2009

Ranolazine attenuates behavioral signs of neuropathic pain

Harry J Gould III, *Louisiana State University Health Sciences Center*

Colleen J Garrett

Renee R. Donahue, *University of Kentucky*

Dennis Paul, *Louisiana State University Health Sciences Center*

Ivan Diamond, et al.

Ranolazine attenuates behavioral signs of neuropathic pain

Harry J. Gould III^{a,c,d}, Colleen Garrett^e, Renee R. Donahue^{e,g}, Dennis Paul^{b,c,d}, Ivan Diamond^f and Bradley K. Taylor^{e,g}

Ranolazine modulates the cardiac voltage-gated sodium channel (Na_v 1.5) and is approved by the FDA in the treatment of ischemic heart disease. Ranolazine also targets neuronal (Na_v 1.7, 1.8) isoforms that are implicated in neuropathic pain. Therefore, we determined the analgesic efficacy of ranolazine in a preclinical animal model of neuropathic pain. Both intraperitoneal and oral administration of ranolazine dose-dependently inhibited the mechanical and cold allodynia associated with spared nerve injury, without producing ataxia or other behavioral side effects. These data warrant clinical investigation of the potential use of ranolazine in the treatment of neuropathic pain. *Behavioural Pharmacology* 00:000–000 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Behavioural Pharmacology 2009, 00:000–000

Keywords: allodynia, hyperalgesia, Na_v 1.7, Na_v 1.8, rat, spared nerve injury

Departments of ^aNeurology, ^bPharmacology, ^cAnesthesiology, ^dPain Mastery Center of Louisiana, Louisiana State University Health Sciences Center, New Orleans, Louisiana, ^eDepartment of Pharmacology, Tulane University Health Sciences Center, ^fGilead Sciences, Palo Alto, California and ^gDepartment of Physiology, University of Kentucky Medical Center, Lexington, Kentucky, USA

Correspondence to Professor Bradley K. Taylor, Department of Physiology, MS508, University of Kentucky Medical Center, Lexington, KY 40536-0298, USA
E-mail: brad.taylor@uky.edu

Received 31 May 2009 Accepted as revised 12 August 2009

Introduction

Ranolazine (Ranexa; Gilead Sciences, Palo Alto, California, USA) was recently approved by the FDA as a cardiac, anti-ischemic, agent (Nash, 2005; Marx and Sweeney, 2006; Pham and Mehta, 2007; Sossalla *et al.*, 2008). Ranolazine blocks skeletal muscle [voltage-gated sodium channel (Na_v 1.4)], cardiac muscle (Na_v 1.5), and neuronal (Na_v 1.7, 1.8) sodium channel subtypes and produces a profound use-dependent blockade of the tetrodotoxin-sensitive, late sodium channel current associated with Na_v 1.7 (Ju *et al.*, 1996a, 1996b; Haufe *et al.*, 2005; Belardinelli *et al.*, 2006; Hale *et al.*, 2008; Rajamani *et al.*, 2008; Wang *et al.*, 2008). Many patients spontaneously report a significant reduction in characteristic signs of neuropathic pain, including burning dysesthesia, in the lower extremities when treated with ranolazine for ischemic heart disease (C. Walker, personal communication). As recent studies link sodium channels to inflammatory pain (Gould *et al.*, 2000, 2004) and several inherited neuropathic pain states (Nassar *et al.*, 2004; Dib-Hajj *et al.*, 2005; Cox *et al.*, 2006; Fertleman *et al.*, 2006; Waxman, 2006; Dib-Hajj *et al.*, 2007; Drenth and Waxman, 2007; Goldberg *et al.*, 2007), we examined whether ranolazine could reduce the mechanical and cold hypersensitivity produced in a preclinical model of acquired peripheral neuropathic pain (Decosterd and Woolf, 2000).

Methods

Subjects

All experiments were conducted in accordance with protocols that were approved and monitored by the Tulane Health Sciences Center and the University of

Kentucky Institutional Animal Care and Use Committee. Male Sprague–Dawley rats, weighing 220–240 g (Harlan Sprague Dawley Inc., Indianapolis, Indiana, USA), were housed one animal per cage and maintained at 25°C and 60% humidity, on a 12 h light/dark cycle beginning at 6 a.m. and allowed free access to food and water.

Procedure

Rats were allowed to acclimate to the testing apparatus by placing them in Plexiglas boxes on a stainless steel grid for 30–60 min. Baseline determinations of responses to mechanical or cold stimuli applied to the lateral aspect of the plantar surface of the hindpaw were measured using von Frey (VF) monofilaments (Stoelting Inc., Wood Dale, Illinois, USA) or acetone, respectively (Decosterd and Woolf, 2000; Taylor *et al.*, 2007; Intondi *et al.*, 2008). Initially, mechanical thresholds were assessed. Hindpaws were stimulated on the lateral aspect of the plantar surface within the distribution of the sural nerve with a series of eight VF monofilaments of logarithmic stiffness. A modified version of the 50% withdrawal threshold was determined using the up–down method of Dixon, modified by Chaplan *et al.* (1994). First, an intermediate monofilament (number 4.31, exerts 2.0 g of force) was gently applied with enough force to bend it. This was repeated up to three times in distinct areas along the lateral paw. In case of a positive response (rapid withdrawal of the paw within 2 s), a smaller filament was tested. If no response was recorded in any of the three different areas, a larger filament was tested.

Paw withdrawal responses to cold stimuli were then measured using an acetone-loaded syringe connected to

PE-90 tubing, flared at the tip to a diameter of 3.5 mm. Surface tension maintained the delivery volume of the acetone drop to 10–12 μ l. The length of time the animal lifted or shook its paw was recorded for the first 30 s. Three observations were averaged.

After the determination of preinjury responses, anesthesia was induced in rats weighing approximately 250 g with 5% isoflurane and maintained for surgical manipulation with 2–3% isoflurane. An incision was made in the skin at the level of the trifurcation of the left sciatic nerve. The overlying muscles were retracted, exposing the common peroneal, tibial, and sural nerves. With care taken to avoid mechanical stimulation of the sural branch, the common peroneal and tibial nerves were ligated with 6.0 silk (Ethicon, Somerville, New Jersey, USA), followed by transection of the intervening 1 mm segment of nerve adjacent to both the proximal and distal ligatures. The muscle was sutured with 5-0 sutures, and the skin was closed with 9 mm metal clips. Sensitivity to stimulation was reassessed 7–14 days postoperatively. Rats that showed signs of allodynia received either vehicle [intraperitoneal (i.p.) injection – propylene glycol and dichloromethane; oral gavage – distilled water titrated to pH 4.0 with dilute acetic acid and sodium hydroxide] or ranolazine (10, 30, and 100 mg/kg), administered in an experimenter-blinded, randomized design. Animals were again tested 15, 30, 60, and 90 min (i.p.) or 30, 60, 90, 120, and 180 min (orally) after drug administration. Occasionally, animals did not develop mechanical allodynia (VF threshold >5.0 g on the nerve-injured side) or exhibit cold allodynia on the day of pharmacological testing after nerve injury. In such cases (5%), testing was either terminated or the data were not included in the final analysis.

To evaluate possible ataxic effects of ranolazine administration, the ability of unoperated animals to remain on an accelerating rotarod was assessed before and after i.p. administration. Rats were placed on the rotarod, set at an initial rotating speed of 4 rpm. Immediately thereafter, speed was increased at a rate of 0.5 rpm every 5 s to a maximum of 40 rpm. Each rat was trained in this procedure three to nine times, until latency to fall was approximately 180 s. Animals that failed to remain on the rotarod for at least 100 s (two) or that remained on the rotarod for more than 300 s (one) were excluded from the study. Testing of a vehicle group ($n = 4$), 30 mg/kg group ($n = 6$), or 100 mg/kg ranolazine group ($n = 5$) began within 1–2 days of training. Triplicate measurements were made before and 30, 60, 90, 120, 150, and 180 min after injection. Values are represented as mean $\pm$ SEM.

Statistical analysis

Significance was determined with a mixed design, two-way analysis of variance with 'drug' as the between-subjects factor and 'time' as the repeated measure. If the

overall F value was significant, further Bonferroni post-hoc tests were conducted to determine the effect of 'drug' relative to vehicle at each time point.

Results

Spared nerve injury increased sensitivity to mechanical and cold stimulation within the ipsilateral sural nerve distribution. As shown in Fig. 1, i.p. administration of ranolazine dose-dependently reduced both mechanical allodynia [$F(3,115) = 5.7$, $P < 0.005$] and cold allodynia [$F(3,115) = 4.3$, $P < 0.05$]. These antiallodynic effects peaked at 15–30 min and lasted for 60–90 min. Vehicle had no effect.

Only the oral route of administration of ranolazine has been approved by the FDA. Therefore, we determined whether oral ranolazine would also reduce spared nerve injury-induced allodynia. As shown in Fig. 2, oral ranolazine dose-dependently reduced both mechanical

Fig. 1

Intraperitoneal (i.p.) administration of ranolazine dose-dependently increases paw withdrawal threshold to a mechanical stimulus (a) and reduces response duration to a cool stimulus (b). Values are represented as mean $\pm$ SEM. The number of animals per group is indicated within the parentheses. * $P < 0.05$ saline versus 100 mg/kg group; * $P < 0.05$ saline versus 100 and 30 mg/kg group; * $P < 0.05$ saline versus 100, 30, and 10 mg/kg group. SNI, spared nerve injury; VF, von Frey.

Fig. 2

Oral administration of ranolazine dose-dependently increases paw withdrawal threshold to a mechanical stimulus (a) and reduces response duration to a cool stimulus (b). Values are represented mean $\pm$ SEM. The number of animals per group is indicated within the parentheses. $^+P < 0.05$ saline versus 100 mg/kg group; $*P < 0.05$ saline versus 100 and 30 mg/kg group; $^*P < 0.05$ saline versus 100, 30, and 10 mg/kg group. SNI, spared nerve injury; VF, von Frey.

allodynia [$F(3,114) = 5.1$, $P < 0.01$] and cold allodynia [$F(3,108) = 4.5$, $P < 0.05$]. These effects peaked at 90 min and lasted for 90–180 min. Vehicle had no effect.

Nonselective sodium channel blockers are an integral part of the pharmacotherapy for peripheral neuropathic pain; however, severe side effects limit their use. Throughout these studies with 10–30 mg/kg ranolazine, we observed no overt signs of adverse effects. To evaluate more subtle effects on behavior, we assessed performance on an accelerating rotarod. Before drug testing, baseline latency was balanced between the three groups (vehicle, 193 ± 10 s; 30 mg/kg, 188 ± 6 s; 100 mg/kg, 189 ± 14 s). Analysis of variance did not show any effect of oral ranolazine treatment [$F(2,60) = 0.6$, NS] at any time point throughout the 3-h test session ($P > 0.05$). For example, when tested at the peak of antihyperalgesic effect (90 min, Fig. 2), rotarod latency in the vehicle, 30, and 100 mg/kg groups was 205 ± 28 s, 225 ± 17 s, and

207 ± 14 s, respectively. These data indicate that oral ranolazine did not produce ataxia.

Discussion

Ranolazine is approved by the FDA in the treatment of ischemic cardiac disease and angina at a dose of 500–1000 mg twice daily (approximately 2000 mg/day for a 70 kg patient). The present experiments used this dosing range in a preclinical model of neuropathic pain. We report that ranolazine reduces nerve injury-induced mechanical allodynia and cold hypersensitivity.

Systemic administration of nonselective sodium channel blockers, such as lidocaine, clearly reduce behavioral signs of neuropathic pain in humans (Backonja, 2001; Devor, 2006). Owing to ubiquitous distribution of the nine known sodium channels in many tissues of the body and the essential role of these channels in the function of excitable membranes, however, recipients risk dangerous cardiac and central nervous system effects. For example, lidocaine produces cardiac arrhythmia and ataxia at the same dosages required to yield analgesia in neuropathic pain models (Challapalli *et al.*, 2005; Gil-Gouveia and Goadsby, 2009). Potentially, these risks could be minimized by using agents that influence just a subset of sodium channel subtypes. One such agent is ranolazine, the actions of which seem restricted to Na_v 1.4, 1.5, 1.7, and 1.8. In this study, ranolazine exhibited antiallodynic efficacy at doses that do not produce significant adverse effects. We suggest that ranolazine may provide a better option for treating neuropathic pain with a greater therapeutic window of efficacy than is currently available with broad-spectrum sodium channel blockers. This remains to be studied in controlled clinical trials.

Neuropathic pain is a multifaceted condition that is driven by a constellation of complex central and peripheral mechanisms. The pharmacotherapy of clinical neuropathic pain is highly variable, perhaps because of large differences in selectivity of a particular medication for a particular pain modality (Zimmerman, 2001; Erichsen *et al.*, 2003; Paul *et al.*, 2008). We speculate that the antiallodynic actions of ranolazine are mediated, at least in part, by Na_v 1.7 and Na_v 1.8 sodium channels located on primary sensory neurons of the peripheral nervous system. Interestingly, in this study, dose–response curves show that ranolazine more potently reduced cold allodynia as compared with mechanical allodynia. For example, 10 mg/kg ranolazine essentially reversed cold allodynia but only partially reduced mechanical allodynia. We speculate that ranolazine is more effective at reducing peripheral mechanisms that drive cold allodynia (such as loss of A- δ suppression of C-fiber input to lamina 1) than those that drive mechanical allodynia (such as enhancement of spontaneous oscillatory activity in A- β input) (Bennett, 1994; Koltzenburg, 1996; Amir *et al.*, 1999,

2002a, 2002b). Further investigation is warranted to determine whether ranolazine could also be an important new option in the management of the cold or mechanical allodynia that is associated with peripheral nerve injury.

Acknowledgements

H.J.G. and D.P. are supported by a grant from Gilead Sciences and by the LSU Health Sciences Center Departments of Neurology and Pharmacology. B.K.T. was supported by grants 5R29DA010356-04, 5K02DA 019656-03, and 5R01 NS045954-04.

Conflicts of interest: H.J.G. is a research consultant for Gilead Sciences. I.D. is Vice President for Neuroscience for Gilead Sciences.

References

- Amir R, Michaelis M, Devor M (1999). Membrane potential oscillations in dorsal root ganglion neurons: role in normal electrogenesis and neuropathic pain. *J Neurosci* **19**:8559–8596.
- Amir R, Michaelis M, Devor M (2002a). Burst discharge in primary sensory neurons: triggered by subthreshold oscillations, maintained by depolarizing afterpotentials. *J Neurosci* **22**:1187–1198.
- Amir R, Liu C-N, Kocsis JD, Devor M (2002b). Oscillatory mechanism in primary sensory neurons. *Brain* **125**:421–435.
- Backonja M (2001). Anticonvulsants and antiarrhythmics in the treatment of neuropathic pain syndromes. In: Hansson PT, Fields HL, Hill RG, Marchettini P, editors. *Neuropathic pain: pathophysiology and treatment, progress in pain research and management (Vol 21)*. Seattle: IASP Press. pp. 285–202.
- Belardinelli L, Shryock JC, Fraser H (2006). Inhibition of the late sodium current as a potential cardioprotective principle: effects of the late sodium current inhibitor ranolazine. *Heart* **92**:6–14.
- Bennett GJ (1994). Neuropathic pain. In: Wall PD, Melzack R, editors. *Textbook of pain (Vol 3)*. Edinburgh UK: Churchill Livingstone. pp. 201–224.
- Challapalli V, Tremont-Lukats IW, McNicol ED, Lau J, Carr DB (2005). Systemic administration of local anesthetic agents to relieve neuropathic pain. *Cochrane Database Syst Rev* **19**:CD003345.
- Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL (1994). Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods* **53**:55–63.
- Cox JJ, Reimann F, Nicholas AK, Thornton G, Roberts E, Springell K, et al. (2006). An SCN9A channelopathy causes congenital inability to experience pain. *Nature* **444**:894–898.
- Decosterd I, Woolf C (2000). Spared nerve injury: an animal model of persistent peripheral neuropathic pain. *Pain* **87**:149–158.
- Devor M (2006). Sodium channels and mechanisms of neuropathic pain. *J Pain* **7**:S3–S12.
- Dib-Hajj SD, Rush AM, Cummins TR, Hisama FM, Novella S, Tyrrell L, et al. (2005). Gain-of-function mutation in Nav 1.7 in familial erythromelalgia induces bursting of sensory neurons. *Brain* **128**:1847–1854.
- Dib-Hajj SD, Cummins TR, Black JA, Waxman SG (2007). From genes to pain: Nav 1.7 and human pain disorders. *Trends Neurosci* **30**:555–563.
- Drenth JPH, Waxman SG (2007). Mutations in sodium-channel gene SCN9A cause a spectrum of human genetic pain disorders. *J Clin Invest* **117**:3603–3609.
- Erichsen HK, Hao JX, Xu XJ, Blackburn-Munro G (2003). A comparison of the antinociceptive effects of voltage-activated Na⁺ channel blockers in two rat models of neuropathic pain. *Eur J Pharmacol* **458**:275–282.
- Fertleman CR, Baker MD, Parker KA, Moffatt S, Elmslie FV, Abrahamsen B, et al. (2006). SCN9A mutations in paroxysmal extreme pain disorder: allelic variants underlie distinct channel defects and phenotypes. *Neuron* **52**:767–774.
- Gil-Gouveia R, Goadsby PJ (2009). Neuropsychiatric side-effects of lidocaine: examples from the treatment of headache and a review. *Cephalalgia* **29**:496–508.
- Goldberg YP, MacFarlane J, MacDonald ML, Thompson J, Dube MP, Mattice MP, et al. (2007). Loss-of-function mutations in the Nav 1.7 gene underlie congenital indifference to pain in multiple human populations. *Clin Genet* **71**:311–319.
- Gould HJ III, Gould TN, England JD, Paul D, Liu ZP, Levinson SR (2000). A possible role for nerve growth factor in the augmentation of sodium channels in models of chronic pain. *Brain Res* **854**:19–29.
- Gould HJ III, England JD, Soignier RD, Nolan P, Minor LD, Liu ZP, et al. (2004). Ibuprofen blocks changes in Nav 1.7 and 1.8 sodium channels associated with complete Freund's adjuvant-induced inflammation in rat. *J Pain* **5**:270–280.
- Hale SL, Shryock JC, Belardinelli L, Sweeney M, Kloner RA (2008). Late sodium current inhibition as a new cardioprotective approach. *J Mol Cell Cardiol* **44**:954–967.
- Haufe V, Camacho JA, Dumaine R, Günther B, Bollensdorff C, Von Banchet GS, et al. (2005). Expression pattern of neuronal and skeletal muscle voltage-gated Na⁺ channels in the developing mouse heart. *J Physiol* **564**:683–696.
- Intondi AB, Dhalgren MN, Eilers MA, Taylor BK (2008). Intrathecal neuropeptide Y reduces behavioral and molecular markers of inflammatory or neuropathic pain. *Pain* **137**:352–365.
- Ju YK, Gage PW, Saint DA (1996a). Tetrodotoxin-sensitive inactivation-resistant sodium channels in pacemaker cells influence heart rate. *Pflügers Arch* **431**:868–875.
- Ju YK, Saint DA, Gage PW (1996b). Hypoxia increases persistent sodium current in rat ventricular myocytes. *J Physiol* **497**:337–347.
- Koltzenburg M (1996). Afferent mechanisms mediating pain and hyperalgesias in neuralgia. In: Jänig W, Stanton-Hicks M, editors. *Reflex sympathetic dystrophy: a reappraisal, progress in pain research and management (Vol 6)*. Seattle: IASP Press. pp. 123–150.
- Marx C, Sweeney M (2006). Mechanism of action of ranolazine. *Arch Intern Med* **166**:1325–1326.
- Nash DT (2005). The case for medical treatment in chronic stable coronary artery disease. *Arch Intern Med* **165**:2587–2589.
- Nassar MA, Stirling LC, Forlani G, Baker MD, Matthews EA, Dickenson AH, et al. (2004). Nociceptor-specific gene deletion reveals a major role for Nav 1.7 (PN1) in acute and inflammatory pain. *Proc Natl Acad Sci USA* **101**:12706–12711.
- Paul D, Gould HJ III, Dunne-Reilly A (2008). Predictive validity of a model of peripheral nerve demyelination-induced neuropathic pain. 12th World Congress on Pain Abstract:PW40.
- Pham DQ, Mehta M (2007). Ranolazine: a novel agent that improves dysfunctional sodium channels. *Int J Clin Pract* **61**:864–872.
- Rajamani S, Shryock JC, Belardinelli L (2008). Block of tetrodotoxin-sensitive, Nav 1.7, and tetrodotoxin-resistant, Nav 1.8, Na⁺ channels by ranolazine. *Channels* **2**:449–460.
- Sossalla S, Wagner S, Rasenack EC, Ruff H, Weber SL, Schöndube FA, et al. (2008). Ranolazine improves diastolic dysfunction in isolated myocardium from failing human hearts—role of late sodium current and intracellular ion accumulation. *J Mol Cell Cardiol* **45**:32–43.
- Taylor BK, Abhyankar SS, Vo N-TT, Kriedt CL, Churi SB, Urban JH (2007). Neuropeptide Y acts at Y1 receptors in the rostral ventral medulla to inhibit neuropathic pain. *Pain* **131**:83–95.
- Wang GK, Calderon J, Wang S-Y (2008). State- and use-dependent block of muscle Nav 1.4, and neuronal Nav 1.7 voltage-gated Na⁺ channel isoforms by ranolazine. *Mol Pharmacol* **73**:940–948.
- Waxman SG (2006). A channel sets the gain on pain. *Nature* **444**:831–832.
- Zimmerman M (2001). Pathobiology of neuropathic pain. *Eur J Pharmacol* **429**:23–37.

AUTHOR QUERY FORM

**LIPPINCOTT
WILLIAMS AND WILKINS**

JOURNAL NAME: FBP

ARTICLE NO: 200385

QUERIES AND / OR REMARKS

QUERY NO.	Details Required	Author's Response
	No queries	