ABT-702 (4-Amino-5-(3-bromophenyl)-7-(6-morpholino-pyridin-3-yl)pyrido[2,3-d]pyrimidine), a Novel Orally Effective Adenosine Kinase Inhibitor with Analgesic and Anti-Inflammatory Properties.

II. In Vivo Characterization in the Rat

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ABSTRACT

Adenosine kinase (AK; EC 2.7.1.20) is a key intracellular enzyme regulating intra- and extracellular concentrations of adenosine (ADO), an endogenous neuromodulator, antinociceptive, and anti-inflammatory autocoid. AK inhibition provides a means of potentiating local tissue concentrations of endogenous ADO, and AK inhibitors may have therapeutic potential as analgesic and anti-inflammatory agents. The effects of ABT-702, a novel, potent (IC\textsubscript{50} = 1.7 nM), and selective non-nucleoside AK inhibitor were examined in rat models of nociception and acute inflammation. ABT-702 was orally effective and fully efficacious to suppress nociception in a spectrum of pain models in the rat, including carrageenan-induced thermal hyperalgesia, the formalin test of persistent pain, and models of nerve injury-induced and diabetic neuropathic pain (tactile allodynia after L5/L6 spinal nerve ligation or streptozotocin injection, respectively.) ABT-702 was especially potent at relieving inflammatory thermal hyperalgesia (ED\textsubscript{50} = 5 \mu mol/kg p.o.). ABT-702 was also effective in the carrageenan-induced paw edema model of acute inflammation (ED\textsubscript{50} = 70 \mu mol/kg p.o.). The antinociceptive and anti-inflammatory effects of ABT-702 were blocked by selective ADO receptor antagonists, consistent with endogenous ADO accumulation and ADO receptor activation as a mechanism of action. The antinociceptive effects of ABT-702 were not blocked by the opioid antagonist naloxone. In addition, ABT-702 showed less potential to develop tolerance to its antinociceptive effects compared with morphine. ABT-702 had no significant effect on rotorod performance or heart rate (at 30–300 \mu mol/kg p.o.), mean arterial pressure (at 30–100 \mu mol/kg p.o.), or exploratory locomotor activity (at \#10 \mu mol/kg p.o.). Thus, ABT-702 is a novel, non-nucleoside AK inhibitor, with a nonopioid, non-nonsteroidal anti-inflammatory drug mechanism of action, which shows antinociceptive and anti-inflammatory activity in vivo.

The purine nucleoside adenosine (ADO) functions as an extracellular signaling molecule within the central and peripheral nervous systems (Ralevic and Burnstock, 1998; Williams and Jarvis, 2000). ADO is released locally at tissue sites in response to adverse conditions (e.g., tissue trauma, pain, seizures, ischemia). Once in the extracellular space, ADO interacts with specific cell-surface receptors, serving as a local autocoid to restore cellular function toward normal (Newby, 1984). Four subtypes of the P1 family of G-protein-coupled ADO receptors have been identified and cloned: A\textsubscript{1}, A\textsubscript{2A}, A\textsubscript{2B}, and A\textsubscript{3} (for review, see Ralevic and Burnstock, 1998). Because ADO has a physiological half-life on the order of seconds (Moser et al., 1989), its actions are highly localized to its site of release.

A large body of experimental animal data and clinical reports has accumulated linking ADO modulation to antinociceptive processes in the brain and spinal cord (for review, see Sawynok, 1999; Kowaluk and Jarvis, 2000). ADO, P1 receptor agonists, and ADO-modulating agents provide antinociceptive effects after systemic, spinal, and local peripheral administration in a spectrum of animal pain models, including models of acute nociceptive (Holmgren et al., 1986; Keil and DeLander, 1992; Kowaluk et al., 1999), inflammatory (Poon and Sawynok, 1998), chemically induced persistent (Malmberg and Yaksh, 1993; Poon and Sawynok, 1995), and neuropathic pain (Sosnowski and Yaksh, 1989; Yamamoto and Yaksh, 1992; Lee and Yaksh, 1996; Kowaluk and Jarvis, 2000).

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ABBREVIATIONS: ADO, adenosine; AK, adenosine kinase; MPO, myeloperoxidase; CPT, cyclopentyltheophylline; DMPX, 3,7-dimethyl-1-propargylxanthine; MPE, maximum protective effect.
Lavand’Homme and Eisenach, 1999; Lynch et al., 1999). In addition, clinical reports indicate that intravenous ADO infusion, administered at doses that exhibited no overt effects on the cardiovascular system, improved pain symptoms in a number of experimental clinical pain models, reduced spontaneous pain and tactile allodynia in patients with neuropathic pain, and reduced the requirement for volatile anesthetic and for postoperative opioid analgesia when administered perioperatively (Sollevi, 1997).

The spinal cord is a key site for ADO-mediated modulation of nociception (for review, see Sawynok, 1999; Kowaluk and Jarvis, 2000). ADO A1 and A2A receptors (Choca et al., 1987), ADO-metabolizing enzymes, and ADO transporters have all been localized to the spinal cord. ADO and its analogs are antinociceptive when administered intrathecally (Sawynok, 1999), and electrophysiological evidence indicates that ADO, acting both pre- and postsynaptically, can modulate primary afferent transmission to neurons of the spinal cord dorsal horn (Salter et al., 1993; Li and Perl, 1994). Supraspinal mechanisms may also contribute to ADO modulation of nociception (Herrick-Davis et al., 1989). In the periphery, the actions of endogenous ADO to inhibit peripheral neurotransmitter release (Fredholm and Dunwiddie, 1988) and to modulate inflammatory processes may also contribute to ADO-mediated antinociception.

ADO is also released at sites of inflammation (Cronstein et al., 1995), and exerts anti-inflammatory effects via multiple mechanisms involving the full spectrum of ADO receptor subtypes (for review, see Firestein, 1996). ADO modulates neutrophil function (A2A receptor), endothelial cell permeability (A1 and A2A receptors), tumor necrosis factor-α production in vitro (A3 receptor) and in vivo, and collagenase (MMP-1) production and gene expression on synoviocytes in vitro (A2B1 receptor). Accordingly, ADO analogs have efficacy in various animal models of inflammation (Firestein et al., 1994; Firestein, 1996).

The identification of compounds that mimic or modulate the antinociceptive and anti-inflammatory actions of ADO represents a potential approach to the treatment of pain and inflammation. Traditionally, the search for such agents has focused primarily on direct-acting ADO receptor agonists. These agents are effective in animal models, but their therapeutic utility has been limited by side effects, in particular, hypotension, bradycardia, and sedation (Williams, 1996). An alternative approach that has received increasing attention is the discovery of compounds that amplify the actions of endogenous ADO by inhibiting the ADO-metabolizing enzyme AK. AK is a key intracellular enzyme regulating intracellular ADO and extracellular AK concentrations (Arch and Newsholme, 1978). Inhibition of AK has the net effect of decreasing cellular reuptake of ADO (Davies et al., 1984), and therefore, of potentiating the local concentration and the effects of ADO in the extracellular compartment. Because the actions of endogenous ADO are highly localized, the effects of AK inhibitors may be more pronounced at tissue sites where pathophysiological changes result in ADO release (Engler, 1987; Britton et al., 1999), thereby limiting systemic side effects.

The present study describes the properties of a novel, potent, and selective AK inhibitor, ABT-702 (Fig. 1; Jarvis et al., 2000), in animal models of pain and acute inflammation. The non-nucleoside structural features of ABT-702 disting-

![Fig. 1. Structure of ABT-702.](image-url)
quickly frozen in liquid nitrogen and placed in a –80°C freezer until assayed for MPO activity. At the time of assay, the hindpaw tissue was placed in centrifuge tubes containing 10 ml of 50 mM potassium phosphate buffer, pH 6.0, with 0.5% hexadecyltrimethylammonium bromide (Sigma Chemical Co.) and homogenized (Brinkman Polytron, setting 6 for 30 s). The homogenate was quickly frozen and thawed for three cycles and then centrifuged for 15 min at 40,000g, 4°C. An aliquot (1 ml) of the supernatant was diluted (1:10, 1:20, and 1:50) and 100 μl of each dilution was added to a 96-well plate. Fifty microliters of a 50 mM potassium phosphate buffer, pH 6.0, solution and 25 μl of the reaction mixture (DMB (O-dianisidine dihydrochlo-
ride, 95.2 mg/30 ml of H2O) and H2O2 (84 ml 30% H2O2/50 ml of H2O; Sigma Chemical Co.) was added to each well. The assay plate was then immediately inserted into a plate reader ( Molecular De-

vices, Sunnyvale, CA) and the change in absorbance at 460 nm was recorded.

In carrageenan-induced thermal hyperalgesia, paw edema, and MPO activity experiments, ABT-702 was administered orally 1 h before carrageenan, unless otherwise noted. In antagonist experiments, ABT-702 (or vehicle) was administered orally 1 h before carrageenan injection, and the antagonists (i.e., theophylline, cyclopentylthiophylline (CPT), 3,7-dimethyl-1-propargylxanthine (DMPX), or vehicle were adminis-
tered (i.p.) 30 min after ABT-702 treatment.

Formalin Test. After a 30-min acclimation period to individual observation cages, 50 μl of a 5% formalin solution was injected s.c. into the dorsal aspect of the right hindpaw and the rats were then returned to the clear observation cages, which were suspended above mirrors. Rats were observed for either a continuous period of 60 min or for periods of time corresponding to phase 1 and phase 2 of the formalin test (Abbott et al., 1985). Phase 1 of the formalin test was defined as the period of time immediately after injection of formalin until 10 min after the formalin injection. Effects on phase 2 of the formalin test were determined using the up-down method of Dixon (1980). A percent maximal protective effect value (% MPE) was calculated for each dose at each pretreatment time according to the following formula: (post-
drug threshold − [baseline threshold])/maximum threshold − [baseline threshold]) × 100%, where maximum threshold was equal to 15 g.

Compound studies began at least 1 week after the nerve ligation surgery. For dose-response experiments, surgically prepared ani-
mals were randomly assigned to treatment groups and tested over a 2- to 3-day period. On test days, animals were placed into the individual chambers and allowed to acclimate for 15 to 20 min, after which baseline scores were determined. Only rats with threshold scores <4.5 g were considered allodynic and used in further testing. Animals were treated with ABT-702 or vehicle, and threshold scores were determined at intervals thereafter. In repeated dosing experi-
ments, ABT-702 (30 μmol/kg i.p.) or vehicle was administered twice daily for 4 days with treatments being separated by approximately 8 h. On day 5, for the morning treatment, both groups received a challenge dose of ABT-702 (30 μmol/kg i.p.).

Streptozotocin-Induced Diabetes and Tactile Allodynia. Di-
abetes was induced using a 75-mg/kg i.p., injection of streptozotocin, and blood glucose levels were assessed 3 to 4 weeks later. Rats with blood glucose levels ≥250 mg/dl (≥14 mM) were considered diabetic and used for further studies. Drug studies began approximately 8 weeks after streptozotocin injection (Lynch et al., 1999). The effects of ABT-702 on tactile allodynia withdrawal thresholds were assessed in these animals in a manner similar to that described above for spinal nerve ligation animals. Only rats with predosing withdrawal thresholds of ≈8.0 g were used in these studies.

Locomotor Activity and Rotorod Performance. Locomotor activity was measured in an open field using photoelectric monitors (AccuScan Instruments, Columbus, OH). Rats were treated with ABT-702 or vehicle, and placed in activity chambers (42 × 42 × 30 cm) 60 min later. Photoelectric breaks were recorded for 30 min and data were collapsed into 10-min intervals for statistical analysis. Rotorod performance was measured using an accelerating rotorod apparatus (Omnitech Electronics, Inc., Columbus, OH). Rats were allowed a 30-min acclimation period in the testing room and then placed on a 9-cm-diameter rod, which increased in speed from 0 to 20 rpm over a 60-s period. The time required for the rat to fall from the rod was recorded, with a maximum score of 60 s. Each rat was given three training sessions before drug treatment. After the training sessions, rats were randomly assigned to treatment groups and injected with either ABT-702 or vehicle. Latencies to fall from the rotorod were determined 30, 60, and 120 min after ABT-702 or vehicle treatment, and these values were used for statistical comparisons.

Heart Rate and Blood Pressure. This study was carried out using the LabPro telemetry system (Dataquest; Data Sciences Inter-
national, Minneapolis, MN). Approximately 1.5 weeks before study and under aseptic conditions, male Sprague-Dawley rats were implanted with indwelling telemetry transmitters connected to a small gel-filled catheter that was secured nonocclusively in the abdominal aorta. Signals emitted by the transmitter were detected by individ-
ual receivers placed under the animal cages, and translated by the system software into blood pressure waveforms and heart rate values. Arterial pressure and heart rate data were sampled at 5-min intervals and serial 10-min averages were determined. Fifteen rats were instrumented, which allowed the use of a randomized crossover design carried out over two separate days to complete the study. A 3-day washout period was allowed between experiments. ABT-702 or vehicle (n = 6 per dose or vehicle group) was delivered orally via gavage at a dose volume of 5 ml/kg. Mean arterial pressure and heart rate were measured beginning 1 h before, and continuing for 6 h following treatment.

Compounds. ABT-702 is 4-amino-5-(3-bromophenyl)-7-(6-mor-
pholino-pyridin-3-yl)pyridin[2,3-d]pyrimidine, and was synthesized as described by C.-H. Lee et al. (C.-H. Lee, M. Jiang, M. Cowart, G. Gfesser, R. Perner, K. H. Kim, Y. G. Gu, M. Williams, M. F. Jarvis, E. A. Kowaluk, A. O. Stewart, and S. S. Bhagwat, submitted). Mor-
phine sulfate was obtained from Mallinkrodt (St. Louis, MO). ADO receptor antagonists and naloxone were obtained from Research Biochemicals International (Natick, MA). ABT-702 was dissolved in a vehicle consisting of 10% dimethyl sulfoxide/54% hydroxypropyl-
β-cyclodextrin in sterile water for i.p. administration and 30% poly-
ethylene glycol 400 for p.o. administration. Polyethylene glycol 400 (30%), dimethyl sulfoxide, and hydroxypropyl-β-cyclodextrin were

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obtained from Sigma (St. Louis, MO). Doses are expressed in micro-
moles per kilogram of free base, and compounds were administered in a final volume of 1 to 3 ml/kg i.p. or 3 to 5 ml/kg p.o.

Statistics. Data analysis was carried out using analysis of vari-
ance (GB-Stat; Dynamic Microsystems, Inc., Silver Spring, MD) as previously described (Kowaluk et al., 1999). Where appropriate, Fisher’s protected least-significant difference was used for post hoc analysis. The level of significance was set at \( P < .05 \). ED\(_{50}\) values were estimated using least-squares linear regression. Data are presented as mean ± S.E.M.

Results

Carrageenan-Induced Thermal Hyperalgesia. In re-
sponse to a noxious thermal stimulus, the withdrawal la-
tency of the carrageenan-injected, inflamed hindpaw was
significantly reduced compared with the contralateral, unin-
flamed hindpaw (Fig. 2A), consistent with the induction of
thermal hyperalgesia by carrageenan, as previously de-
scribed (Hargreaves et al., 1988). ABT-702 dose dependently
and fully relieved carrageenan-induced inflammatory hyper-
algesia when administered orally (ED\(_{50}\) = 5 \( \mu \)mol/kg, Fig.
2A) or intraperitoneally (ED\(_{50}\) = 0.6 \( \mu \)mol/kg) before carra-
geenan. Under the same conditions, ABT-702 had no effect on
the withdrawal latency of the contralateral, uninflamed paw
(Fig. 2A), indicative of a specific antihyperalgesic effect of
ABT-702. The antihyperalgesic effects of ABT-702 (30
\( \mu \)mol/kg p.o.) persisted when it was administered up to 6 h
before carrageenan, which represents an interval of 8 h be-
fore thermal hyperalgesia testing (data not shown). ABT-702
also relieved thermal hyperalgesia when it was administered
1 h after carrageenan injection (ED\(_{50}\) = 10 \( \mu \)mol/kg p.o.).
ABT-702 was more potent than morphine (ED\(_{50}\) = 30
\( \mu \)mol/kg p.o.) or the nonsteroidal anti-inflammatory drug ibuprofen (70 \( \mu \)mol/kg p.o.) at relieving thermal hyperalgesia
when orally administered before carrageenan (Fig. 2B).

The antihyperalgesic effects of ABT-702 (10 \( \mu \)mol/kg p.o.) were reversed by the nonselective ADO receptor antagonist theophylline (10 mg/kg i.p.), the ADO \( A_1 \) selective antagonist CPT (10 mg/kg i.p.), and the ADO \( A_{2A} \) receptor-selective antagonist DMPX (1 mg/kg i.p.) (Fig. 3).

ABT-702 (30 nmol) also fully relieved carrageenan-induced
thermal hyperalgesia after intraplantar injection locally at the site of inflammation (Table 1). The effects of intraplantar
ABT-702 (30 nmol) were selective for the inflamed paw be-
cause ABT-702 injection into the contralateral (control) paw
had no effect on the paw withdrawal latency of the inflamed
paw (Table 1). This dose of ABT-702 (30 nmol) also had no
effect on the paw withdrawal latency of the contralateral
(control) paw when injected into either the inflamed or con-
trol paw (Table 1).

Carrageenan-Induced Paw Edema. The acute anti-in-
flammatory effects of ABT-702 were assessed in the carra-
geenan-induced paw edema model in the rat. ABT-702 dose
dependently reduced paw edema after oral administration
(ED\(_{50}\) = 70 \( \mu \)mol/kg, Fig. 4A), exhibiting similar potency and
efficacy when it was administered 1 h before (Fig. 4A) or 1 h
after (Fig. 4B) carrageenan. ABT-702 also dose dependently
reduced neutrophil accumulation at the site of inflammation,
as reflected by the reduction of MPO activity in the paw
(ED\(_{50}\) = 60 \( \mu \)mol/kg p.o., Fig. 4A). The effects of ABT-702
performed when ABT-702 (100 \( \mu \)mol/kg p.o.) was adminis-
tered up to 9 h before carrageenan (data not shown). ABT-
702 showed similar potency and efficacy to ibuprofen and
denisolone when administered orally 1 h after carrageenan
(Fig. 4B).

ABT-702 (100 \( \mu \)mol/kg p.o.) mediated edema reduction was
completely reversed by the nonselective ADO receptor antag-
osist theophylline (10 mg/kg i.p.), and partially reversed by
either the ADO \( A_1 \) selective antagonist CPT (10 mg/kg i.p.),
or the ADO \( A_{2A} \) receptor-selective antagonist DMPX (10
mg/kg i.p.) (Fig. 5).

Rat Formalin Test. In the rat formalin test, 5% formalin
was injected into the right hindpaw to induce a characteris-
tic biphasic flinching response. ABT-702 dose dependently
reduced both the acute (phase 1) and persistent (phase 2)
phases of formalin-evoked flinching (Fig. 6A). ABT-702 was
effective after both oral (ED\(_{50}\) = 60 \( \mu \)mol/kg in phase 2, Fig.
6) and intraperitoneal (ED\(_{50}\) = 15 \( \mu \)mol/kg in phase 2) ad-
ministration. ABT-702 was somewhat less potent than mor-
phine (ED\(_{50}\) = 20 \( \mu \)mol/kg) in blocking phase 2 nocicep-
tive behavior, but markedly more potent and more effective
than the NSAID ibuprofen (ED\(_{50}\) >500 \( \mu \)mol/kg p.o.) (Fig.
6B). The antinociceptive effects of ABT-702 (100 \( \mu \)mol/kg p.o.) in phase 2 of the formalin test (27 ± 3 flinches, \( P < .05 \)
versus vehicle response of 67 ± 7 flinches, \( P < .05 \), \( n = 6 \))
were significantly attenuated by the nonselective ADO recep-
tor antagonist theophylline (10 mg/kg i.p., 44 ± 4 flinches,
The antinociceptive effects of ABT-702 in the formalin test were completely reversed by the nonselective ADO receptor antagonist theophylline (10 mg/kg i.p.), and by the ADO A1 receptor-selective antagonist CPT (10 mg/kg i.p.), but not by the ADO A2A receptor-selective antagonist DMPX (1 mg/kg i.p.). (Fig. 9).

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these diabetic rats with similar potency and efficacy as was observed in the L5/L6 spinal nerve ligation model (ED50 = 5 μmol/kg i.p., ABT-702 was administered 1 h before carrageenan; antagonists were administered 30 min later. Paw edema was assessed 2 h after carrageenan. Data are mean ± S.E.M. *P < .05 versus respective vehicle control, †P < .05 versus respective ABT-702/vehicle group (n = 6).}

**Effects on Motor Activity.** ABT-702 had no significant effect on motor coordination at 30 to 300 μmol/kg p.o. as measured by the ability of rats to run on an accelerating rotating rod (rotorod assay). ABT-702 also had no effect on spontaneous exploratory activity of rats in a novel open field at 10 μmol/kg p.o., but significantly reduced spontaneous exploratory activity at 30 μmol/kg p.o. (25 ± 3% of vehicle response, P < .05 versus vehicle, n = 6), 100 μmol/kg p.o. (50 ± 6% of vehicle response, P < .05 versus vehicle, n = 6) and 300 μmol/kg p.o. (25 ± 3% of vehicle response, P < .05 versus vehicle, n = 6). Rats were fully awake, responsive to stimuli, and retained the righting reflex, consistent with their ability to perform the rotorod test at all doses tested. While ABT-702 had no significant effects on rotorod performance, its effects on locomotor activity occurred with an ED50 value that was 6-fold higher than that required to reduce thermal hyperalgesia. As such, the potency of ABT-702 to reduce locomotor activity (ED50 = 30 μmol/kg p.o.) was similar to its potency to reduce nociception in the formalin (ED50 = 60 μmol/kg p.o) and L5/L6 spinal nerve ligation (ED50 = 50 μmol/kg p.o) assays.

**Cardiovascular Effects.** The cardiovascular effects of ABT-702 were examined using conscious, freely behaving rats with similar potency and efficacy as was observed in the L5/L6 spinal nerve ligation model (ED50 = 5 μmol/kg i.p., Fig. 7A). The effects of ABT-702 were maximal within 30 min of acute administration. The antiallodynic effects of ABT-702 (30 μmol/kg i.p.) in the diabetic rats [88 ± 8% MPE, P < .05 versus vehicle response (19 ± 12% MPE), n = 6] were blocked by the nonselective ADO receptor antagonist theophylline (20 μmol/kg i.p., 35 ± 15% MPE, n = 6, P < .05 versus ABT-702 alone). Theophylline alone (20 μmol/kg i.p., 17 ± 15% MPE, n = 6) had no significant effect on nociceptive behaviors compared with vehicle (19 ± 12% MPE, n = 6).

**Fig. 5.** Effect of ADO receptor antagonists on the reduction of carrageenan-induced paw edema by ABT-702. The effect of ABT-702 (100 μmol/kg p.o.) was reversed by the nonselective ADO receptor antagonist theophylline, by the ADO A1 receptor-selective antagonist CPT (10 μmol/kg i.p.), and by the ADO A2A receptor-selective antagonist DMPX (1 mg/kg i.p.). ABT-702 was administered 1 h before carrageenan; antagonists were administered 30 min later. Paw edema was assessed 2 h after carrageenan. Data are mean ± S.E.M. *P < .05 versus respective vehicle control, †P < .05 versus respective ABT-702/vehicle group (n = 6).

**Fig. 6.** A, ABT-702 (p.o.) significantly reduced nociceptive behaviors in both phase 1 (0–10 min) and phase 2 (30–50 min) of the formalin test in rats. B, effects of orally administered ABT-702 (●), compared with ibuprofen (▲) and morphine (■), on phase 2 (30–50 min after formalin) of the rat formalin test. Data are mean ± S.E.M. *P < .05 versus respective vehicle control (n = 6).

**Fig. 7.** A, effect of ABT-702 on nerve injury-induced tactile allodynia of the hindpaw in rats (Chung model) after oral (●) and i.p. (■) administration is compared with the effect of i.p. ABT-702 to relieve streptozotocin-induced tactile allodynia (▲). B, effects of i.p. ABT-702 (●) compared with i.p. morphine (▲) and i.p. ibuprofen (■). Compounds were administered 2 h before (p.o.) or 30 min before (i.p.) tactile allodynia testing. A response in the 80 to 100% range represents the response range for normal rats. Data are mean ± S.E.M. *P < .05 versus vehicle (n = 6).
later. Data are mean ± S.E.M. *P < .05 versus vehicle (n = 6 per group).

**Fig. 8.** Duration of action of ABT-702 (100 μmol/kg p.o.) compared with vehicle (●) to relieve nerve injury-induced tactile allodynia in the rat. Data are mean ± S.E.M. *P < .05 versus vehicle (n = 6 per group).

rats instrumented with telemetry transmitters. After oral administration, ABT-702 produced no significant change in mean arterial pressure when administered to conscious rats at 30 or 100 μmol/kg (data not shown). ABT-702 produced a modest reduction in mean arterial pressure at 300 μmol/kg p.o., compared with vehicle; however, mean arterial pressure did not fall below baseline values in ABT-702-treated rats (Fig. 11). Heart rate was not significantly altered at any dose tested.

**Fig. 9.** Effect of ADO receptor antagonists on the relief of nerve injury induced tactile allodynia in the rat by ABT-702. The effects of ABT-702 (100 μmol/kg p.o.) were reversed by the nonselective ADO receptor antagonist theophylline (10 mg/kg i.p.) and by the ADO A1 receptor-selective antagonist CPT (10 mg/kg i.p.), but not by the ADO A2A receptor-selective antagonist DMPX (1 mg/kg i.p.). ABT-702 was administered 2 h before tactile allodynia testing; antagonists were administered 30 min later. Data are mean ± S.E.M. *P < .05 versus respective vehicle/vehicle group. tP < .05 versus respective ABT-702/vehicle group (n = 6 per group). V, vehicle; A, ABT-702; T, theophylline; C, CPT; D, DMPX.

ABT-702, like other AK inhibitors (Kowaluk et al., 1999), has demonstrated analgesic effects in acute thermal threshold tests (e.g., the mouse hot-plate test, Jarvis et al., 2000), it does so at greater than antihyperalgesic doses. ABT-702 also showed equivalent antihyperalgesic activity when administered either before or after carrageenan-evoked tissue injury. The latter effect may be more relevant to the clinical setting where analogics are administered after injury.

Consistent with the endogenous anti-inflammatory actions of ADO (Firestein, 1996), ABT-702 also dose-dependently reduced carrageenan-evoked paw edema and neutrophil accumulation (reflected by MPO activity) at the site of inflammation. ABT-702 equi-effectively reduced paw edema whether it was administered before or after carrageenan, consistent with its antihyperalgesic effects. Interestingly, antihyperalgesia by ABT-702 was evident at doses providing minimal overt reduction of paw edema, suggesting that subtle anti-inflammatory actions that were not detected with the paw edema assay, as well as central mechanisms, may contribute to the antihyperalgesia produced by ABT-702. ABT-702 also significantly reduced paw edema, and decreased radiographic and histological evidence of joint destruction in the rat adjuvant arthritis model of chronic inflammation.

**Fig. 10.** Effect of subchronic administration of ABT-702 or morphine on nerve injury-induced tactile allodynia in rats. ABT-702 was equally effective to relieve tactile allodynia on day 5 in rats injected twice daily for 4 days with ABT-702 (30 μmol/kg i.p., A) or with vehicle (V). Data are mean ± S.E.M. *P < .05 versus vehicle/morphine group (n = 6 per group). In contrast, morphine (21 μmol/kg i.p.) was significantly less effective to relieve tactile allodynia on day 5 in Chung rats treated twice daily for 4 days with morphine (M, 21 μmol/kg i.p.) than in rats treated twice daily for 4 days with vehicle. Data are mean ± S.E.M. tP < .05 versus vehicle/vehicle group.


**Discussion**

The present data demonstrate that the novel AK inhibitor ABT-702 is orally effective to reduce nociception in a variety of rat models of inflammatory and neuropathic pain. ABT-702 exhibited an acute onset (∼1 h) and a prolonged duration of action (8 to 11 h) in these models and was especially potent to fully block inflammatory thermal hyperalgesia (Greaves et al., 1988). ABT-702 had a selective antihyperalgesic, rather than analgesic, effect in this model. Although...
Similarly, the effects of ABT-702 to relieve acute thermal pain in the mouse hot-plate test involved A1, but not A2A, receptor activation (Jarvis et al., 2000). These data suggest differing control mechanisms for the relief of inflammatory pain compared with neuropathic and acute physiological pain by ABT-702.

The observed role of ADO A1 receptor activation in the antinociceptive effects of ABT-702 across pain models is consistent with evidence implicating the activation of spinal ADO A1 receptors in antinociception (Sawynok, 1999). Studies with ADO receptor-selective agonists and antagonists have demonstrated a pharmacology for spinal antinociception that is primarily ADO A1 receptor mediated (Keil and DeLander, 1999; Lee and Yaksh, 1996; Poon and Sawynok, 1998; Sawynok, 1999). ADO A1 receptors are localized to the dorsal horn of the spinal cord (Choca et al., 1987). In addition, electrophysiological studies in intact rats indicate that ADO A1 receptor agonists modulate acutely evoked and inflammation-evoked responses of spinal cord dorsal horn neurons (Reeve and Dickenson, 1995). In the central nervous system, ADO activates A1 receptors to inhibit cAMP production, increase K+ currents, and decrease Ca2+ currents (Kowaluk and Jarvis, 2000). Presynaptically, ADO inhibits neurotransmitter and neuropeptide release, including glutamate, substance P, and calcitonin-gene related peptide (Santicioli et al., 1992). Postsynaptically, ADO suppresses sensory transmission as a result of the activation of K+ conductances and membrane hyperpolarization (Salter et al., 1993; Li and Perl, 1994). All of these actions of ADO have been implicated as key mediators of the central sensitization characterizing inflammatory and neuropathic pain states.

The involvement of both ADO A1 and A2A receptors in the anti-inflammatory effects of ABT-702 is consistent with the role of these ADO receptor subtypes in the inhibition of neutrophil function and inflammatory mediator-evoked vascular leakage by endogenous ADO (Firestein, 1996). The anti-inflammatory effects of locally released endogenous ADO may also account, in part, for the ADO A2A receptor-mediated component underlying ABT-702 anti-hyperalgesia in the carrageenan model. Consistent with such a peripheral mechanism, ABT-702 relieved carrageenan-induced thermal hyperalgesia when administered locally at the site of carrageenan inflammation. Similar local, peripheral antinociceptive effects have been observed with the prototypical AK inhibitor 5’-amino,5’-deoxyadenosine in the rat formalin test.
The possibility that spinal A2A receptors also contribute to ABT-702-mediated antihyperalgesia cannot be ruled out, given the presence of ADO A2A receptors in the dorsal spinal cord (Choca et al., 1987), as well as pharmacological evidence suggesting involvement of ADO A2A receptors in spinal antinociception (Sawynok, 1999).

Previous efforts to develop direct-acting ADO agonists as therapeutic entities have been hampered by their mechanism-based cardiovascular and sedative side effects (Williams, 1996). ABT-702 had no effects on motor coordination, as reflected by rotorod performance, at doses providing maximal antinociception in all animal models studied, including models of nociceptive, inflammatory, and neuropathic pain. ABT-702 also had no significant effect on exploratory locomotor activity at doses providing relief of carrageenan-induced thermal hyperalgesia (≤10 μmol/kg i.p.), although effects were observed at higher doses. ABT-702 had no effect on mean arterial pressure in rats at oral doses providing maximal antinociception in rat carrageenan-induced thermal hyperalgesia, and at doses providing near-maximal antinociception in the formalin and neuropathic pain models after oral administration. A modest reduction in mean arterial pressure was observed at the maximally effective oral dose in the latter two models. No effects were seen on heart rate at any dose tested. Thus, ABT-702 provided antihyperalgesic effects at doses devoid of classical ADO-mediated side effects.

Consistent with its in vitro pharmacology (Jarvis et al., 2000), the antinociceptive effects of ABT-702 can be differentiated from classical analgesics and anti-inflammatory agents, morphine and ibuprofen. ABT-702 was markedly more potent than ibuprofen to relieve carrageenan-induced thermal hyperalgesia, and was more potent and effective than ibuprofen in the formalin test. ABT-702 fully relieved tactile alldynia in the Chung model, whereas ibuprofen was completely ineffective in this model. The antinociceptive effects of ABT-702 do not involve opioid systems because the antinociceptive effects of ABT-702 in both a chronic neuropathic pain model and in an acute thermal nociceptive test (Jarvis et al., 2000) were not attenuated by the opioid receptor antagonist naloxone. The observation that the antinociceptive effects of ABT-702 and morphine in the formalin test are additive is also consistent with distinct mechanisms of action for ABT-702 and morphine. Importantly, ABT-702 was also shown to have less potential to develop tolerance to its antinociceptive effects than morphine.

In summary, the novel, potent, and selective non-nucleoside AK inhibitor ABT-702 is orally effective to ameliorate nociceptive behaviors in a spectrum of well characterized animal models of pain, including inflammatory pain, chemically induced persistent pain, and neuropathic pain. It is especially potent to relieve the hyperalgesia of inflammatory pain, at doses devoid of effects on blood pressure, heart rate, and motor activity. Thus, ABT-702 is a novel non-nucleoside AK inhibitor that shows antinociceptive and anti-inflammatory activity in vivo.

References


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