Kinetic gait and subjective analysis of the effects of a tachykinin receptor antagonist in dogs with sodium urate–induced synovitis

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Objective—To examine the ability of preemptive administration of a proprietary neurokinin-1 (NK₁) receptor antagonist to attenuate limb dysfunction associated with monosodium urate–induced synovitis in the stifle joints of dogs.

Animals—16 clinically normal adult mixed-breed dogs (8 males and 8 females).

Procedures—A crossover study was conducted in 2 phases. Dogs were assigned to 2 groups (8 dogs/group) and orally administered an NK₁ receptor antagonist (3 mg/kg) or a control substance once daily for 4 days. Synovitis was then induced in the left stifle joint by intra-articular injection of monosodium urate. Investigators were not aware of treatment group assignments. Dogs were evaluated by use of subjective lameness scores during standing, walking, and trotting and by use of ground reaction force data 3, 6, 9, 12, and 24 hours after urate injection. After a 21-day washout period, the experiment was repeated with each dog administered the other treatment and injected with monosodium urate in the contralateral stifle joint.

Results—No significant differences were detected between the NK₁ receptor antagonist and control treatments with regard to peak vertical force, vertical impulse area, or subjective evaluations of lameness during standing, walking, or trotting, except during walking 24 hours after monosodium urate injection.


Osteoarthritis is the most common cause of chronic pain and inflammation in domestic dogs. Twenty percent of all dogs are believed to be affected, which makes osteoarthritis a common clinical problem.¹ The NSAIDs are currently the basis of treatment and, as such, the most commonly prescribed class of drugs in veterinary medicine. It is estimated that >10 million dogs receive NSAIDs each year, and almost 19,500 adverse effects of NSAIDs in dogs have been reported to the FDA Center for Veterinary Medicine since its inception in 1997.² Adverse effects of the NSAIDs can affect the gastrointestinal tract and reproductive, renal, and hematopoetic systems and are contraindicated in animals that are hypovolemic or pregnant or that have gastrointestinal, renal, or hepatic disease.³ Additionally, there are many situations in which NSAIDs alone do not provide adequate relief for severe pain, and adjunctive treatment is warranted.

Currently, other drug classes, including opioid receptor agonists and N-methyl-D-aspartate receptor antagonists, are used in lieu of or in combination with NSAIDs for the treatment of animals with osteoarthritis.⁴ Results are variable and, even in combination with NSAIDs, quite often do not provide adequate relief from pain. These drugs also carry a risk of adverse neurologic effects or adverse effects for the gastrointestinal tract or cardiovascular system.⁵ Thus, despite the numerous NSAIDs approved for use in veterinary medicine, and even though there are other therapeutic options available to veterinarians for control of pain in animals, a need still exists for additional safe and efficacious treatment strategies for pain attributable to osteoarthritis.

Inhibition of NK₁ receptors may provide another option. The NK₁ receptors are found in the CNS as well as the peripheral nervous system.⁶ Peripherally, binding of NK₁ receptors by substance P, a neuropeptide synthesized by sensory nerves, results in an increase in local vascular permeability, vasodilation, and chemotraction of leukocytes.⁷ These same inflammatory changes are evident during development of osteoarthritis.³ Locally, NK₁ receptors play a role in the induction of pain attributable to inflammation, whereas NK₁ receptors in the dorsal horn of the spinal cord contribute to maintenance of pain attributable to arthritis.⁸ Inhibition of NK₁ receptors before induction of pain attributable to arthritis in rats (acute arthritis induced by injection of...
carrageenan) results in anti-inflammatory and analgesic effects. To our knowledge, NK receptor antagonist compounds have not been evaluated for efficacy in modulating pain in dogs.

The objective of the study reported here was to examine the analgesic effects of a proprietary NK receptor antagonist in dogs with monosodium urate-induced synovitis in a stifle joint. Our hypothesis was that administration of the NK receptor antagonist would result in a significant improvement in vertical ground reaction forces and subjective lameness scores in the affected limb, compared with results for a control substance.

Materials and Methods

Animals—Sixteen purpose-bred mixed-breed dogs (8 males and 8 females) were obtained from an animal supplier for use in the study. Dogs weighed 18 to 32 kg. Dogs were screened for underlying systemic or orthopedic disease by use of Dinofilaria immitis antigen tests, fecal examinations, CBCs, serum biochemical analyses, urinalyses, and evaluations of radiographic views of the hip and stifle joints. Exclusion criteria included pregnancy; fractious nature; systemic or active disease of any organ system; intra-articular injections within 90 days of the onset of the study; previous joint surgery; arthrocentesis within 30 days of the onset of the study; treatment with any topically or systemically administered pharmaceutical or biologic within 14 days of the onset of the study; or administration of glucosamine, chondroitin sulfate, or injectable corticosteroids within 30 days of the onset of the study.

All dogs were housed in a climate-controlled animal housing facility at the University of Georgia. Dogs were administered routine vaccinations and anthelmintics >14 days before initiation of the study. Dogs had ad libitum access to a maintenance diet and water. The study was approved by the University of Georgia Animal Care and Use Committee (animal use protocol No. A2006-10038-M1).

Study design—A single crossover study was conducted in 2 phases. Dogs were assigned to 2 groups (8 dogs/group; 4 males and 4 females/group). Baseline physical and subjective lameness examinations were performed and ground reaction forces were obtained twice on 2 separate days between days 6 and 4 before an injection of monosodium urate into a stifle joint. Physical examinations, CBCs, and serum biochemical analysis were also performed once during the period 6 to 4 days before urate injection and again after the final collection of ground reaction force data and subjective lameness evaluation at the end of the study.

Dogs of one of the groups were administered a proprietary NK receptor antagonist (3 mg/kg, PO, q 24 h for 4 days), whereas dogs of the other group were administered a control substance (sodium bicarbonate) in a gelatin capsule orally every 24 hours for 4 days. Dogs were observed for 10 minutes after treatment, then twice daily thereafter for signs of adverse events, including an anaphylactic reaction, ataxia, excess ocular discharge, excessive salivation, coughing, diarrhea, recumbency, ataxia, convulsions, reluctance to move, rapid or labored breathing, muscle tremors, or any other abnormalities.

The last dose of the NK receptor antagonist or control substance was administered on the morning of day 0. Two hours later, all dogs were anesthetized by administration of propofol (4 to 8 mg/kg, IV). Arthrocentesis through the patellar tendon was used to collect synovial fluid from the left stifle joint. The needle was left in place, and 1 mL of a solution of monosodium urate (10 mg/mL) was injected intra-articularly. One investigator (JPP) performed subjective clinical lameness evaluations during standing, walking, and trotting (maximum range of scale, 0 to 11) in dogs at 3, 6, 9, 12, and 24 hours after urate injection (Appendix). This investigator also obtained ground reaction force measurements at those same time points. The investigator was not aware of the treatment administered to each dog.

Dogs with cumulative subjective lameness scores of 11, that vocalized as a result of pain after recovery from anesthesia, or that had dramatic changes in behavior consistent with pain after injection of the urate solution were deemed to be in excessive pain and were immediately withdrawn from the study; these dogs were administered an NSAID and opioid, as necessary, to control signs of pain.

After a 21-day washout period, dogs that received the NK receptor antagonist during the first phase received the control substance during the second phase and vice versa. The second phase of the study was conducted identically to the first, except that synovitis was induced and evaluated in the contralateral stifle.

Evaluation of ground reaction forces—Ground reaction force data were collected by use of 2 force plates in series and a dedicated computer and software, as described elsewhere. All trials were performed at a trotting speed of 1.70 to 2.00 m/s and an acceleration of ±0.50 m/s² by 1 of 2 experienced handlers. Each dog was trotted by the same handler within each phase of the study. Trials were accepted only when the ipsilateral limbs made contact with the same plate without the dog pulling on the lead or having extraneous movement of the head. The 1st acceptable trial for each evaluation of each dog was included for analysis. Clinical evaluations and acceptance of ground reaction force data were performed by an investigator (JPP) who was not aware of the treatment administered to each dog.

Statistical analysis—Repeated-measures ANOVAs were used to evaluate the effects of treatment (NK receptor antagonist or control substance) on peak vertical force, vertical impulse area, and subjective lameness evaluations. The outcome variable used in the statistical analysis was the change from baseline value at each time point (3, 6, 9, 12, and 24 hours after urate injection). Values of P < 0.05 were considered significant.

Results

No adverse effects or changes in laboratory values were evident in any dogs during the study. A consistent, severe synovitis, as indicated by an increase in all measures of lameness and by the development of a severe effusion of the injected stifle joint, was induced in all but 1 dog in both phases of the study (Table 1). One
dog administered the control substance during the sec-
ond phase failed to develop a consistent lameness after
urate injection. This single failure was most likely at-
tributable to extra-articular injection of urate. Data for
this dog during the second phase were excluded.

Synovial fluid was aspirated before every urate
injection, and except for the aforementioned dog that
potentially received an extra-articular injection, there
were no complications for any of the intra-articular
injections. None of the dogs had severe signs of pain
to warrant rescue analgesia.

No significant differences were detected between
the NK_1 receptor antagonist and control treatments for
4 outcome measures (peak vertical force [T] = 0.81 at 12
hours and 0.88 at 24 hours], vertical impulse area [T] = 0.91 at 12 hours and 0.85 at 24 hours], or subjective
lameness evaluation during standing or trotting) at any
time point. A significant difference was found between
the NK_1 receptor antagonist and control treatments for
subjective lameness during walking only at 24 hours
after urate injection.

Discussion

Analysis of data obtained during the study reported
here failed to provide subjective or objective evidence
that preemptive administration of an NK_1 receptor an-
tagont (3 mg/kg, PO, q 24 h for 4 days) attenuated limb
dysfunction caused by the acute inflammatory response
to intra-articular injection of monosodium urate, com-
pared with preemptive administration of a control sub-
stance. To the authors’ knowledge, this is the first time
this method of induced inflammation has been used to
evaluate an NK_1 receptor antagonist in dogs. Urate-in-
duced synovitis causes an acute inflammatory reaction
that stimulates pain and inflammation, which leads to
dramatic increases in synovial concentrations of prosta-
glandin E_2 and infiltration of leukocytes.10

Urate-induced synovitis has been used in numerous
studies11-15 to evaluate the analgesic effects of vari-
ous NSAIDs and ketamine.11-15 In 1 study,11 investiga-
tors found significant differences for 2 concentrations
of meloxicam, compared with results for a control treatment,
with only 6 subjects/group by use of a 2-way crossover
design. Power calculations on objective gait data obtained
at various time points confirmed that committing a type
II error in the study reported here was highly unlikely.

Other studies of NK_1 receptor antagonists in rodents
have yielded promising evidence for alleviation of neuropathic
pain25-28; thermal inflammation25, and facial,21 surgical,22
and urinary tract pain.26,27 Concurrent use of an NK_1
receptor antagonist and gabapentin resulted in a syner-
gistic effect.25 Analysis of the results reported here suggested
that it is likely the NK_1 receptor antagonist plays only a
small role in the initiation of pain in dogs with urate-in-
duced synovitis.

On the basis of unpublished studies of the NK_1
receptor antagonist, 3 mg/kg administered orally once
daily was determined to provide sufficient blood con-
centrations to induce receptor antagonism with a mini-
mal risk of adverse effects in dogs. Therefore, it was un-
likely that the amount of the NK_1 receptor antagonist or
frequency of administration was a possible explanation
for the lack of effect.

However, species differences in sensitivity, uniden-
tified neuroanatomic differences, drug metabolism, or
receptor specificities between rodents and canids may
have contributed to the lack of efficacy for the study
reported here. Analgesic effects of NK_1 receptor an-
tagonts have been reported for inflammation induced
in rodents to evaluate this class of drug, neuropathic
pain induced by streptozocin and chronic constric-
tion injury,25 synovitis induced by intra-articular injection
of carrageenan,27 and peripheral inflammation caused
by injection of phorbol myristate acetate.28 Thus, those
methods may be more appropriate for evaluating the
NK_1 pathway in dogs. To our knowledge, investigation
of the effects of NK_1 receptor antagonists in dogs has
been limited to their gastrointestinal tract and cardio-
vascular effects.27-30 In those studies, the bioavailability
of an NK_1 receptor antagonist after IV and oral admin-
istration was identified. With regard to analgesic proper-
sties, NK_1 receptor antagonists can potentiate the neuro-

day
nal responses of cats to direct application of substance P (both centrally and peripherally), direct neuronal excitation, and noxious cutaneous stimulation via thermal and mechanical (pinch) stimuli. Analysis of results of those studies in cats suggests that substance P is not involved in the initial mediation of nociceptive inputs but perhaps is involved in the regulation of these inputs. Thus, NK<sub>1</sub> receptor antagonists may not be useful in the prevention of nociception but may modify the potentiation of the initial pain reflex and affect prolonged transmission of pain. This may explain why the NK<sub>1</sub> receptor antagonist failed to attenuate the pain response during induced acute synovitis in dogs.

It has also been reported<sup>13,34</sup> that there is variation in the potency and selectivity of NK<sub>1</sub> receptor antagonists. There is evidence that the analgesic effects of these drugs may not be entirely attributable to their action on NK<sub>1</sub> receptors.<sup>35,36</sup> Therefore, some care must be taken when studies of other compounds are used to predict the efficacy of a new compound without in vivo experimental data from mammals. Researchers must also be careful not to generalize a class of drug, especially a new one, on the basis of test results for only one of the members of that class of drug.

Despite evidence of potential as an analgesic, the NK<sub>1</sub> receptor antagonist tested in the study reported here did not yield subjective or objective evidence of analgesic effects in dogs with monosodium urate-induced synovitis, compared with results for a control substance. However, other methods for pain induction, such as the aforementioned streptozocin and chronic constriction injury method to induce neuropathic pain,<sup>23,31</sup> intra-articular injection of carrageenan,<sup>24</sup> and SC injection of phorbol myristate acetate,<sup>25</sup> may be more appropriate for use in testing the potential of this NK<sub>1</sub> receptor antagonist as an analgesic in the future. Methods of pain induction that activate primarily NK<sub>1</sub> receptors or, perhaps, observations of synergism when other analgesics are used in combination with NK<sub>1</sub> receptor antagonists may be more appropriate in detecting attenuation of signs of acute inflammatory pain in dogs. Analysis of the results of this study does not support the use of NK<sub>1</sub> receptor antagonists in the treatment of dogs with signs of pain induced by an acute inflammatory reaction.

References


**Appendix**

Description of the scales used for the subjective clinical lameness evaluation of dogs with monosodium urate–induced synovitis of a stifle joint.

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Score and description</th>
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</thead>
<tbody>
<tr>
<td>Standing (posture)</td>
<td>0 = Normal stance. 1 = Slightly abnormal stance (favors limb but it remains on the ground).</td>
</tr>
<tr>
<td>Walking</td>
<td>0 = No lameness; weight bearing observed for all strides.</td>
</tr>
<tr>
<td></td>
<td>1 = Mild, subtle lameness with partial weight bearing.</td>
</tr>
<tr>
<td></td>
<td>2 = Obvious lameness with partial weight bearing.</td>
</tr>
<tr>
<td></td>
<td>3 = Obvious lameness with intermittent weight bearing.</td>
</tr>
<tr>
<td></td>
<td>4 = Full non–weight-bearing lameness.</td>
</tr>
<tr>
<td>Trotting</td>
<td>0 = No lameness; weight bearing observed for all strides.</td>
</tr>
<tr>
<td></td>
<td>1 = Mild, subtle lameness with partial weight bearing.</td>
</tr>
<tr>
<td></td>
<td>2 = Obvious lameness with partial weight bearing.</td>
</tr>
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