Rufinamide Attenuates Mechanical Alloodynia in a Model of Neuropathic Pain in the Mouse and Stabilizes Voltage-gated Sodium Channel Inactivated State

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ABSTRACT

Background: Voltage-gated sodium channels dysregulation is important for hyperexcitability leading to pain persistence. Sodium channel blockers currently used to treat neuropathic pain are poorly tolerated. Getting new molecules to clinical use is laborious. We here propose a drug already marketed as anticonvulsant, rufinamide.

Methods: We compared the behavioral effect of rufinamide to amitriptyline using the Spared Nerve Injury neuropathic pain model in mice. We compared the effect of rufinamide on sodium currents using in vitro patch clamp in cells expressing the voltage-gated sodium channel Nav1.7 isoform and on dissociated dorsal root ganglion neurons to amitriptyline and mexiletine.

Results: In naive mice, amitriptyline (20 mg/kg) increased withdrawal threshold to mechanical stimulation from 1.3 (0.6–1.9) (median [95% CI]) to 2.3 g (2.2–2.5) and latency of withdrawal to heat stimulation from 13.1 (10.4–15.5) to 30.0 s (21.8–31.9), whereas rufinamide had no effect. Rufinamide and amitriptyline alleviated injury-induced mechanical allodynia for 4 h (maximal effect: 0.10 ± 0.03 g (mean ± SD) to 1.99 ± 0.26 g for rufinamide and 0.25 ± 0.22 g to 1.92 ± 0.85 g for amitriptyline). All drugs reduced peak current and stabilized the inactivated state of voltage-gated sodium channel Nav1.7, with similar effects in dorsal root ganglion neurons.

Conclusions: At doses alleviating neuropathic pain, amitriptyline showed alteration of behavioral response possibly related to either alteration of basal pain sensitivity or sedative effect or both. Side-effects and drug tolerance/compliance are major problems with drugs such as amitriptyline. Rufinamide seems to have a better tolerability profile and could be a new alternative to explore for the treatment of neuropathic pain.

What We Already Know about This Topic

• Neuropathic pain remains a poorly treated chronic pain condition.
• Rufinamide is an antiepileptic drug that is thought to produce its effect against seizures through reducing sodium channel activity.

What This Article Tells Us That Is New

• Rufinamide may produce, in part, an effect against neuropathic pain by interfering with the sodium channel subtype Nav1.7 implicated in pain transmission.

PAIN is essential for survival as it serves as an alert to engage protective behavior. Neuropathic pain, caused by a lesion or disease of the somatosensory nervous system, affects 7% of the population1 and possesses no protective purpose.

Sodium channels are major targets for the development of new drug to treat neuropathic pain.2 Nerve injury changes the expression of sodium channels3 which affects peripheral nerve hyperexcitability and ectopic discharges along the nerve, in the dorsal root ganglion or at the injury site.4,5 They are composed of a α-pore forming subunit associated to one or two β-modulating subunits. Nine genes encode for the α-subunits, Nav1.1–1.9.6

Current therapy for neuropathic pain involves adjuvant medications—not primarily developed for this purpose—such as anticonvulsants, antidepressants, or local anesthetics.7 Tricyclic antidepressants are considered as first-line treatment in different international guidelines.8 Their mode of action does not seem to be linked to their antidepressant actions as acknowledged by their faster onset.9 Amitriptyline was shown to interact with sodium channels as exemplified by its
cardiac toxicity and this target could also play a role in pain modulation.\textsuperscript{10} Mexiletine, a sodium channel blocker and an oral analog of local anesthetics has been used in the treatment of neuropathic pain\textsuperscript{11} but its tolerance on long-term therapy raises considerable questions as shown by a median discontinuation of treatment of 43 days in a recent study.\textsuperscript{12} Rufinamide is an antiepileptic drug licensed for Lennox-Gastaut syndrome, a refractory type of epilepsy.\textsuperscript{13} It is considered to inhibit sodium channels, stabilizing its inactive form, and reducing the firing of sodium-dependent action potentials.

Since the discovery that loss-of-function mutations in SCN9A, the gene encoding for Nav1.7 isoform, are associated with congenital insensitivity to pain,\textsuperscript{14} it has become a potential target for treatment. Moreover, gain-of-function mutations SCN9A are associated with familial pain syndromes (erythromelalgia and paroxysmal extreme pain disorder)\textsuperscript{15} and in subset of patients with idiopathic small nerve fiber neuropathy or generalized pain syndromes.\textsuperscript{16–17} Nav1.7 is expressed in sensory, sympathetic, and myenteric fibers.\textsuperscript{18–20} It exhibits slower recovery from fast inactivation\textsuperscript{21,22} compared with other tetrodotoxin-sensitive channels Nav1.4 and 1.6 and slower inactivation at potentials close to the membrane resting potential, thus contributing to the large ramp current during slow depolarization.\textsuperscript{23} Nav1.7 is thought to play an important role in “boosting” the depolarization of small diameter nociceptive neurons.

In the present study, we investigated the analgesic effect of rufinamide on the spared nerve injury (SNI) model of neuropathic pain and amitriptyline was used as a positive control. Our null hypothesis was that treated and control groups show the same behavior. We also explored the effect of rufinamide on heterogeneously expressed Nav1.7 channels and used mexiletine and amitriptyline as control. We finally tested the effect of rufinamide on dorsal root ganglia neurons. For electrophysiological studies, our null hypothesis was that the drugs do not change the measured parameters, which were $V_{1/2}$ of activation and steady-state inactivation, frequency-dependent inhibition and $t_{1/2}$ of recovery from inactivation.

### Materials and Methods

#### Drugs

Rufinamide (R8404), amitriptyline (A8404), and mexiletine (M2727) were purchased from Sigma (Buchs, Switzerland). For behavioral experiment, rufinamide was dissolved in dimethylsulfoxide (DMSO) and then mixed with 1 × phosphate buffered saline to the desired concentration. Control was 30% DMSO in 1 × phosphate buffered saline. Doses (5, 10, 25, 50 mg/kg) were chosen according to the therapeutic ones used in epilepsy models in mice (rufinamide was effective in the maximal electroshock test (effective dose 23.9 mg/kg orally) and in the pentylenetetrazol induced seizure test (54 mg/kg, intraperitoneally)).\textsuperscript{24} Amitriptyline was dissolved directly in sterile 0.9% saline and doses were chosen according to previous studies in neuropathic pain models. Drugs were administered intraperitoneally.

#### Animal Experiments

All experiments were approved by the Committee on Animal Experimentation of the Canton de Vaud, Lausanne, Switzerland, in accordance with Swiss Federal law on animal care and the guidelines of the International Association for the Study of Pain.\textsuperscript{25} 5-week-old C57BL/6 male mice (Charles River, l’Abresle, France) weighting 20–25 g at the start of experiment were housed in the same room, 5 per cage, at constant temperature of 21°C and a 12/12 dark/light cycle. No other animals were housed in that room. Mice had ad libitum access to water and food.

#### Surgery

SNI surgery\textsuperscript{26–27} on mice\textsuperscript{28} was performed under 1.5–2.5% isoflurane (Abbott AG, Baar, ZG, Switzerland) anesthesia. Briefly, the left hindlimb was immobilized in a lateral position and slightly elevated. Incision was made at mid-thigh level using the femur as a landmark and a section was made through the biceps femoris in the direction of point of origin of the vascular structure. The three peripheral branches (sural, common peroneal, and tibial nerves) of the sciatic nerve were exposed without stretching nerve structures. Both tibial and common peroneal nerves were ligated using a 6.0 silk suture and transected together. The sural nerve was carefully preserved by avoiding any nerve stretch or nerve contact.

#### Behavior

For all the behavioral experiments, the observer was blinded to the treatment applied.

**Mechanical Sensitivity.** Animals were habituated to the testing environment daily for at least 2 days before baseline testing. The room temperature and humidity remained stable for all experiments. For testing mechanical sensitivity, animals were put under inverted plastic boxes on an elevated mesh floor and allowed 10 min for habituation before the threshold testing. Mechanical allodynia was tested using a series of von Frey hairs with logarithmically incrementing stiffness (0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, and 2.56 g). The filaments were applied perpendicularly to the plantar surface 1–2 s. The 50% withdrawal threshold was determined using Dixon’s up–down method.\textsuperscript{29} The cutoff was set at 30 s to avoid tissue damage.

Activity was quantified with the Activ-meter (Bioseb, Vitrolles, France). The total activity (summation of immobile, slow and fast activity given by the software) of naive animals in their home cage was measured during the 4 h...
following injection of rufinamide (50 mg/kg) and amitriptyline (10 mg/kg). It was compared with the activity after saline injection. All experiments for activity were performed between 5 and 9 PM.

A five-point sedation score from 0 to 4 points was used for rufinamide (50 mg/kg) and amitriptyline (10 mg/kg), 0 = normal behavior, normal locomotion, 1 = awake, slow locomotion, 2 = no locomotion, eyes half closed, still responding to righting reflex, 3 = asleep, eyes closed, still responding to righting reflex, 4 = no righting reflex, adapted from Boast et al.30

**Experimental Design**

For drug effect on naïve animals, eight animals per group were used to assess mechanical withdrawal threshold and heat withdrawal latency. For the Activ-meter, six animals were used in a cross-over design for rufinamide and amitriptyline.

Normal mechanical threshold was assessed before surgery without difference between groups. SNI surgery was performed and 1 week later allodynia-like behavior was tested before intraperitoneal injection of rufinamide. Two series of experiments were done, the first one compared rufinamide 25 mg/kg and 50 mg/kg with DMSO 30% (n = 10 per group, 9 for DMSO) and the second one compared rufinamide 5 mg/kg and 10 mg/kg with DMSO 30% (n = 8 per group) at 20-40-60-120-240 min and 24 h. After a washout period of 1 week the animals of the first series were tested with amitriptyline 10 or 20 mg/kg or saline at 60-120-240 min and 24 h after intraperitoneal injection (n = 9 per group for amitriptyline 20 mg/kg and 10 per group for amitriptyline 10 mg/kg and saline).

Plasma levels of the drug were assessed at 120 min after injection of 50 mg/kg rufinamide. Mice (n = 3) were anesthetized with isoflurane and 1 ml of blood was collected intracardially. Drug levels were analyzed by the pharmaceutical monitoring laboratory of Lavigny, Switzerland.

**Electrophysiology**

Rufinamide was dissolved in DMSO at 10 mM as stock solution and diluted daily at desired concentration in the extracellular medium. As control, the same DMSO concentration was used (1% for 100% for 100 mM, to 5% for 500 mM). Higher concentration could not be achieved without increasing DMSO content. Amitriptyline and mexiletine were dissolved in extracellular medium directly. Higher concentration was used (1% for 100 1% for 100 mM, to 5% for 100 5% for 500 mM). Percentage inhibition was calculated as (peak_{vehicle} - peak_{drug})/ peak_{vehicle} × 100 for each cell and then mean inhibition for each drug and concentration was calculated. Other protocols are shown as inserts in the figures. The linear ascending segment of the current-voltage relationship was used to estimate the reversal potential for each trace before obtaining the voltage-dependent activation curve. Voltage dependence of activation and steady-state inactivation curves were individually fitted with Boltzmann relationships, y(V_m) = 1/(1 + exp[(V_m - V_{1/2})/K]) in which y is the normalized current or conductance, Vm is the membrane potential, V_{1/2} is the voltage at which half of the channels are activated or inactivated, and K is the slope factor. The value of t_{1/2} of recovery from inactivation was calculated by interpolation from a linear relation between the two points juxtaposing half recovery (y_1 < 0.5 < y_2), using the relation x = (0.5 - [y_2 - y_1]x_1)/[x_2 - x_1]) × (x-x_2)/(y_2 - y_1). For use-dependent block, the percentage of decrease of current was calculated between the 1st and 50th pulse.

For *ex-vivo* recordings, dorsal root ganglion neurons were collected from adult C57BL/6 mice (4–8 weeks old). Briefly, L4 and L5 dorsal root ganglion neurons were harvested and digested in Liberase blendzyme thermolysin medium (Roche, Indianapolis, IN) 0.5 U/dorsal root ganglion with 12 μM EDTA in 5 ml Complete Saline Solution (in mM, NaCl 137, KCl 5.3, MgCl2-6H2O 1, Sorbitol 25, HEPES 10, CaCl2 3, and pH adjusted to 7.2 with NaOH) for 20 min at 37°C. Neurons were further digested with Liberase blendzyme TL with EDTA in Complete Saline Solution with papain (30 U/ml) for 10 min. Finally neurons were suspended in dorsal root ganglion medium mix (89% made at room temperature using pClamp software, version 10.2, and a VE-2 amplifier (Alembic Instruments, Montreal, Quebec, Canada). The sampling rate was 30 kHz. Data were smoothed and analyzed using Clampfit software version 10.2.0.12 (Axon Instruments, Union City, CA) and KaleidaGraph (Synergy Software, Reading, PA). Whole-cell patch clamp recordings were conducted using an internal solution containing (in millimole per liter (mM)) CsCl 60, Cesium aspartate 70, EGTA 11, MgCl2 1, CaCl2 1, HEPES 10, and Na2-adenosine triphosphate 5, pH adjusted to 7.2 with CsOH; and an external solution containing NaCl 50, n-methyl-D-glutamine-Cl 80, CaCl2 2, MgCl2 1.2, CsCl 5, HEPES 10, and glucose 5, pH adjusted to 7.4 with CsOH. Holding potential was −100 mV. The values were not corrected for liquid junction potential. Pipette resistance was ranging from 2 to 4 MOhm. Only data from cells having stable access resistance over the duration of the experiment were used; cells for which signs of poor voltage-clamp control, such as delayed inflections of the current or discontinuities in the peak sodium current (I max) versus Vm curve, were not analyzed. Around 15% of sealed cells were lost. Data were filtered after acquisition using Boxcar 9 points. Peak currents were measured with a single 10 ms pulse protocol to −10mV from the holding potential. Percentage inhibition was calculated as peak_{vehicle} - peak_{drug}/ peak_{vehicle} × 100 for each cell and then mean inhibition for each drug and concentration was calculated. Other protocols are shown as inserts in the figures. The linear ascending segment of the current-voltage relationship was used to estimate the reversal potential for each trace before obtaining the voltage-dependent activation curve. Voltage dependence of activation and steady-state inactivation curves were individually fitted with Boltzmann relationships, y(V_m) = 1/(1 + exp[(V_m - V_{1/2})/K]) in which y is the normalized current or conductance, Vm is the membrane potential, V_{1/2} is the voltage at which half of the channels are activated or inactivated, and K is the slope factor. The value of t_{1/2} of recovery from inactivation was calculated by interpolation from a linear relation between the two points juxtaposing half recovery (y_1 < 0.5 < y_2), using the relation x = (0.5 - [y_2 - y_1]x_1)/[x_2 - x_1]) × (x-x_2)/(y_2 - y_1). For use-dependent block, the percentage of decrease of current was calculated between the 1st and 50th pulse.

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DMEM/F-12, 10% bovine serum albumin, 1% penicillin/streptomycin) supplemented with 1.5 mg/ml of trypsin inhibitor and 1.5 mg/ml of purified bovine serum albumin. Mechanical dissociation was performed using a pipetman and neurons were plated on poly-D-lysine-coated coverslips and incubated 12 h before recording to allow recovery and adhesion of neurons. Neurons were only recorded for 12 more hours to prevent long-term culture phenotypic changes and neurite outgrowth that degrades space clamp. Small neurons (diameter < 30 µm) were recorded using an EPC-10 amplifier (HEKA Electronics, Lambrecht, Germany) and Patchmaster/Fitmaster software for data acquisition/analysis. The sampling interval was 20 µs and a 5 kHz filter was used in all experiments. Experiments were carried out in the whole-cell patch clamp configuration. Extracellular solution contained (in mM) NaCl 30, tetraethylammonium—Cl 110, KCl 3, CaCl$_2$ 1, MgCl$_2$ 1, HEPES 10, Glucose 10, CdCl$_2$ 0.1; pH was adjusted to 7.3 using Tris base, osmolarity was adjusted to 320 mOsm/l with sucrose. The pipette solution contained cerebrospinal fluid 140, NaCl 10, MgCl$_2$ 2, CaCl$_2$ 0.1, EGTA 1.1, HEPES 10, pH was adjusted to 7.2 with CsOH and osmolarity was adjusted to 310 mOsm/l. Pipettes were pulled from Borosilicate glass (World Precision Instruments, Sarasota, FL) and had a resistance < 3 MΩm, when filled with the pipette solution. Capacity transients were canceled and series resistance was compensated to around 90%. Leakage current was digitally subtracted online using hyperpolarizing control pulses, applied after the test pulse, of one-fourth test pulse amplitude (P/4 procedure). For current density measurements, membrane currents were normalized to the membrane capacitance which was calculated from the integral of the transient current in response to a brief hyperpolarizing pulse of 10 mV from the holding potential.

Once in whole-cell configuration, cells were held at −60 mV for 5 min to dialyze the cell with CsF solution (fluoride shifts Nav1.8 steady-state activation and inactivation to hyperpolarized potentials) to reach Nav1.8 stable biophysical properties and to inactivate Nav1.9 current and was further clamped at −80 mV for 2 more minutes. Whole-cell Na currents were elicited by a series of 100 ms test pulses ranging from −80 to +40 mV in increments of 5 mV at a frequency of 0.33 Hz. Test pulses were preceded by a prepulse of 3 s at −120 mV. Normalized conductance (G/Gmax) was fitted as described for in vitro recordings and $V_{1/2}$ and slope factor were extracted from the equation. Steady-state inactivation curves were measured from a holding potential of −120 mV using 500 ms prepulses to the indicated potentials followed by a test pulse to 0 mV. Again, $V_{1/2}$ and slope factors were obtained as mentioned for in vitro recordings.

Recovery from inactivation curves was obtained with a standard two-pulse protocol consisting of a depolarizing pulse from a holding potential of −120 to 0 mV for 50 ms to inactivate the channels, followed by a variable duration step (from 0.05 to 3276.8 ms) back to −120 mV to promote recovery. The availability of the channels was assessed with a second test pulse at 0 mV and the ratio of the second pulse versus the first was plotted against the recovery interval. The $t_{1/2}$ of recovery was calculated as mentioned previously.

Statistics

Behavioral Statistics. For the time course and drug effect on mechanical allodynia after nerve injury three experiments were done separately: (1) rufinamide 25 mg/kg, rufinamide 50 mg/kg, and DMSO 30%; (2) rufinamide 5 mg/kg, rufinamide 10 mg/kg, and DMSO 30%; and (3) amitriptyline 10 mg/kg, amitriptyline 20 mg/kg, and saline. The log values of withdrawal thresholds were assessed for each experiment using an Anova two-ways with Bonferroni correction for repeated measures from preinjection to 24 h after injection. For the development of allodynia, baseline and preinjection were compared by using the Wilcoxon matched-pairs signed rank test (Bonferroni’s correction for multiple testing) because baseline values are skewed. For clarity purposes on figure 1, a mean value of both DMSO groups is used and values are presented as mean ± SD also for baseline. For the drug effect on naïve animals, data were analyzed with Kruskal-Wallis test and Dunn’s correction for multiple testing. The numerical data are presented as median with 95% CI. Electrophysiological Statistics. Data are presented as mean ± SD and were analyzed using paired student t tests for drug effect.

All hypotheses were challenged using two-tailed testing and P value less than 0.05 was used as the level of significance. Statistical analysis was performed using Prism 5 for windows, version 5.03, GraphPad Software, San Diego, CA.

Results

1. Behavior

1.1 Rufinamide Reduces Mechanical Allodynia after SNI. All animals showed allodynia before injection of amitriptyline (P < 0.05, preinjection vs. baseline for all groups). Rufinamide significantly and dose-dependently alleviated SNI-induced allodynia (fig. 1A), with maximal effect from 0.10 ± 0.03 (mean ± SD) to 1.99 ± 0.26 g. The effect was seen already 20 min following injection, peaked at 60 min, lasted for at least 4 h, but had faded 24 h after drug administration. At the highest dose of rufinamide, allodynia-like behavior was completely reversed. The vehicle DMSO showed a tendency for anti-allodynic effect but the values did not reach statistical significance in multiple testing.

1.2 Amitriptyline Reduces Mechanical Allodynia after SNI. All animals showed allodynia before injection of amitriptyline (P < 0.05 preinjection vs. baseline for all groups). Amitriptyline alleviated the allodynic behavior from 60 to 240 min after injection and the effect had disappeared at 24 h (fig. 1B) with maximal effect from 0.25 ± 0.22 to 1.92 ± 0.85 g. There was no difference between 10 and 20 mg/kg.

Anesthesiology 2013; 118:160–72
Rufinamide Reduces Neuropathic Pain in the Mouse

1.3 Amitriptyline But Not Rufinamide Affects Basal Sensitivity. Rufinamide (50 mg/kg) did not modify basal mechanical sensitivity of naive animals or heat withdrawal latency. We therefore did not test lower doses (fig. 2, A and B). On the other hand, amitriptyline at 20 mg/kg increased withdrawal threshold for innocuous mechanical stimulation with von Frey hairs from 1.3 (0.6–1.9) (median and 95% CI) to 2.3 g (2.2–2.5) and increased withdrawal latency on heat stimulation compared with saline from 13.1 (10.4–15.5) (median, 95% CI) to 30.0 s (21.8–31.9). We therefore tested amitriptyline at 10 mg/kg and also observed antinoceptive effect on heat stimulation (withdrawal threshold from 10.5 [7.2–11.7] to 25.3 [16.4–27.7]), but no statistically significant difference on non-noxious mechanical stimulation (fig. 2, A and B).

Animals injected with rufinamide 50 mg/kg did not lower their total activity measured over 4 h after injection with the Activ-meter as compared with saline-injected controls.
Amitriptyline decreased total activity statistically significantly compared with saline-injected controls (fig. 2C). Amitriptyline increased the score of sedation from 0 (saline group) to 2(0–3) (median, range, n = 8). Rufinamide did not change the score (0).

1.4 Rufinamide Plasma Level Corresponds to Therapeutic Level for Epileptic Patients. At peak effect for mechanical allodynia, the range of plasma level for rufinamide was 68–86 mM.

2. Effect of Rufinamide on Nav1.7 Channel Compared with Amitriptyline and Mexiletine

2.1 Rufinamide Reduces Nav1.7 Peak Current. Rufinamide reduced \( I_{Na} \) induced by a single pulse depolarization using human embryonic kidney 293 cells stably expressing Nav1.7 (fig. 3). The most substantial reduction obtained with rufinamide was 28.3%, at a concentration of 500 µM. The drug could not be dissolved at higher concentration. A concentration of 100 µM was used for the rest of the testing to avoid the high DMSO concentration used for 500 µM. With high concentration of amitriptyline and mexiletine a complete inhibition of \( I_{Na} \) could be obtained and EC50 was used for the following experiments (fig. 3).

2.2 Rufinamide Shifts Steady-State Inactivation of Nav1.7. The voltage dependence of activation was examined using a series of 10 ms depolarizing test pulses from −80 to +85 mV from a holding potential of −100 mV. Rufinamide had no effect on voltage dependency of activation for Nav1.7 sodium channel, nor did amitriptyline and mexiletine. No statistically significant changes were seen in \( V_{1/2} \) of activation. Slopes were slightly altered by rufinamide and mexiletine (fig. 4). For the steady-state inactivation experiments, cells were given a 500 ms conditioning pulse at voltages between −130 and −10 mV from a holding potential of −100 mV followed by a 20 ms test pulse. Normalized sodium currents (\( I_{Na}/I_{max} \)) measured during test pulses were plotted against conditioning voltage. Rufinamide shifted the steady-state inactivation relationship to more hyperpolarized value with a \( V_{1/2} \) of inactivation shifting from −81.8±4.4 to −86.7±4.9 mV. The control drugs had a similar effect with shift of \( V_{1/2} \) of inactivation, from −78.9±2.8 to −88.4±1.1 mV for amitriptyline and from −79.8±3.0 to −91.4±2.6 mV for mexiletine. The slopes of steady-state inactivation curves were not influenced by any of the tested drugs (fig. 4).

2.3 Rufinamide Prolongs the Recovery from Fast Inactivation of Nav1.7. Effects on the recovery from fast inactivation was examined with a standard double-pulse protocol consisting of a depolarizing pulse to −10 mV to inactivate the channels followed by a variable duration (0.25–2000 ms) step to the holding potential of −100 mV to promote recovery. The availability of the channels at the end of the recovery interval was assessed with a standard test pulse. The ratios of response of second/first pulse were plotted versus the recovery interval. The \( t_{1/2} \) of recovery was interpolated. It was statistically significantly prolonged for the three tested drugs (fig. 5).

2.4 Rufinamide Shows Use-dependent Inhibition of Nav1.7. Frequency-dependent or use-dependent blocking refers to the accumulation of channels in inactivated state when subjected to a train of depolarizing pulses at high frequency. We applied a series of 50 pulses at varying frequencies (2, 5, 10, 25, 50 Hz) and plotted the normalized current against the pulse number. Rufinamide at 100 µM increased the use-dependent block at all frequencies tested, except 2 Hz. Amitriptyline and mexiletine also increased the use-dependent block, even at 2 Hz (fig. 6).

Fig. 3. Drugs inhibit voltage-gated sodium channel Nav 1.7 peak current. A, Percentage reduction of peak current after single pulse stimulation. B, Example of traces with the drug concentrations used afterwards in the biophysical properties testing, respectively, 100, 10, and 100 µM for RUF, AMI, and MEX. Transients were blanked. AMI = amitriptyline; MEX = mexiletine; RUF = Rufinamide.
3. Rufinamide Influences INa in Dorsal Root Ganglion Neurons

We then wanted to validate the effect of rufinamide using dissociated mouse dorsal root ganglion neurons which contain also other Nav channels and the β-subunits. We first observed that rufinamide at 100 µM consistently induced a statistically significant 10.1% mean reduction in peak sodium current densities from 956 ± 396 to 850 ± 339 pA/pF (P < 0.05) despite a great variability in absolute values of current density (fig. 7A). We then assessed voltage dependence of activation and inactivation of the sodium current on the dorsal root ganglion with step protocols. The global effect of rufinamide on dorsal root ganglion was similar to the one observed using human embryonic kidney 293 cells expressing only Nav1.7. The voltage dependence of activation was unchanged and the inactivation curve was shifted with statistical significance toward more hyperpolarized potentials, from a V1/2 of activation/inactivation of −64.4 ± 16.8 mV to −69.4 ± 17.1 mV (P < 0.0001) (fig. 7B). Finally we observed that rufinamide also delayed t1/2 of recovery from inactivation from 2.58 ± 2.12 to 6.24 ± 5.04 ms (P < 0.05) (fig. 7C).

Discussion

We here demonstrate that rufinamide alleviates mechanical allodynia-like behavior in the SNI model of neuropathic pain in mice. Its effect is comparable to amitriptyline, but with no interference on basal sensitivity and activity tests.
We also show that rufinamide modulates Nav1.7. It stabilizes the channel in its inactivated state similarly to amitriptyline and mexiletine, and delays its recovery from inactivation. By the observation of rufinamide effect on total sodium currents recorded in dorsal root ganglion neurons, we finally validated a potential peripheral mechanism of action of rufinamide for the treatment of neuropathic pain.

Effect of Rufinamide on Mechanical Allodynia after SNI in Mice

To our knowledge, this is the first trial testing rufinamide in a model of neuropathic pain.

Amitriptyline is a first-line treatment for clinical neuropathic pain. Amitriptyline alleviates neuropathic pain-like behavior in the chronic constriction injury and spinal nerve ligation models but failed to affect mechanical allodynia in these models or on paw pressure hypersensitivity in a rat diabetes-related pain model. In rats, amitriptyline decreased mechanical allodynia 3–5 days after SNI but not after 2–4 weeks. When administered perisurgically for 1 week, amitriptyline failed to prevent the development of mechanical allodynia in rodents.

Despite diverging results explained by the different sensory modalities tested, timing, dose, and administration route or species/genetic background, the SNI model remains a robust neuropathic pain model in rodents. In rats, mechanical allodynia following SNI does not respond to moderate doses of morphine, gabapentin, carbamazepine, MK-801, lidocaine, lamotrigine, or rofecoxib. Other groups showed a transient effect of high dose of morphine (6 mg/kg, effect < 3 h), mexiletine (37 mg/kg, < 1 h) or gabapentin (100 mg/kg, < 5 h) and tocainide. Side-effects and sedation are rarely mentioned but with high doses, many of the tested drugs in SNI could impair basal sensitivity.

Rufinamide alleviates dose-dependently mechanical allodynia in this model, without inducing any changes in sedation or affecting basal sensitivity. Amitriptyline reduced allodynia, but also modified basal pain sensitivity and sedation score, which could participate in its anti-allodynic effect. Amitriptyline has been shown previously to change locomotor activity in rodents attributable to sedation, ataxia, changes in nociception, depression, or anxiety. In one study, amitriptyline did not change locomotor activity in the chronic constriction injury model despite reducing allodynia.
We are in agreement with others who showed an increase in thermal latency after acute amitriptyline treatment.\textsuperscript{45,50}

**Rufinamide Has the Potential of a New Treatment for Neuropathic Pain**

As first-line therapy for the treatment of neuropathic pain, clinical guidelines propose tricyclic antidepressants (amitriptyline), serotonin and norepinephrine reuptake inhibitors ( duloxetine and venlafaxine) or anticonvulsants targeting \( \alpha_2\)-\( \delta \) subunit of calcium channels ( gabapentin and pregabalin).\textsuperscript{8,51} The most effective antidepressants in the treatment of neuropathic pain have sodium channel blocking properties,\textsuperscript{52} which may contribute to their analgesic activity.\textsuperscript{10,53} Sodium channel blockers as first-line evidence-based treatment recommendation have not yet been suggested except for two specific conditions: carbamazepine
In clinical practice, the efficacy of amitriptyline on neuropathic pain is variable.\(^5\)\(^,\)\(^6\) Amitriptyline is well known for its side-effects, predominantly sedation, hypotension, and anti-cholinergic effects, considerably reducing patient’s compliance.\(^7\) In particular, sedation has been known for a long time even at “light” dosage (50 mg).\(^8\)\(^,\)\(^9\) For rufinamide, in a study on Lennox-Gastaut syndrome, the incidence of adverse events for somnolence or vomiting was more common in the rufinamide-treated group,\(^1\) but causing only 2 or 3 patients out of 74 to withdraw from the study, respectively.

Drug interaction is also a major issue for pain therapy. Rufinamide presents favorable pharmacokinetic parameters; it is well absorbed orally and is not a substrate of cytochrome p450 system, thereby reducing its potential interactions. It is however a mild inducer of CYP3A4.\(^6\) Rufinamide may be a mood-stabilizing molecule with anxiolytic properties\(^6\) that could be an added value considering the large proportion of psychiatric mood-disorders encountered in chronic pain patients.\(^5\) The toxicity studies in rodents show a greater safety ratio than other anticonvulsants.\(^2\)\(^,\)\(^3\) Na channels are still a major target in the development of new analgesic drugs,\(^2\)\(^,\)\(^6\) but rufinamide already being on the market, might offer a new treatment opportunity in the pain field, whereas other drugs trying their way through clinical trials have failed.\(^6\)\(^,\)\(^5\) Rufinamide offers a valuable alternative to the current first-line treatments for the management of neuropathic pain.

### Site of Action of Rufinamide

The site of action of rufinamide is unknown. Its effects on biophysical properties of sodium currents are similar to amitriptyline and mexiletine. Amitriptyline and mexiletine apparently interact with residues on the DIV\(^,\)\(^6\)\(^,\)\(^6\) DIIIS6 (domain III segment 6), and DIVS6 (domain IV segment 6) segments may jointly form parts of the amitriptyline/local anesthetic receptor.\(^6\)\(^,\)\(^6\)

Following the recent report of the crystal structure of the voltage-gated sodium channel, we hope new mechanistic knowledge will be gained in drug-channel interactions.\(^6\)\(^9\)

We demonstrated the action of rufinamide on the peripherally expressed Nav1.7 isofrom of sodium channel but we do not intend to show any specific Nav1.7 blocking properties. Indeed the drug is used in the treatment of epilepsy and therefore should also act on centrally expressed sodium channels. Rufinamide showed no relevant interaction with monoaminergic binding sites in radioligand binding studies and no interactions with benzodiazepine or \(\gamma\)-aminobutyric acid receptors, 5-HT1 and 5HT2 receptors, \(\alpha\)- or \(\beta\)-adrenoceptors, or human recombinant metabotropic glutamate receptor subtypes 1b, 2, or 4 (mGluR1b, mGluR2, mGluR4). However, an inhibitory effect of rufinamide at the mGluR5 subtype was observed at 100 \(\mu\)M.\(^6\)\(^0\) mGluR5 is upregulated in the dorsal root ganglia and spinal cord after spinal nerve ligation (but not after partial sciatic nerve ligation)\(^6\)\(^0\) and peripheral mGluR5.

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**Fig. 7.** Effects of rufinamide on freshly dissociated dorsal root ganglion neurons. A, RUF at 100 \(\mu\)M induced a 10% reduction in sodium peak current density \((P = 0.0084, n = 7,\) horizontal bars represent mean values). B, It significantly shifted the SSI curve to a hyperpolarizing direction \((V_{1/2}\) of inactivation from \(-64.4 \pm 16.8\) to \(-69.4 \pm 17.1\) mV, \(P < 0.0001, n = 6\) without changing activation properties \((V_{1/2}\) of activation from \(-40.6 \pm 8.4\) to \(-43.4 \pm 5.1\) mV, \(P = 0.17, n = 7\) ). C, RUF also prolonged recovery from inactivation with half-time \((t_{1/2}\) for CTRL and RUF of, respectively, 2.58\(\pm\)2.12 and 6.24\(\pm\)5.04 ms, \(P = 0.0028, n = 6\). Values are mean \(\pm\) SD. CTRL = control; RUF = Rufinamide; SSI = steady-state inactivation.
agonists can produce thermal hyperalgesia. In neuropathic pain, mGluR5 antagonists mostly show an effect on thermal sensitivity but not on mechanical allodynia. The magnitude of effect mGluR5 antagonist on mechanical allodynia is below 40% of recovery toward baseline values for systemic administration on spinal nerve ligation model or chronic constriction injury in rats and 66% reduction for intrathecal delivery with a shallow dose–response curve following spinal nerve ligation. Antagonizing mGluR5 could prevent the development of mechanical allodynia after sciatic nerve constriction injury but not reverse it. Altogether, the effects of mGluR5 antagonists are indeed not as potent as the complete reversal of established mechanical allodynia through rufinamide. Therefore, we suggest mGluR5 is not the major target for rufinamide.

Therapeutic plasmatic concentration for epilepsy (20–200 µM) and plasmatic concentration in our study at the time of anti-allodynic effect (range 68–86 µM) are in the range of concentration used for in vitro testing (100 µM). Rufinamide at the concentration we used does not completely block the current but globally the channel is less excitable. After nerve injury, hyperexcitability and ectopic discharges at the neuroma or in the dorsal root ganglion might be affected by the modulation of Na channel properties by rufinamide whereas there is no effect on nociception on a naïve nerve. We therefore suggest the anti-allodynic effect of rufinamide is related to its Na channel blocking properties.

**Limitations of the Study**

**Differential Effect of Rufinamide, Amitriptyline, and Mexiletine on Nav1.7 Sodium Channel.** We used the ED50 (half maximal effective concentration) of amitriptyline and mexiletine, 10 µM and 100 µM, respectively. The plasma concentrations of these two drugs are typically around 0.3 µM and 2.3–9.3 µM. Rufinamide was used at 100 µM, attributable to its low solubility in patch clamp solution. Our study is not intended to compare the effect size of the drugs on the different biophysical properties. The low solubility of rufinamide impeded a comparison of the three drugs at their ED50 values. The effect on peak current on Nav1.7 as well as on dorsal root ganglion neurons is low but nonetheless statistically significant and reproducible.

**Effect of DMSO as Control**

DMSO was used to dissolve rufinamide despite the potential neurotoxicity with prolonged administration at high dose. It was also used as a treatment option in osteoarthritis but only with relative efficacy on pain scores. We did not see any effect of DMSO on naïve animal sensitivity behavior regarding toxicity and compared the anti-allodynic effect of rufinamide with DMSO.

**Conclusion and Future Directions**

We here show that rufinamide dose-dependently alleviates neuropathic pain behavior in the SNI model in mice. We show in vitro electrophysiological data that rufinamide induces a hyperpolarizing shift in the steady-state inactivation curve, a use-dependent block and a delay in recovery from inactivation from Nav1.7-mediated current and ex vivo data that the same stabilizing effect on inactivation is also present in dorsal root ganglion neurons. Sodium channels blockers still belong to the potential targets to treat neuropathic pain but often do not come on the market for toxicity or side-effects issues. Rufinamide is currently on the market and could therefore be used in clinical studies in the pain field rapidly. With the low rate of success from current chronic pain therapy, a new drug would be highly valued.

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Rufinamide Reduces Neuropathic Pain in the Mouse


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