Intrathecal Substance P-Saporin in the Dog

Efficacy in Bone Cancer Pain

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ABSTRACT

Background: Substance P-saporin (SP-SAP), a chemical conjugate of substance P and a recombinant version of the ribosome-inactivating protein, saporin, when administered intrathecally, acts as a targeted neurotoxin producing selective destruction of superficial neurokinin-1 receptor–bearing cells in the spinal dorsal horn. The goal of this study was to provide proof-of-concept data that a single intrathecal injection of SP-SAP could safely provide effective pain relief in spontaneous bone cancer pain in companion (pet) dogs.

Methods: In a single-blind, controlled study, 70 companion dogs with bone cancer pain were randomized to standard-of-care analgesic therapy alone (control, n = 35) or intrathecal SP-SAP (20–60 µg) in addition to standard-of-care analgesic therapy (n = 35). Activity, pain scores, and videography data were collected at baseline, 2 weeks postrandomization, and then monthly until death.

Results: Although the efficacy results at the 2-week postrandomization point were equivocal, the outcomes evaluated beyond 2 weeks revealed a positive effect of SP-SAP on chronic pain management. Significantly, more dogs in the control group (74%) required unblinding and adjustment in analgesic protocol or euthanasia within 6 weeks of randomization than dogs that were treated with SP-SAP (24%; P < 0.001); and overall, dogs in the control group required unblinding significantly sooner than dogs that had been treated with SP-SAP (P < 0.01).

Conclusion: Intrathecal administration of SP-SAP in dogs with bone cancer produces a time-dependent antinociceptive effect with no evidence of development of deafferentation pain syndrome which can be seen with neurolytic therapies.

What We Already Know about This Topic

• Destruction of the spinal cord cells with receptors to substance P by intrathecal injection of a substance P-saporin conjugate reduces experimental pain behavior in animals

• Companion dogs that spontaneously develop bone cancer have been proposed as a closely approximating preclinical model of human bone cancer pain

What This Article Tells Us That Is New

• In 70 companion dogs with bone cancer pain randomized to receive intrathecal substance P-saporin or standard-of-care therapy alone, owner-requested unblinding for inadequate pain control occurred significantly sooner in animals receiving standard-of-care therapy alone, suggesting an analgesic effect of substance P-saporin

• Although behaviors consistent with development of deafferentation pain syndromes did not occur in any animals, motor effects including ataxia were observed in some

PATIENTS with advanced cancer commonly experience life-altering pain.1 Severe pain is particularly associated with cancer-induced bone destruction. During advanced stages of disease, activities of daily living and overall quality of life can be greatly impacted. Opioids are the mainstay of treatment; however, their use is often complicated by dose-limiting side effects.2 In the last days of life, some patients undergo non-selective chemical or surgical neuroablative interventions and palliative sedation. These issues associated with managing pain in human cancer patients are precisely mirrored in canine patients, where bone cancer is commonly associated with severe pain that is refractory to conventional pain therapeutics.3–8 Novel analgesics and innovative procedures with greater efficacy and fewer side effects are clearly needed in both species.

Bone cancer pain is a unique persistent pain state that changes with the evolution of the disease.9 Using animal models that are specific to bone cancer is an important approach to identifying novel treatments for the
Although rodent models of bone cancer have been instrumental in elucidating the pathophysiology of bone cancer pain, the very rapid disease progression that occurs in these models makes translational interpretation of efficacy of novel compounds challenging. A large animal model of naturally occurring bone cancer, which closely mirrors the progression of clinical disease in humans and is observable in the animal’s natural environment, has proven to be a useful model of human clinical bone cancer pain.

The spontaneous development of bone cancer is common in companion dogs and bears striking resemblance to bone cancer in humans. In the current work, we explore the use of substance P-saporin (SP-SAP) as an approach to control spontaneous bone cancer pain in companion dogs. SP-SAP is a chemical conjugate of substance P and a recombinant version of the ribosome-inactivating protein, saporin. The conjugate recognizes substance P receptor-bearing neurons in vivo and in vitro and binds to its target on the cell surface. The conjugate is internalized and saporin breaks away from the targeting agent. It inactivates ribosomes, which leads to protein inhibition and ultimately cell death. Cells without the cell surface marker are not affected. Thus, when administered intrathecally, SP-SAP acts as a targeted neurotoxin producing selective destruction of superficial neurokinin-1 receptor (NK1r)-bearing cells in the spinal dorsal horn. In a companion article in this journal, long-term examination of lumbar NK1r-bearing cells after intrathecal SP-SAP administration in a canine model demonstrated a dose-dependent safety profile for this agent at doses which lead to significant reduction in NK1r-bearing dorsal horn neurons. The goal of this project was to provide efficacy data that intrathecal SP-SAP could provide effective pain relief and improve function in dogs with bone cancer. The hypothesis was that there would be an analgesic effect that appears over time after a single intrathecal bolus injection of SP-SAP.

Materials and Methods

The protocol was reviewed and approved by the University Institutional Animal Care and Use Committee (Philadelphia, Pennsylvania), and the consent form was reviewed and approved by the Privately Owned Animal Protocol Review Committee (Philadelphia, Pennsylvania). A schematic overview of the protocol is presented in figure 1.

Population

Seventy companion dogs with appendicular bone cancer, confirmed via history, physical, and radiographic examination, were enrolled (fig. 2). Animals with clinically significant abnormalities identified on screening complete blood count and serum biochemistry profile were not included. In addition, dogs with any clinically significant neurologic disease or history of unexplained coagulopathy were not included. All dogs were on a consistent standard-of-care analgesic regimen for at least 2 weeks before enrollment. This regimen was set by the referring veterinarian, who optimized analgesia for each dog individually by using a variety of standard-of-care interventions such as nonsteroidal anti-inflammatory drugs, tramadol, and gabapentin before referral to the study. This regimen was then maintained as the baseline analgesic regimen for each dog in the study. Thus, each dog was maintained on a stable, consistent baseline analgesic regimen, but baseline regimens could vary from dog to dog.

Outcome Measures

The study was designed with two primary and three secondary outcomes. The first primary outcome was: time to owner’s request for unblinding and additional intervention for their pet. Intervention was defined as additional analgesics or euthanasia. Unblinding also occurred at spontaneous death of a dog. The second primary outcome was: the number of dogs in each group that required unblinding by 6 weeks postrandomization. The three secondary outcomes included an assessment at 2 weeks postrandomization of (1) owner-completed pain scores on the Canine Brief Pain Inventory (BPI), (2) veterinarian-assessed lameness based on blinded video analysis, and (3) daytime activity based on the output of an accelerometer-based activity monitor.

Baseline Assessment

Data regarding the animal’s activity level using the activity monitor, level of discomfort using the Canine BPI, and degree of lameness documented via videography were collected just before randomization.

Randomization

Sequentially numbered opaque-sealed envelopes were used to allocate dogs to intrathecal SP-SAP injection or no injection. Thus, study personnel were unaware as to which group an animal would be assigned as it made its way through the screening process.

Blinding

To maintain owner blinding, all dogs, regardless of treatment group, were admitted to the hospital for randomization. All dogs had the fur clipped over the IV catheter and intrathecal injection sites. Only the dogs randomized to SP-SAP then underwent general anesthesia and intrathecal injection. All dogs were maintained in the hospital overnight to allow for full recovery from general anesthesia for the SP-SAP dogs, and all dogs were discharged from the hospital the following day to continue with the same oral analgesic regimen that they had been using before randomization. Thus, the owners were unaware as to which group their dog was assigned.

SP-SAP Injection

Dogs were premedicated with 0.3 mg/kg hydromorphone subcutaneously. Anesthesia was induced with thiopental...
Anesthesia was maintained with isoflurane in oxygen at 1.3 mean alveolar concentration. Once the dogs were at a steady state (approximately 20 min after induction), the intrathecal injection was performed. The intrathecal injection site was prepped (chlorhexidine solution) in a sterile manner. Dogs with forelimb tumors had percutaneous placement of a 20-gauge 1.5-inch spinal needle (B-D Quincke Type Point Spinal Needle; Becton Dickinson and Company, Franklin Lakes, NJ) into the intrathecal space (bevel facing caudad) at the level of the cisterna magna. Proper needle placement was verified by the flow of clear cerebrospinal fluid. SP-SAP was injected into the cisternal space through the spinal needle, followed by 0.1 ml/kg sterile saline, and then the needle was removed. Based on laboratory beagle data, the following SP-SAP dosing scheme was used: 10–15 kg dogs received 20 µg (200 µl) SP-SAP; 16–30 kg dogs received 40 µg (400 µl) SP-SAP; and dogs greater than 30 kg received 60 µg (600 µl) SP-SAP.\(^\text{17}\)

**Re-evaluation**

The dogs returned 2 weeks after randomization and then at monthly intervals thereafter until death. Activity, Canine BPI, and videography data were collected at each visit. If at other times.

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**Fig. 1.** Dogs were randomized to standard-of-care analgesics alone or standard-of-care analgesics in addition to intrathecal substance P-saporin injection. Owners were blinded to treatment group. Data were collected at baseline, 2 weeks after randomization, and monthly thereafter until the dog’s death. After the 2-week postrandomization assessment, unblinding occurred when the owner requested additional intervention for chronic pain management. At that point, dogs that had already received substance P-saporin had adjustments made to the standard-of-care therapy to improve pain control, and dogs that had not yet received substance P-saporin were offered substance P-saporin injection.
speculated and sunburst periosteal reactions associated with it.

A 9-yr-old, male, mixed-breed dog was evaluated for a right antebrachium in a 9-yr-old, male, mixed-breed dog diagnosed with osteosarcoma. There is an expansile moth-eaten lytic lesion in the distal radial metaphyseal region with change in lameness score, and (6) change in activity counts, postrandomization, (3) change in Canine BPI pain severity score, (5) change in Canine BPI pain interference score. To account for the nonnormality of the data, the Mann–Whitney test was used to compare the percent change in pain severity and pain interference scores from baseline to 2 weeks postrandomization between control and SP-SAP dogs.

**Change in Daytime Activity Counts 2 Weeks after Randomization.** Activity counts occurring between 1 AM and 5 AM, when most dogs would be asleep, were excluded from the analysis. Total daytime activity counts for the 7 days before randomization (baseline period) were compared with total daytime activity counts of days 7 through 14 after randomization (endpoint period). Because total activity count data are not normally distributed, nonparametric methods of data analysis were used. For each group, the Wilcoxon signed-rank test was used to compare total activity counts for the baseline and endpoint periods. The Mann–Whitney test was used to compare the percent change in total activity counts between groups.

**Number of Dogs with Improved Lameness 2 Weeks after Randomization.** A board-certified orthopedist, blinded to treatment group and visit, reviewed the videos and rated lameness using an 11-point numerical rating scale with 0 = "sound" and 10 = "could not be more lame." Dogs were coded as responders if there was a decrease in lameness score of 1 or more from the randomization visit to the 2-week visit. Fisher exact test was used to determine whether there was a significant difference in the number of responders between the SP-SAP and control groups.

Because six comparisons were made between treatment and control groups, such as (1) time from randomization to unblinding, (2) number of dogs unblinded at 6 weeks postrandomization, (3) change in Canine BPI pain severity score, (4) change in Canine BPI pain interference score, (5) change in lameness score, and (6) change in activity counts, a bonferroni correction was applied to the uncorrected overall critical P value of 0.05. The corrected overall critical P value was 0.008. All analyses were performed using STATA statistical software (Version 11; StataCorp LC, College Station, TX).

**Results**

Demographic and baseline characteristics of the 70 dogs that were randomized to control or SP-SAP groups are presented in table 1. All dogs treated with SP-SAP recovered uneventfully from general anesthesia and intrathecal injection and were discharged from the hospital within 24–36 h after the injection. In an interim evaluation of the data, it was discovered that there was a disproportionate incidence of hind limb weakness and ataxia occurring 5–7 weeks after injection in the dogs receiving cisternal injections of SP-SAP (i.e., dogs

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**Fig. 2.** Lateral (A) and anterior-posterior (B) views of the right antebrachium in a 9-yr-old, male, mixed-breed dog diagnosed with osteosarcoma. There is an expansile moth-eaten lytic lesion in the distal radial metaphyseal region with change in lameness score, and (6) change in activity counts, postrandomization, (3) change in Canine BPI pain severity score, (5) change in Canine BPI pain interference score. To account for the nonnormality of the data, the Mann–Whitney test was used to compare the percent change in pain severity and pain interference scores from baseline to 2 weeks postrandomization between control and SP-SAP dogs.

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**Statistical Analysis**

**Primary Outcome**

**Time from Randomization to Unblinding.** Time to unblinding was determined by the use of the Kaplan–Meier product-limit method, and log-rank analysis was used to compare the failure curves between the two treatment groups (control vs. SP-SAP).

**Number of Dogs Requiring an Unblinding by 6 Weeks Postrandomization.** The Fisher exact test was used to compare the number of control versus SP-SAP dogs for which unblinding occurred within 6 weeks of randomization.

**Secondary Outcome**

**Change in Canine BPI Scores 2 Weeks after Randomization.** The Canine BPI‡ is a publicly available owner-completed questionnaire designed to quantify the severity and impact of chronic pain in companion dogs. The Canine BPI contains four questions pertaining to the severity of pain (the responses for these questions can be averaged to provide a pain severity score) and six questions pertaining to how the pain interferes with the dog’s typical activities (the responses to these question can be averaged to provide a pain interference score). To account for the nonnormality of the data, the Mann–Whitney test was used to compare the percent change in pain severity and pain interference scores from baseline to 2 weeks postrandomization between control and SP-SAP dogs.

Efficacy of Substance P-Saporin in Canine Bone Cancer

At this point, SP-SAP dosing for dogs with front limb tumors was decreased by 50%, and no further occurrences were documented. Therefore, in the SP-SAP group, all dogs with hind limb tumors (n = 11) received the original protocol dose of SP-SAP; 19 dogs with front limb tumors received the original protocol dose of SP-SAP; and 5 dogs with front limb tumors received 50% of the protocol dose of SP-SAP for their bodyweight (i.e., 20 µg for dogs weighing 16–30 kg and 30 µg for dogs weighing >30 kg).

An intention-to-treat analysis was maintained, and all dogs randomized to the SP-SAP group were analyzed in that group regardless of the dose reduction in five dogs. Because these dogs were at home with their owners and were evaluated for the study purposes at monthly intervals, it is not possible to detail the nuances of onset and progression of the motor dysfunction. However, hind limb neurological deficits were documented a median of 46 days (range, 37–53 days) after SP-SAP injection. Six of the seven dogs were euthanized within 8 days of this documentation and had neurologic deficits at the time of euthanasia. One dog had resolution of ataxia 18 days after documented recognition of the signs, lived for 6 months after SP-SAP injection, and died without evidence of neurologic deficits. One dog, in the SP-SAP group, died acutely 2 days after intrathecal injection. The dog was riding in the car with the owner at the time of death and acutely collapsed. Necropsy did not identify a cause of death. All other dogs lived to the primary endpoint and were followed until death.

Time from Randomization to Unblinding

Kaplan–Meier product-limit method and log-rank analysis revealed that dogs receiving standard-of-care therapy alone were unblinded significantly (uncorrected \( P = 0.002 \)) sooner than dogs that received SP-SAP in addition to standard-of-care therapy (fig. 3).

Table 2. Interim Evaluation of 42 Dogs Receiving the Protocol Dose of Substance P-Saporin and the Occurrence of Hind Limb Ataxia and Weakness 5–7 Weeks after Injection

<table>
<thead>
<tr>
<th>Substance P-Saporin Dose</th>
<th>Cisternal Injection (Front Limb Tumor)</th>
<th>Lumbar Injection (Hind Limb Tumor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 µg (for dogs 16–30 kg)</td>
<td>1 occurrence/4 injections (25%)</td>
<td>0 occurrences/1 injection (0%)</td>
</tr>
<tr>
<td>60 µg (for dogs &gt;30 kg)</td>
<td>6 occurrences/26 injections (23%)</td>
<td>0 occurrences/11 injections (0%)</td>
</tr>
</tbody>
</table>

* One or two of nine other purebreds. † Histopathology was not available on five dogs.

NSAIDs = nonsteroidal antinflammatory drugs.
Number of Dogs Requiring Unblinding by 6 Weeks Postrandomization

Twenty-six dogs (74%) in the control group required unblinding within 6 weeks of randomization and eight SP-SAP dogs (24%) required unblinding. This was a statistically significant difference between groups (uncorrected \( P = 0.001 \)).

Change in Canine BPI Pain Scores

Dogs in the control group had a median 6% increase (i.e., deterioration) in pain severity score from baseline to 2 weeks postrandomization, whereas SP-SAP dogs had a median 0% change in pain severity score from baseline to 2 weeks postrandomization. The difference between the two groups was not statistically significant on Mann–Whitney test (uncorrected \( P = 0.93 \)). Dogs in the control group had a median 8% increase (deterioration) in pain interference score from baseline to 2 weeks postrandomization, whereas SP-SAP dogs had a median 5% decrease (improvement) in pain interference score from baseline to 2 weeks postrandomization. The difference between the two groups was not statistically significant on Mann–Whitney test (uncorrected \( P = 0.43 \)).

Change in Activity

For dogs in the control group, there was a decrease in total activity counts between the baseline (median, 617,124) and endpoint (median, 493,150) periods. For dogs in the SP-SAP group, there was an increase in total activity counts between the baseline (median, 588,083) and endpoint (median, 602,714) periods. These changes, however, were not statistically significant (uncorrected \( P = 0.02 \)).

Number of Dogs with Improved Lameness

Thirty sets of randomization and 2-week postrandomization videos were available for review from dogs in the control group and 34 sets of randomization and 2-week postrandomization videos were available for review from dogs in the SP-SAP group. There were five dogs in the control group and one dog in the SP-SAP group that could not be videotaped because of the request for euthanasia, pathologic fracture, or disease progression over the 2 weeks after randomization. One dog (3%) in the control group was a responder for improved lameness, whereas six dogs (18%) in the SP-SAP group were responders for improved lameness at 2 weeks postrandomization. This difference between groups was not statistically significant (uncorrected \( P = 0.06 \)).

Discussion

The diversity of etiology of severe pain seen at the end of life necessitates novel effective therapies. We presented the use of SP-SAP in a canine model with a complex pain state that parallels the human disease presentation.7,8 This approach demonstrated the time-dependent efficacy and some of the challenges associated with the selective destruction of superficial NK1r-bearing cells in the spinal dorsal horn in complex pain states.

The companion canine population is a novel additional step in validating drug safety and efficacy in human translational therapy. The degree of medical surveillance of dogs is second only to that of humans. Dogs' health is observed in detail on a day-to-day basis, and ailments are attended by veterinary specialists with the use of all of the diagnostic approaches of modern medicine.21 Dogs develop cancer about twice as frequently as humans, and the presentation, histology, and biology of many canine cancers closely parallel those of humans.21,22 Dogs share the environment with people and thus the potential environmental risk factors for disease, and have comparable responses to cytotoxic agents. Large body size simplifies biologic sampling, and shorter overall lifespan allows for spontaneous development and time course of disease within a time-frame reasonable for efficient data collection. In the case of dogs with bone cancer, they 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, which allows enough time to evaluate effectiveness of novel antinociceptive agents through evolution of the pain process. Therefore, testing in these dogs may alleviate some of the inconsistencies found when translating drugs from induced models to humans, as well as provide data to support the use of new drugs in the canine population.23 The results of the study described here are useful in informing subsequent clinical trial designs by elucidating potential issues around such things as choice of efficacy measures, timing of primary outcome, and informing potential adverse event profiles. An additional advantage is that the canine model has a long history of use in defining the safety and kinetics of spinally delivered drugs. Accordingly, the ability to define safety, kinetics, and efficacy in the same nonhuman species provides an important model for the development of novel intrathecal agents.24
Six efficacy outcomes were evaluated in this study, two primary and four secondary. The secondary outcomes which were evaluated at 2 weeks postrandomization were not statistically significant, whereas the primary outcomes which were evaluated at 6 weeks and beyond were statistically significant supporting a time-dependent positive effect of SP-SAP on chronic pain control. Two weeks after randomization, changes in pain scores, activity counts, and lameness were in the direction one would expect if SP-SAP is effective in managing chronic pain; the magnitude of the change, however, was not statistically significant. On average, pain scores deteriorated, activity levels significantly decreased, and lameness worsened in the 2 weeks after randomization in the control group; whereas these parameters either improved or remained unchanged in the SP-SAP group. The expected response, but lesser magnitude and thus lack of statistical significance between groups, could be attributable to several factors.

It is possible that 2 weeks postinjection is too soon for ribosomal inactivation to progress to cell death. In rodent studies, SP-SAP produces a loss of cell function within 4 days and cell death within approximately 7 days. In normal beagles, 15–150 μg intrathecal SP-SAP produces a pronounced decrease in the number of NK1r-bearing cells in the dorsal horn 1 month after administration. In that study, only two dogs (injected with 45 μg SP-SAP) were sacrificed before 28 days. In these dogs, evaluated at 7 days postinjection, there was no change in the number of NK1r-bearing cells compared with controls. These results were confirmed in the companion article published in this journal issue, where significant reductions in dorsal horn NK1r-bearing cells were noted after high-dose lumbar intrathecal SP-SAP at 3 months, but not 7 days. The 2-week evaluation point for the secondary outcomes in current study was chosen based on these data suggesting cell death occurs between 7 and 28 days after injection, and the fact that a 2-week time point would maximize the likelihood that dogs randomized to the control group would remain stable enough to not warrant additional intervention before that endpoint. It was not possible to evaluate these outcomes beyond 2 weeks, because it was the only postrandomization time point at which all owners were still blinded to their dog’s treatment group, and no adjustments in baseline pain management regimens had been made. In further canine studies, it is advisable to evaluate these secondary outcome measures at a 3- to 4-week postinjection time point and increase sample size to accommodate the potential for some dogs in the control group necessitating unblinding before that time point.

It is also possible, however, that there was a masking of some positive effects at 2 weeks due to the unexpected development of motor dysfunction in seven dogs in the SP-SAP group and the 50% dose reduction that was implemented for the subsequent five dogs with forelimb tumors that were treated with SP-SAP. The cause of motor dysfunction is unclear. In the laboratory beagle safety study, drug was delivered via implanted catheter in the unanesthetized animal. In those studies, there was transient, dose-dependent motor dysfunction documented in some dogs 3–4 h after injection and lasted 1–3 days. At the highest dose, half of the animals developed a persistent hind limb motor deficit requiring euthanasia. In the current study, dogs with bone cancer did not exhibit signs of motor dysfunction during the 24-h postinjection hospitalization period, but rather developed motor dysfunction over 5–7 weeks after injection. It has been noted that, unlike the rat, the dog spinal cord can display NK1r-bearing motor neurons in the motor horn, and a small reduction in deeper motor neurons has been reported at high doses in segments proximal to drug delivery. Although it is possible that NK1r-bearing motor neurons were deleted resulting in the motor signs documented in this study, it seems unlikely because only dogs with forelimb tumors, and therefore cisternal injections, developed motor signs. It is possible that the motor dysfunction was due to an effect on NK1r-bearing cells in the brain. There is abundant distribution of NK1 in the central nervous system which is paralleled by a wide variety of behavioral (e.g., locomotion, grooming, wet-dog shakes, hind paw tapping) and autonomic (e.g., cardiovascular, respiratory) functions that are linked to activation or blockade of these receptors in a variety of mammalian models. No studies reported, to date, directly explain the signs and time course of the motor dysfunction noted in this study, and no cardiovascular or respiratory effects were documented; however, this is clearly an area that needs more investigation.

One adverse event that can be seen with neurolytic therapies, deafferentation pain syndromes, did not manifest in any of the dogs in this study. Complete or partial interruption of afferent nerve impulses can lead to central sensitization with patients experiencing abnormal sensory phenomena such as allodynia, hyperalgesia, dysesthesias, and hyperpathia. In animals, deafferentation often leads to self-mutilation, biting the region in which they might feel painful, or paresthetic sensations. Self-mutilation did not occur in any of the dogs in this study, suggesting a lack of development of deafferentation pain syndromes. The absence of this effect is not completely unexpected as there are several studies suggesting ablation of central sensitization by SP-SAP.

In summary, the current results demonstrate an analgesic effect that appears over time after a bolus injection of intrathecal SP-SAP. Significantly, more dogs in the control group required unblinding (adjustment in analgesic protocol or euthanasia) within 6 weeks of randomization than dogs in the SP-SAP group; and overall, dogs in the control group required unblinding sooner than dogs treated with SP-SAP. The positive analgesic effects of SP-SAP documented in these dogs with a complex pain state that closely parallels the human condition provide proof-of-concept data that encourages further investigation into the use of intrathecal SP-SAP for chronic pain control. Although this modality has potential application to a variety of chronic pain states, it is of particular interest for palliative care at the end of life, when ideally interventions are minimized
while preserving quality of life. It is hoped that results in this canine population will inform the design of future canine studies and clinical trials as well as be predictive of future human experience.

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References
31. Choi J, Koehrn FJ, Sorkin LS: Carrageenan induced phosphorylation of Akt is dependent on neurokinin-1 expressing neurons in the superficial dorsal horn. Mol Pain 2012; 8:4