Dose Response of Intrathecal Adenosine in Experimental Pain and Allodynia

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Background: Intrathecal adenosine reduces areas of mechanical hypersensitivity and provides analgesia in patients with neuropathic pain. Adenosine also causes side effects, yet its dose response for either efficacy or side effects has not been examined in double blind studies. We studied two doses of intrathecal adenosine in humans with experimental hypersensitivity and the ability of the adenosine receptor antagonist, aminophylline, to reverse adenosine's effects.

Methods: Following Internal Review Board approval and written informed consent, 35 volunteers were studied. Five volunteers were studied to confirm the stability of a new method of inducing hypersensitivity with capsaicin. The remaining 30 volunteers received, in a randomized, double-blind manner, saline, or adenosine, 0.5 or 2.0 mg, by intrathecal injection 40 min after areas of allodynia and hyperalgesia were established from capsaicin. Two hr later, volunteers were randomized to receive intravenous saline or aminophylline, 5 mg/kg.

Results: Topical capsaicin with intermittent heating resulted in stable areas of allodynia and hyperalgesia. Intrathecal adenosine, but not saline, reduced areas of allodynia and hyperalgesia from capsaicin, with no differences between doses. Side effects occurred in 1, 2, and 6 volunteers receiving saline, 0.5 mg and 2.0 mg adenosine, respectively. Aminophylline failed to reverse adenosine's effects.

Conclusions: There is no difference in efficacy to experimental hypersensitivity between the largest approved dose of intrathecal adenosine and a dose 25% this size, but side effects are more common with the larger dose. Failure of aminophylline to reverse adenosine's effects could reflect inadequate concentrations at receptors in the spinal cord after intravenous injection.

INTRATHECAL injection of an adenosine agonist was first tested in animals for antinociception in 1984[1], and following preclinical safety studies, an A1 adenosine agonist was first tested intrathecally in humans for analgesia 11 yr later in 1995.[2] Although the human trial revealed a potent antiallodynic effect of the adenosine analog, no further studies were reported. Intrathecal adenosine itself was first studied after preclinical safety testing by Sollevi *et al.* in 1998 in Sweden[3] and the American formulation tested by us in 2002.[4,5] These initial studies, which were all single dose or open-label trials, suggested that intrathecal adenosine and adenosine analogs fail to reduce acute nociceptive pain in humans, but reduce areas of hypersensitivity (hyperalge-

sia and allodynia) that is induced experimentally with topical or intradermal application of irritants. The effective dose range has not, however, been determined in these studies, or in open-label experience in patients.[6]

Fewer than 100 humans have been reported who have received intrathecal adenosine, and no serious side effects have been observed. The most common side effects are headache and backache, and these have been suggested in open label trials to be more common in patients with neuropathic pain than in normal volunteers.[6] The dose-dependency of these side effects has been suggested in a small open label series,[4] but not formally determined. The primary purpose of the current randomized, double blind study was to determine whether efficacy or the incidence of side effects differed between the maximum allowable dose of the American formulation of intrathecal adenosine (2.0 mg) and what we anticipated to be a threshold dose (0.5 mg) for antihypersensitivity.

A secondary purpose was to determine whether intravenous infusion of an adenosine receptor antagonist, aminophylline, would reverse efficacy or side effects from intrathecal adenosine. We used, in a randomized, double-blind manner, a dose of aminophylline which was previously shown to reverse analgesia from peripherally administered adenosine.[7] To test adenosine's effect and its reversal in the same volunteer, we employed a recently described method of inducing hypersensitivity by topical capsaicin combined with intermittent heating of the sensitized skin,[8] reported to result in stable areas of hypersensitivity over several hours.

Materials and Methods

Following Food and Drug Administration, Institutional Review Board, and General Clinical Research Center Protocol Review Committee approval, two clinical trials were performed. The first trial was an open-label study of the stability of areas of allodynia and hyperalgesia induced by topical capsaicin followed by intermittent heating, as recently described.[8] This study was performed in the General Clinical Research Center, and all volunteers gave written consent on a day previous to study, at which time they were familiarized with testing procedures (touch of skin with von Frey filaments and cotton wisps). A 4 cm^2 Peltier-controlled thermode was placed on the mid forearm volar skin and maintained at 45°C for 5 min. During that period, volunteers rated pain, if present, on a 0–10 verbal scale at 1 min intervals. Areas of hyperalgesia to probing with a 225 mN von Frey

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filament and allodynia to cotton wisp stroking were determined, then capsaicin cream was placed on the same area with an occlusive dressing. Von Frey filament probing in normal skin resulted in mild prickly pain, whereas cotton wisp stroking resulted in nonpainful light touch. During the next 30 min, volunteers were asked to rate pain, if present, on a 0–10 verbal scale at 5 min intervals. Areas of hyperalgesia and allodynia were determined after removing the capsaicin. At 40, 80, 120, 160, and 200 min thereafter, the Peltier-controlled thermode was placed on the same area of original stimulation and maintained at 40°C for 5 min. At the end of each of these periods, volunteers were asked to rate pain, if present, on a 0–10 verbal scale. Areas of hyperalgesia and allodynia were determined immediately before and after each application of the thermode. Subjects did not observe the area of sensory testing during the study. The study ended 40 min after the last thermode stimulation.

The second study utilized this method of inducing and maintaining hypersensitivity to examine adenosine and aminophylline. Thirty healthy volunteers were recruited, written informed consent was obtained, and, on a day previous to study, they were trained to rate pain consistently in response to 5 s randomized heat stimuli, between 38 and 51°C, using a 2 cm^2 Peltier-controlled thermode. On the day of the study the volunteer came to the General Clinical Research Center, having had nothing to eat or drink after midnight. A peripheral intravenous catheter was inserted into a vein in an upper extremity and lactated Ringer's solution infused at 1.5 ml · kg^{-1} · h^{-1} for the duration of the study. Areas of hyperalgesia and allodynia were induced on the lateral calf by skin heating and topical capsaicin as described above. They then were randomized to receive intrathecal saline (2 ml) or adenosine (Adenoscan, Fujisawa Pharmaceutical Co., Deerfield, IL), 0.5 or 2.0 mg, diluted in normal saline (n = 10 per group). Injections were performed using a #27 Whitacre spinal needle at the L3–L4 or L4–L5 interspace, with the volunteer in the lateral position. They were then positioned supine with the head of the bed elevated for their comfort.

Areas of hyperalgesia and allodynia surrounding the capsaicin-treated skin were determined after each "rekindling" at 40 min intervals. The primary outcome measure was area of allodynia at 120 min after intrathecal injection, the time of peak adenosine effect.[4] In addition, pain report at the end of each rekindling was recorded. Immediately after the 120 min determinations, volunteers received, in a randomized, double-blind manner, intravenous aminophylline, 5 mg/kg or an equal volume of saline over 20 min (n = 5 for aminophylline and n = 5 for saline in each intrathecal dose group). The effect of intravenous drug administration was determined after the next two rekindlings (20 min and 60 min after the end of intravenous infusion, respectively).
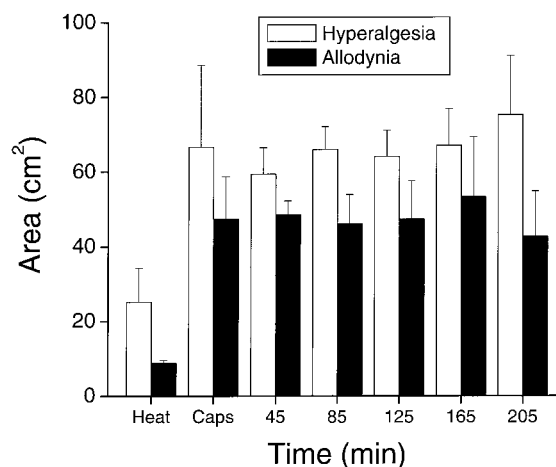
Fig. 1. Areas of hyperalgesia to von Frey filament testing (open bars) and allodynia to cotton wisp testing (filled bars) in volunteers after a 5 min period of heating skin to 45°C (heat) followed by 30 min of application of topical capsaicin (caps), followed by application of heat (40°C) for 5 min every 40 min for 205 min. Each bar represents the mean ± SE of five individuals.

Volunteers were questioned for presence of side effects at 15 min intervals throughout the study. Blood pressure, heart rate, and oxyhemoglobin saturation were monitored at the same intervals throughout the study, with the exception of heart rate, which was also monitored at 5 min intervals during and for 20 min after cessation of the intravenous infusion.

Statistics

Data are presented as mean ± SE. The effect of treatment within each group was determined by one-way analysis of variance (ANOVA) for repeated measures on the raw data. The effect of drug treatments were compared by two-way ANOVA for repeated measures on the raw data. To account for changes within the saline placebo group, groups were also compared by two-way ANOVA for repeated measures on percent change from control. The incidence of side effects among groups was compared by Fisher exact test. $P < 0.05$ was considered significant.

Results

In the validation study there were two men and three women, 31–45 yr in age, including one black and four white individuals. Initial heating of the skin for 5 min at 45°C resulted in mild pain (3.7 ± 0.5 on a 0–10 scale), as did the 30-min application of topical capsaicin (3.2 ± 1.1 at the end of the 30 min). Pain at the end of each 5 min, 40°C rekindling was greatest at the first 40 min time point (4.8 ± 0.9), gradually declining to 1.9 ± 0.3 at the 200 min time point. In contrast, areas of hyperalgesia and allodynia at the end of each rekindling were remarkably stable (fig. 1), with a coefficient of
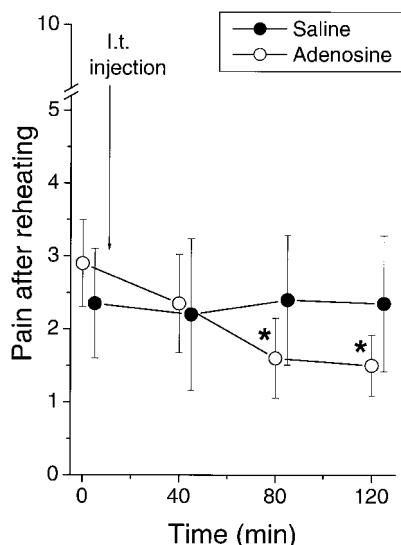
Fig. 2. Pain (0–10 verbal scale) at the end of each 5 min reheating of capsaicin-exposed skin in volunteers treated with intrathecal saline (open circles) or adenosine (closed circles; 0.5 and 2.0 mg doses combined). Each symbol represents the mean ± SE of 10 volunteers receiving saline and 20 volunteers receiving adenosine. * *P* < 0.05 compared to control (time 0).

variation within each individual for this greater than 3 h time period of 20 ± 4% for area of hyperalgesia and 29 ± 6% for area of allodynia. As a population, the coefficients of variation were 13 ± 3% and 14 ± 4% for areas of hyperalgesia and allodynia, respectively.

In the randomized dose-response and reversal study there were 17 women and 13 men, with age 30 ± 1.5 yr, height 171 ± 1.9 cm, and weight 74 ± 2.6 kg. Six individuals were black, one was Hispanic, and 23 were white. There were no sex or racial differences in responses to capsaicin or drug treatments, although the study was not designed or powered to test for such differences. Intrathecal saline had no effect on pain at the end of each rekindling (fig. 2). In contrast, intrathecal adenosine reduced pain at the end of the 80 and 120 min rekindling periods (fig. 2), although there were no differences between adenosine doses.

Areas of hyperalgesia and allodynia were unaffected by intrathecal saline. In contrast, intrathecal adenosine reduced areas of both measures of hypersensitivity (fig. 3). For hyperalgesia, the effect of 0.5 mg adenosine just missed significance (*P* = 0.052), whereas for allodynia both doses significantly reduced area. There was no significant difference between adenosine doses for areas of either hyperalgesia or allodynia on the raw data. When using % change from control, similar results were obtained: For hyperalgesia, only 2 mg adenosine differed from saline (but did not differ from 0.5 mg adenosine). For allodynia, both 0.5 and 2 mg adenosine differed from saline, but there was no difference between adenosine doses.

Aminophylline infusion had no effect on blood pressure (data not shown), but significantly increased heart
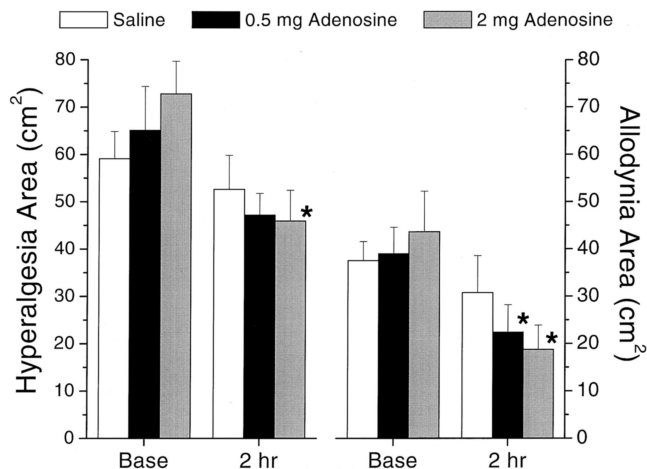

Fig. 3. Areas of hyperalgesia (left) and allodynia (right) at the end of capsaicin and heat treatment (Base) and 2 h after intrathecal injection of saline (open bars), 0.5 mg adenosine (filled bars), or 2 mg adenosine (light gray bars). Each column represents the mean ± SE of 10 volunteers. *P < 0.05 compared to Base.

rate by 8 ± 2 beats/min. No dysrhythmias were noted, and no volunteer experienced nausea or other symptoms during intravenous infusion of either saline or aminophylline. Aminophylline had no effect on areas of hyperalgesia or allodynia (fig. 4).

Only one volunteer receiving intrathecal saline experienced side effects, described as mild back stiffness 24 h after injection. No volunteer receiving intrathecal saline experienced side effects during the 4 h study itself. In contrast, headache and back or groin ache occurred in volunteers receiving intrathecal adenosine. Two volunteers in the 0.5 mg adenosine group had side effects: headache in one volunteer from 15 min to 2 h after injection and discomfort in the back of the legs in an-
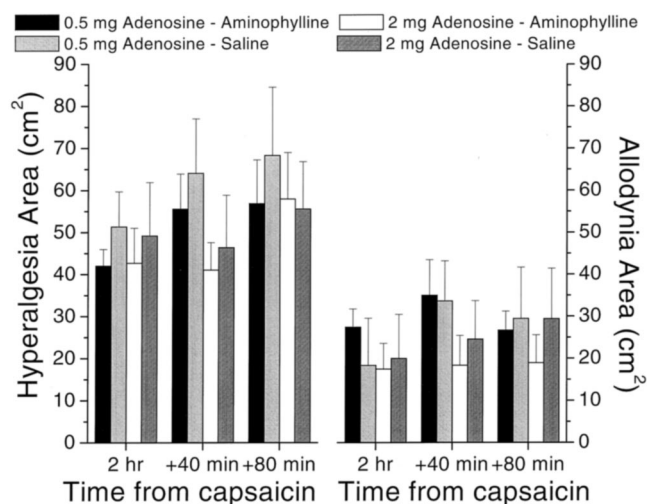

Fig. 4. Areas of hyperalgesia (left) and allodynia (right) 2 h after intrathecal injection of adenosine (0.5 or 2.0 mg) in volunteers receiving intravenous saline or aminophylline, begun at time 2 h on this figure. No significant difference within groups over time.

other volunteer for less than 15 min beginning 30 min after injection. Six volunteers in the 2 mg adenosine group had side effects: headache in 3 volunteers at 15–45 min after injection period, and discomfort and cramping in legs or groin in 3 volunteers at 15–60 min after injection. The incidence of side effects was greater in the 2 mg adenosine group than saline, but was similar in the 0.5 mg adenosine group to saline. All side effects were described by the subjects as mild and disappeared before the intravenous saline or aminophylline infusion commenced.

Discussion

Key observations during the introduction of a new drug into clinical practice are dose responses for efficacy and side effects and reversal with specific antagonists. This is the first double blind, placebo controlled trial to compare doses of intrathecal adenosine in this regard, and the results suggest that the dose response for this agent to reduce hypersensitivity, at least in the experimental pain setting, is less than 0.5 mg. This is in agreement with an open label trial in patients with neuropathic pain, in which there was no difference in efficacy between 0.5 and 1 mg intrathecal adenosine.[6]

We observed, similar to the original report,[8] stable areas of hyperalgesia and allodynia for several hours using this simple method of capsaicin application and repeated heating of the skin. The originators of this method have previously demonstrated efficacy of intravenous lidocaine,[9] magnesium,[10] and remifentanil[11] using this model, but failure of intravenous adenosine.[12] The current results, with efficacy of intrathecal adenosine, suggest that adenosine's site of action is likely in the spinal cord. Of course there may be many differences in pathophysiology and response to therapy between hypersensitivity induced for a few hours by capsaicin and that occurring in patients with years of chronic pain. However, studies in rodents, nonhuman primates, and humans demonstrate that capsaicin treatment induces central sensitization with a pharmacology similar to that observed in peripheral nerve injury models of neuropathic pain.[13–15]

We focused in the current study on the time of peak drug effect. It is conceivable that doses that are on the plateau for peak drug effect could nonetheless differ in duration of effect, with longer duration from larger doses. We previously reported a duration of action of up to 24 h from a single, 2 mg intrathecal adenosine dose in normal volunteers with capsaicin-induced hypersensitivity,[4] but did not determine the duration of drug action in the current study, other then that it was still present 200 min after injection. In an open label study of patients with neuropathic pain, intrathecal adenosine was active in 12 of 14 cases with a duration of 12 h to 4 days

(median = 1 day) after 0.5 mg and 10 h to 6 days (median = 1 day) after 1 mg.[6] The reasons for the long duration of intrathecal adenosine, despite a half life in human cerebrospinal fluid of less than 2 h,[4,6] are uncertain. In rats with peripheral nerve injury-induced hypersensitivity there is also a long duration of effect from intrathecal adenosine, not due to prolonged residence time in cerebrospinal fluid, increased receptor number, or G protein coupling efficiency.[16] Regardless of the mechanism, we believe it is likely, based on the open label trials, that this dose range of intrathecal adenosine (0.5–2 mg) is also on the plateau for duration as well as peak effect.

Backache has been observed in volunteers receiving intrathecal adenosine,[3,5] as well as in patients,[6] and is of unknown etiology. Preclinical studies fail to demonstrate neurotoxicity from large doses of intrathecal adenosine over prolonged periods in rats and dogs,[17,18] suggesting this is unlikely a neurotoxic effect. It has been proposed that vasodilation by adenosine may cause a migrainous-like symptom focally in the spinal cord,[6] and this perhaps could also explain headache that occasionally accompanies adenosine injection. The time course of these side effects in the current study (15 min to 2 h after injection) is consistent with previous reports,[3,5,6] as well as the incidence of approximately 50% with the 2 mg dose.[5] The current small study demonstrates a higher incidence of these side effects after 2 mg adenosine than saline control, but no difference between saline and 0.5 mg adenosine. Because we observed no difference in efficacy between the two doses in this model of acute hypersensitivity from capsaicin, we hypothesize that a dose range of 0.5 mg or less be adequate in patients with chronic pain. Whereas side effects in this study in volunteers were mild, backache has been reported to be severe in patients with neuropathic pain receiving intrathecal adenosine,[6] justifying an attempt to reduce the incidence of this side effect. Finally, as regards safety, we observed no effect of intrathecal adenosine on cardiovascular variables, no volunteer experienced focal neurologic symptoms, other than the aches described above, and no volunteer experienced any effects beyond 24 h after injection.

The phosphodiesterase inhibitor, aminophylline, effectively antagonizes adenosine receptors, and aminophylline has been used to investigate mechanisms of spinal purinergic antinociception in animals.[19] We could not administer aminophylline intrathecally to humans, since no preclinical toxicity testing of this agent has been performed. However, intravenous aminophylline was reported in a double-blind study to reverse analgesia from systemic adenosine in humans.[7] Intravenous infusion of aminophylline reverses coronary vasospasm associated with dipyridamole stress testing,[20] consistent with dipyridamole's effect to increase extracellular adenosine concentrations and aminophylline's adenosine receptor

blockade. Because aminophylline induces central side effects and passes readily into cerebrospinal fluid (Physicians Desk Reference, 56th Edition, 2002), we anticipated that it might reverse the effects of intrathecal adenosine. Its failure to do so could reflect inadequate concentrations in the spinal cord, analogous to the inability of low doses of systemic naloxone to reverse epidural morphine analgesia after surgery.[21] We did not test in the current study whether aminophylline would reduce side effects from intrathecal adenosine, since these had disappeared prior to the intravenous infusion.

In summary, intrathecal adenosine, 0.5 and 2 mg, reduces areas of mechanical hyperalgesia and allodynia following the application of heat and topical capsaicin in volunteers, with no differences in efficacy between doses. Side effects, however, were more common with the larger dose than with saline control. These data suggest that doses of intrathecal adenosine of 0.5 mg or less should be investigated for the treatment of pain.

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