Chapter 18
Selective Ablation of Nociceptive Neurons for Elimination of Hyperalgesia and Neurogenic Inflammation

Gabriel C. Tender, M.D., Stuart Walbridge, B.S., Zoltan Olah, Ph.D., Laszlo Karai, M.D., Michael Iadarola, Ph.D., Edward H. Oldfield, M.D., and Russell R. Lonser, M.D.

INTRODUCTION

New treatment approaches directed at mechanisms of neuropathic pain have led to the description of the vanilloid receptor 1 (VR1) (2), a molecular integrator for pain (8). Nociceptive (Type 2 Aδ-fiber and C-fiber) neurons expressing this receptor mediate hyperalgesia, neurogenic inflammation, and neuropathic pain (5, 7). Resiniferatoxin (RTX) is a VR1 agonist that induces calcium-mediated cell death within minutes of binding to VR1-expressing neurons (6). Because VR1 is expressed only by this subset of nociceptive neurons, targeting and RTX-induced ablation of these cells may permit selective elimination of pathological pain (4), while preserving tactile sensation (Aα-, Aβ-, and Type 1 Aδ-fiber neurons) and motor neurons. To analyze whether this unique selective toxicity for VR1-expressing neurons can be used to eliminate hyperalgesia and neurogenic inflammation without affecting normal tactile sensation and motor function, we infused RTX unilaterally in primate trigeminal ganglia.

MATERIALS AND METHODS

Resiniferatoxin

For trigeminal ganglion infusion, 0.1 mg/ml of RTX solution containing Tween 80 and 0.05% ascorbic acid in phosphate buffered saline (PBS) was used.

Survival Surgery

Four adult Rhesus primates underwent a right middle-fossa surgical approach for intraganglionic infusion of RTX (n = 3) or vehicle (n = 1). Animals were induced, intubated, and placed under general endotracheal anesthesia. The temporal scalp was incised in a U-shaped fashion, and a myocutaneous flap was based over the pinna of the ear. A craniectomy was performed, centered over the external auditory canal. The dura mater was elevated off of the cranium base. Under direct visualization, a blunt 32-gauge needle was introduced 3 to 4 mm into the trigeminal ganglion. Convective infusion (4 μl/min) of 20 μl of RTX or vehicle was performed (1). After completing the infusion, the needle was removed and the musculocutaneous flap was closed in layers. The animal was awakened and extubated. All animals were observed twice daily for medical problems or neurological deficits throughout the study period (up to 3 months).

Sensory Testing

A short-term survival (4 wk) animal (experimental n = 1) was tested at 1 week postoperatively. Long-term survival (12 weeks) animals (experimental n = 2, control n = 1) were tested at 1, 4, and 7 weeks postoperatively. The animals were transferred from their housing cage to a transparent observation cage where they could be observed from

various angles by multiple observers for accurate data accrual (i.e., number of blinks, number of eye wipes, and duration of squinting) during testing.

All animals underwent specific testing of trigeminal ganglion–mediated corneal tactile sensation and hyperalgesia. Normal corneal sensation was assessed after the animal was momentarily immobilized in the observation cage and 0.5 ml of normal saline at room temperature (18ºC) was applied to the right eye. Immediately after application, the animal was released and the number of blinks during a 3-minute period was recorded. Testing was repeated in the left eye in an identical manner. Corneal hyperalgesia was assessed after the animal was momentarily immobilized in the observation cage and 0.5 ml of 0.01% capsaicin was applied to the right eye. The number of blinks, the number of eye wipes, and the duration of squinting (in seconds) during a 3-minute period were recorded. Testing was repeated for the left eye in an identical manner.

Neurogenic Inflammation Testing

Immediately before being killed, animals were tested for neurogenic inflammation. Under anesthesia, all facial hair was removed with Nair (Carter Products, New York, NY) and 3% capsaicin cream was applied to the entire scalp and face. Ten minutes after application of the capsaicin cream, 1 mg/kg of 0.1% Evans Blue solution in PBS was administered intravenously. Evans Blue extravasation (indicating nociceptive neuron–mediated neurogenic inflammation) was recorded photographically.

Gross and Histological Analyses

Animals were killed by an intravenous overdose of 90 mg/kg of pentobarbital at either 4 weeks (n = 1) or 12 weeks (n = 3). All animals were perfused with PBS followed by 4% paraformaldehyde. The eyes and trigeminal ganglia were removed bilaterally and placed in 4% paraformaldehyde. Five-micron-thick axial sections throughout the entire trigeminal ganglia were cut on a cryostat after fixation in paraffin. These sections were stained with hematoxylin and eosin, or used for immunohistochemical analysis.

Immunohistochemical Analysis

The VR1-expressing cells in the trigeminal ganglia were identified by immunohistochemistry using a 1:500 dilution of a human anti-VR1 antibody (Affinity Bioreagents, Golden, CO). The number of VR1-immunoreactive cells was analyzed for the bilateral trigeminal ganglia. Cell counts of VR1-immunoreactive cells were obtained from 10 randomly chosen high-power fields from the trigeminal ganglia of each side.

Statistical Analysis

Statistical analysis was performed using commercially available software, and Student’s paired t test with a P value threshold of less than 0.05 was used to determine statistical significance.

RESULTS

Sensory Testing

Normal Tactile Sensation

Consistent with the preservation of tactile sensation, there was no difference in response to corneal saline application, as analyzed by the number of blinks on the RTX-treated, vehicle-treated, or untreated sides (mean number of blinks ¡Ó standard deviation, SD: 18.8 ¡Ó 2.3, 18.0 ¡Ó 2.6, and 18.4 ¡Ó 3.3, respectively; $P > 0.05$; Fig. 18.1A).

Hyperalgesia

Consistent with the elimination of nociceptive neuron¡Vmediated hyperalgesia, there was a dramatic reduction in the number of blinks after corneal application of capsaicin on the side of RTX-treated ganglia compared with the vehicle-treated and untreated sides (mean number of blinks ¡Ó SD: 25.7 ¡Ó 4.4 versus 106.6 ¡Ó 20.8 and 112.8 ¡Ó 19.7, respectively; $P < 0.001$; Fig. 18.1B). This effect was substantiated by the significant reduction in the number of eye wipes on the side of the RTX-treated ganglia compared with the vehicle-treated and untreated sides (mean number of eye wipes ¡Ó SD: 1.4 ¡Ó 0.8 versus 19.3 ¡Ó 2.5 and 16.7 ¡Ó 2.7, respectively; $P < 0.001$; Fig. 18.1C). A significant reduction in the duration of squinting further supported the decreased hyperalgesic response on the side of the RTX-treated ganglia compared with the vehicle-treated and untreated sides (mean duration of squinting: 1.4 ¡Ó 0.6 s versus 11.4 ¡Ó 1.6 s and 14.8 ¡Ó 1.7 s, respectively; $P < 0.01$; Fig. 18.1D). These effects were durable and sustained throughout the postoperative period ($P < 0.01$; Fig. 18.1E).

Clinical Evaluation

All animals were ambulatory within 4 hours and resumed normal feeding habits within 12 hours of surgery. None of the animals had any neurological deficits or other clinical signs of toxicity throughout the duration of observation (up to 3 months). Specifically, mastication was not affected and animals did not experience weight loss during the study period. Symmetrical and appropriate eye blinking was preserved in all animals.

Neurogenic Inflammation

Because activation of nociceptive neurons leads to the release of neurotransmitters (substance P, calcitonin gene¡Vrelated peptide, adenosine triphosphate) that promote inflammation by the release of mediators (bradykinin, serotonin, protons, and others) from neighboring non-neuronal cells and vascular tissue, resulting in neurogenic inflammation, we tested the animals to see whether this phenomenon was blocked by RTX. To elicit this response over the entire trigeminal-innervated territory, we applied 3% capsaicin to the scalp and face of control ($n = 1$) and experimental ($n = 3$) animals just before killing (12 wk after treatment). A cutaneous inflammatory response occurred within 10 minutes of application, as evidenced by the reddening of the entire face and scalp in the control animal, and selective reddening of the hemiface and scalp on the untreated side in experimental animals. Intravenous injection of Evans Blue (1 mg/kg) confirmed and further delineated the regions of neurogenic inflammation by extravasating into the inflamed areas (Fig. 18.1F).

Gross and Histological Findings

Gross and histological examination of the corneas revealed no evidence of keratitis or abrasions. Examination of the oral mucosa showed no signs of injury. Histology showed preservation of the normal architecture of the RTX-treated trigeminal ganglia and lack of non-specific toxicity.

Immunohistochemistry

VR1-immunohistochemistry (Fig. 18.2A and B) revealed uniform ablation of VR1-immunoreactive neurons (mean decrease, 79.3 ¡Ó 2.8%) in the RTX-treated ganglia compared with the vehicle-treated and untreated ganglia (mean number of VR1-immunoreactive cells per high-power field ¡Ó SD: 7.3 ¡Ó 1.6 versus 38.3 ¡Ó 6.8 and 40.6 ¡Ó 6.1, respectively; $P < 0.001$; Fig. 18.2C). There was no difference between vehicle-treated and untreated ganglia in the control animal ($P > 0.05$).

DISCUSSION

Sensory neurons can be divided into four types: Aƒ Ñ-, Aƒ Ò-, Aƒ Ô-, and C-fiber neurons (3). Aƒ Ñ-fiber and Aƒ Ò-fiber neurons mediate normal sensation (e.g., proprioception and light touch), whereas Aƒ Ô-fiber and C-fiber neurons primarily mediate noxious stimuli and are defined as nociceptive neurons (Fig. 18.3). On the basis of electrophysiological characteristics, the Aƒ Ô-fiber neurons are subdivided into two types (5). Type 1 Aƒ Ô-fiber neurons do not express VR1 and have a high threshold for heat activation, whereas Type 2 Aƒ Ô-fiber neurons express VR1 and have a low threshold for heat activation. Because C-fiber and Type 2 Aƒ Ô-fiber neurons express VR1, they are selectively ablated by intraganglionic RTX infusion, which eliminates hyperalgesia, neurogenic inflammation, and the cellular mediators of neuropathic pain. Because Aƒ Ò-fiber and Type 1 Aƒ Ô-fiber neurons do not express VR1, they are not destroyed by RTX infusion, and normal tactile sensation, perception of noxious heat, and acute pain sensation are preserved.

These results demonstrate that perfusion of trigeminal ganglia with RTX effectively and selectively ablates VR1-immunoreactive nociceptive neurons and eliminates hyperalgesia and neurogenic inflammation, while maintaining normal tactile sensation and motor function. Thus, intraganglionic perfusion with RTX may provide a new site-specific, physiologically based treatment for neuropathic pain syndromes, including trigeminal neuralgia.

References

1. Bobo RH, Laske DW, Akbasak A, Morrison PF, Dedrick RL, Oldfield EH: Convection-enhanced delivery of macromolecules in the brain. Proc Natl Acad Sci U S A 91:2076¡V2080, 1994.
2. Caterina MJ, Schumacher MA, Tominaga M, et al: The capsaicin receptor: A heat-activated ion channel in the pain pathway. Nature 389:816¡V824, 1997.
3. Julius D, Basbaum AI: Molecular mechanisms of nociception. Nature 413:203¡V210, 2001.
4. Karai L, Brown DC, Mannes AJ, Connelly ST, Brown J, Gandal M, Wellisch OM, Neubert JK, Olah Z, Iadarola MJ: Deletion of vanilloid receptor 1-expressing primary afferent neurons for pain control. J Clin Invest 113:1344¡V1352, 2004.
5. Nagy I, Rang H: Noxious heat activates all capsaicin-sensitive and also a sub-population of capsaicin-insensitive dorsal root ganglion neurons. Neuroscience 88:995¡V997, 1999.
6. Olah Z, Szabo T, Karai L, Hough C, Fields RD, Caudle RM, Blumberg PM, Iadarola MJ: Ligand-induced dynamic

membrane changes and cell deletion conferred by vanilloid receptor 1. J Biol Chem 276:11021¡V11030, 2001.
7. Szallasi AB, Blumberg PM: Resiniferatoxin, a phorbol-related diterpene, acts as an ultrapotent analog of capsaicin, the irritant constituent in red pepper. Neuroscience 30:515¡V520, 1989.
8. Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D: The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21:531¡V543, 1998.

<DFIG>

FIG. 18.1 Sensory and neurogenic inflammation testing. A, there was no difference in mean (all testing) number of blinks during 3 minutes after corneal application of saline on the RTX-treated, vehicle-treated, and untreated sides ($P > 0.05$; error bars, SD). Corneal hyperalgesia was blocked on the RTX-treated side, as demonstrated by a significant reduction ($P < 0.001$) in the mean (all testing); B, number of blinks; C, number of eye wipes; and D, duration of squinting (in seconds) during 3 minutes after application of capsaicin. E, elimination of hyperalgesic sensation on the RTX-treated side (bottom), compared with the untreated side (top) was durable during the study ($n = 2$; mean blinks after capsaicin application; $P < 0.001$). F, Evans Blue extravasation (blue), demonstrating nociceptive neuron-mediated neurogenic inflammation, was blocked on the RTX-treated side (right-side) of the face and scalp (white).
FIG. 18.2 Immunohistochemistry for VR1. Corresponding sections from the A, untreated and B, RTX-treated trigeminal ganglia showing selective ablation of VR1-immunoreactive neurons. C, the mean number of VR1-immunoreactive cells per high-power field was reduced in the RTX-treated ganglia compared with the untreated ganglia ($n = 2$; $P < 0.001$; error bars, SD).
FIG. 18.3 Schematic drawing demonstrating the various types of sensory neurons (A*f*Ò-, A*f*Ô-, and C-fiber neurons), the presence of VR1 expression, and sensory modalities mediated by the specific neuronal subtypes. A*f*Ô-fiber neurons are divided into two types (Type 1 and 2) on the basis of their expression of VR1. Nociceptive neurons (A*f*Ô- and C-fiber neurons) expressing VR1 receptors can be selectively destroyed using the excitotoxic agonist, RTX.