized by the release of prostaglandins. Compounds were dosed 30 min before carrageenan. Four hours after the carrageenan injection the animals were euthanized and the pouch was lavaged with phosphate-buffered saline containing 1000 units heparin/mL, and the lavage fluid was added to ice-cold methanol to a final concentration of 30% methanol. The levels of immunoreactive prosta-
glandins were determined by enzyme immunoassay as described above. Percentage of inhibitions was calculated by comparing the response in animals that received vehicle to those that received compound.

**Carrageenan-Induced Paw Edema in Rats.** Hind paw edema was induced in male rats as described by Winter et al. (1962). Male Sprague-Dawley rats weighing between 170 and 190 g were dosed with experimental compound p.o. by gavage 1 h before the subplan-
tar injection of 0.1 mL of 1% sodium carrageenan (Sigma-Aldrich) into the right hind paw. Right paw volumes (milliliters) were measured immediately after injection of carrageenan for baseline volume measure-
ments using a Buxco plethysmograph (Buxco Electronics Inc., Troy, NY). After 3 h, right paws were remeasured and paw edema calculated for each rat by subtracting the zero time reading from the 3-h reading. Data are reported as percent inhibition and were ana-
lized when appropriate by analysis of variance; p < 0.05 was con-
sidered statistically significant.

**Adjuvant Arthritis.** Male Lewis rats (150–180 g) were weighed, and both hind paw volumes were measured using a Buxco plethysmograph (Buxco Electronics Inc.). The acute arthritic response was initiated on day 0 by injecting rats subcutaneously in the right hind paw with a suspension of heat-killed, desiccated *Mycobacterium butyricum* (0.5 mg in 0.1 mL of light mineral oil) as described by Stoerk et al. (1954) and Weichman (1989). On day 15, rats were again weighed and both hind paws remeasured. Animals demonstr-
ing a typical secondary inflammation as evidenced by swelling of the left paw were randomized into groups of 8 to 10 animals. ABT-963, celecoxib, or vehicle was orally administered once daily for 13 consecutive days. On day 30, body weights and left hind paw volumes were measured, and the differences between day 0 and day 30 was calculated for both body weight and paw. Paw edema was evaluated for significance from control adjuvant edema using Dun-
ett’s multiple comparison test where p < 0.05 was considered sta-
tistically significant. MRI images were taken at initiation of dosing and after the last dose (Jacobson et al., 1999).

**Carrageenan-Induced Thermal Hyperalgesia.** Carrageenan-induced hyperalgesia was induced by injecting 0.1 mL of a 1% solu-
tion of carrageenan in physiological saline into the plantar surface of the right hindpaw of the rat as previously described by Hargreaves et al. (1988). Paw withdrawal latencies of both injured and uninjured paws to thermal stimulation was determined 2 h later using a commercially available paw thermal stimulator (UARDG, Depart-
ment of Anesthesiology, University of California, San Diego, La Jolla, CA), modeled after the one described by Hargreaves et al., (1988). Paw withdrawal latencies were calculated as the mean of the two shortest latencies. Sensitivity to acute thermal stimulation in naive rats was also assessed as a measure of acute thermal nocicep-
tion.

**Receptor Binding.** The selectivity of ABT-963 as a COX-2 inhi-
bitor was evaluated in a receptor binding selectivity screen of 75 receptors, enzymes, and ion channels by the use of standard proto-
col (CERE, Celle l’Evescault, France) as described previously (Jarvis et al., 2000).

**CNS Safety.** CNS safety was evaluated by the use of standard behavior methods (Porsolt and Partners Pharmacology, Paris, France). ABT-963 was evaluated for potential CNS effects in the following tests: locomotor stimulation/depression, motor coordina-
tion (rotating treadmill, mouse, 3–100 mg/kg p.o., reference com-
pound diazepam), hypnentic potentiation, proconvulsant and anticon-
vulsant activity, nociception (hot-plate, mouse; and tail-flick, rat). Effects on rectal temperature were also measured (mouse, 3–100 mg/kg p.o., reference compound chlorpromazine). The gross physi-
ological, behavioral, and toxic effects ABT-963 were assessed in the mouse Irwin test (Irwin, 1968).
Inhibition rat models of inflammation and pain

Carbon of the alkoxy chain yielded ABT-963, a potent and soluble compound that demonstrated improved selectivity in whole blood assays (Table 1) and an improved pharmacokinetic profile as described below.

Characterization of ABT-963

Selective Inhibition of Cyclooxygenase-2 in Vitro. As described above, COX-2 was assessed using LPS challenged blood for 24 h. As shown in Table 1, ABT-963 was very selective in these assays with an IC50 value of 17 nM for COX-2 and 4.7 μM for COX-1. A selectivity ratio (COX-2/COX-1) of 276 was calculated from this data (Fig. 3). ABT-963 was also found to be a potent inhibitor of PGE2 formation with an IC50 value of 130 nM in the IL-1-stimulated WISH COX-2 assay. Platelet eicosanoid production was also inhibited at micromolar concentrations (Table 1).

The potency of ABT-963 against isolated enzymes was also assessed in partially purified preparations of human COX-2 and COX-1 expressed in baculovirus. The enzymatic reaction was initiated with the substrate arachidonic acid and the production of PGE2 assessed by enzyme immunoassay. Inhibition of COX-2 by several classes of selective inhibitors has been shown to be time-dependent, whereas COX-1 inhibition is not (Copeland et al., 1994). This was also seen with the early pyridazinone inhibitors such as A-282904. COX-2 inhibitory potencies have been shown to be assay condition-dependent and have been the source of considerable confusion in the literature (Grossman et al., 1995). In this system, ABT-963 yielded an IC50 value of 2 μM with no inhibition of COX-1 at 300 μM. Thus, the compound gave a selectivity ratio of >150 in this assay. In comparison, celecoxib gave an IC50 value of 12 nM against COX-2 and 4 μM against COX-1 with a resulting selectivity ratio of 333. Rofecoxib gave an IC50 value of 190 nM against COX-2 with no significant inhibition at 100 μM COX-1. Although this type of assay has

<table>
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<th>Inhibitor</th>
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<tr>
<td></td>
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<td></td>
<td>IC50 (μM)</td>
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<tr>
<td>A-241611</td>
<td>0.185 (0.080–0.41)</td>
<td>29.0 (22–39)</td>
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<tr>
<td>A-282904</td>
<td>0.011 (0.003–0.052)</td>
<td>0.467 (0.393–0.551)</td>
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<tr>
<td>ABT-963</td>
<td>0.130 (0.080–0.190)</td>
<td>12.6 (4.4–25)</td>
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<tr>
<td>Celecoxib</td>
<td>0.015 (0.007–0.034)</td>
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<td>0.068 (0.043–0.110)</td>
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ND, not determined.

* Values represent means (95% confidence intervals) from three to six observations.

Alkoxyl-Containing Pyridazinone Inhibitors

A 4-butoxy-substituted compound, A-282904 (Fig. 1), was found to have excellent anti-inflammatory and analgesic properties in vitro and in vivo (Tables 1 and 2). In particular, this compound was more potent for COX-2 inhibition than A-241611. However, this compound still suffered from poor solubility. The addition of a tertiary alcohol to the terminal alkoxy chain yielded ABT-963, a potent and selective COX-2 inhibitor, that had significantly increased solubility compared with the earlier compounds as well as celecoxib and rofecoxib (Fig. 2). Furthermore, ABT-963 demonstrated improved selectivity in vivo in both inhibiting prostaglandin production and inflammation in the rat in acute models with oral doses of 10 to 30 mg/kg (Table 2).

Given the activity of A-241611 in vivo in acute models, we examined the compound in a more chronic established adjuvant arthritis model (Stoerk et al., 1954). At an oral dose of 1 mg/kg given over a 2-week period, A-241611 produced a 73% inhibition of paw edema and was comparable with celecoxib (Table 2). In addition, A-241611 reduced both paw tissue damage and bone destruction as assessed by MRI (data not shown).

Although the initial anti-inflammatory activity of A-241611 was very encouraging in both acute and chronic models in the rat, further characterization of the compound revealed some deficiencies. One challenge was aqueous solubility. A-241611 and other compounds in the series required dosing in PEG-400 to achieve good oral bioavailability and efficacy. Another issue was the very slow elimination half-life for A-241611 of greater than 15 h. Accordingly, chemical modifications were sought in A-241611 that might give more aqueous soluble compounds and improved clearance profiles.

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<td>A-282904</td>
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<td>ABT-963</td>
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ND, not determined.

* Values represent means (95% confidence limits).
not been predictive of selectivity in vivo, it does indicate that ABT-963 is a direct inhibitor of human COX-2.

**Additional Selectivity Studies.** ABT-963 was examined in a battery of receptor binding, enzyme, and ion channel assays at a concentration of 10^(-6)M (CEREP). No significant activity was found in any of these assays.

**Inhibition of Prostaglandin Formation in the Rat.** The primary in vivo assay used in the discovery of ABT-963 was the carrageenan air pouch model. ABT-963 was a potent inhibitor of PGE_2, yielding an ED_{50} value of 0.4 mg/kg after oral dosing (Fig. 4A).

**Rat Carrageenan Paw Edema Assay.** The rat carrageenan paw edema assay was used by the project as a primary indicator of anti-inflammatory activity. This model has been used classically to discover anti-inflammatory compounds (Winter et al., 1962). ABT-963 was active in the model and showed a dose-dependent inhibition of the edema as shown in Fig. 4B. Celecoxib and ABT-963 were nearly equipotent and rofecoxib was less potent (Table 2).

**Inflammatory Thermal Hyperalgesia.** Another hallmark of prostaglandin production is hyperalgesia and pain. Cyclooxygenase inhibitors are effective in a number of animal models of pain and we used the carrageenan-induced hyperalgesia model to determine ABT-963 antinociceptive activity (Hargreaves et al., 1988). As shown in Fig. 5, ABT-963 was effective (ED_{50} = 3.1 mg/kg) in the model with...
activity similar to rofecoxib (ED$_{50}$ = 4.6 mg/kg) and celecoxib (ED$_{50}$ = 1.9 mg/kg) (Table 2).

**Established Adjuvant-Induced Arthritis in the Rat.** ABT-963 was examined in the established adjuvant assay in rats, a chronic model of inflammation and tissue destruction (Stoerk et al., 1954; Weichman, 1989). ABT-963 inhibited the edematous response with potency similar to both rofecoxib and celecoxib (Fig. 6A). MRI was used to analyze the effects of ABT-963 on bone and soft tissue. Representative images for each compound (3-mg/kg dose), and positive and negative controls are illustrated in Fig. 6B. The compound gave very good inhibition of bone and tissue destruction as shown in Fig. 6B and quantified in Fig. 6C.

**Pharmacokinetics in the Rat.** The pharmacokinetic profile of ABT-963 was evaluated in rats (Table 3). The pharmacokinetic behavior of ABT-963 after a 3-mg/kg intravenous dose in rat was characterized by a plasma elimination half-life of 4.9 h. Initial volume of distribution value ($V_d$) was modest (0.5 l/kg). Plasma clearance value was very low (0.14 l/h · kg). ABT-963 was rapidly absorbed from a solution formulation (PEG-400) at low doses but more slowly at higher doses, with peak plasma concentrations observed from 1 to 6 h after dosing. Peak plasma concentrations averaged 1.74 μg/ml after a 3-mg/kg oral dose in the rat. The plasma elimination half-life was similar to that noted following intravenous dosing, averaging 5.2 h in the rat. Bioavailability of ABT-963 from the aqueous solution formulation was 93% in the rat.

**Gastric Safety Profile.** The gastric safety of ABT-963, celecoxib, and rofecoxib was evaluated in a rat model of gastric prostaglandin production for their ability to reduce the levels of prostaglandins. For the prostaglandin measurements, the compounds were given to fasted animals at a dose of 10 mg/kg, and the animals were euthanized 4 h later. ABT-963, rofecoxib, and celecoxib did not significantly change prostaglandin production in the stomach relative to vehicle controls, whereas indomethacin gave 66% inhibition.

A second model in which the compounds were examined for gastric safety used the dog. In these experiments, the compounds were given orally for 4 days and then on the 5th day, endoscopy was performed to access the integrity of the duodenum and the stomach. Standard views were used that included both the proximal and distal duodenum, the pyloric antrum, greater curvature, and the cardia. Significant damage to the stomach and the duodenum was seen in the indomethacin (10-mg/kg)-treated animals. In the ABT-963-treated animals (20 mg/kg, plasma level at endoscopy 21.8 μM) three animals gave normal endoscopy. In the fourth animal, a few small punctate blemishes were seen. Celecoxib (20 mg/kg, plasma level at endoscopy 0.49 μM) showed these type of lesions in two of four dogs, whereas rofecoxib (20 mg/kg, plasma level at endoscopy <0.05 μM) gave numerous small lesions in all four dogs. These small lesions were not ulcers and thus are not the same as seen for indomethacin where three of the four dogs had frank ulceration (Fig. 7). It is unclear what the significance of the small lesions was. However, in this study the gastrointestinal safety of ABT-963 was equivalent or superior to both marketed selective COX-2 inhibitors, especially in light of the plasma concentrations that were determined from blood samples taken at the time of endoscopy.

**CNS Safety Pharmacology.** Doses of 3 to 100 mg/kg p.o. of ABT-963 caused no significant effects on locomotor activity, motor coordination, hypnotic potentiation, pentylentetrazol seizures, nociception, or rectal temperature. Together, these results suggest that ABT-963 had no clear CNS effects in rats and mice in the dose range tested.

**Cardiovascular Safety.** ABT-963 was administered as a series of three, 30-min intravenous infusions (0.2, 2, and 20 mg/kg). At the end of each infusion period, respective plasma concentrations of ABT-963 achieved were 0.31 ± 0.12, 2.36 ± 0.54, and 31.24 ± 2.11 μg/ml. Sixty minutes after termination of infusion plasma concentration declined to 15.02 ± 0.65 μg/ml.

**Efficacious Levels in the Animal Models of Inflammation Were in the Range of 0.25 to 1 μg/ml.** In the anesthetized dog model and at the highest plasma concentrations tested (31.2 ± 2.1 μg/ml), ABT-963 produced modest effects on mean arterial pressure (MAP) but had no physiologically relevant effects (compared with vehicle controls) on any other parameter measured, including heart rate, cardiac output, pulmonary arterial pressure, indexes of cardiac contractile function, systemic and pulmonary vascular resistance, the QT interval corrected for heart rate (Bazett’s QTcB), and the PR interval. During the high dose infusion, ABT-963 produced a 25% reduction in MAP during the last 10 min of the infusion period. During the subsequent 60-min post-treatment period, MAP tended to remain at these levels but was not significantly different from vehicle controls. Thus, plasma concentrations of ABT-963 as high as 31 μg/ml or 30- to 124-fold over the efficacious concentration produced little to no effect on cardiovascular and hemodynamic function in the anesthetized beagle dog.

**Discussion**

Prostaglandins modulate the pain and edema observed in a variety of inflammatory disorders. These compounds also play a role in protection of the gastric lining, hemodynamic functions such as platelet aggregation, as well as having a role in normal renal function. The first enzyme in the pros-
taglandin biosynthetic pathway is prostaglandin H synthase, and this enzyme catalyzes two activities, cyclooxygenase and hydroperoxidase (Smith, 1992). Usually, both activities are termed cyclooxygenase. There are two isozymes of cyclooxygenase, termed COX-1 and COX-2 (Herschman, 1996). A wealth of work indicates that COX-1 is constitutively expressed in most tissues, whereas COX-2 is not normally expressed but is induced by cytokines, hormones, and growth factors (Smith, 1992; Herschman, 1996).

Until recently, most clinically used inhibitors of prostaglandin formation inhibit both isozymes of cyclooxygenase. The result of inhibiting both isozymes of the enzyme is clinically significant inhibition of pain and inflammation accompanied by a significant incidence of gastrointestinal distress and renal complications. The gastrointestinal distress seen in the clinic can be modeled in rats or dogs by giving high doses of conventional nonsteroidal anti-inflammatory drugs. Experimental compounds with greater selectivity for COX-2 versus COX-1 have markedly attenuated gastrointestinal damage in rodents. The recent approval of COX-2-selective

**Table 3**

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<th>Intraavenous Dose</th>
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agents for use in human disease has been accompanied by initial safety results indicating a clear advantage for selective agents versus mixed inhibitors (Capone et al., 2003). This and other evidence indicates that of the two known isozymes, COX-1 is responsible for gastric protection and hemodynamic balance, whereas COX-2 produces prostaglandins in inflammation and cytokine/growth factor induced processes.

The discovery of agents that selectively inhibit COX-2 and not COX-1 has proven challenging. This difficulty is derived from three sources. First, the active sites of the two proteins are very similar. There are few amino acid changes in the surface amino acids that form the active site pocket (Garavito et al., 2002). One important difference may be the substitution of Val 509 for Ile. This change may open a pocket that allows for some flexibility of binding. Other differences in the second layer of amino acids allows for some increase in size of the right-hand side of the active site (Garavito et al., 2002). The active site also is very hydrophobic, which allows very little hydrophilicity to be incorporated in the inhibitors (Garavito et al., 2002; K. Stewart, unpublished observations).

The second source of difficulty in providing selective agents is providing agents that do not inhibit prostaglandin formation by inhibiting the release of arachidonic acid. Arachidonic acid is a substrate for both COX-1 and COX-2 and therefore agents that inhibit arachidonic acid are nonsellective.

The third difficulty is accessing the compound’s selectivity. Several methods had been published using different cells and stimuli with standard inhibitors (Grossman et al., 1995; Laufer et al., 1999). Based upon this, our approaches to assess in vitro selectivity for COX-2 versus COX-1 evolved over the course of discovering ABT-963. Initially, the early series selectivity values were determined in cell assays using the WISH cell line, which upon IL-1 stimulation, COX-2 is dramatically induced and large amounts of PGE2 are formed (Hulkower et al., 1997). COX-1 activity was determined in platelets, which only express COX-1 (Pairet and Engelhardt, 1996). The later alkoxy and hydroxy alkoxy series were discovered using two whole blood assays performed essentially as described by Patrignani et al. (1994). In our hands, the clotting COX-1 assay and the ionophore whole blood assay for COX-1 gave similar results for compounds that inhibit COX-1. A number of studies examining COX-2 expression in LPS-stimulated whole blood and product formation profiles indicated that a 24-h assay was required to selectively measure COX-2 inhibition (S. Majest and R. Bell, unpublished data) in this assay.

In vitro, ABT-963 selectively inhibits recombinant human COX-2 enzyme as well as COX-2-driven cellular production of prostaglandins while leaving platelet COX-1 thromboxane production intact at concentrations where COX-2 is completely inhibited. In whole blood assays, ABT-963 is particularly potent and selective. Compared with other COX-2 inhibitors such as rofecoxib and celecoxib, ABT-963 was 11-fold more potent against COX-2 than celecoxib, whereas rofecoxib was 30-fold weaker against COX-2. ABT-963, celecoxib, and rofecoxib were all equally active at inhibiting COX-1. Thus, the selectivity ratios in blood were 157 for ABT-963, six for rofecoxib, and 14 for celecoxib. Etoricoxib has been described in the literature (Riendeau et al., 2001). This compound has potencies that are in the same range as ABT-963; however, it seems to be more potent in the rat assays. From the published selectivity data, ABT-963 seems to be comparable with this agent.

The present in vivo data show that ABT-963 is an effective anti-inflammatory agent. The carrageenan air pouch model had been previously used to assess in vivo inhibition of both leukotrienes and prostaglandins (de Brito, 1989). More recently, Seibert et al. (1994) showed that the lining of the carrageenan-challenged air pouch contains a significant level of induced COX-2 enzyme and implied that the majority of the PGE2 formed was from COX-2. This model gave good reproducibility and selective agents gave nearly complete inhibition of PGE2. ABT-963 inhibited prostaglandin formation in the air pouch model with oral doses of less than 1 mg/kg (ED50 = 0.3 mg/kg). This potency was similar to that seen with both rofecoxib and celecoxib. The compound also inhibited prostaglandin-driven inflammation and pain in acute rat models. In the carrageenan paw edema model, ABT-963 was effective at reducing the edema caused by the release of prostaglandins with an ED50 of 1.9 mg/kg. The compound was also effective in the carrageenan-induced hyperalgesia model with an ED50 of 3.1 mg/kg. These data confirm the results with earlier selective agents that COX-2-derived prostaglandins are involved in the inflammatory reaction in these models (Penning et al., 1997; Chan et al., 1999).

ABT-963 was also examined in an established adjuvant arthritis model in the rat. The established model exhibits pronounced soft tissue and synovial inflammation between day 16 and 30 and is accompanied by a marked progression of periosteal reactions, pannus formation, internal bone inflammation, fibrosis in the joints, and end-stage ankylosis (Weichman, 1989). Classical paw edema measurements were made as well as examinations of paw architecture using MRI. The compound was equally effective on paw swelling as measured by plethysmography and MRI. In addition, MRI analysis showed that COX-2 inhibition arrested the progression of the disease at the time of

Fig. 7. Endoscopy of dogs treated with either indomethacin or rofecoxib. Dogs were treated with compound for 4 days and then they were fasted for 24 h before the endoscopy.
initial dosing. Similar results were also seen for indomethacin, suggesting that, in the rat, prostaglandins play a significant role in the progression of bone loss seen in this model (Weichman, 1989).

ABT-963 was examined in a dog model of cardiovascular safety and the data from these experiments suggest that ABT-963 may not have cardiovascular effects in humans. Only a minor effect was seen at the highest plasma level, which was 30- to 124-fold over the efficacious levels in the models of inflammation.

Most importantly, ABT-963 dosed daily in dogs for 4 days at doses 20-fold above effective anti-inflammatory doses gave no gastrointestinal damage as assessed by endoscopy. These data, although preliminary, indicate that ABT-963 has the potential to have increased gastric safety. The higher level of exposure of the animals to the drug coupled with the increased selectivity further suggest that ABT-963 will have improved gastric safety in humans. In summary, ABT-963 has a preclinical anti-inflammatory and safety profile that suggests that this compound may be safe and effective in humans. Continued clinical evaluation of ABT-963 will determine the human safety and efficacy profile of this compound.

References


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