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The Vanilloid Agonist Resiniferatoxin for Interventional-Based Pain Control

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Abstract

The idea of selectively targeting nociceptive transmission at the level of the peripheral nervous system is attractive from multiple perspectives, particularly the potential lack of non-specific (non-targeted) CNS side effects. Out of the multiple TRP channels involved in nociception, TRPV1 is a strong candidate based on its biophysical conductance properties and its expression in inflammation-sensitive dorsal root ganglion neurons and their axons and central and peripheral nerve terminals. While TRPV1 antagonists have undergone extensive medicinal chemical and pharmacological investigation, for TRPV1 agonists nature has provided an optimized compound in RTX. RTX is not suitable for systemic administration, but it is highly adaptable to a variety of pain problems when used by local administration. This can include routes as diverse as subcutaneous, intraganglionic or intrathecal (CSF space around the spinal cord). The present review focuses on the molecular and preclinical animal experiments that form the underpinnings of our clinical trial of intrathecal RTX for pain in advanced cancer. As such this represents a new approach to pain control that emerges from a long line of research on capsaicin and other vanilloids, their physiological actions, and the molecular biology of the capsaicin receptor TRPV1.

Keywords

Analgesia; hyperalgesia; cancer pain; osteosarcoma; canine; capsaicin; intrathecal; cerebrospinal fluid; spinal cord; dorsal root ganglion; proprioceptor; C-fiber; A-delta fiber; vanilloid receptor; calcium cytotoxicity; ion channel

1. INTRODUCTION

This review examines the development and use of vanilloid agonists, in particular resiniferatoxin (RTX), to control intractable pain conditions. It examines the main studies of the underlying observations on RTX actions and the animal translational research that form the basis of the present human clinical trial of intrathecal RTX for intractable pain in patients with advanced cancer. Capsaicin is the prototypical vanilloid agonist, which has been used as a research tool for many decades and is now being used clinically. For

example, the 8% patch (Qutenza®) was recently approved for clinical use. This topical route of application has been intensively investigated for a variety of different pain conditions [1–3]. However, for other routes of administration, such as the intrathecal space, the greater potency of RTX makes it a more tractable compound. Early studies with intrathecal capsaicin appeared to be somewhat confounded by non-specific toxicities, in part attributable to high concentrations of vehicle [4–7]. With RTX lower concentrations of vehicle are needed and the propensity for toxicity due to high amounts of vehicle or vehicle-drug interactions [8] is reduced. We have also extensively explored the route of direct intraganglionic administration of RTX in rat and monkey. Here, too, where contact with the sensory neurons is immediate and occurs in a confined space, reducing unwanted toxicities is a primary concern. Prior to the *in vivo* studies, experiments performed *in vitro* using calcium imaging also clearly demonstrated the superiority of RTX in producing a prolonged channel open time, thereby leading to effective calcium cytotoxicity [9]. Thus, for all of the routes of administration explored by our laboratory: peripheral [10], intrathecal [11,12], intraganglionic [11,13], or perineural [14], RTX was the drug of choice.

Conceptually, vanilloid agonists represent a viable and versatile approach to pain control. There is one main proviso: in order to inactivate the nerve ending or axon, vanilloid agonists need to be applied close to the site of action: the axon or neuronal perikarya (e.g., intrathecally or intraganglionically) or directly into the peripheral site of tissue injury or pain generation. Thus, agonists are used in an interventional fashion. This property addresses one of the problems with devising a treatment strategy for pain, which is that it can occur in any part of the body, in any tissue, and the amount of tissue involved can be either very small or very large. In many cases systemic treatment with an orally bioavailable analgesic may not be desirable. The benefit of local vanilloid agonist application is that it allows drug administration to be tailored to the pain problem the patient presents with. One constraint of this attribute is that not all pain problems are amenable to local drug administration. Thus, there is an appropriate set of acute and chronic pain problems that can be addressed with RTX, yet the range of potential condition that can be treated is quite broad.

The insight to develop RTX for control of intractable pain conditions emerged directly from live cell microscopic examination of stably transfected cell lines expressing a TRPV1eGFP fusion protein [15,16]. The impetus to carry this forward into human clinical trials emerged from our preclinical studies in a large animal model, specifically canine osteosarcoma and osteoarthritis [11,12]. The former studies provided the necessary mechanistic insights into how to use RTX as a therapeutic agent; the latter study showed that this compound worked exceptionally well on clinical pain, that is, not just in rodent models, but also against pain from naturally occurring cancer. At the conclusion of these studies, a dedicated effort was made to assemble the various elements necessary for a Phase I clinical trial in humans including the chemistry and manufacture of RTX, formulation and stability testing, toxicological studies, generation and review of the clinical protocol and the investigational new drug application, and patient recruitment.

2. BACKGROUND

In 1986 we adapted the complete Freund adjuvant (CFA) model of disseminated inflammation, in which CFA was injected into the base of the tail, to produce a reversible, unilateral inflammation in order to examine neuroplasticity induced by persistent nociceptive activity [17–19]. One of the main questions we addressed was the ability of peripheral inflammation from intraplantar injection of CFA-saline emulsion or carrageenan to alter the molecular properties of second order spinal cord neurons, in particular, neuropeptide gene expression. In parallel, we investigated the effects of peripheral inflammation on behavioral parameters such as hyperalgesia and allodynia. These studies evolved into a broader examination of the anatomy, physiology of nociceptive systems and the molecular transcriptional control processes regulating dynorphin gene expression in particular [27,29–33].

While the CNS remained a focus, it was evident that, to complete the circuit, the primary afferent neurons and the abnormal environment produced by inflammatory agents also required investigation. Shortly after the cloning of TRPV1 in 1997 [20], which initially was called the vanilloid receptor 1, we assembled a full length TRPV1 cDNA from a rat dorsal root ganglia cDNA library [15] as part of our broader effort to obtain a more complete understanding of the initial steps in the pain pathway [21,22]. The pathway starts with damaged or inflamed peripheral tissue, nociceptive signals are transduced by nerve endings of dorsal root ganglion neurons and transmitted to the central nervous system by the first set of synaptic connections in the dorsal spinal cord. The nerve endings of primary afferent nociceptors are immersed in the inflammatory environment and TRPV1 is capable of integrating multiple signals from this environment through sensitivity to heat, acidic conditions and second messenger signaling generated by additional receptors for algogenic chemicals on the afferent endings [21–23]. We, like many groups, considered TRPV1 to be a major transducer of inflammatory, as well as thermal pain. Since TRPV1 is one of the very first molecules in the pain pathway, and contributes to depolarizing nociceptive nerve endings, we began to investigate the molecular and cellular mechanisms regulating TRPV1 using the new technique of live cell imaging by making a TRPV1 fusion with enhanced green fluorescent protein (TRPV1eGFP). We examined the effects of agonist stimulation [15], treatment with anandamide and low pH [16], phosphorylation by PKC isoforms [24], and the regulation of TRPV1 in plasma membrane and endoplasmic reticulum compartments [9,25], however, this review mainly examines the group of studies that formed the route to human clinical use of RTX [11,12,15].

3. RTX IS A MECHANISM-BASED TREATMENT

The TRPV1eGFP fusion construct was used in live cell imaging experiments to explore the cellular location of the receptor and observe the results of capsaicin activation *in vivo*. After several iterations of imaging platforms and constructs, dual wavelength experiments were performed to image both the fluorescent TRPV1 and the mitochondria (with the vital dye Mitotracker Red). Transfection of the fluorescent TRPV1 fusion protein disclosed prominent localization in the endoplasmic reticulum (ER) and comparatively less in the plasma membrane Fig. (1a) [15,16], and no localization in the mitochondria. The COS7 cells grow

as a thin flat sheet and the ER exists as a lattice of thin tubular structures compared to the mitochondria, which are elongate, but much thicker than the ER. Upon addition of RTX there is a rapid and remarkable remodeling of the ER lattice: within seconds it fragmented into spherical vesicles Fig. (1) and Table 1 and by 60 seconds vesiculation was complete.

Fig. (1). TRPV1eGRP fusion protein was expressed in COS7 cells, The green fluorescent protein can be seen in the endoplasmic reticulum, a lattice of thin tubular structures, and the golgi apparatus. In this confocal section, the plasma membrane is only weakly labeled compared to the ER although at points where the cell attaches to the culture dish the plasma membrane TRPV1 can be seen more clearly [15,16]. The mitochondria are labeled in red with a vital dye. They are seen as elongate thick tubular structures. Addition of RTX causes a rapid fragmentation of the ER and the mitochondria. Within seconds both intracellular organelles form spherical vesicles Fig. (1) and Table 1 and by 60 seconds vesiculation is complete.

The same rapid vesiculation occurred with the mitochondria. The time course of the fragmentation-vesiculation corresponded to the rise in free intracellular calcium. Subsequent to vesiculation of intracellular organelles, a progressive cellular deterioration ensued. This was characterized by nuclear blebbing and loss of plasma membrane [25]. The first nuclear bleb was observed at 2 min and by 10 min all cells examined (N=30) exhibited at least one bleb. The disintegration process culminates in cellular lysis in about 45 minutes. RTX treatment also caused a loss of plasma membrane; this occurred coincident with the intracellular organelle changes and was detectable microscopically [15,16,25] with live cell imaging and electrophysiologically. The electrophysiological endpoint measured was a decrease in cell capacitance. The loss of cell membrane (and associated receptors) may partially underlie earlier observation obtained with RTX treatment such as a decrease in [3H]RTX binding upon exposure to agonist. Such observations are likely a combination of desensitization, the membrane loss that vanilloid agonists produce [25] and outright cell death [15].

(Table 1). Laser confocal microscopy was carried out in Cos7 cells after 24 hrs of transfection with TRPV1eGFP. Other agonists like capsaicin, olvanil and the endocannabinoid-endovanilloid anandamide can also provoke the lytic changes [15,16].

At the time these experiment were performed this type of intracellular fragmentation had not been described for vanilloid agonists. However, such a vesiculation process had been observed with a calcium ionophore (ionomycin), in fact, the organelle fragmentation and cell lysis produced by RTX appeared nearly indistinguishable from that produced by ionomycin [15,16,26]. These data suggested that RTX-induced cytotoxicity was due to opening of the TRPV1 ion channel and consequent rapid rise in free intracellular calcium ($[Ca^{++}]_i$). Both capsaicin and RTX produce a rapid rise in $[Ca^{++}]_i$. Live cell calcium imaging showed that the difference was due to the high potency of RTX, which caused a larger and more sustained increase in $[Ca^{++}]_i$ than capsaicin at about 1/500th the dose [9]. It was this insight into a specific mechanism: to specifically kill pain-transducing primary afferent neurons, that formed the basis for proceeding from cellular level work into preclinical *in vivo* investigations with the aim of therapeutic application in humans.

The RTX induced cell and axonal lysis appeared to be a very effective mechanism to rapidly produce a specific lesion of the TRPV1 subpopulation of nociceptive neurons. This clearly defined mechanism allowed us to articulate a well-defined pathway for preclinical studies on pain control using peripheral, intraganglionic and intrathecal routes of administration. While earlier studies on capsaicin had shown a long-term depletion of spinal cord substance P and long-term reduction in nociception [4], subsequent work suggested that the initial results were due to non-specific damage to the spinal cord [5–7]. Also, the depletion of substance P was not directly linked to a neuronal degeneration process, which makes explicit formulation of a therapeutic strategy difficult. In contrast, our *in vitro* results with RTX clearly indicated the mechanism and hence the routes of administration that could be exploited for therapeutic purposes. The transition to clinical trial was made through experiments in both rat and canine models.

4. MODELS AND ROUTES OF ADMINISTRATION

4.1. Peripheral Administration

4.1.1. Intraplantar Injections—As mentioned above, pain can occur at many sites in the body, so the questions of what pain indications to concentrate on and where to administer RTX were subjects of much debate. In rats, we explored peripheral injections (into the hind paw foot pad, intraplantar), intraganglionic and intrathecal routes of administration [10–13]. These were possible routes for treatment of post-operative pain, bodily or nerve injuries, pain problems localized to one or a few dermatomes, or more widespread pain from metastatic disease, respectively. The objective was to demonstrate that the pain signal could be intercepted before it reached the spinal cord.

Intraplantar or intrathecal administration of RTX produced an elevation in paw withdrawal latency (PWL) in the non-inflamed state. Injection of carrageenan into the hind paw produced a thermal hyperalgesia, as indicated by the decrease in PWL. Injection of RTX reversed the thermal hyperalgesia and increased the thermal withdrawal latency. The table contains data from one dose of RTX, 250 nanograms (ng), and one time point following carrageenan. A more complete presentation of the dose-response and time course data can be found in references 10 and 11.

(Table 2). The dose for the peripheral injection was 250 nanograms administered by direct intraplantar injection. In this experiment the cutoff was 16 sec, which was used to avoid thermal burn in the analgesic animals. Testing was done 24 hrs post-RTX. Note that even in the hyperalgesic state RTX treatment caused the latency to reach cutoff. Doses as low as 62.5 ng also caused the latency to reach cut-off [10]. Intrathecal RTX also causes an elevation in thermal nociceptive withdrawal threshold. In the vehicle group (column 3) there is hyperalgesia after intraplantar carrageenan. RTX (100–200 ng) reversed the hyperalgesia but did not cause all of the animals to reach cutoff, which in this experiment was 14 sec. Testing was performed with a radiant thermal stimulus in unrestrained rats. *Criterion for significance was $P < 0.05$; # the cutoff was 16 sec.

With the peripheral route of administration, as the dose increased, the duration of RTX analgesic action was extended in time. The effect on duration was mainly seen in the

number of successive days the animals reached the 16 sec cutoff. The overall duration at the doses used was approximately 18 days, with the most robust effects occurring in the first 7 days. Thus, RTX injected peripherally is highly efficacious, has a prolonged duration of action yet is reversible over time.

The actions of peripherally administered RTX on primary afferents were also manifested by blockade of *central* neuro-chemical endpoints. Peripheral inflammation rapidly induces expression of the *c-fos* gene and subsequent translation of the mRNA into protein [27, 28]. Immunocytochemical staining of spinal cord sections with a c-Fos antibody (raised against the conserved midportion that contains the DNA binding domain) labeled second order neurons in laminae I and V [29,30,31]. Counts of labeled neurons showed that RTX treatment could block the inflammation-induced elevation of c-Fos in both laminae [10]. In response to the peripheral inflammatory challenge, rats pretreated with RTX exhibited decreases of 60 to 70% in the number of c-Fos positive neurons in lamina I and in lamina V. These data support the idea that the TRPV1-positive primary afferent neurons, many of which are CGRP-containing, make contact with second order noci-responsive neurons [32]. Additionally, many of the c-Fos positive second order neurons are also immunopositive for NMDA receptors [33] and exhibit a dynorphin up-regulation following inflammation [31]. Thus, the afferent input activates not only physiological responses but molecular adaptations as well, and it is likely that both responses contribute to establishment of central sensitization or the fine-tuning of the sensitization process. Taken together these data support the idea of using RTX for postoperative pain control either at the time of surgery or in a preemptive fashion.

4.2. Intraganglionic and Intrathecal RTX: Rat and Monkey Studies

4.2.1. Intraganglionic—The *in vitro* actions of RTX to produce calcium cytotoxicity focused our attention on using nociceptive neuronal cell deletion as a therapeutic approach. Cell deletion seemed appropriate for patients with advanced pain problems, as might be encountered in metastatic cancer or other chronic intractable pain problems that involve one or several dorsal root ganglia or spinal segments.

For the rat and the monkey, we injected the trigeminal ganglion. For the rat we used a stereotaxic approach [11]. For the monkey a direct injection procedure was used. This involved making a craniectomy centered over the external auditory canal, elevating the dura mater off the skull base, and, by direct visual guidance, introducing a blunt 32-gauge needle 3 to 4 mm into the trigeminal ganglion. Twenty μ l of a 0.1 μ g/ μ l RTX solution was infused over a 5-minute period. Thus, the total dose in the monkey was 2 μ g and in the rat we explored 0.02 and 0.20 μ g doses; the PBS, Tween-80, ascorbic acid vehicle was the same in both cases [12].

Both the afferent and efferent functions of the TRPV1-containing primary neurons were affected: the afferent function was evaluated via the capsaicin-induced eye wipe response and the efferent function using capsaicin-induced plasma extravasation of intravenously administered Evans blue. In both species we obtained the ipsi- and contralateral ganglia for histological and immunohistological evaluations. In monkey and rat intratrigeminal RTX injection produced a unilateral block of the eyewipe response. The effect was rapid in onset

being observed as soon as 24 hrs after the microinjection in the rat. The blockade was permanent: in the rat loss of capsaicin eye wipe lasted for 350 days and in the monkey out to 4 months (at which point the experiment was terminated). The efferent function was also blocked. In both rat and monkey intratrigeminal RTX blocked plasma extravasation over the entire trigeminal distribution. In some rats TRPV1-positive cell deletion was incomplete in the mandibular division, which is located more laterally than the ophthalmic and maxillary divisions, suggesting that the injection needle was more medially placed. However with proper placement, TRPV1-expressing neurons in all three divisions could be ablated. Nonetheless, in all the rats small islands of blue skin were present. This is consistent with the idea that some neurons survived, even though the surrounding cells were lesioned. This suggests that a subpopulation of trigeminal neurons have calcium clearance mechanisms that render them relatively resistant to the toxic effects of RTX [9,11]. Nonetheless, in the rat, RTX injections administered peripherally produce a profound loss of responsiveness to noxious thermal stimulation, which is consistent with the idea of a broad-spectrum sensitivity of noxious thermally-responsive nerve endings to RTX-induced calcium cytotoxicity [39].

(Table 3). Intraganglionic RTX suppressed capsaicin eye wipe and deleted ganglionic TRPV1 containing neurons. These effects were permanent. Rats were tested out to 350 days and displayed no recovery of capsaicin-induced eye wipe. Monkeys were tested for up to 4 months, again, without recovery of the eye wipe response. Afferents subserving protective functions such as the *blink reflex* were retained [11,13,34]. *The differences in numbers of cells are due to endpoints of counting (e.g. cells/ high power field versus total cells counted in representative sections) between the two experiments. Despite the difference in ganglionic size and injection technique, the percent decrease was similar and not all cells were killed, which in part is a reflection of the structural complexity of this three-part ganglion. More details can be found in [11,13].

4.2.2. Intrathecal RTX: Attenuation of Thermal Nociception—The intraganglionic approach is ideal for pain problems that are localized to one or several dermatomes, especially if the problem is lateralized. However, many chronic pain problems present with a more widespread, bilateral distribution such as pain in advanced cancer. In patients with such disseminated presentations, using a ganglion-by-ganglion treatment strategy may not be practical. Intrathecal administration would reach multiple dorsal roots and affect large zones of the body. In the rat and human we inject mainly into the lumbar cistern; in the dog RTX injections were made into either the lumbar cistern for hind limb tumors or the cisterna magna for forelimb tumors. Injections into the lumbar cistern will expose the cauda equina and theoretically nerve roots from human S5 to ~L1 can be affected if the solution containing the RTX is well distributed. After intrathecal injection, both the rat and dog became less sensitive to noxious thermal stimulation with a radiant heat source. Increasing doses of RTX produced an increasing analgesic effect, although the dose-response appears fairly steep. In the rat, significant increases in withdrawal threshold were seen at 100 ng injected into the CSF at the L3–L4 interspace, and at 200 ng (~0.7 µg/kg) some animals reached the 14 sec cutoff. In the initial dose-ranging study in dogs, RTX was delivered into the cisterna magna and the fore paws were tested with a radiant thermal stimulus similar to

the rodent Hargreaves test. Administration of 1.2 or 3 µg/kg RTX intracisternally to dogs caused nearly complete loss of sensitivity to noxious thermal stimulation on the first test, conducted on post-injection day 2, which was sustained out to day 12 [12]. RTX also attenuated inflammatory thermal hyperalgesia when injected intrathecally to rats with a hind paw peripheral inflammation. Thus, responses to painful thermal stimulation in both baseline and hyper-excitable states are blocked by RTX.

In both rat and dog the zone of target engagement was spatially limited. The lumbar intrathecal injections affected hind paw responsiveness, but spared the fore paw and vice-versa for the intracisternal route in the dog. This suggests that moderate to high doses of RTX do not mix well in the rostro-caudal dimensions of the intrathecal space to any substantial degree. However, it must be noted that no specific attempts were made to mix the injected drug solution with the rat or dog CSF, alter the baricity of the drug product or the position of the client owned animals. Thus, more work is needed to explore specific aspects of the injection procedure to optimize drug distribution in the lumbar intrathecal space. In the rat, higher doses (2,000 ng) given by lumbar puncture could reduce forepaw thermal sensitivity and sensitivity of the spinal trigeminal system as assessed by capsaicin-induced eyewipe. These observations show that the spatial extent of the RTX distribution can be manipulated by increasing the dose and, likely, by deliberate mixing with CSF upon injection.

5. CANINE OSTEOSARCOMA

5.1. Intrathecal RTX for Canine Bone Cancer Pain

5.1.1. General Considerations—It is interesting to ask the question: Do the models (thermal pain/thermal hyperalgesia) have predictive value for treating pain from clinical cancer as encountered in osteosarcoma? The apparent lack of predictive value of the various animal models as guidelines for analgesic drug development has been the subject of numerous reviews. While there is no simple answer, this was a very real concern for the RTX program: purposely deleting a set of neural inputs is not a typical pharmacological paradigm and if the intrathecal route is used, certainly not a reversible step. Thus, proceeding directly from an observation of reduced thermal nociception in the rat or dog to the human seemed premature. We did obtain an idea of safety and efficacy in the models but these results still left a gap in knowledge about how well RTX ameliorated clinical cancer pain. We thought it advisable to obtain data from a “transitional model” that was more akin to human clinical conditions before purposely deleting nociceptive neurons in the human. In conjunction with Dorothy Ci-mino Brown, a veterinary surgeon at University of Pennsylvania School of Veterinary Medicine and a long-term collaborator, pain associated with naturally occurring canine osteosarcoma was identified as a clinical condition that, potentially, could be treated with intrathecal RTX. Canine osteosarcoma is usually localized to one limb initially and the effects of the pain on locomotion and the activities of daily living are evident and fairly straightforward to observe and quantify [12]. Furthermore, the pain is difficult to control satisfactorily with opioids, NSAIDS or a combination of agents. All of these aspects are very similar to the human counterpart. Bases on these

considerations, we reasoned that strong analgesic activity in canine veterinary patients should provide strong predictive value for performance in human clinical trials.

Following initial dose-ranging using intracisternal RTX and thermal stimulation of the fore paw [12] (as noted above), a series of client dogs with intractable pain from naturally occurring osteosarcoma was recruited under a clinical protocol [12]. All of the animals had intractable osteosarcoma that had become unresponsive or intolerant to conventional pain management (NSAIDs, opioids, steroids); most of the animals were candidates for euthanasia because of poor pain control [12]. The results from these large animal studies demonstrated a very strong analgesic drug effect and provided a powerful motivation to proceed to the human clinical trial.

5.1.2. Procedural Aspects of Administration—Intrathecal RTX acutely activates the pain fibers before it produces calcium cytotoxicity that in turn yields a transiently inactive or permanently chemically-severed axon or actual loss of the neuronal perikarya. Therefore, before the inactivation or axotomy occurs the intrathecal injection of RTX is acutely painful. Because of the acute pain the injection is performed under general anesthesia. The dogs were pre-medicated with hydromorphone, general anesthesia was induced with thiopental, the trachea intubated, and anesthesia maintained with isoflurane; hemodynamics, respiratory rate and body temperature were monitored. Animals were observed overnight in the hospital and discharged to home the next day. Re-evaluation occurred at 2, 6, 10, and 14 weeks after RTX, or longer; the longest post-injection survival was 9 months. The caregivers assessed the animals' discomfort level using a visual analog scale (VAS). Since our initial publications, the canine model has been studied more extensively and further validated in animals with osteoarthritis [36,37], or osteosarcoma [38].

5.1.3. Acute Hemodynamic Effects—There was an acute hemodynamic response to intrathecal RTX but, while strong, it was relatively short-lived. In normal control animals, at 5 min after injection the mean arterial blood pressure increased from 79 mmHg to 130.8 ± 3.5 mmHg and decreased to ~ 116 mmHg by 15 min and to ~ 90 mmHg by 60. After 2 hrs there was no significant difference from control. Heart rate also increased from 88.8 to 138.5 ± 4.33 beats/min at 5 min and decreased to approximately 110 beats/min by 60 and remained at about this level out to 240 min. The dogs with bone cancer also showed elevations in the same range, although on average the basal heart rate was higher 122 beats/min. These data show that the peak alterations in hemodynamic parameters occur during the first 15 min after injection. Considering that the activation of the pain system was bilateral, synchronous, and involved multiple spinal segments the degree of change did not reach a physiologically abnormal level [12,35].

5.1.4. Acute Effects on Body Temperature—RTX injection also produced a transient decrease in body temperature, which was monitored every 30 min for 4 hrs and again at 18 hrs post-intubation; at the latter time, the body temperature was normal. Despite the low body temperature (net decrease of 2.2°C from the point of extubation out to 4 hrs), animals exhibited panting for several hours post-injection, a behavior consistent with an increase in body temperature. Recovery was generally uneventful and blood and urine collected before and 2 weeks after RTX injection showed no clinically significant increases or decreases.

These studies indicated that the peak hemodynamic were relatively short lived (5 to 10 min) as was the hypothermic effect, and that RTX did not produce any long-term alteration in blood or urine chemistries.

5.1.5. Analgesic Actions—The behavioral effects were consistent with a profound analgesic action. In these studies the pain levels were rated by the owners using a visual analog scale at 2, 6, 10 and 14 weeks. The average VAS rating preinjection was 53 on a 100 mm scale and the rating went to an 8.0 after RTX at two weeks and remained at this level till 14 weeks. These animals were at the end stages of their disease: at two weeks there were 18 animals and by 14 weeks only 4 remained alive, nonetheless, their pain rating remained low throughout the 14 week period. Having the caregiver perform the rating obviously yields an indirect measure, but it is based on an assessment of the activities of daily living (ADL) which reflects a multitude of readily apparent endpoints like ease of locomotion and weight bearing on the affected limb, as well as subtle judgments of behavior that only come from living with the dog on a daily basis. For example, there were no reports of changes in “personality” of the animals, suggesting that higher order CNS functions were unaffected. Lastly there were no reports of bladder or bowel dysfunction. The longest period of survival was 9 months and no long-term negative effects of RTX on ADL were apparent although the analgesic action was sustained throughout.

In addition to the marked analgesic action, for 66% of the animals the owners decreased or discontinued completely the animal’s concurrent analgesic medications. This is quite remarkable considering the progressive course of the disease. In humans with cancer the incidence of pain increases greatly in the last two months of life. Since most of the animals died of their primary disease, the maintained low pain ratings indicate that the pain control from intrathecal RTX remained effective despite disease progression.

5.1.6. Histological Observations in DRG—Lastly, we obtained the dorsal root ganglia from some of the animals treated with RTX. While there are no available antibodies that recognize canine TRPV1, standard histology of DRG paraffin sections clearly showed evidence of neuronal loss. We observed numerous nodules of Nageotte, which are seen as rosettes of proliferating small satellite cells with small basophilic nuclei that surround the disintegrating neuronal cell bodies. Neurons with larger diameter perikarya were unaffected even in the immediate vicinity of one of more degenerating neurons [11,12]. These histological observations reflect the behavioral data where the analgesic action of RTX occurs without affecting other somatic sensations and proprioception.

6. HUMAN CLINICAL TRIAL

6.1. Preparatory Steps for the Human Clinical Trial

6.1.1. Investigational New Drug (IND) Application—The above preclinical rat and dog studies demonstrated both the efficacy and safety of intrathecal RTX. Additional preparations for the intrathecal trial in humans included the chemistry and manufacture of a clinical grade of RTX that was free of volatile organic contaminants, formulation of a stable injectable form of RTX, evaluation of the stability under various storage conditions, and design and execution of Food and Drug Administration (FDA) compliant toxicology studies

in two species and both sexes. The clinical protocol was also written, reviewed and approved. All of the data and documents were assembled into an Investigational New Drug application to the FDA. All of these steps were made possible by a long-term inter-Institute collaborative effort between the NIDCR and the National Institute of Drug Abuse (NIDA). Once all these regulatory elements and the appropriate reviews were completed we proceeded to the Phase I trial.

6.2. Aspects of the Study

It is too early to report on patient responses, but elements of the recruitment and eligibility criteria are useful to consider. Clearly there are many similarities to the canine study in terms of design, patient selection, and general route of administration. In dogs the standard compartment for CSF sampling is the cisterna magna, whereas for humans it is the lumbar intrathecal space where the cauda equina can be found. We injected at the L3–L4 interspace, thereby potentially exposing dorsal roots from L3 down to S5 to the RTX solution. Thus, patients with pelvic involvement have a chance for pain relief whereas patients with pain above the abdomen are very unlikely to benefit from the treatment and are excluded. All patients must be diagnosed with histological documentation of cancer and are not receiving benefit from palliative treatments to relieve pain or are obtaining inadequate pain relief at maximally tolerated opioid doses or are experiencing dose-limiting side effects. Such problems occur with increasing frequency at the end stages of the disease [40–42]. The protocol does not require the patients to cease their opioid drug usage. In fact, alterations in opioid consumption post-RTX injection are one of the endpoints that are followed. There are also several exclusion criteria. The most obvious are related to potential complications with the lumbar puncture procedure such as coagulation problems due to anticoagulants, bone marrow dysfunction or low platelet count. The presence of tumors or other types of spinal structural problems that might impair the flow of CSF or impede catheter placement were also exclusionary criteria. Patients with advanced brain pathology or increased intracranial pressure were also excluded. More details of the inclusion and exclusion criteria can be found at <http://clinicalstudies.info.nih.gov/detail/A_2009-D-0039.html> or at <<http://www.clinicaltrials.gov>>.

This study is also designed to examine the pharmacokinetics (PK) of RTX in CSF and the consequences of the RTX axonopathy in terms of CSF proteomics by periodic removal of CSF within the first 24 hrs. In the dog studies we determined the RTX PK and these measurements showed that RTX is rapidly cleared from the CSF with a half-life of between 5 and 15 min depending on the dose administered. We expect approximately the same kinetics in the human. The catheter is left in place for 24 hrs and, for proteomic analyses, we sample at 4, 12, and 24 hrs. At these times we expect to detect proteins from axons or oligodendrocytes as the TRPV1 positive A δ and C-fiber axons degenerate. One objective is to determine if the clinical response correlates with one of the proteomic endpoints; another is to determine if the amounts of proteins released into the CSF increase with increasing doses of RTX. These measurements may yield one or more biomarkers for RTX actions, provide insight into dose-response relationship and when the plateau phase is being approached.

CONCLUSIONS

This chapter reviewed the initial basic molecular and physiological research underlying the neurobiology of TRPV1-RTX interactions. These basic observations provided the insight into how to use RTX clinically for treatment of cancer pain. This is the condition where we have progressed the furthest. However, there are a variety of other chronic and acute pain problems ranging from spinal stenosis to post-operative pain in which RTX could be used via a variety of routes of administration [10–14]. RTX is obviously a highly effective treatment for intractable cancer pain as seen with the canine osteosarcoma. Nearly all the dogs recruited to the study were candidates for euthanasia because of poor pain control. All the animals returned home after treatment and lived with a much higher quality of life subsequent to RTX treatment. The results of these canine studies, in particular the strength of the analgesic effect and the lack of apparent side effects at therapeutic doses, provided the impetus to proceed with the human clinical trials.

The route from identification of mechanism to clinical trial consumed several years of work. During this period, TRPV1 antagonists were developed and many have undergone clinical trials. These compounds have encountered two problems that have dampened, if not eliminated, their utilization as new analgesics. The first is a tendency, to varying degrees, for different antagonists to produce a rise in core body temperature. The system that the antagonists work on is not completely clear [43–45]. The second is predictable: the sensations of noxious heat are blocked, leading to the perception that hot objects or liquids are only warm. This lack of feedback raised a safety issue for people on chronic antagonist regimens consuming or exposing themselves to dangerously hot liquids. Since the entire body loses sensitivity to damagingly hot temperatures, there is no capacity to sample the “thermal environment” since no body part retains thermal sensitivity. Similar to the antagonists, intrathecal RTX produces the same insensitivity to hot thermal stimuli. The major difference is that, because RTX is administered locally, the effects are spatially delimited on the body surface and this leaves some areas of the body available for thermal environmental sampling. Both rat and dog studies showed that, if the dose is raised high enough, a loss of thermal sensation occurs throughout the body. Such an overdose effect can be obtained from either lumbar or cisternal sites of administration. Thus, optimizing the RTX administration procedure and dose is an important endeavor.

The intrathecal space is only one body compartment into which RTX can be administered and we, and others, have explored peripheral, perineural and intraganglionic sites of administration. All of these routes have potential target patient populations. The ability to tailor the treatment to the patient’s pain problem is one of the most exciting aspects of using a procedure-based analgesic like RTX. Lastly, orally active TRPV1 analgesics would still be useful therapeutic agents and new medicinal chemistry efforts are in progress targeting allosteric sites on TRPV1 that might avoid problems encountered with antagonists of the orthosteric capsaicin binding domain [8]. It is important to note that blocking TRPV1 or removing the TRPV1-containing afferents does not remove all pain sensation: pinch, pressure and mechanical pain transduction are intact, however, a large swath of the pain spectrum is inhibited. As we pointed out in two letters [46,47], retention of some pain sensibility is an important component for bodily protection, thus the local application aspect

of RTX or the imposition of an “activity-dependent filter” using an allosteric mechanism may provide the necessary balance between algescic and analgesic processes.

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Fig. 1.
Rapid Fragmentation of the ER and Mitochondria after RTX

Table 1

Time Course of Cellular Remodeling Events after Addition of 1 nM RTX

Cellular Compartment and Effect	Time
Endoplasmic reticulum Fragmentation and Vesiculation	Initiation: 6 sec Complete: 60 sec
Mitochondrial Fragmentation and Vesiculation	Initiation: 6 sec Complete: 60 sec
Nuclear Membrane Blebbing	First bleb observed: 2 min One bleb in all cells: 10 min
Plasma Membrane Cell Lysis	Approximately 45 min

Table 2

Analgesic Actions of Peripherally or Intrathecally Administered RTX

	Baseline PWL	Post RTX Injection PWL	Inflammation Hyperalgesic PWL	Inflammation PWL post-RTX
Peripheral route	6.7 ± 0.5 s	16.0* ± 0.0 [#] s	3.4 ± 0.8 s	16.0* ± 0.0 [#] s
Intrathecal route	8.3 ± 1.0 s	13.2* ± 0.6 s	3.5 ± 2.1 s	10.6* ± 2.0 s

Table 3

Intraganglionic RTX: Similar Responses of Rat and Monkey to

	Capsaicin Eye Wipes Contralateral-Side: no RTX	Capsaicin Eye Wipes Ipsilateral Side: after RTX	TRPV1 + Neurons in TG Contralateral	TRPV1 + Neurons in TG Ipsilateral
Intraganglionic RTX (Rat)	31.0 ± 4.3	~1.0	604 ± 68	123 ± 36 80% decrease
Intraganglionic RTX (Monkey)	19.3 ± 2.5	1.4 ± 0.8	38.3 ± 6.8*	7.3 ± 1.6* 81% decrease