Target engagement and histopathology of neuraxial resiniferatoxin in dog

Abstract

Objective To evaluate target engagement of intracisternally (IC) delivered TRPV1 agonist, resiniferatoxin (RTX), as measured by primary afferent and dorsal horn substance P immunoreactivity (sP-IR), histopathology and thermal escape latencies in dogs.

Study design Prospective experimental trial.

Animals Fourteen adult male Beagle dogs, weighing 10.3–13.2 kg; 11 dogs surviving to scheduled euthanasia.

Methods Anesthetized dogs were randomly assigned to be administered IC RTX (3.6 µg, 0.1 mL kg⁻¹) in a hyperbaric (hRTX, n = 6), normobaric (nRTX, n = 4) vehicle or a hyperbaric vehicle (hVehicle, n = 4). Over 16 days, animals were examined for thoracic and pelvic limb paw thermal withdrawal latencies and neurologic function. Spinal cords, trigeminal ganglia and dorsal root ganglia (DRGs) were assessed for morphologic changes and sP-IR.

Results IC RTX in anesthetized dogs resulted in a < 1 hour increase in blood pressure. Acute reactions leading to euthanasia within 8 hours occurred in three dogs (two hRTX, one nRTX). All other animals recovered with normal neurologic, bowel and bladder function. Final groups were: vehicle n = 4, hRTX n = 4 and nRTX n = 3. Animals in nRTX and hRTX showed increases in escape latencies in thoracic paws and, to a lesser extent, in pelvic paws, correlating to a loss of sP-IR in cervical cord with smaller reductions in thoracic and lumbar cord. In animals surviving to euthanasia, thickening of the arachnoid membrane (predominantly in the cervical region) was the most consistent change. This change, present in controls, was interpreted to be vehicle related. There was no evidence of structural changes in brain and spinal cord.

Conclusions and clinical relevance IC RTX produced localized loss of spinal and DRG sP with a corresponding thermal analgesia, absent motor impairment or spinal pathology. Loss of three animals emphasizes the need to refine the use of this promising therapeutic modality in managing companion animal pain.

Keywords antinociception, dorsal horn, intracisternal, intrathecal, substance P.

Introduction

Capsaicin and a variety of structural analogues activate small peptidergic sensory afferents in vitro (Theriault et al. 1979; Akagi et al. 1980; Bucsis & Lembeck 1981; Gamse et al. 1981; Franco-Cereceda et al. 1987; Iadarola & Mannes 2011) and in vivo (Yaksh et al. 1980; Jhamandas et al. 1984; Go & Yaksh 1987; Aimone & Yaksh 1989; Iadarola & Mannes 2011). The membrane target for this effect was identified as the transient receptor potential V1 (TRPV1) receptor (Caterina et al. 1997). Activation of this channel results in influx of cations (Caterina et al. 1997; Bevan et al. 2014), leading to loss of C-fibers and associated cell bodies, which express TRPV1 channels located in the dorsal root ganglion (DRG) or trigeminal ganglia (Iadarola & Mannes 2011). In rodents, spinal application of
these analogues results in an enduring anti-
nociception (Yaksh et al. 1979; Jhamandas et al.
1984). As toxicity is limited to the nociceptors that
express TRPV1 receptors, other systems mediating
light touch and proprioception would be unaltered.
Resiniferatoxin (RTX) obtained from the Euphorbia
resinifera plant acts on TRPV1 with a potency that is
several orders of magnitude greater than that noted
with capsaicin (Sallais & Blumberg 1990; Iadarola
& Mannes 2011). Of interest, the ameliorating ef-
fects of intracisternal (IC) and lumbar intrathecal
RTX on pain has been shown in dogs with osteoar-
thritis and osteosarcoma (Karai et al. 2004; Brown
These clinically important effects of neuraxial RTX
are considered to reflect a loss of small afferent
function. The present work addresses the organizing
hypothesis that IC RTX will produce a local change in
thoracic limb pain thresholds and an associated loss
of substance P (sP) in the cervical DRG and spinal
cord.

Materials and methods
These studies were carried out according to protocols
approved by the Institutional Animal Care and Use
Committee (IACUC) of the University of California,
San Diego. The facilities involved in these studies
have Association for Assessment and Accreditation of
Laboratory Animal Care accreditation.

Animals
Fourteen adult male Beagle dogs (Marshal Farms
USA Inc., NY, USA; 8–24 months, 10.3–13.2 kg)
were studied. The dogs were individually housed in
an IACUC-approved vivarium on a 12/12-hour
day–night cycle with ad libitum access to food and
water. All animals underwent physical examination
by the veterinarian (TMH) to confirm health status.
Neurologic and ophthalmologic examinations were
performed 4 days before IC injection.

Table 1: Summary of resiniferatoxin (RTX) study formulations for intracisternal administration in Beagle dogs.

<table>
<thead>
<tr>
<th>RTX formulation</th>
<th>n</th>
<th>RTX dose (µg)</th>
<th>Volume (mL)</th>
<th>Concentration RTX (µg mL⁻¹)</th>
<th>Tween 80 (µL mL⁻¹)</th>
<th>Dextrose (mg mL⁻¹)</th>
<th>Baricity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>4</td>
<td>0</td>
<td>1.0</td>
<td>0</td>
<td>30</td>
<td>50</td>
<td>1.0299</td>
</tr>
<tr>
<td>Normobaric 3.6 µg (0.1 mL g⁻¹)</td>
<td>4</td>
<td>36</td>
<td>1.0</td>
<td>36</td>
<td>70</td>
<td>0</td>
<td>1.0142</td>
</tr>
<tr>
<td>Hyperbaric 3.6 µg (0.1 mL g⁻¹)</td>
<td>6</td>
<td>36</td>
<td>1.0</td>
<td>36</td>
<td>30</td>
<td>50</td>
<td>1.0299</td>
</tr>
</tbody>
</table>

n, number of dogs.

Dose and volume delivered based on 10 kg Beagle.

1 Different for two animals in hRTX group contained 17 µg mL⁻¹ Tween 80 and 41 µg mL⁻¹ dextrose; baricity approximately 1.0268.

Study design
Animals were randomly assigned based on order of
receipt to be administered a single IC injection of RTX
(3.6 µg, 0.1 mg mL⁻¹ kg⁻¹) in a normobaric formulation (group nRTX, n = 4) or a hyperbaric formulation (group hRTX, n = 6) (Table 1). Two of the hRTX animals were administered formulations that contained the appropriate RTX doses but were slightly less hyperbaric and were included in hRTX.

Test article formulation
Resiniferatoxin (Lot number: BH-202; RTI International,
NC, USA) was obtained as a white solid (certificate of analysis: 99.87%). RTX for injection was prepared by serial dilution of stock solutions of RTX (200 µg mL⁻¹) prepared in phosphate buffered saline (PBS) and Tween 80 (70 mg mL⁻¹) with a final pH of 4–6 (Table 1). Dextrose was added to render the injectate hyperbaric, and the Tween 80 concentration was reduced.

Resiniferatoxin solutions were drawn into five catheters to assess retention with a 5-minute resi-
dency time. Analysis of the catheter fluid revealed absorption to the catheter (data not shown).

Intracisternal drug delivery
Following a 10-day acclimation, the animal was food
fasted overnight. Before surgery, the dog was admin-
istered intramuscular (IM) atropine (0.04 mg kg⁻¹;
Atropine Sulfate 0.54 mg mL⁻¹, VetOne; MWI, ID,
USA). Anesthesia was induced with intravenous (IV)
propofol (5–8 mg kg⁻¹; Propoflo 28, 10 mg mL⁻¹;
Zoetis, MI, USA). After orotracheal intubation, anes-
thesia was maintained with iso-

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