Anesthesiology
1999; 91:824–32

Preclinical Toxicity Screening of Intrathecal Adenosine in Rats and Dogs

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Background: **Intrathecally administered adenosine receptor agonists have antinociceptive effects in animals, suggesting that intrathecal adenosine might provide analgesia in humans. The authors performed preclinical neurotoxicity studies to define the safety of intrathecally administered adenosine in rats and dogs.**

Methods: **Eighteen rats with long-term intrathecal catheters received daily injections of saline or 100 μg adenosine for 4 days and were observed for general behavior and thermal nociception before being killed on day 6. Nine beagle dogs were prepared with long-term, lumbar intrathecal catheters and infused continuously with saline or adenosine, 2.4 mg/day for 48 h, then 7.2 mg/day for 26 days. Animals were then anesthetized and perfused with preservative and their spinal cords were examined systematically.**

Results: **No disturbances in neurologic function were detected in either animal species. intrathecal adenosine caused transient sedation in rats and increased muscle tone in dogs, resolving with continued exposure to drug. Neither adenosine- nor saline-treated rats or dogs showed acute thermal analgesia. Adenosine groups did not differ from saline groups regarding histopathology, although a moderate fibrotic and inflammatory reaction was noted in both, and protein concentrations in cerebrospinal fluid were increased in both.**

Conclusion: **The current study in rats and dogs failed to provide behavioral or histologic evidence of neurotoxicity from intrathecal administration of adenosine. This provides evidence for the presumption of safety of adenosine in this dose range, and supports phase I safety trials of acute intrathecal adenosine administration in humans. (Key words: Analgesics; neurotoxicity; pain; spinal injection.)**

BEHAVIORAL studies in rodents have shown that intrathecal administration of adenosine A_1 receptor agonists (including adenosine itself) produces a modest antinociception in models of acute stimulation[1–4] and diminishes the hyperalgesia and allodynia that arises secondary to tissue or nerve injury or loss of spinal inhibition.[5–10]

The mechanisms underlying this spinal antinociceptive effect are not understood fully. In the spinal cord, adenosine-like immunoreactivity has been shown in the substantia gelatinosa, where primary afferent neurons transmitting noxious sensory information terminate.[11] intrathecal injection of A_1 and nonselective adenosine agonists may produce analgesia in part by a reduction of substance P release.[12] This suggests a direct effect on small afferent terminals (but see Vasko and Ono[13]). Adenosine may also act to diminish excitatory amino acid release.[14] intrathecally administered A_1 receptor agonists have a suppressive effect on dorsal horn neurons sensitized by subcutaneous formalin[15] or mustard oil application to a region of skin adjacent to their receptive fields.[16]

These findings in animals suggest that intrathecal injection of adenosine receptor agonists might be useful in treating neuropathic pain states. Anecdotal reports from Sweden show reduction in pain and allodynia in patients with neuropathic pain by intrathecal injection of the A_1 agonist, R-PIA.[17] More recently, it has been reported that intrathecal injection of adenosine can diminish hyperalgesia in human models of experimental, facilitated processing.[18] Interestingly, adenosine lacks antinociceptive effects to noxious thermal stimuli in rats[19] or humans[18]

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Received from the Departments of Anesthesiology, Wake Forest University, Winston-Salem, North Carolina, and the University of California, San Diego, California. Submitted for publication October 7, 1998. Accepted for publication May 5, 1999. Supported by grant GM48085 from the National Institutes of Health, Bethesda, Maryland, and the Max Kade Foundation, Vienna, Austria.

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but is effective in rat models of mechanical hypersensitivity.[20]

Because adenosine is an endogenous nucleoside that occurs in all cells of the body, and because it is available commercially in a preservative-free formulation for injection, one would expect it to lack neurotoxicity after intrathecal injection. However, before human study can begin, appropriate preclinical toxicity assessments are mandatory[21] because other endogenous substances have shown neurotoxicity after intrathecal injection in pharmacologic doses.[22,23] Preclinical toxicity screening has been performed by Gordh and Sollevi for the Swedish formulation of injectable adenosine (personal communication, December 1997), but this formulation differs from that available in the United States; in that, it contains mannitol instead of normal saline. Repeated administration, followed by a detailed behavioral examination and evaluation of cerebrospinal fluid (CSF) chemistry and histology in at least two animal species have been undertaken with clonidine,[24] opioids,[25] and neostigmine[26] before U.S. regulatory approval and administration to humans. The purpose of this study was to use these same methods to assess the behavioral and toxicologic effects on the spinal cord from intrathecal adenosine compared to a saline control in rats and dogs with long-term, implanted intrathecal catheters.

Methods

The studies were approved by the Institutional Animal Care and Use Committees of the Wake Forest School of Medicine, Winston-Salem, North Carolina (for studies in rats) and the University of California, San Diego, California (for studies in dogs).

Drug

The commercially available, American preparation of adenosine (MW: 267.2 Da; ADENOCARD, Fujisawa USA, Deerfield, IL) was used. This solution contains adenosine, 3 mg/ml, in 9 mg/ml sodium chloride in water solution without preservatives, with a pH between 5.5 and 7.5

Rat Studies

Animal Preparation. Male Sprague-Dawley rats (Harlan-Industries, Indianapolis, IN), (260–370 g; N = 18) were used in this study. Rats were maintained after surgery in individual cages with *ad libitum* food and water and in a 12-h light–dark cycle. Food and water intake were not measured. Body weight was assessed before implantation of the intrathecal catheter and at the beginning and discontinuation of drug treatment. intrathecal catheters were inserted according to a modification of the method described by Yaksh and Rudy[27] during anesthesia with 2–4% halothane in oxygen–air. Polyethylene catheters were inserted through a small incision in the atlantooccipital membrane and passed 8 cm caudally to the level of the lumbar enlargement. To confirm correct placement of the catheters, 10 μl lidocaine, 2%, was injected, followed by a 10-μl saline flush, 0.9%, the day after surgery. All animals in which a bilateral motor block of the hind limbs developed within 30 s were included in the study. After surgery and testing with lidocaine, the animals recovered for at least 5 days. Animals with a deficit in fore or hind limb function or other obvious neurologic damage were excluded from the study.

Drug Administration. Rats were randomized to receive daily bolus injections of either 100 μg adenosine (n = 12) or 0.9% saline (n = 6). All animals were injected with the same volume (33 μl) intrathecally over 1 min, followed by 10 μl saline to flush the catheter dead space, and all injections were given at the same time of day (8:00–10:00 AM). The dosage of drug administered was limited by the drug concentration of the marketed formulation. Although somewhat arbitrary, the volume chosen (43 μl) is rarely exceeded in laboratory studies in rats, and approximates the entire spinal CSF volume of a rat. This dose is five times that necessary to reduce mechanical hypersensitivity after nerve injury in rats.[20] Each group received a single bolus injection once daily on four sequential days. On day 6 the animals were killed.

Measurements. Arousal, motor coordination, and general behavior were assessed daily for 1 h after treatment. Arousal scores were assessed on a seven-point scale, as previously described,[26] ranging from −3 (comatose) to +3 (maximal excitation). Motor impairment was evaluated by observation of placing reflex and ambulation ability, as previously described.[28] Episodes of urination after intrathecal injection were noted.

Antinociception to noxious heat was measured using a commercially available device, as previously described.[29] A radiant heat source was focused on the plantar surface of the hind paw every 15 min for 1 h, and the latency to paw withdrawal was measured (average of two determinations). In the absence of a response, a 30-s cutoff was used to limit possible tissue damage after exposure to the stimulus. Data are expressed as percent of the maximum possible effect (% MPE), where % MPE = 100 × (postdrug response − predrug response) / (cutoff time [30 s] − predrug response).

Histopathology. At completion of the study on day 6, the animals were killed by induction of a deep anesthesia, followed by left ventricle cannulation and perfusion with phosphate buffer, then 4% paraformaldehydrate. After removal of the vertebral column, the spinal cords were removed with the catheters in place. After fixation in formalin, blocks were embedded in paraffin, sectioned at 10-μm thickness, and stained with hematoxylin–eosin. Coded sections from the vicinity of the catheter tip from all animals were evaluated in random order by a neuropathologist, without knowledge of drug treatment group. Particular attention was given to the presence or absence of fibrosis or other reactions around the catheter, inflammation in the subarachnoid space, and spinal cord parenchyma damage, as evidenced by the presence of demyelination or gliosis. Inflammation and fibrosis were graded as absent (0), mild (1), moderate (2) or severe (3).

Dogs

Animal Model. Adult male (n = 5) and female (n = 4) beagles weighing 10–14 kg (Marshall Farms, USA, Inc., North Rose, NY) were used in these investigations. Dogs were acclimated to the laboratory environment for a minimum of 5 days before the start of the study. During the acclimation period, animals underwent a physical examination and were screened for good health through clinical blood profiles. Animals were fed dry dog food, with the exception of the night before scheduled surgery and clinical chemistry blood collection. Tap water was available *ad libitum.*

Surgery was performed approximately 96 h before intrathecal treatment. A prophylactic treatment of sulfamethoxazole–trimethoprim was administered, 15–25 mg/kg orally, twice daily for 5 days, beginning 48 h before surgery. During halothane anesthesia, the cisterna magna was exposed, a small incision was made through the dura, and a PE10 catheter (Intramedic, Becton Dickinson, Franklin Lakes, NJ) was inserted and advanced caudally, approximately 41 cm to a level corresponding to the L3–L4 segment. Dexamethasone sodium phosphate (0.25 mg/kg, intramuscularly) was administered just after spinal catheter insertion to provide postoperative analgesia and to reduce inflammation around the catheter after implantation, which could interfere with neurotoxicologic assessment. Catheters were tunneled subcutaneously and caudally to exit on the upper back at the level of the scapula; incisions were closed; and anesthesia was discontinued. Dogs were previously acclimated to a pump vest 48 h before surgery. After recovery, the dogs were refitted with the vest and attached to an infusion pump (Disetronic Medical Systems, Inc., Minneapolis, MN).

Drug Administration. Animals were randomized to receive intrathecal infusion of either saline or adenosine. In animals receiving adenosine, infusion was begun with a concentration of 1.0 mg/ml for 48 h, then infusion continued with a concentration of 3.0 mg/ml (undiluted commercially available solution) for the remainder of 28 days. Infusion rate was constant at 2.4 ml/day in all animals. Thus, total solution exposure was 192 mg adenosine during this time, or an equivalent volume of saline.

Measurements. Animals were observed twice daily for morbidity–mortality, signs of reaction to treatment, general behavior, the presence of stool and urine, and overt signs of toxicity. Rectal temperatures were determined daily. Body weights were determined on days −5, −3, 1, 5, 7, then at 4-day intervals and on the day of necropsy. Specific behavioral indices (arousal, muscle tone, and coordination) were assessed twice daily, as previously described.[26]

Heart rate and blood pressure, measured using a tail cuff manometer (Dinamap 8100; Critikon Company LLC, Tampa, FL), and respiratory rate were recorded at specified time points for each dosing interval.

The thermally evoked skin twitch response was measured using a probe with approximately 1 cm^2 surface area maintained at 62.5°C (±0.5°C) with a feedback controller. The probe was applied to shaven lumbar areas of the back, resulting in a brisk contraction of the local, underlying musculature within 1–3 s of probe placement. Failure to respond within 6 s was cause to remove the probe and assign that value as the latency.

Cisternal CSF clinical chemistry[26] and concentrations of adenosine were assessed on the day of surgery, at the time of catheter placement, and on day 28, the day of killing, by percutaneous puncture of the cisterna with a 22-gauge (3.8 cm) spinal needle.

Concentrations of adenosine were measured in CSF and plasma samples using high-pressure liquid chromatography (HPLC) separation, coupled with fluorescence detection after derivation, as previously described.[30] Absolute assay sensitivity was 0.1 pmol, with an interassay coefficient of variation at 10 pmol of 8%.

Histopathology. On the last treatment day, 28 days after initiation of drug infusion, dogs were killed. Ani-

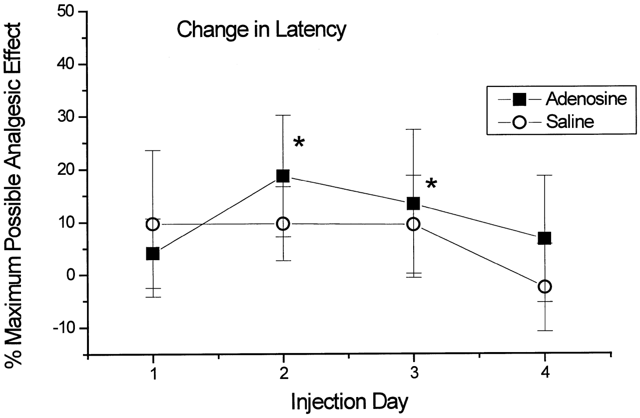

Fig. 1. Effect on paw withdrawal to a noxious heat stimulus of intrathecal injection of saline or adenosine in rats (100 μg) on days 1, 2, 3, and 4. Increase in response latency is expressed as the percentage of the maximum possible effect. **$P < 0.05$ **compared to baseline by one-way analysis of variance (ANOVA) for repeated measures on the raw data.

mals received a large anesthetic dose of sodium pentobarbital (35–50 mg/kg). The trachea was intubated and dogs were manually ventilated to maintain adequate oxygenation during percutaneous puncture of the cisterna magna for CSF withdrawal. The chest was opened and a whole body perfusion of saline (4 l), followed by 10% formalin (4 l) was conducted at a perfusion pressure of 120 mm Hg *via* a cannula in the aortic arch. After fixation, the dura was exposed and dye was injected through the catheter to confirm catheter integrity, to aid in visualizing the position of the intrathecally catheter, and to determine the spread of the dye around the catheter. The spinal cord was then removed in four blocks of vertebral levels of cervical, thoracic, lumbar (with PE10 catheter tip region), and lumbar, below the PE10 catheter tip region and were placed in formalin, embedded in paraffin, and prepared and examined, as described previously, for the rat studies.

Statistics

Comparisons between groups were accomplished with paired or unpaired Student *t* tests as appropriate. Comparisons over time were accomplished with a one-way repeated measures analysis of variance (ANOVA). Histopathology rankings between vehicle and adenosine groups were accomplished with nonparametric statistics (*e.g.*, Kruskal–Wallis). Critical values corresponding to a *P* value of <0.05% were considered significant.

Results

Rat Studies

Behavior. Daily intrathecal injections of vehicle or drug were well-tolerated by all rats, and none of the animals exhibited excitation or pain behavior during injection of the drug. Unlike saline, intrathecal injection of 100 μg adenosine produced a minimal degree of thermal antinociception, shown as a small and transient, but significant, increase of paw withdrawal latency compared to baseline on days 2 and 3 of drug exposure only. However, paw withdrawal latencies to the thermal stimulus did not differ between the saline- and adenosine-treated animals at any time (fig. 1). Withdrawal latency before intrathecal injection did not change during the four days of treatment in either group, indicating no prolonged sensory or motor blocking effects of the drug.

Adenosine did not induce motor dysfunction (table 1). In contrast to saline-treated rats, adenosine-treated rats had higher sedation scores after intrathecal injection on each day of treatment. The onset of sedation was within 5–10 min after injection. Sedation always disappeared before the subsequent injection 24 h later. Increased frequency of urination occurred in all adenosine-treated animals within a short latency period after injection and occurred after every adenosine injection during the 4-day interval, resolving before the next injection.

All rats survived treatments as scheduled, and there were no abnormal behaviors observed on days of testing or thereafter. All animals showed normal gait and appeared well-groomed throughout the study period. Measurement of body weight indicated a mild decrease after surgery and further decrease after intrathecal treatment (table 2). However, body weight did not differ between the saline and adenosine group at any time.

Table 1. Motor Effects and Sedation in Rats

	Day 1	Day 2	Day 3	Day 4
Placing score: 2/1/0				
Adenosine (n = 12)	9/3/0	8/4/0	8/4/0	9/3/0
Saline (n = 6)	6/0/0	6/0/0	6/0/0	6/0/0
Ambulation score: 2/1/0				
Adenosine (n = 12)	9/3/0	6/6/0	8/4/0	9/3/0
Saline (n = 6)	6/6/0	6/6/0	6/6/0	6/6/0
Sedation score −1/0				
Adenosine (n = 12)	12/0	10/2	9/3	8/4
Saline (n = 6)	0/6	0/6	0/6	0/6

Placing and ambulation scores do not differ on any day between saline and adenosine. For placing and ambulation, a score of 2 is normal. For sedation, 0 is absence of sedation. Sedation differs on each day between saline and adenosine animals (Fisher exact test).

Table 2. Body Weight in Rats Receiving Intrathecal Drug Injections

	Presurgery (Day −5)	Predrug (Day 1)	Postdrug (Day +6)
Adenosine (n = 12)	322 ± 36	295 ± 30	285 ± 37
Saline (n = 6)	305 ± 16	300 ± 15	288 ± 18

There were no differences between groups at any time by two-way analysis of variance for repeated measurements.

Histopathology. Catheters were identified in the intrathecal space of all rats, with the tip in the lumbar region. Visual inspection of the tissue revealed no gross abnormalities. Histologic examination showed moderate fibrosis and inflammation in most rats (table 3), but treatment groups did not differ in the incidence or severity of these changes. Inflammation was exclusively of a chronic type, limited to the leptomeninges and the catheter site. In two animals (one of each group), the inflammation spread into a margin of nervous tissue, without neuronal damage. No signs of demyelination or gliosis were observed in any rat.

Dogs Studies

Behavior. All animals survived dosing and sampling without neurologic deficits. There were no systematic changes in body temperature, weight, arousal, and coordination scores in either saline- or adenosine-treated animals (data not shown), nor in heart rate (fig. 2, upper panel) or blood pressure (fig. 2, lower panel). A small but statistically significant increase in muscle tone was noted on the first two days of infusion of adenosine (1 mg/ml), which manifested as mild truncal and hind limb stiffness, but no other effect on muscle tone in either group during the 28 day trial

Skin twitch latency before the initiation of infusion was 2.2 ± 0.9 s and 3.4 ± 0.5 s for saline and adenosine groups, respectively. Continuous infusion of intrathecal adenosine or saline did not result in any significant increase in skin twitch latency during the 28 day period.

Table 3. Summary of Rat Histopathology: Inflammation and Fibrosis Observed at the Level of the Catheter Tip in Rats Injected Daily for 4 Days with Saline or 100 μg Adenosine

	Adenosine (n = 12)		Saline (n = 6)	
Grade	Fibrosis	Inflammation	Fibrosis	Inflammation
0	4	4	2	2
1	4	2	2	2
2	4	5	2	0
3	0	1	0	2

Comparison of rank order of response between adenosine and saline revealed no difference between groups (Kruskal-Wallis test). 0 = normal, no reaction; 1 = mild reaction; 2 = moderate reaction; 3 = severe reaction.

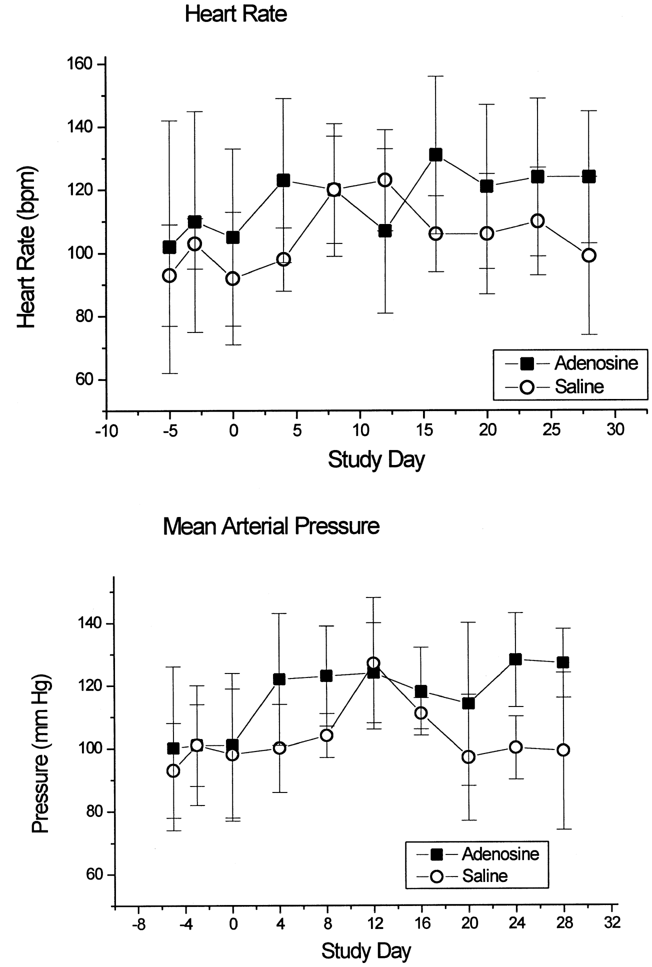

Fig. 2. Time course of heart rate (*upper*) and mean arterial blood pressure (*lower*) assessed over 28 days in dogs receiving long-term intrathecal infusions of adenosine (7.2 mg · 2.4 ml^{-1} · day^{-1}) or saline (2.4 ml/day). Each point represents the mean and SD. No significant differences were seen from baseline in either group.

Cerebrospinal Fluid Analyses. Adenosine concentrations in cisternal CSF at the time of surgery (before catheter placement) was 6,250 ± 940 fmol/50 μl. After 28 days of infusion, the cisternal CSF adenosine concentration was 22,400 ± 7200 fmol/50 μl. There was a slight increase in protein for both the vehicle and the adenosine groups during the 28 days, but protein concentrations did not differ at any time between groups (table 4).

Histopathology. At the time of killing, all spinal cords appeared normal to gross inspection, and all catheters were observed to lie within the intrathecal space at the

Table 4. Summary of Cisternal Cerebrospinal Fluid Protein, Glucose, and Specific Gravity before Implantation and after 28 Days of Infusion with Saline or Adenosine (3 mg/ml; 100 μl/h)*

	Saline		Adenosine (3 mg/ml)	
	Pre	Post 28 days	Pre	Post 28 days
Protein (mg/dl)	15 ± 4	31 ± 8*	23 ± 10	49 ± 15*
Glucose (mg/dl)	72 ± 5	74 ± 4	65 ± 4	61 ± 4
Specific gravity	1.005 ± 0.000	1.005 ± 0.000	1.005 ± 0.000	1.006 ± 0.000

Comparisons of adenosine *versus* saline with pre or post samples were not statistically different ($P > 0.10$) for any measure.

* $P < 0.05$ pre *versus* post; paired *t* test.

level of the L2–L3 spinal segment. Histologic examination revealed the expected fibrotic–inflammatory response at margins in contact with the intrathecal catheter, but was otherwise normal in both groups (fig. 3). Thickening and fibrosis of the dura confined to the catheter area were seen in saline- and adenosine-treated animals, but without inflammatory cells invading tissue. The degree of fibrosis and inflammation did not differ among treatment groups (table 5). There was no evidence of demyelination, gliosis, or neuronal damage in the underlying spinal cord in any dog.

Discussion

Repeated bolus delivery in the rat or 28-day infusion in the dog of the U.S. commercially available concentration of adenosine evoked no behavioral, chemical, or histologic evidence of toxicity. The analysis of CSF obtained from the cisternal membrane at the time of killing is of importance because concentrations of protein, specific gravity, and glucose are measures of acute and chronic inflammatory responses.[31] Previous studies using intrathecal catheters have indicated that the 28-day implant results in a modest but statistically significant increase in protein and glucose concentrations that are comparable to those observed in the current study.[26] These observations support the hypothesis that intrathecal adenosine in the models as delivered is without evident toxicity.

Test Models

An important question in the current work relates to the sensitivity of these models to show toxicity. Previous work with the rodent model using repeated dosing has shown no untoward reaction in systematic studies with a variety of opiates,[32] droperidol,[33] neostigmine,[26] or the A_1 adenosine receptor agonist, R-PIA.[34] In contrast, significant irreversible motor dysfunction and histopathologic indices of injury after even a single injection have been noted with several peptides, including somatostatin[22] and dynorphin.[23] In the dog, long-term intrathecal administration of baclofen,[35] morphine, alfentanil, sufentanil,[25] neostigmine,[26] brain-derived nerve growth factor,[36] and, in the current study, adenosine failed to produce toxicity. In contrast, behavioral dysfunction has been observed after the spinal delivery of amitriptyline and ketamine (Yaksh, Provencher, Eisenach, unpublished information, March 1999). These data jointly indicate that these animal models are able to show a pathologic effect of an intrathecally delivered agent.

The robustness of the preclinical safety assessment depends on the concentration of drug to which the spinal tissue is exposed (as opposed to simply the total dosage delivered) and the duration of drug exposure. Considerable data suggest that an important variable in defining toxicity is the actual concentration of drug at the spinal surface, as exemplified by the experience with intrathecal lidocaine, which shows a concentration-dependent neurotoxicity.[37] With lidocaine, neurotoxicity is manifested in the nerve roots at lower concentrations and in the cord itself at higher concentrations. Although we did not examine the cauda equina in these studies, nerve roots at their entry site in the cord showed no evidence of toxicity from intrathecal adenosine.

Table 5. Summary of Dog Histopathology: Fibrosis/Inflammation in Dogs after Continuous Infusion with Saline or 300 μg Adenosine/h for 28 Days (Cervical, Thoracic, Lumbar 1, and Lumbar 2)

Saline Animals	Pathology Score	Adenosine Animals	Pathology Score
1	1, 1, 1+, 1	1	1, 1, 1, 1
2	1, 1+, 1+, 1	2	1+, 1+, 1+, 1
3	1, 2, 1+, 1	3	2, 2, 2, 2
4	2, 2, 1+, 1	4	2, 2+, 2+, 1+
		5	1, 1, 1, 1

Four-point scale for fibrosis/inflammation: 1 = minimal; 4 = serious inflammation/infection. Comparison of rank order of response between adenosine and saline revealed no difference between groups (Kruskal-Wallis test).

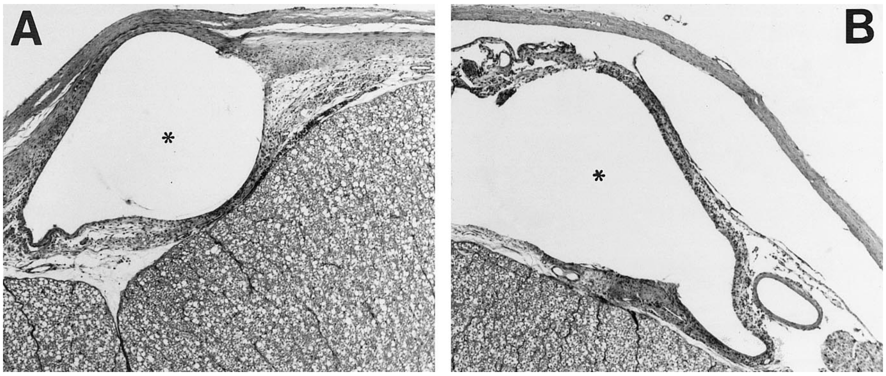

Fig. 3. Examples of light micrographs of intrathecal catheters and spinal cords in (*A*) control and (*B*) adenosine-treated (3 mg/ml) dogs. There was mild to moderate inflammation caused by the catheters that was not significantly different between groups. * Note inflammatory response that defines the catheter tract.

Extensive pharmacokinetics have not been described with intrathecal adenosine, although preliminary studies in humans suggest a half-life of adenosine in CSF of approximately 1 to 2 h, much longer than in plasma.[18] In the rat, the effect of a single bolus of intrathecal adenosine against mechanical hypersensitivity after nerve injury lasts more than 18 h,[20] although drug concentrations were not measured in that study. In dogs, adenosine was administered as a continuous intrathecal infusion in the current study. Sampling of lumbar CSF in one animal measured before and then at 8, 24, 48, and 96 h after initiation of the intrathecal infusion of adenosine were 0.1, 2.1, 10, 26, and 31 μmol/l, suggesting sustained elevation of adenosine concentrations more than 200 times over baseline were likely to have been achieved in CSF near the catheter tip.

Relevance of Preclinical Model for the Human

Duration of Exposure. The initial intended use of intrathecal adenosine is by single bolus delivery. This bolus paradigm was used in the rodent model. Although a useful paradigm, a difficulty with bolus delivery is that it may underestimate exposure if the clearance of drug is very fast or if there are unappreciated differences in the kinetics of the model. For this reason, continuous infusion was administered in dogs to achieve a steady state concentration at or near the catheter tip that will approach the concentration of the drug that is being delivered and that is maintained for the infusion interval. The current studies showed no untoward consequence of a nominal 1-month intrathecal drug exposure, an exposure that considerably exceeds that anticipated for intrathecal adenosine use in humans in initial trials (single administration).

Concentrations. As noted previously, we believe that the principal variable concerning local spinal toxicity relates to injectate drug concentration. Two variables define the local concentrations to which the human spinal cord will be exposed.

1. Injectate concentrations: In both models, the spinal cord was exposed to the maximum concentrations available in the commercial preparation: 3 mg/ml. The tissue exposure at the catheter tip will be limited by that delivered concentration.
2. Local spinal dilution: The bolus delivery of a small volume of injectate into a large CSF volume will further dilute drug concentration. Based on apparent dilution of polar molecules, such as morphine, shortly after intrathecal delivery in humans and dogs, it has been estimated that the local dilution volume in a dog is approximately 3 ml and is approximately 10 ml in a human (see Sabbe *et al.*[25] and Yaksh *et al.*[26]). Although there is no specific nomogram relating the human and dog intrathecal volumes, this difference suggests that, for any given concentration, the spinal cord in the dog model will be exposed to an approximately threefold higher concentration than would be seen in the human spinal cord after bolus delivery of the same concentration.

Spinal and Supraspinal Redistribution

Intrathecal delivery of an agent may induce neurologic effects by spinal or supraspinal actions. Measurement of adenosine concentrations after extended infusion intervals revealed significant increases in cisternal adenosine concentrations (0.12 μg/ml), as compared to those resting levels of adenosine that reflect endogenous release (0.04 μg/ml), indicating a supraspinal movement of the drug. Based on the lumbar sampling studies, these cisternal concentrations at steady state with continuous infusion represent approximately 1.4% of the lumbar levels (*i.e.*, a dilution factor of approximately 70). In the dog model, the typical lumbar to cisternal ratio with continuous lumbar infusion of a large, poorly metabolized molecule, such as the calcium channel blocker SNX-111, was 1:5 to 1:10 (Yaksh and Provencher, unpublished observations, and Yaksh *et al.*[36]). The greater reduction in CSF concentrations along the neuraxis of adenosine compared to SNX-111 may reflect rapid adenosine clearance by deaminases or kinases.

Physiologic Effects of Intrathecal Adenosine

Spinal Cord Blood Flow. Adenosine is known to be a potent vasodilator, and its meningeal vasodilating action has been suggested to cause lumbar pain in one of the volunteers who received 2,000 μg mannitol-containing adenosine solution in Sweden.[18] Similarly, increased spinal cord blood flow was observed from the A_1 agonist R-PIA in rats.[38] Adenosine itself has been administered in high doses systemically[39] and regionally[40] to the spinal cord of rabbits and attenuated ischemic injury associated with aortic occlusion. Proposed neuroprotective mechanisms of adenosine included local vasodilatation and decrease in the metabolic activity of the neural tissue. Although the influence of intrathecal adenosine itself on spinal cord blood flow has not been studied in any animal model at present, it seems unlikely that intrathecal adenosine would reduce spinal cord blood flow in light of the aforementioned studies. Whether intrathecal adenosine, applied to the surface of the cord, could result in vasodilatation of superficial vessels and a "steal" phenomenon during conditions of hemodynamic instability was not tested in the current study, but is worthy of future investigation.

Sedation. Adenosine induced sedation in the rats. This probably is attributable to cephalad spread and subsequent central action of the drug. The large injectate volume in the rat studies could have led to rapid ascent of the drug. The dogs received continuous infusion of adenosine at a rate of 100 μl/h, and no sedation was observed. These differences could be explained by the volume of distribution of the drug in the CSF, which is considerably greater in dogs than in rats, and in humans than in dogs.

Urination. Increased frequency of urination was observed with intrathecal adenosine in rats. This may reflect an increased urine formation, although volume of urine was not recorded in the current study. In previous works, using a volume-evoked cystometrography model in the rat, it has been shown that intrathecal adenosine agonists increase the volume necessary to evoke the micturition reflex (synergic bladder contraction and sphincter relaxation) without altering the strength of the contraction in the rat. This suggests a suppression of the afferent limb of reflex sensitivity.[41]

Antinociceptive Effects. Intrathecal adenosine did not produce analgesia to an acute thermal stimulus in either animal species. This lack of effect may reflect several factors. First, the current models used acute thermal stimuli (hot plate and skin twitch). Although intrathecal adenosine agonists have been shown to be modestly effective in such models, effectiveness of adenosine agonists are most readily noted in models of facilitated processing, both in rats[19] and in humans.[18] Second, the lack of effect may reflect rapid metabolism of adenosine by either kinases or deaminases (see *Spinal and Supraspinal Redistribution*). Third, the study may have been inadequately powered to observe an antinociceptive effect. Power analysis revealed that there was adequate power to detect an effect of at least 40% MPE, although a smaller effect could have been missed.

In summary, long-term administration of intrathecal adenosine failed to produce behavioral, chemical, or histologic evidence of neurotoxicity in these two well-established animal models. Animals were exposed to doses and durations of drug exposure greatly in excess of single bolus intrathecal administration proposed for human use (<2 mg). These results jointly provide evidence for the presumption of safety of adenosine in this dosage range and support phase I safety trials in humans.

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