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Physiologic and Antinociceptive Effects of Intrathecal Resiniferatoxin in a Canine Bone Cancer Model

Dorothy Cimino Brown, D.V.M., Michael J. Iadarola, Ph.D.,† Sandra Z. Perkowski, V.M.D., Ph.D.,‡ Hardam Erin, D.V.M.,§ Frances Shofer, Ph.D.,‖ Karai J. Laszlo, M.D.,# Zoltan Olah, Ph.D.,** Andrew J. Mannes, M.D.††*

Background: **Resiniferatoxin is a potent capsaicin analog. Intrathecal administration leads to selective, prolonged opening of the transient receptor potential V1 ion channel, which is localized mainly to C-fiber primary afferent nociceptive sensory neurons. Following work in laboratory animals, the authors explored the use of intrathecal resiniferatoxin to control spontaneous bone cancer pain in companion (pet) dogs.**

Methods: **Normal canine population: Behavioral testing was performed to establish baseline paw withdrawal latency; subsequently, general anesthesia was induced and resiniferatoxin was administered intrathecally while hemodynamic parameters were recorded. Behavior testing was repeated for 12 days after administration of resiniferatoxin. Clinical canine population: Twenty companion dogs with bone cancer pain were recruited. The animal's baseline level of discomfort and analgesic use were recorded. Resiniferatoxin was administered intrathecally and hemodynamic parameters were monitored while the dogs were under general anesthesia. Dogs were reevaluated up to 14 weeks after resiniferatoxin administration.**

Results: **Normal canine population: In the first minutes after resiniferatoxin injection, there were significant ($P < 0.05$) increases in mean arterial blood pressure and heart rate from baseline. Two days after injection, limb withdrawal latencies increased to the point of cutoff in the dogs that received at least 1.2 μg/kg resiniferatoxin. Clinical canine population: From baseline, there were significant ($P < 0.05$) increases in mean arterial blood pressure and heart rate after resiniferatoxin injection. Comfort scores were significantly improved at 2, 6, 10, and 14 weeks after resiniferatoxin administration ($P < 0.0001$). There was decreased or discontinued use of supplemental analgesics in 67% of the dogs 2 weeks after resiniferatoxin administration.**

Conclusions: **Intrathecal resiniferatoxin elicits transient hemodynamic effects. In controls, a profound and sustained blockade of thermal stimuli is produced in a dose-dependent fashion. Similar administration in dogs with bone cancer produces a prolonged antinociceptive response.**

SEVENTY-FIVE to 90% of patients with advanced cancer experience significant, life-altering, cancer-induced pain.[1] Severe pain is especially associated with tumors involving bone destruction. Pain intensity and frequency tend to increase during the advanced stages of the disease, often greatly impacting the activities of daily living and overall quality of life. Opioids can be very effective in many patients and remain the mainstay of treatment for severe pain.[2] However, it has been difficult to dissociate their analgesic actions from their troublesome and often dose-limiting side effects. Furthermore, there are patients who experience severe pain despite optimal medical management, especially in the last days of life. These patients may undergo nonselective chemical or surgical neuroablative interventions or palliative sedation. At this stage, therapies must be compatible with maintaining quality of life at the end of life. Novel analgesics and innovative procedures with greater efficacy and fewer nonspecific side effects are clearly needed.

The difficulty with developing innovative therapeutic approaches for bone cancer pain management results, in part, from the fact that bone cancer pain seems to be a unique persistent pain state that changes with the evolution of the disease.[3] The development and characterization of animal models of chronic pain that are specific to bone cancer is an important approach to improving the probability of identifying novel treatments for the condition.[4,5] Conclusions drawn from studies performed in rodent bone cancer models may be difficult to translate to human application in light of the fact that these models involve induced tumors that generate very rapid disease progression in inbred populations and artificial environments. A large animal model of naturally occurring bone cancer that more closely mirrors the diversity and progression of clinical disease in humans and is observable in the animal's natural environment is likely to be a very useful model of human clinical bone cancer pain and to provide a more specific paradigm for assessing the efficacy of novel analgesics. Many spontaneous diseases that occur in veterinary patients are excellent and underutilized preclinical models of human disease and, in many cases, offer distinct advantages over studying induced disease in purpose-bred animals.[6–16]

Spontaneous bone cancer is common in companion (pet) dogs and bears striking resemblance to bone cancer in humans. In both species, osteosarcoma is histologically indistinguishable and has the same biologic behavior and disease progression.[6] Dogs with bone cancer (osteosarcoma and others) have an evolution of bone cancer pain that parallels that which occurs in people. The frequency and intensity of the pain tends to increase over weeks or months. This is manifested in the need to give analgesics and increase or change the dose to allow

* Associate Professor of Surgery, ‡ Assistant Professor of Anesthesiology, § Anatomic Pathologist, ‖ Epidemiologist, School of Veterinary Medicine, University of Pennsylvania. †† Anesthesiologist, Pain and Palliative Care Service, † Chief, Neuronal Gene Expression Unit, # Researcher, Neuronal Gene Expression Unit, Pain and Neurosensory Mechanism Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland. ** CSO and President, Acheuron Hungary LTD., Szeged, Hungary.

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Address reprint requests to Dr. Brown: Department of Clinical Studies–Philadelphia, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6010. Address electronic mail to: dottie@vet.upenn.edu.

continued weight bearing on the affected limb and improve the activity of the animal. As the disease progresses, weight bearing produces frequent episodes of breakthrough pain that are more difficult to control even with large doses of opioids. In the current article, we explore the use of intrathecal resiniferatoxin as an approach to control intractable spontaneous bone cancer pain in companion dogs.

Resiniferatoxin is a highly potent capsaicin analog obtained from the latex of the *Euphorbia resinifera* plant.[17,18] Transient receptor potential V1, the receptor for capsaicin and its analogs, is a mixed Na^+/Ca^{2+} ion channel. Vanilloid agonist (resiniferatoxin or capsaicin) binding opens the calcium ion channel, and excessive activation causes calcium cytotoxicity. When applied to the sensory neuron perikarya, the prolonged calcium influx induced by resiniferatoxin specifically deletes *only* the sensory neurons that express the transient receptor potential V1 ion channel. Thus, intrathecal resiniferatoxin administration leads to selective targeting and permanent deletion of the transient receptor potential V1-expressing C-fiber neuronal cell bodies in the dorsal root ganglia.[19,20] Loss of these C-fiber neurons interrupts the transmission of pain information from the body to second-order spinal cord neurons, which in turn convey the information to the brain. At the same time, noxious and nonnoxious mechanosensation, proprioception, and locomotor capability are retained.

The current report demonstrates the viability of this agonist approach to control intractable spontaneous bone cancer pain. First, preliminary work in laboratory animals confirming behavioral effects, safety, and an appropriate dose range in the canine population is presented. Then, work demonstrating the safety and efficacy as well as excellent therapeutic behavioral potential of resiniferatoxin in a cohort of companion dogs (n = 20) with intractable spontaneous bone cancer pain is presented. These results in companion dogs with the similar nature and progression of bone cancer pain seen in people suggest that resiniferatoxin holds promise as a new pain-selective neurolytic drug.

Materials and Methods

The protocols were reviewed and approved by the University of Pennsylvania Institutional Animal Care and Use Committee and the School of Veterinary Medicine Companion Animal Protocol Review Committee, Philadelphia, Pennsylvania.

Normal Canine Population

Demographics. Five clinically normal laboratory dogs were screened (physical examination, complete blood count, chemistry screen, urinalysis and fecal flotation) and quarantined for 2 weeks.

Catheter Placement. Animals were anesthetized, the trachea was intubated, and the back of the neck and head was shaved, aseptically prepared (chlorhexidine solution), and draped using sterile technique. The dura dorsal to the cisterna magna was exposed, and a small incision was made (1–2 mm). A catheter (20/24 Micro Catheter System; Sims Portex Inc., Keene, NH) was inserted into the intrathecal space and passed caudally a distance of approximately 40 cm (corresponding with L3–L4), and a second intrathecal catheter was inserted in a similar fashion caudally 4 cm (corresponding with C1–C2). The appropriate length of catheter to be tunneled intrathecally was determined by measuring the length of the catheter needed to reach from the atlantooccipital space to the dorsal spinal process of the appropriate vertebral spinal body. Intrathecal placement and function of the catheters was confirmed by withdrawal of clear cerebrospinal fluid. The catheters were capped and tunneled subcutaneously to exit at the upper left interscapular area, the incision was closed, and the animal recovered from general anesthesia.

Behavioral Testing. On postoperative day 2, paw withdrawal latency behavioral testing was performed. The dogs were acclimated to the apparatus before catheter placement. Briefly, the unrestrained animal was placed on a glass-top table, and a focused radiant halogen heat source was positioned under a paw. The intensity of the stimulation light during testing was 50% of maximum intensity for all dogs. When the dog lifted its limb, the time in seconds was recorded, and the heat source was terminated. A maximum exposure time of 20 s was allowed to prevent injury to the animal.

Resiniferatoxin Injection. After baseline behavioral testing, general anesthesia was induced with thiopental (2- to 4-mg/kg boluses given until the dog was just sedated enough for tracheal intubation) and maintained with isoflurane and oxygen. A dorsal pedal artery catheter was placed, allowing direct blood pressure monitoring and blood withdrawal. Instruments were placed to continuously monitor the electrocardiogram, direct blood pressure, and end-tidal inhalant anesthetic concentration. Each dog was maintained at a constant end-tidal concentration (1.0–1.2 minimum alveolar concentration) for the duration of the anesthetic episode. Intrathecal catheter placement was confirmed by the withdrawal of cerebrospinal fluid, and resiniferatoxin was administered (table 1) *via* the lumbar catheter. Hemodynamic parameters (heart rate, direct arterial blood pressure) were recorded, cerebrospinal fluid was withdrawn from the cervical intrathecal catheter, and blood was withdrawn from the catheter in the dorsal pedal artery at 0, 5, 10, 15, 30, 60, 120, and 240 min after resiniferatoxin injection. Cerebrospinal fluid and plasma were stored at −80°C until processing. Two hundred forty minutes after resiniferatoxin administration, all catheters were removed, and the dogs recovered from general anesthesia.

Table 1. Normal Dog Demographics and Resiniferatoxin Dose

Dog No.	Breed	Sex	Weight, kg	Age, yr	Resiniferatoxin Dose, μg/kg
1	Beagle	F	11.5	4	0.1
2	Mixed breed	M	21	3	0.1
3	Beagle	F	8.5	5	1.2
4	Hound mix	M	25	3	1.2
5	Shepherd mix	F	20	6	3.0

Postprocedure. Behavior testing was repeated 2, 5, 7, 10, and 12 days after resiniferatoxin administration. Two weeks after resiniferatoxin administration, urine was collected for analysis, and blood was drawn for complete blood count and chemistry screening. The dogs were then euthanized, and tissues were collected and prepared for histopathology.

Clinical Canine Population

Twenty companion dogs with intractable bone cancer pain, confirmed *via* history and physical and radiographic examinations, were enrolled in an open-label trial of intrathecal resiniferatoxin.

For these dogs, discomfort due to the underlying condition had become unresponsive to conventional pain management (nonsteroidal antiinflammatory agents, steroids, opioids) or the dogs were intolerant to conventional medications.

Baseline Data. A complete blood count (erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, leukocytes, neutrophils, lymphocytes, monocytes, eosinophils) and chemistry screening (glucose, blood urea nitrogen, creatinine, phosphorus, calcium, sodium, potassium, chloride, carbon dioxide, total protein, albumin, globulin, alanine aminotransferase, alkaline phosphatase, total bilirubin, cholesterol) were performed. Data regarding the animal's function, level of discomfort, and analgesic use were obtained from the dog's caregiver (in all cases the dog's owner). The caregiver was asked to measure his or her dog's discomfort level using a visual analog score. In this assessment, the caregiver documented the dog's discomfort level by placing a mark on a 10-cm line, where the left end of the line represents no pain and the right end represents extreme pain.

Resiniferatoxin Injection. The dogs were premedicated with hydromorphone (0.1 mg/kg intramuscular), general anesthesia was induced with 2- to 4-mg/kg boluses of thiopental until the trachea could be intubated, and anesthesia was maintained with isoflurane and oxygen. All dogs were monitored with continuous electrocardiography and indirect blood pressure monitoring every 5 min while anesthetized. The animal was shaved, and a sterile field was prepared by scrubbing with chlorhexidine solution. A 20-gauge, 1.5-in spinal needle (B-D Quincke Type Point Spinal Needle; Becton Dickinson and Company, Franklin Lakes, NJ) was placed percutaneously into the cisterna magna (for forelimb tumors) or the intrathecal space of L5–L6 (for hind limb tumors). Position was verified by the flow of clear cerebrospinal fluid, 1 ml cerebrospinal fluid was drained, and resiniferatoxin was injected slowly (1.2 μg/kg, 100 μg/ml solution), followed by a 0.01-ml/kg flush of sterile saline. The spinal needle was then withdrawn. Because resiniferatoxin stimulates transient receptor potential V1-sensitive neurons at the same time that it causes excessive transmembrane calcium influx, general anesthesia was maintained throughout this initial excitation phase (45–60 min after the injection), a time course similar to that seen *in vitro*,[21] and then the dog recovered from general anesthesia.

Recovery. Rectal body temperature was recorded before induction, at extubation, and during recovery until the dog could maintain a body temperature of at least 100°F for at least 30 min. The dogs were observed in the hospital overnight and discharged home on the following day.

Reevaluation. The dogs returned 2, 6, 10, and 14 weeks after resiniferatoxin administration for reevaluation of their health, function, level of discomfort, and analgesic use. The caregiver was again asked to measure his or her dog's discomfort level using a visual analog score.

Necropsy. After spontaneous death or euthanasia, the dogs underwent full necropsy to determine the extent of primary and metastatic disease.

Histopathology. Tissues retrieved during the necropsies were fixed in 10% buffered formalin for 24 h and processed in paraffin. Sections (5 μm thick) were stained with hematoxylin and eosin.

Statistical Analysis

Differences over time in heart rate, blood pressure, temperature, and visual analog score were evaluated by analysis of variance with repeated measures. $P < 0.05$ was considered significant. Values are reported as mean ± SEM. Analyses were performed using SAS statistical software (version 9.1; SAS Institute Inc., Cary, NC).

Results

Normal Population

Demographics are presented in table 1. All dogs recovered uneventfully from intrathecal catheter placement and were normal on physical examination including neurologic examination before baseline thermal sensitivity testing and resiniferatoxin treatment. Methods developed for measurement of cerebrospinal fluid of resiniferatoxin concentrations were reported in detail previously.[22]

Resiniferatoxin Administration. There was a significant increase in mean arterial blood pressure from 79.0

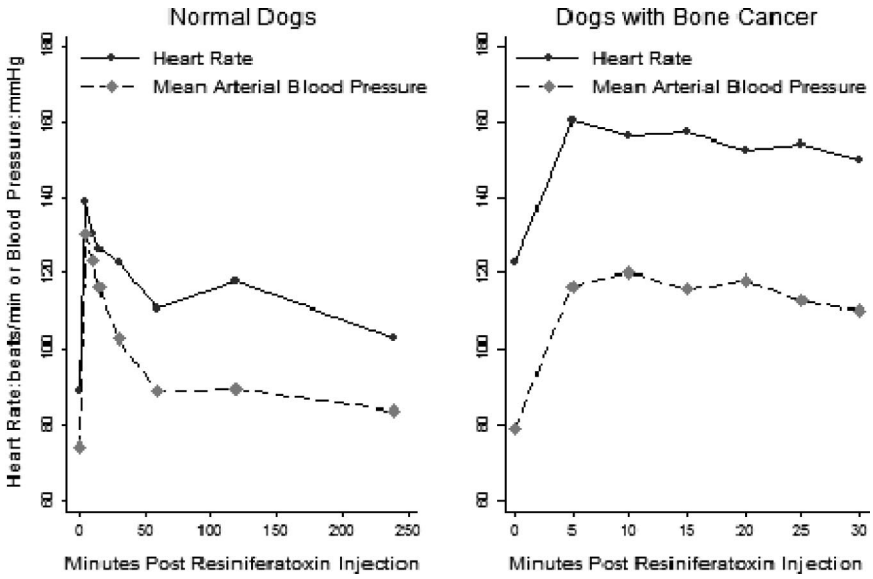

Fig. 1. Average mean arterial blood pressure and heart rate of 5 normal dogs and 20 dogs with bone cancer, during general anesthesia, after intrathecal administration of resiniferatoxin. Normal dogs were monitored for 240 min, and dogs with bone cancer were monitored for 30 min. Normal dogs received 0.1 μg/kg (n = 2), 1.2 μg/kg (n = 2), and 3.0 μg/kg (n = 1) resiniferatoxin. All dogs with bone cancer received 1.2 μg/kg of resiniferatoxin. Time 0 represents the minute before intrathecal injection.

to 130.8 ± 3.46 mmHg from baseline to 5 min after resiniferatoxin injection ($P < 0.0001$). This increase remained significantly different from baseline through 120 min after resiniferatoxin injection (fig. 1). There was a significant increase in heart rate from 88.8 to 138.5 ± 4.33 beats/min from baseline to 5 min after resiniferatoxin injection ($P < 0.0001$). This increase remained significantly different from baseline through 240 min after resiniferatoxin injection (fig. 1). The peak within 5 min and then steady decrease in heart rate and blood pressure through 60 min followed by a slow return toward baseline through 240 min coincided with the previously reported concentration of resiniferatoxin in the cerebrospinal fluid, which was approximately 100 ng/ml 5 min after intrathecal injection and constantly decreased to less than 1 ng/ml within 60 min, where it remained through 240 min after injection.[22] Dog 3 was recovered from anesthesia at 30 min after resiniferatoxin injection because of an inability to withdraw cerebrospinal fluid from the cisternal catheter for pharmacokinetic measurements. This dog appeared extremely dysphoric and demonstrated symptoms consistent with acute pain (vocalizing, paddling, thrashing) after awakening. The dog was sedated with diazepam and hydromorphone and subsequently had an uneventful recovery over the following 40 min. All other dogs remained successfully instrumented for the full 240 min after resiniferatoxin administration and recovered uneventfully.

Behavioral Testing. Two days after resiniferatoxin administration, all dogs were grossly normal on physical examination, including an extensive neurologic examination. Compared with pretreatment values, limb withdrawal latency on day 2 was substantially increased, to the point of cutoff, in the dogs that received at least 1.2 μg/kg resiniferatoxin. The increased latency behavior persisted on days 5, 7, 10, and 12 (fig. 2).

Clinical Laboratory Testing. Blood and urine collected before and 2 weeks after resiniferatoxin administration revealed no clinically significant increases or decreases of parameters out of the normal range.

Necropsy. At necropsy, the only significant gross findings related to the injections were a small amount of subdural hemorrhage extending approximately 3 cm from the third to fourth lumbar vertebral segments in dog 2 and a focal 0.6 × 0.5 × 0.1-cm area of white firm subdural material surrounding the dorsal second lumbar spinal cord segment in dog 4. On histology, one dog had moderate subacute to chronic focal myelomalacia at C2, and three dogs had mild to moderate focal subacute wallerian degeneration associated with cervical or thoracic spinal segments or both. These findings are consistent with intrathecal catheter placement.[23,24] Resiniferatoxin-related effects were evident in the dorsal root ganglia of the treated animals (fig. 3). One month after intrathecal injection of resiniferatoxin, degenerating neurons are in the process of being replaced by rosettes of proliferating satellite cells. Neurons with larger cell

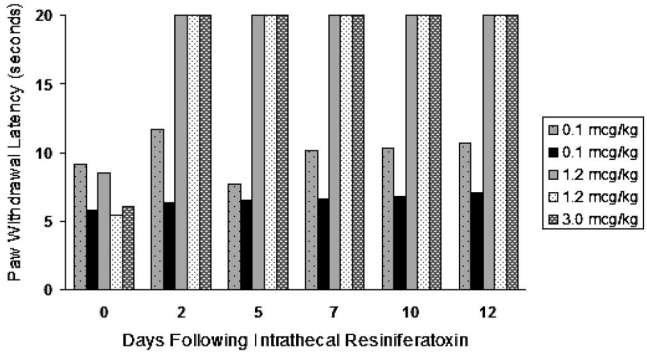

Fig. 2. Latency of forelimb withdrawal from heat of five normal dogs at baseline (day 0) and after intrathecal resiniferatoxin injection. Dogs received 0.1 μg/kg (n = 2), 1.2 μg/kg (n = 2), and 3.0 μg/kg (n = 1) resiniferatoxin. The heat source was discontinued at 20 s to prevent thermal injury to the foot pad.

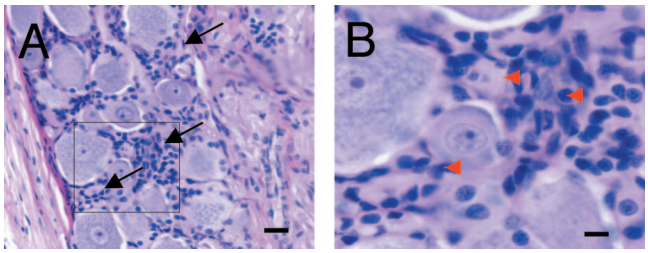

Fig. 3. Dorsal root ganglia 28 days after intrathecal resiniferatoxin administration. Damaged and dead neurons are replaced by proliferating satellite cell colonies called nodules of Nageotte (*black arrows*), which can be seen as collections of small cells with small basophilic nuclei forming rosette-like structures around disintegrating neuronal bodies (*red arrowheads*). *Bars*: 100 μm (*A*) and 25 μm (*B*).

body diameters remain unaffected, even in the immediate vicinity of a degenerating neuron. These histologic findings reflect the observed, profound analgesic effect that occurs in the clinical population and the retention of other sensory and proprioceptive functions.

Clinical Population

Demographics. These animals were predominantly older (mean age, 9.3 ± 0.5 yr), large-breed (mean weight, 41.5 ± 2.9 kg) dogs (10 females, 10 males). Diagnoses of the primary bone tumors included osteosarcoma (n = 16, 80%), histiocytic sarcoma (n = 2, 10%), hemangiosarcoma (n = 1, 5%), and liposarcoma (n = 1, 5%). Bones involved included the humerus (n = 9, 45%), scapula (n = 3, 15%), radius (n = 3, 15%), and, with 1 each (5%), femur, tibia, ulna, ischium, and rib. Dogs had clinical signs on average 9.3 ± 1.3 weeks before presentation for resiniferatoxin treatment.

Resiniferatoxin Injection. There was a significant increase in mean arterial blood pressure from 79.0 to 116.8 ± 3.46 mmHg from baseline to 5 min after resiniferatoxin injection ($P < 0.001$). This increase remained significantly different from baseline through 30 min after resiniferatoxin injection (fig. 1). There was a significant increase in heart rate from 122.8 to 160.3 ± 2.9 beats/min from baseline to 5 min after resiniferatoxin injection ($P < 0.001$) This increase also remained significantly different from baseline through 30 min after resiniferatoxin injection (fig. 1).

Recovery. Immediately after extubation, all dogs began panting heavily and continued to do so for several hours. Although this is a behavior consistent with thermal regulation, no dog was hyperthermic after resiniferatoxin administration. As the panting persisted, the dogs became significantly hypothermic for several hours, decreasing from 99.4 to 97.2 ± 0.82°F at the time of extubation to 240 min after extubation (normothermia is 100.5–102.5°F) (fig. 4). Nineteen of the 20 dogs recovered uneventfully and were discharged from the hospital the day after resiniferatoxin administration. One dog had preexisting laryngeal paralysis that the owner had declined to treat; this worsened secondary to anesthesia, intubation, and the panting seen on recovery, and the owner opted for euthanasia of the dog during the recovery period rather than surgical treatment of the laryngeal paralysis.

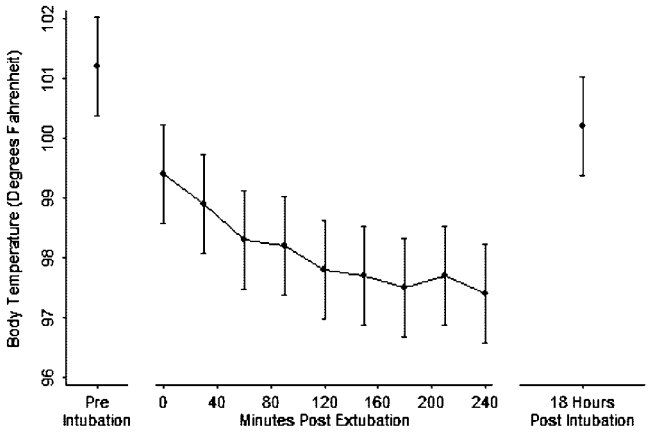

Fig. 4. Average (± SEM) body temperature of 19 dogs with bone cancer before and during recovery from intrathecal administration of 1.2 μg/kg resiniferatoxin, which was administered during general anesthesia. Time 0 represents tracheal extubation.

Follow-up. One other dog was euthanized before the 2-week reevaluation point. In this dog, there was rapidly progressing primary tumor growth the week after treatment, and the size of the tumor (associated with the humerus) prohibited the dog from getting up and walking unassisted, which prompted the owner to request euthanasia.

Pain Scores. For the 18 dogs that survived for at least 2 weeks after resiniferatoxin administration, caregivers, on average, reported significantly improved comfort levels using the visual analog score at 2, 6, 10, and 14 weeks after resiniferatoxin administration ($P < 0.0001$ for all time points) (fig. 5). The visual analog score from 3 of these dogs has been reported previously. Improved comfort level was also demonstrated by the decreased or discontinued use of analgesics in 12 of the 18 dogs (table 2). No dogs from this group seemed to have any long-

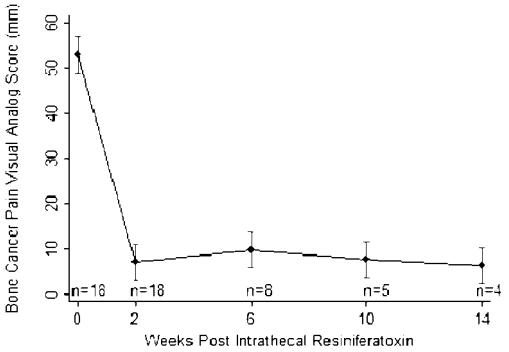

Fig. 5. Average (± SEM) visual analog score (millimeters) of dogs with bone cancer at baseline and after intrathecal administration of 1.2 μg/kg resiniferatoxin. Time 0 represents the day before general anesthesia and intrathecal injection. n represents the number of dogs still alive at each time point.

Table 2. Analgesic and Antiinflammatory Medication History of Dogs with Bone Cancer

Dog No.	Before Resiniferatoxin Administration	2 Weeks after Resiniferatoxin Administration
1	100 mg carprofen every 12 h 10 mg prednisone every 12 h	2.5 mg prednisone every 12 h
2	10 mg piroxicam every 24 h	10 mg piroxicam every 48 h
3	90 mg codeine every 8 h 10 mg piroxicam every 24 h	None
4	20 mg prednisone every 12 h	20 mg prednisone every 12 h
5	450 mg etodolac every 12 h	450 mg etodolac every 12 h
6	75 mg carprofen every 12 h 5 mg butorphanol every 12 h	None
7	20 mg piroxicam every 24 h	None
8	100 mg carprofen every 12 h	None
9	100 mg deracoxib every 24 h	100 mg deracoxib every 24 h
10	120 mg codeine every 8 h	None
11	100 mg deracoxib every 24 h	None
14	100 mg deracoxib every 24 h 30 mg codeine every 6 h	50 mg deracoxib every 24 h
15	100 mg carprofen every 12 h	75 mg carprofen every 12 h
16	75 mg carprofen every 12 h	75 mg carprofen every 12 h
17	25 mg deracoxib every 12 h 2.5 mg butorphanol every 8 h	2.5 mg butorphanol every 8 h
18	150 mg acetaminophen every 6 h 30 mg codeine every 6 h	None
19	150 mg deracoxib every 24 h	150 mg deracoxib every 24 h
20	15 mg piroxicam every 24 h	15 mg piroxicam every 24 h

Etodolac, carprofen, piroxicam, and deracoxib are nonsteroidal antiinflammatory drugs. Acetaminophen is a nonopiate, nonsalicylate analgesic and antipyretic. Butorphanol is an opiate partial agonist. Codeine is an opiate agonist.

term negative effects from resiniferatoxin administration, and all deaths were due to the progression of local or metastatic disease.

Discussion

The diverse etiology of severe pain seen at the end of life necessitates novel effective therapies. We describe the use of resiniferatoxin in canine bone cancer patients, a clinical population that closely parallels the human disease presentation. This treatment demonstrated the safety and pronounced and prolonged efficacy of selective C-fiber neurolysis.

Intrathecal resiniferatoxin elicited a profound and sustained blockade of thermal stimuli in the normal control animals at a dose of 1.2 μg/kg. Similar administration of the same dose in the companion animals with bone cancer produced an antinociceptive response that was prolonged, with effects often lasting until the death of the animal.

An unexpected result was the dogs' behavior during postoperative recovery from intrathecal resiniferatoxin administration. Immediately after extubation, all dogs panted excessively, appearing to be attempting to cool themselves as if they had an increased body temperature; however, no dog was hyperthermic immediately after resiniferatoxin administration. All dogs subsequently became significantly hypothermic for up to several hours after extubation. Temporally, it seemed that the panting that occurred immediately at extubation was the result of intrathecal resiniferatoxin administration and the hypothermia was secondary to heat loss from the panting. It was not until the panting ceased that the body temperature slowly began to increase back to the normal range. The hypothermia seen in these dogs persisted much longer than is normally seen in dogs undergoing general anesthesia for a cerebrospinal fluid tap, which typically resolves within an hour or two at the most. In addition to the panting, in an attempt to cool themselves, the dogs repeatedly crawled or rolled off of the warming blankets onto which they were routinely placed while recovering from general anesthesia. Hypothermia has been reported after subcutaneous resiniferatoxin administration in awake rats; however, the response was more acute (within 30 min of resiniferatoxin administration) in this species, and these animals exhibited heat-conserving behaviors in response to the hypothermia (huddling, piloerection, and operant responding for heat).[25] It is possible that the differences in the hypothermic response to resiniferatoxin administration are due to differences in mechanism between the species, in the route of administration (subcutaneous in the rat and intrathecal in the dog), the impact of general anesthesia in the dogs, or some combination of these. The hypothermic response in the rat is postulated to be due to activation, followed by damage to the warm thermoreceptors, or the vanilloid receptors on those neurons.[25] The mechanism of the hypothermic response in the dog has not been studied. It is difficult to hypothesize what effects may be elicited if resiniferatoxin is

given intrathecally to humans; however, it is possible that the patient may experience transient hypothermia, which, based on the dog data, would be accompanied by sweating (panting in dogs is the human equivalent of sweating). This is consistent with the diaphoresis seen with the oral ingestion of capsaicin (chili peppers) by humans.

There seems to be transient and intense activation of nociceptive primary afferent neurons and axons associated with the intrathecal injection of resiniferatoxin, and it is unlikely that it can be administered without an anesthetic. If the hemodynamic data parallel the pain response, the peak effects are seen at 5 min and resolve over 60 min. The hemodynamic response to intrathecal resiniferatoxin, although significant in both groups, was somewhat blunted in the dogs with bone cancer. This is likely due to the fact that the dogs with bone cancer were premedicated with an opioid before anesthetic induction and the control dogs were not. One control animal that recovered from anesthesia at 30 min had behaviors consistent with pain. Although the pain responded to analgesics, it is not clear whether sufficient analgesics can be given to suppress the response to the peak effects in an awake patient. However, the benefits of reduced pain and reduced need for subsequent medication, as seen in this trial, may outweigh the increased risks of an anesthetic in this population.

Another concern with this technique is the potential subsequent development of a deafferentation pain syndrome seen with other less selective neurolytic therapies. This has been described as severe pain referred to the area treated with or without the recovery of sensory function. This complication prevents the routine use of these therapies in patients with chronic, nonmalignant pain (*i.e.*, chronic pancreatitis).[26] However, behavior consistent with the presence of such a syndrome was not observed in any of the dogs in this study. Resiniferatoxin treatment leads to selective excision of pain sensing neurons. It is associated with proliferation of satellite cells and macrophages, and these cells are replacing the dead neurons, but without formation of significant scar tissue. The later can lead to the above-mentioned denervation-associated pain syndromes by inadvertent compression of surviving fibers. The presence of large-diameter neurons is consistent with the preserved proprioception in both the control and bone cancer patient groups. More experience with intrathecal resiniferatoxin in dogs and humans is needed to further evaluate the possibility of such a response.

There are several elements of the study design that should be highlighted. First, only five purpose-bred dogs were used in the behavioral testing phase of this study. One of the aims of this part of the study was to determine what dose of resiniferatoxin would have an effect on the VR1 receptor in the dog based on thermal sensitivity. Although five is a small number, it seemed to be sufficient to determine a rough threshold dose of resiniferatoxin that interferes with thermal sensation and thus has an impact on the complement of transient receptor potential V1 receptor–expressing neurons in the dog. A more precise threshold dose could potentially be determined if a larger number of dogs were used. In addition, these five dogs were not of the same breed, age, or size, which could lead to some variability in the results. However, this heterogeneity in the purpose-bred dog population made the results more generalizable to the clinical canine population to which the threshold dose was applied.

Second, this was an open-label, unblinded study in companion dogs experiencing severe pain that was no longer responsive to conventional therapy. As in preliminary human studies with unrelieved symptoms, it would be ethically questionable to subject a control group to a therapy that was known to be ineffective. With the efficacy of resiniferatoxin preliminarily established in dogs with end-stage disease in this study, a double-blind, randomized, controlled (against standard of care) trial of dogs newly diagnosed with bone cancer is planned.

An additional aspect to consider is that the dog's discomfort level was recorded using a caregiver's assessment. A trained observer is necessary to reliably measure pain in animals. For acute pain in companion animals, *e.g.*, postsurgical pain, a trained observer can disentangle pain from sedation, anxiety, and the effects of medication. This is typically a veterinarian or a veterinary nurse. For chronic pain, the veterinarian relies on the caregiver to be the trained observer. The caregiver, who has daily contact with the dog, can report the effects of chronic pain on the dog's activities of daily living (sleeping, eating, climbing stairs, jumping on the bed, and so forth). In human pain scoring, self-report is considered the accepted standard but obviously is not an option in a canine trial. The inability to verbalize pain is not a problem exclusive to this type of study; it is also a difficult or impossible task for cognitively impaired people. With the growing cognitively impaired population, healthcare providers increasingly depend on caregivers' reports to assess and manage cognitively impaired older adults living in community settings. These assessments are often based solely on caregivers' perceptions through direct observations. Visual or graphic scales, similar to that used in this study, are often used to assess pain quantitatively in these situations.[27] More complex, behavior-based, caregiver-completed scales exist for the cognitively impaired population. For example, the Hospice Approach Discomfort Scale describes nine behaviors, such as frightened facial expression or tense body language.[28] Based on these same principles, more complex, behavior-based assessment tools are being validated for caregiver appraisal of chronic pain conditions in companion dogs and will be used in the subsequent randomized, controlled trial of resiniferatoxin.

Finally, although the companion dogs in this report were treated for primary bone cancer pain, metastatic disease is the most common cause of cancer related pain in people. Although the clinical course of primary bone tumor pain in the dog seems to parallel the clinical course of metastatic disease to bone in people, these pain experiences exhibit only a partial overlap in responses to treatment. However, because the bony destruction seems responsible for the unique pain state associated with bone cancer, whether it is primary or metastatic, the two pain conditions are clearly related.

The companion canine population is a novel additional step in validating drug safety and efficacy with human translational therapy. Dogs are seen in the veterinary clinic with the natural progression of disease, having greater variability in presentation that may have characteristics not seen in carefully created models. Dogs with bone cancer have an evolution of bone cancer pain that parallels the human presentation, with the frequency and intensity of the pain increasing over weeks or months. This is manifested in the need to give analgesics and increase or change the dose to allow continued weight bearing on the affected limb and improve the activity of the animal. As the disease progresses, weight bearing produces frequent episodes of breakthrough pain that are more difficult to control even with large doses of opioids, often leading to euthanasia within several months of the diagnosis. This evolution of bone pain over weeks to months better approximates the human condition than induced rodent models and allows enough time to evaluate the effectiveness of novel antinociceptive agents through the evolution of the pain process. For this reason, outcome in dogs with spontaneous disease may better approximate actual outcome in human trials. Testing in these dogs may alleviate some of the inconsistencies found when translating drugs from induced models, generally performed in rodents, to humans, as well as provide data to support the use of new drugs in the canine population.[29]

Palliative care at the end of life is the time when therapies and interventions should be effective and nonburdensome. Treatments that allow discontinuing medications while preserving function will improve a patient's quality of life. As seen in this study, intrathecal administration of resiniferatoxin is consistent with this approach. It is hoped that results in this canine population will be predictive of future human experience. However, this will only become apparent if intrathecal resiniferatoxin progresses to human clinical trials.

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