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RESEARCH PAPER

Target engagement and histopathology of neuraxial resiniferatoxin in dog

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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; Bucsics & 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

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these analogues results in an enduring antinociception (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 (Szallasi & Blumberg 1990; Iadarola & Mannes 2011). Of interest, the ameliorating effects of intracisternal (IC) and lumbar intrathecal RTX on pain has been shown in dogs with osteoarthritis and osteosarcoma (Karai et al. 2004; Brown et al. 2005, 2015; Iadarola & Mannes 2011). 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.

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 residency 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 administered 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, anesthesia was maintained with isoflurane (Fluriso; VetOne; MWI) at an end-tidal isoflurane concentration (F_E'Iso) of 2% that was adjusted as required, delivered in 50% O_2:50% nitrous oxide for ≥ 1 hour via a circle rebreathing system. The animal was constantly monitored intraoperatively, including end-tidal carbon

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

RTX formulation Dose (volume)	*n*	RTX dose (μg)*	Volume (mL)*	Concentration RTX (μg mL^{-1})	Tween 80 (μL mL^{-1})	Dextrose (mg mL^{-1})	Baricity
Vehicle	4	0	1.0	0	30	50	1.0299
Normobaric 3.6 μg (0.1 mL kg^{-1})	4	36	1.0	36	70	0	1.0142
Hyperbaric 3.6 μg (0.1 mL kg^{-1})	6	36	1.0	36	30†	50†	1.0299†

n, number of dogs.
*Dose and volume delivered based on 10 kg Beagle.
†Diluent for two animals in hRTX group contained 37 μg mL^{-1} Tween 80 and 41 μg mL^{-1} dextrose; baricity approximately 1.0268.

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