Phase I Safety Assessment of Intrathecal Injection of an American Formulation of Adenosine in Humans

James C. Eisenach, M.D., * *David D. Hood, M.D.,*† *Regina Curry, R.N.*‡

Background: Preclinical studies of intrathecal adenosine suggest it may be effective in the treatment of acute and chronic pain in humans. A phase I safety trial of the intrathecal injection of a mannitol-containing formulation of adenosine in Sweden showed a considerable incidence of backache. We performed a phase I safety trial of intrathecal injection of the American formulation of adenosine, which lacks mannitol.

Methods: Following US Food and Drug Administration and institutional review board approval and written informed consent, 65 volunteers were studied in two trials: an open-label, dose-escalating trial with intrathecal adenosine doses of 0.25–2.0 mg (25 subjects) and a double-blind, placebo-controlled trial of adenosine, 2 mg (40 subjects). Blood pressure, heart rate, end-tidal carbon dioxide, and sensory, motor, and reflex neurologic functions were systematically examined for 24 h after injection, and volunteers were contacted by telephone at times up to 6 months after injection.

Results: Intrathecal adenosine did not affect blood pressure, heart rate, end-tidal carbon dioxide, or neurologic function. Headache was reported by 10 and back pain was reported by 8 of 30 subjects exposed to adenosine in the second double-blind trial, whereas none of these symptoms was reported by the 10 saline-treated subjects.

Conclusion: These data support further investigation of intrathecal adenosine for analgesia in humans and suggest that this agent does not produce a high incidence of severe side effects.

TESTING of the intrathecal injection of novel classes of pharmaceuticals in humans may have practical consequences by leading to use of these agents for spinal analgesia. Additionally, such tests with spinal application can prove the concept of activity of drug classes to treat clinical pain and lead to development of these drugs by spinal or systemic administration. Agents acting on adenosine A_1 receptors are examples of one such class. Studies in animals show the presence of A_1 receptors on spinal cord neurons,[1] electrophysiologic evidence that their activation reduces C fiber–evoked activity,[2] and behavioral evidence that intrathecal injection of A_1 agonists reduces responses to acute noxious stimulation[3] and reduces hypersensitivity in models of chronic pain.[4]

The current study examines the safety of intrathecal injection of the marketed American formulation of adenosine (Adenoscan; Fujisawa Pharmaceutical Co., Deer-field, IL) in human volunteers. We concluded it was ethical to examine intrathecal adenosine after completion of neurotoxicity studies in rats and dogs.[5] Study design of initial trials in humans is as important as preclinical assessment in safe introduction of novel agents into clinical practice. Studies typically consist of three phases, beginning with a phase I trial to assess safety, focusing on expected side effects, determining maximum tolerated dose, and screening for unexpected side effects.[6] We report the initial phase I experience with intrathecal adenosine (Adenoscan) in humans. A dose-escalating, open-label phase Ia trial examined the effects up to the maximum dose supported by endotoxin unit testing of this formulation, followed by a double-blind, placebo-controlled phase Ib trial of this dose. Endotoxin unit testing is required of all agents for parenteral use and is used as a measure of extremely minor contamination of the product. Marketed parenteral medications are manufactured to meet a standard of maximum such contamination as determined by endotoxin unit testing. In the case of the marketed formulation of adenosine in the US, the maximum dose that could be administered and meet this endotoxin unit testing limit is 2 mg. This was the maximum dose used in the current study.

Materials and Methods

Protocols were reviewed and approved by the Food and Drug Administration and Wake Forest University School of Medicine Institutional Review Board and General Clinical Research Center protocol committee (Winston-Salem, NC). Healthy (American Society of Anesthesiologists physical status I) adult volunteers taking no prescription medications were recruited by advertising within the community, using Institutional Review Board–approved wording and targeting all sectors of the population. Consent was obtained in a three-stage process: the study was initially described to the volunteer, the volunteer was given the informed consent document to review, the informed consent document was reviewed in detail with the volunteer by one of the investigators, all questions were answered, and written informed consent obtained. Consent was verbally confirmed on the day of study, and participants were asked whether they had any additional questions at that time. Risks included in the informed consent document were pain and bruising from needle insertion, post–dural puncture headache, effects on blood pressure, heart rate, or respiration, and unforeseen risks, including paralysis. Because a previous study with a Swedish for-

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* FM James, III Professor of Anesthesiology, † Associate Professor of Anesthesiology, ‡ Research Nurse.

Address correspondence to Dr. Eisenach: Department of Anesthesiology, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, North Carolina 27157. Address electronic mail to: eisenach@wfubmc.edu. Reprints will not be available from the authors. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

mulation of adenosine noted backache or leg pain in volunteers receiving intrathecal adenosine,[7] volunteers were warned that back or leg pain could occur. Women of childbearing potential underwent a serum pregnancy test and used an effective birth control method.

There was a payment to the volunteers for study participation. Payment amount was determined by the Institutional Review Board and was divided according to the schedule of procedures performed so that in the event of dropout, partial payment was available for the portion of the study completed. Total payment was $400 for the phase Ia trial and $350 for the phase Ib trial.

For each trial, volunteers arrived at the General Clinical Research Center on the morning of the study, having had nothing to eat or drink since midnight. An 18- or 20-gauge intravenous catheter was inserted in a forearm vein, and lactated Ringer's solution was infused at $1.5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for the duration of the study. After baseline measurements, a No. 27 Whitacre tipped spinal needle was inserted at a lower lumbar interspace, and spinal injection of saline or adenosine was administered in a 2-ml solution. All but six injections were performed with the volunteer in the lateral position. Subjects were then positioned supine with the head of the bed elevated for their comfort.

The focus of both trials was on safety. Blood pressure was monitored noninvasively at 5-min intervals for 15 min after injection and then at 1, 2, 3, 4, and 6 h after injection. Heart rate and oxyhemoglobin saturation were monitored using a pulse oximeter continuously for 60 min after injection and then at the same intervals as blood pressure until 6 h after injection. A screening neurologic examination, along with questioning for qualitative symptoms, was performed before and at 45, 90, 150, 210, and 240 min and 24 h after injection. This consisted of examining deep tendon reflexes of both the arms and the legs, light touch, and extension–withdrawal strength. In addition, volunteers were asked at these times about other symptoms, including sedation, anxiety, nausea, gastrointestinal- and bladder-related symptoms, dizziness, or extremity weakness. They were also questioned about any symptoms not in this list. In addition, they were asked to report any unusual symptoms, including weakness, numbness, or sedation, at any time they occurred. Volunteers were allowed to ambulate beginning 2 h after spinal injection and left the General Clinical Research Center 6 h after injection, accompanied by a responsible adult. They returned the following day for neurologic evaluation and questioning about symptoms. Then, they were contacted by telephone and questioned about symptoms daily for 5 days, weekly for 1 month, and after 3 and 6 months.

Phase Ia Trial

In this open-label study, five consecutive groups of volunteers received intrathecal preservative-free adeno-

sine (Adenoscan) diluted to a 2-ml volume with preservative-free normal saline in doses of 0.25, 0.5, 1.0, 1.5, or 2.0 mg. This marketed formulation of adenosine has an adenosine concentration of 3 mg/ml in preservative-free normal saline, with no other constituents. Escalation to the next dose level was only allowed in the absence of predefined severe side effects in two of five volunteers at the lower dose level, and the study was to be halted with any evidence of neurotoxicity in any individual. Efficacy to acute noxious stimulation was screened by Peltier-controlled thermode heating of the skin of the lower leg and is described elsewhere.[8] A second lumbar puncture was performed with a 27-gauge Whitacre needle at the same interspace as the first 1 h after adenosine injection.

Phase Ib Trial

In this double-blind, placebo-controlled trial, 40 volunteers were randomly assigned to receive either 2 ml saline or 2 ml adenosine, 2.0 mg, diluted in preservative-free normal saline. Efficacy to mechanical hypersensitivity was screened by testing punctate and brush sensations of the skin after intradermal capsaicin injection and is described elsewhere.[8] A second lumbar puncture was performed with a 27-gauge Whitacre needle at the same interspace as the first, either 0.5, 1, 2, 4, or 24 h after adenosine injection.

Statistics

Unless otherwise indicated, data are presented as mean ± SE. Continuous variables were tested over time by one-way repeated-measures analysis of variance followed by a Dunnett test. Incidence of side effects was compared between saline and adenosine groups using the Fisher exact test. $P < 0.05$ was considered significant.

Results

Phase Ia Trial

Ten women and 15 men were studied, including 1 African-American, 1 Hispanic, 1 Asian, and 22 white subjects. Their average age was 33 ± 1.4 yr, average height was 180 ± 6.2 cm, and average weight was 69 ± 0.9 kg. There was no effect of adenosine at any dose on blood pressure, heart rate, end-tidal carbon dioxide, oxyhemoglobin saturation, neurologic examination, or level of sedation or anxiety. Adenosine produced no gastrointestinal or urinary symptoms. No weakness was noted by the volunteers, and all were able to ambulate and met discharge criteria at the end of the study. There were no symptoms at long-term follow-up.

Some volunteers described headache, backache, or leg ache after adenosine administration. Headache was described as mild and pressure-like, was not described as painful, and was described with no apparent difference
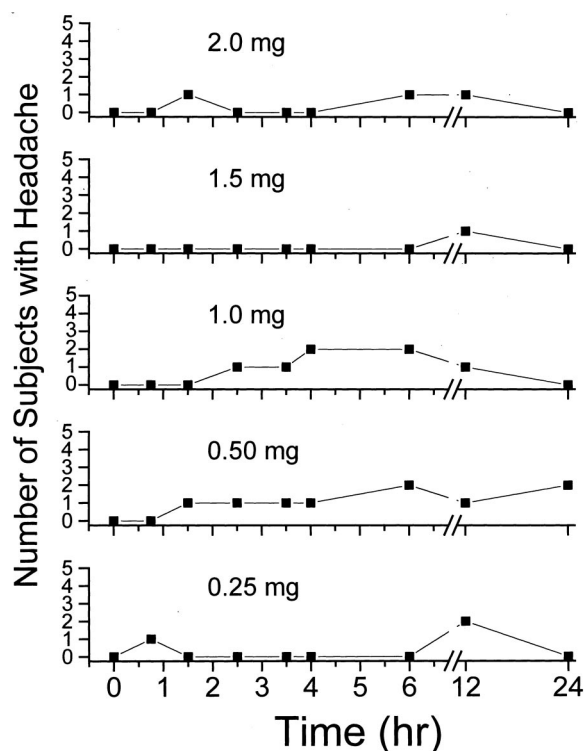
Fig. 1. Number of subjects with headache over time in volunteers in the phase Ia trial after different doses of intrathecal adenosine at time 0. Each symbol represents the number of subjects with headache in a group of five volunteers.

among adenosine doses (fig. 1). When questioned, no volunteer requested treatment for the headache. There were no long-term headaches consistent with post–dural puncture headache in either study.

Volunteers also noted backache or leg ache, described usually as a tightness or cramp, located in the lower back or posterior thighs (fig. 2). There was an apparent dose dependency in that backache or leg ache occurring within the first 6 h after injection was present in one volunteer at the 0.25-mg dose, two volunteers at the 1.5-mg dose, and four volunteers at the 2.0-mg dose. Only two individuals rated the sensation as painful. One volunteer at the 1.5-mg dose experienced acute local back pain at the site of spinal injection, noted when moving from the lateral to supine position after the injection, and rated it as 7 out of 10 for pain. Within 1 min, it was reduced to a rating of 1 out of 10, lasting 30 min. Another individual at the 1.5-mg dose experienced low back pain surrounding the site of injection and rated it as 1 out of 10, lasting 60 min. No one at the 2-mg dose rated the sensation as painful, and no volunteer requested analgesics for the ache when questioned by the investigator.

We further examined symptoms in this study by time of onset, reasoning that onset of a symptom within 6 h after injection was more likely to be related to drug than late-occurring symptoms. Mild headache and backache were common 6–24 h after spinal injection, with an

incidence of 40–100% for those with either of the two symptoms, without difference in incidence among adenosine doses. Early headache occurred in four volunteers, early backache occurred in two volunteers, and early leg ache occurred in four volunteers. The time course of headache differed from backache or leg ache in that its onset was later (65 ± 40 *vs.* 5 ± 2.9 min; $P < 0.05$) and was longer in duration (9.3 ± 6.3 *vs.* 1.0 ± 0.7 h; $P < 0.05$).

Phase Ib Trial

Nineteen women and 21 men were studied, including 5 African-American, 3 Asian, and 32 white subjects. The saline and adenosine groups were similar in age (31 ± 2.6 *vs.* 31 ± 1.3 yr), height (156 ± 12 *vs.* 165 ± 5.7 cm), and weight (67 ± 1.2 *vs.* 68 ± 0.7 kg, respectively). Neither adenosine nor saline affected blood pressure, heart rate, end-tidal carbon dioxide, oxyhemoglobin saturation oximetry, neurologic examination, or level of sedation or anxiety, and neither produced gastrointestinal or urinary symptoms or weakness. As in the phase Ia study, all volunteers were able to ambulate and met discharge criteria at the end of the study. There were no symptoms at long-term follow-up.

Headache occurred in 33% of volunteers receiving adenosine and 0% of volunteers receiving saline ($P = 0.08$). As in phase Ia, headache was described as mild,
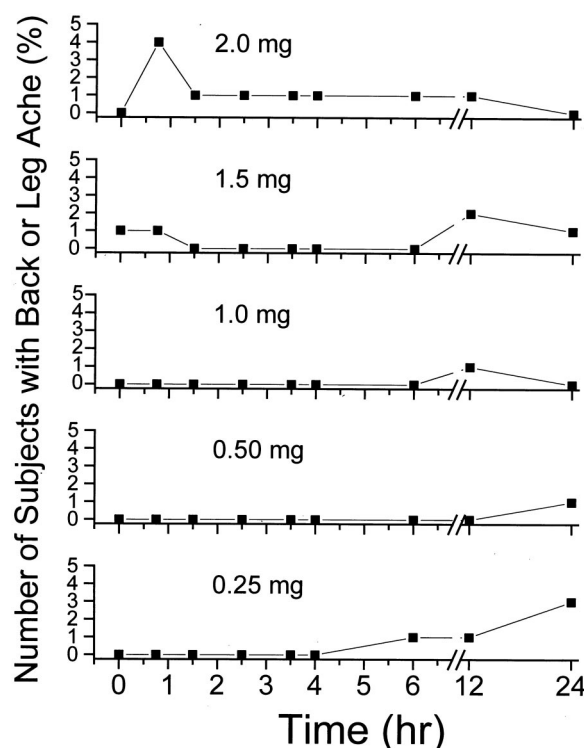

Fig. 2. Number of subjects with backache or leg ache over time in volunteers in the phase Ia trial after different doses of intrathecal adenosine at time 0. Each symbol represents the number of subjects with backache or leg ache in a group of five volunteers.

and no volunteer accepted the offer of analgesic treatment for the headache. Backache occurred in 23% and leg ache occurred in 3% of volunteers receiving adenosine and 0% of volunteers receiving saline (P = not significant). Six individuals characterized the backache as painful, assigning it a score of 1, 2, 2, 3, 4, or 6 out of 10. One individual described a unilateral posterior thigh ache that was not painful. None accepted the offer for analgesic treatment. Subsequent questioning revealed no long-term backaches.

When divided as in the previous study by onset of symptoms, there were no headaches or leg aches beginning 6 h after injection or later. Nine individuals (30%) had mild backache during this late (6–24 h) period, similar to the overall incidence of 32% in the first study for backache at this time period. As in the first study, there was a significant difference in the timing of headache and backache or leg ache in the periinjection period (fig. 3). The average time to onset of symptoms from injection was longer for headache (21 ± 4 min) than for backache or leg ache (8.6 ± 1.9 min; P < 0.05).

Discussion

These data provide several guides to further clinical trials with intrathecal adenosine for analgesia. First, this initial safety study did not observe side effects typical of other spinal analgesics, including local anesthetics (nonspecific numbness, motor blockade, hemodynamic instability), opioids (nausea, sedation, respiratory depression), α_2-adrenergic agonists (sedation, hypotension, bradycardia), or neostigmine (nausea, hallucinations). One might have expected some of these side effects because adenosine is an important sleep-inducing neurotransmitter in the brainstem,[9] and movement of aden-

Fig. 3. Incidence of backache or leg ache (dashed line) or headache (solid line) in the phase Ib trial after intrathecal adenosine, 2 mg, at time 0.

osine in cerebrospinal fluid could result in delayed sedation. In addition, systemically administered adenosine has profound effects on cardiac rhythm and rate, and it is conceivable that extremely rapid systemic absorption could have similar effects. This would be unlikely, given the known absorption rates of several similar compounds from the intrathecal space and the small doses used in the current study. Other side effects, especially nausea, are difficult to predict from preclinical studies, and these phase I data are comforting in that they indicate that such side effects are unlikely from adenosine.

However, because the incidence of a side effect from the study of only 55 individuals could have been as high as 5% and still be missed in this small sample size, the use of intrathecal adenosine should still be considered investigational, and careful monitoring should be applied. For example, initial small studies of intrathecal morphine failed to show respiratory depression, which occurs at dangerous levels in less than 1% of cases, and several fatalities occurred before this was recognized and appropriate monitoring was instituted.

These data also indicate that a dose range of 0.25–2.0 mg adenosine as a single bolus in hypobaric solution is well-tolerated by normal humans, although the same limitations regarding the certainty of this statement apply as indicated. The maximum bolus dose allowed under Food and Drug Administration regulations with this formulation is 2.0 mg. Although preclinical toxicity studies observed no neurotoxicity at much larger doses, this 2.0-mg maximum dose reflects specifications of purity of the marketed formulation. As noted in the introduction, one measure of purity is endotoxin unit contamination, and a maximum amount of such contamination is allowed for any formulation administered by intravenous bolus. The maximum allowable endotoxin unit contamination is much lower for intrathecal than intravenous injections. In this case, the certified purity of adenosine (Adenoscan) intended and marketed for intravenous use supports a maximum intrathecal dose of 2.0 mg. In addition to this maximum allowable dose, these regulations raise a second practical note for further studies.

The maximum allowable endotoxin unit dose for a human is determined by the dose of drug to be administered. Adenosine is marketed in the US in two formulations by Fujisawa Pharmaceutical Company: Adenoscan for myocardial scanning and Adenocard for dysrhythmia treatment. Because many more milligrams of adenosine are used for the myocardial scanning indication of Adenoscan than for the dysrhythmia-treating indication of Adenocard, the endotoxin unit testing for Adenoscan supports a larger number of milligrams of drug for acute administration. Thus, although Adenocard might be more convenient for spinal administration, given its cheaper cost and smaller volume, the maximum dose of this formulation would be less than 0.2 mg adenosine to meet the endotoxin unit specifications. If
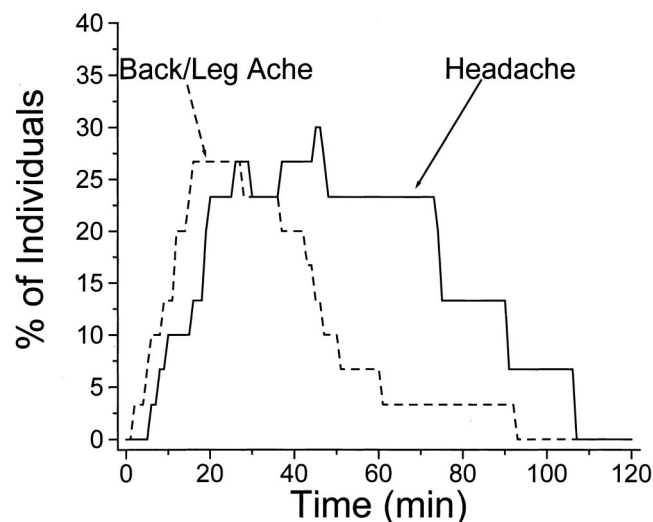
greater doses are to be used, regulations indicate that Adenoscan is the appropriate formulation to use.

Although the first human use of a new agent or known agent by a new route of administration should be under an open-label, dose-escalating protocol, there are clear problems with this study design. Lack of double blinding and placebo control greatly weaken testing for efficacy, which is not the focus of these studies. However, clear descriptions of expected side effects to volunteers and the knowledge that all volunteers will receive active drug could increase the observed incidence of such side effects. The current study supports this concept because 80% of volunteers in the open-label trial who received 2.0 mg intrathecal adenosine experienced leg ache, compared with 3% in the placebo-controlled, blinded study.

Future studies of intrathecal adenosine clearly should include assessment of headache and backache or leg ache. Although the current studies were underpowered to distinguish the incidence of these side effects (27–33%) from placebo, the observation that they were never observed after placebo injection suggests they are likely to be drug related. The cause of these symptoms, assuming they are related to adenosine injection, is unknown. Difference in time of onset of symptoms (lower backache or leg ache preceding headache) is consistent with movement of adenosine in cerebrospinal fluid. One could speculate that local vasodilatation produced by adenosine, spreading in cerebrospinal fluid to cerebral sites within 20–30 min after injection, could result in headache of later onset than could a local effect in the lumbar region, as previously suggested.[7] None of the volunteers in the current study had a history of chronic headaches, including migraine, but it is conceivable that such individuals would be at higher risk for having a headache, or having a more painful headache, than those without such disorders.

It is conceivable that backache or leg ache could reflect toxicity of adenosine. We believe this is unlikely, given the preclinical safety profile of this agent and the mild, transient nature of these symptoms. We are aware that local anesthetics can cause such backache or leg ache or pain, especially intrathecal lidocaine[10] and epidural 2-chloroprocaine[11] in ambulatory patients. This is often severe pain in patients, as it is in volunteers,[12] in contrast to the mildness of the symptoms with adenosine. We originally hypothesized that the back pain previously observed after intrathecal injection of the Swedish formulation of adenosine[7] reflected the tonicity or other actions of mannitol of that solution, which contains isotonic mannitol. However, the current data suggest that adenosine in preservative-free saline may also produce this effect.

Finally, it should be recognized that the clinical experience with intrathecal adenosine is extremely small. The current studies exposed only a small number of healthy individuals to adenosine. The Swedish experience is well less than 300 subjects (Alf Sollevi, M.D., Professor of Anaesthesiology and Intensive Care, Karolinska Hospital, Stockholm, Sweden, written communication, July 2000), and although no unexpected or long-lasting side effects have occurred, prudence dictates that currently, this compound for intrathecal administration should be used only in carefully controlled investigations.

In summary, intrathecal injection of the American formulation of adenosine is well-tolerated in volunteers. In 55 healthy individuals studied under open-label and double-blind, controlled conditions, adenosine did not affect cardiovascular, respiratory, or neurologic function. The only subjective side effects that these early studies indicate may be associated with this compound are transient headache and lower backache or leg ache. These data support further hypothesis-based trials of the efficacy of this agent in the treatment of acute and chronic pain.

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