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Inhibition of c-Kit signaling is associated with reduced heat and cold pain sensitivity in humans

Marta Ceko^{a,1,2}, Nevena Milenkovic^{a,2}, Philipp le Coutre^b, Jörg Westermann^b, Gary R. Lewin^{a,*}^aMax-Delbrück-Center for Molecular Medicine, Berlin, Germany^bDepartment of Haematology, Oncology and Tumor Immunology, Charité-University Medicine Berlin, Campus Virchow-Klinikum, Berlin, Germany

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ABSTRACT

The tyrosine kinase receptor c-Kit is critically involved in the modulation of nociceptive sensitivity in mice. Ablation of the c-Kit gene results in hyposensitivity to thermal pain, whereas activation of c-Kit produces hypersensitivity to noxious heat, without altering sensitivity to innocuous mechanical stimuli. In this study, we investigated the role of c-Kit signaling in human pain perception. We hypothesized that subjects treated with Imatinib or Nilotinib, potent inhibitors of tyrosine kinases including c-Kit but also Abl1, PDGFR α , and PDGFR β , that are used to treat chronic myeloid leukemia (CML), would experience changes in thermal pain sensitivity. We examined 31 asymptomatic CML patients (14 male and 17 female) receiving Imatinib/Nilotinib treatment and compared them to 39 age- and sex-matched healthy controls (12 male and 27 female). We used cutaneous heat and cold stimulation to test normal and noxious thermal sensitivity, and a grating orientation task to assess tactile acuity. Thermal pain thresholds were significantly increased in the Imatinib/Nilotinib-treated group, whereas innocuous thermal and tactile thresholds were unchanged compared to those in the control group. In conclusion, our findings suggest that the biological effects of c-Kit inhibition are comparable in mice and humans in that c-Kit activity is required to regulate thermal pain sensitivity but does not affect innocuous thermal and mechanical sensation. The effect on experimental heat pain observed in our study is comparable to those of several common analgesics; thus modulation of the c-Kit pathway can be used to specifically modulate noxious heat and cold sensitivity in humans.

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1. Introduction

Heat pain threshold is often used as a clinical outcome measure of analgesic efficacy in human patients [3,8,16,55]. Heat pain threshold varies among individuals, but there is evidence that this variation is partly determined by genetic factors [35]. Inflammation and injury lead to mechanical and heat hyperalgesia in humans, and the latter is primarily due to the sensitization of primary afferent nociceptors [57]. Nerve growth factor (NGF) not only regulates the development of nociceptors [27] but is also an important regulator of their functional properties in adults

[17,28,47]. By binding to its high-affinity receptor tyrosine kinase (RTK), trkA, NGF can acutely sensitize sensory neurons to heat and capsaicin [14,46]. NGF produces rapid heat hyperalgesia when injected [11,43], and increases the number of sensory neurons that respond to noxious heat [52]. The physiological levels of NGF in the adult organism also set the thermal sensitivity of nociceptors [24]. NGF is not the only growth factor that can regulate the functional properties of C-fibers. Another RTK, c-RET, also regulates the functional properties of heat-sensitive IB4-positive nociceptors that, in the adult animal, do not express trkA receptors [29,50,53]. The c-RET receptor binds glial-derived nerve growth factor (GDNF); in concert with its co-receptor GFR α 2, it can form a receptor for neurturin, a factor that regulates the heat sensitivity of IB4 positive nociceptors [53]. Recently, another signaling system, stem cell factor (SCF) and its RTK receptor c-Kit, was shown to regulate nociceptor function [31]. The c-Kit receptor is expressed by a small number of developing sensory neurons [18,22], and this expression is maintained in about 20% of adult sensory neurons [31]. Genetic

* Corresponding author. Address: Max-Delbrück-Center for Molecular Medicine, Molecular Physiology of Somatic Sensation, Robert-Rössle Strasse 10, 13125 Berlin Buch, Germany. Tel.: +43 30 9406 2430; fax: +43 30 9406 2793.

E-mail address: glewin@mdc-berlin.de (G.R. Lewin).

¹ Current address: Division of Intramural Research, National Center for Complementary and Alternative Medicine (NCCAM), Bethesda, MD, USA.

² M.C. and N.M. contributed equally to this work.

ablation of the c-Kit receptor in mice results in hyposensitivity to noxious heat, paralleled by reduced heat sensitivity of nociceptive C-fibers [31]. Activation of the c-Kit receptor using its natural ligand SCF produced marked heat hyperalgesia, without altering the behavioral response of mice to innocuous mechanical stimuli.

In humans, c-Kit/SCF signaling is best known for controlling the proliferation, survival, differentiation, and migration of mast cells, hematopoietic stem cells, melanocytes, and germ cells [2,5,37]. Mutations in the c-Kit transduction pathway are strongly linked to the occurrence of malignant tumors, including gastrointestinal stromal tumors [42,56,59]. Small-molecule c-Kit tyrosine-kinase inhibitors have proved very effective for the treatment of these tumors [10,19]. In chronic myeloid leukemia (CML), a reciprocal translocation between chromosomes 9 and 22 results in the chimeric fusion protein BCR-ABL. BCR-ABL is characterized by a deregulated activity of the Abelson tyrosine kinase, a highly oncogenic protein responsible for the development of the disease [26,44]. Imatinib (Glivec, Novartis) and Nilotinib (Tasigna, Novartis), are tyrosine-kinase inhibitors used as first-line treatment in CML. Nilotinib is also used to treat CML patients with intolerance to or resistance against other tyrosine kinase inhibitors [1]. Imatinib and Nilotinib also potently inhibit the c-Kit kinase [6,39]. Because Imatinib and Nilotinib are routinely used for CML therapy at doses that inhibit the c-Kit receptor, we designed a study to determine whether such treatments are associated with analgesia in humans.

2. Methods

2.1. Study participants

A total of 31 ambulant, asymptomatic, chronic-phase CML patients (17 male, 14 female; mean age 49.7 ± 2.2 years) were recruited and tested as outpatients at the Campus Virchow Klinikum, Charité Universitätsmedizin, Berlin, Germany. A cohort of disease-free age-matched controls (mean age 51.6 ± 1.3 years; not significantly different from the CML cohort, $P = .36$, Student *t* test) were recruited in parallel, in total 39 healthy male and female individuals (12 male and 27 female, not significantly different from the CML cohort, $\chi^2 P = .22$). Study participants did not have medical conditions known to affect temperature or touch sensitivity (diabetes mellitus, polyneuropathy, multiple sclerosis, stroke, endogenous psychosis and other major psychiatric disorders, alcohol disease, major liver and kidney disorders), nor were they receiving treatment known to affect temperature and touch sensitivity (painkillers, antipsychotic drugs, epilepsy drugs, chemotherapeutics, chronic dialysis). Written informed consent was obtained in accordance with the Declaration of Helsinki, and the study was approved in full by the Charité Ethics Committee. CML patients in this study were receiving Imatinib or Nilotinib treatment, apart from 1 female patient who was on a 480-mg daily dose of a new-generation tyrosine kinase inhibitor INNO-406 (Table 1). Because INNO-406 and Imatinib/Nilotinib exhibit similar affinity for the c-Kit receptor [23], this patient was included in the study pool. Of the remaining 30 patients, 21 (9 male and 12 female) were receiving Imatinib treatment (mean daily dose, 480 mg; Table 1), and 9 (7 male, 2 female) were receiving Nilotinib treatment (mean daily dose 600 mg; Table 1). Only those patients on stable drug treatment for more than 2 months were included in the study.

2.2. Assessment of pre-existing occasional pain

Prevalence and degree of pre-existing occasional pain were assessed using a short questionnaire addressing occurrence, type, intensity, anatomical site, and duration of any type of occasional

Table 1

Demographic characteristics of study patients.

Patient	Sex	Age (y)	Treatment	Daily dose (mg)
1	Female	67	Imatinib	400
2	Male	69	Nilotinib	NA*
3	Female	42	Imatinib	400
4	Male	33	Imatinib	400
5	Male	56	Imatinib	400
6	Female	37	Imatinib	500
7	Female	59	Nilotinib	800
8	Male	53	Imatinib	400
9	Male	49	Nilotinib	800
10	Female	44	Imatinib	NA*
11	Female	40	Imatinib	400
12	Female	63	Nilotinib	200
13	Male	35	Nilotinib	800
14	Female	72	Inno-406	480
15	Male	60	Imatinib	400
16	Male	67	Nilotinib	400
17	Male	35	Nilotinib	400
18	Male	35	Imatinib	600
19	Female	32	Imatinib	400
20	Female	41	Imatinib	400
21	Female	46	Imatinib	400
22	Male	62	Nilotinib	800
23	Female	65	Imatinib/Nilotinib	NA*
24	Male	43	Imatinib	800
25	Male	54	Nilotinib	600
26	Male	46	Nilotinib	600
27	Female	48	Imatinib	400
28	Male	67	Imatinib	400
29	Female	46	Imatinib	400
30	Male	39	Imatinib	400
31	Male	71	Imatinib	400

* NA, information on exact daily dose not available. Estimated daily dose based on study sample: 400 to 800 mg (Imatinib) or 200 to 800 mg (Nilotinib).

pain over the last 12 months (eg, headache, neck and shoulder pain, back pain, joint pain, or pain in other body regions). In addition, subjects were asked to rate their current pain state on an 11-point numerical rating scale (NRS; 0 = no pain, 10 = worst pain imaginable).

2.3. Tactile acuity determination

We used a grating orientation task to assess the subject's tactile acuity [4]. Subjects were asked to name the orientation of grooved surfaces lightly pressed against the distal phalanx of the index finger and the little finger. This test is considered to be a rigorous measure of passive tactile spatial acuity, in that it requires the subject to distinguish between 2 stimuli that differ only in respect to spatial orientation, whereas the applied contact area, force, and pressure are kept constant, thereby avoiding nonspatial cues that might bias the outcome [20]. We followed the procedure as previously described [4] and as subsequently applied by us in genetic studies [13]. Briefly, square-wave gratings of equal groove and ridge widths were placed on the distal finger segment either parallel or transverse to the axis of the finger, and subjects, with their eyes closed, were asked to report the orientation of the stimulus. A cube with 6 grating widths (1 per surface) ranging from 6 to 0.75 mm was used for stimulus presentation. Beginning with the easiest detectable grating orientation, the grating width was stepped down after 2 sequential correct responses, and stepped up after a single incorrect response. The test was terminated after the 13th reversal point, with average of gratings widths at reversal points 4 through 13 taken as the individual tactile acuity threshold, that is, the width of the grooves with orientations that the subject could reliably perceive, which is inversely related to tactile spatial acuity.

2.4. Thermal sensory testing

Thermal sensitivity was measured using the TSA-II Thermal Sensory Analyzer (Medoc Advanced Medical Systems, Ramat Yishai, Israel). A 30 × 30-mm thermode, with a baseline temperature of 32°C was placed in full contact on the volar aspect of the left forearm. For assessment of innocuous thermal sensitivity (ie, detection threshold), the temperature first gradually decreased, then increased, and subjects were asked to press a button as soon as they perceived the temperature shift from neutral to cold and from neutral to warm, respectively. Detection thresholds were determined 4 times, and the average was taken for analysis. Next, thermal heat and cold pain thresholds were assessed. The testing procedure was the same as for innocuous thermal sensitivity, except that subjects were now asked to press the button as soon as they experienced the temperature shift from hot to painfully hot, and from cold to painfully cold, respectively. Pain thresholds were determined 3 times, and the average was taken for analysis.

2.5. Animal experiments

All animal experiments were performed in adult female C75BL/6 mice (20–25 g) (Charles River Laboratories, Willmington, MA) in accordance with the National Academy of Science “Guide for the Care and Use of Laboratory Animals,” and were approved by the local animal care committee (Landesamt für Arbeitsschutz, Gesundheitsschutz und Technische Sicherheit, Berlin, Germany). Animals were housed 6 per cage with a bedding of wood sawdust and maintained on a 12-hour light/dark cycle (light on 8 AM to 8 PM), with free access to food pellets and water. Animals were treated with Imatinib mesylate or Nilotinib (both Sequoia Research Products, Pangbourne, UK). Both drugs were diluted in 10% dimethyl sulfoxide (DMSO; in 1 × phosphate-buffered saline solution). Control animals received 10% DMSO (in 1 × PBS). Solutions were administered in a volume of 100 µL by means of a straight animal ball-tipped feeding needle (oral gavage [p.o.]). The needle was introduced into the mouth laterally, the solution injected, and the needle gently withdrawn. Drug and vehicle were administered in the morning, and sensory testing was performed in the afternoon of the same day. Paw withdrawal latency to thermal noxious stimuli was measured using the Hargreaves radiant-heat plantar test (IITC Life Science, Woodland Hills, CA). Animals were placed in elevated Plexiglas chambers (10 × 10 × 22.5 cm; tempered glass surface) and allowed to acclimate. A high-intensity light beam was focused onto the plantar surface of the hind paw. The intensity of the stimulus was adjusted to achieve an average baseline paw withdrawal latency of approximately 4 to 6 seconds in naive mice. The cut-off time was 20 seconds to prevent tissue damage. Quick hind paw withdrawals from the hot beam (with or without licking of the hind paw), after calm behavior before testing, were counted as a response. Paw movements associated with locomotion or weight shifting were not included. Baseline values were taken once a day for 3 days. During each session, paw withdrawal latency was determined for both paws, 5 times per paw. Sensitivity to mechanical stimuli was measured using the dynamic plantar aesthesiometer (Ugo Basile, Milan, Italy). Animals were placed in elevated chambers (15 × 15 × 22.5 cm; wire mesh floor) and allowed to acclimate. A small-diameter, blunt metallic filament was applied onto the plantar surface of the hindpaw with an increasing force (0.5 g/s with a maximum of 20 g for 10 seconds). The force at which animals showed paw withdrawal (see above) was recorded. Baseline values were taken on 3 consecutive days. Paw withdrawal latency was determined for both paws, 3 times per paw.

2.6. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). The nonparametric Mann–Whitney *U* test (1-sided *P* value, $\alpha = .025$) was used to compare thermal and tactile data, age distribution, and pre-existing pain states among groups. The χ^2 test was used to compare sex distribution and occurrence of pre-existing occasional pain.

3. Results

In a previous study, we had obtained some evidence that Imatinib treatment of mice leads to an elevation in baseline heat pain threshold [31]. However, because the majority of CML patients are now treated with the second-generation tyrosine kinase inhibitors [1], we tested the effects of oral dosing of Imatinib and Nilotinib on baseline heat and mechanical pain thresholds in mice. We found that oral dosing with Imatinib and Nilotinib produced a robust and significant elevation of the paw withdrawal latency to radiant heat (Fig. 1A). The elevation of paw withdrawal threshold was statistically significant on the fourth day after Imatinib administration and on the third day after oral dosing with nilotinib ($P < .05$ with an analysis of variance with post hoc Bonferroni test). The same animals were also tested for changes in the force required to evoke paw withdrawal using a dynamic plantar aesthesiometer, but there was no significant effect of either Imatinib or Nilotinib treatment on paw withdrawal threshold (Fig. 1B).

We next investigated, in a group of asymptomatic chronic-phase CML patients, the effects of prolonged exposure to a c-KIT tyrosine kinase inhibitor on thermal pain sensitivity, and normal thermal and tactile sensation.

General current pain state, and prevalence and degree of occasional pain, were comparable between groups. General current pain state was rated 1.83 ± 0.33 NRS in the Imatinib/Nilotinib-treated group, compared to 1.41 ± 0.27 NRS in controls ($P > .40$; Mann–Whitney *U* test). In the Imatinib/Nilotinib-treated group, 34% of subjects reported the existence of occasional pain in the last 12 months (compared to 56% in controls, $P > .40$; χ^2 test).

3.1. Imatinib/Nilotinib treatment is associated with decreased sensitivity to painful thermal stimuli

Both heat and cold pain threshold were significantly elevated in the Imatinib/Nilotinib-treated group (Fig. 2A). Mean heat pain threshold was $46.58^\circ\text{C} \pm 0.31^\circ\text{C}$ in the Imatinib/Nilotinib-treated group compared to $45.22^\circ\text{C} \pm 0.31^\circ\text{C}$ in controls ($P < .005$; Mann–Whitney *U* test). Mean cold pain threshold was $7.69^\circ\text{C} \pm 1.53^\circ\text{C}$ in the Imatinib/Nilotinib-treated group compared to $11.96^\circ\text{C} \pm 1.50^\circ\text{C}$ in controls ($P < .05$; Mann–Whitney *U* test). We also investigated whether the higher daily doses were associated higher thermal thresholds for heat and cold pain, respectively. We did in fact observe that higher heat pain thresholds were associated with increasing dose, and that cold pain thresholds were lower in patients treated with higher daily doses of Imatinib, nilotinib, or INNO-406 (Fig. 2B, C). Thus there was a significant correlation between heat and cold pain threshold and daily dose (Spearman correlations for heat pain $r = 0.39$, for cold pain $r = -0.35$; $P < .05$ 1-sided comparison). We also investigated whether disease duration correlated with increases in heat or cold pain thresholds, but we found no significant correlation in our dataset (Spearman correlations for heat pain $r = 0.036$, $P > .2$, for cold pain $r = 0.012$, $P > .2$).

3.2. Imatinib/Nilotinib treatment does not affect innocuous thermosensation

Sensitivity to innocuous warming and cooling, expressed as absolute change (Δ) from the baseline temperature (32°C), did

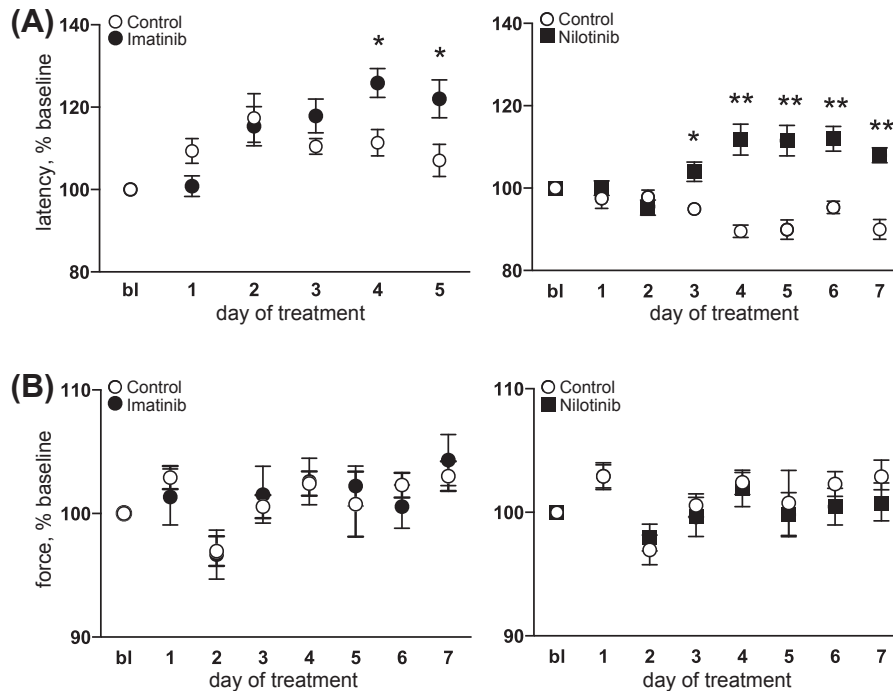


Fig. 1. Effect of Imatinib and Nilotinib on acute noxious thermal and mechanical sensitivity in mice. (A) Attenuated sensitivity to noxious thermal stimulation (Hargreaves radiant heat) in adult female C75BL/6 mice treated orally with Imatinib (left panel; 0.1 mg/g per day, $n = 24$ animals per group) or Nilotinib (right panel; 0.1 mg/g per day, $n = 6$ animals per group). (B) Unchanged sensitivity to noxious mechanical stimulation in adult female C75BL/6 mice treated orally with Imatinib (left panel; 0.1 mg/g per day, $n = 24$ animals per group) or Nilotinib (right panel, 0.1 mg/g per day, $n = 6$ animals per group). Data are expressed as mean \pm standard error of the mean. All controls are 10% dimethyl sulfoxide in phosphate-buffered saline solution. * $P < .05$ vs control, ** $P < .01$ vs. control; 2-way analysis of variance, Bonferroni post hoc tests.

not differ significantly between groups (Fig. 3A). Cold detection threshold was $\Delta -1.99^\circ\text{C} \pm 0.29^\circ\text{C}$ in the Imatinib/Nilotinib-treated group, compared to $\Delta -1.46^\circ\text{C} \pm 0.20$ in the control group ($P > .15$; Mann–Whitney U test). Warm detection threshold was $\Delta +2.25^\circ\text{C} \pm 0.21$ in the Imatinib/Nilotinib-treated group, compared to $\Delta +1.90^\circ\text{C} \pm 0.24^\circ\text{C}$ in the control group ($P > .30$; Mann–Whitney U test).

3.3. Imatinib/Nilotinib treatment does not alter tactile sensitivity

Tactile acuity, as measured using the grating orientation test on the dominant hand, did not differ significantly between groups. Tactile acuity threshold was 2.21 ± 0.23 mm in the Imatinib/Nilotinib-treated group, compared to 2.11 ± 0.12 mm in controls ($P = .84$; Mann–Whitney U test) for the index finger, and was 2.74 ± 0.23 mm in the Imatinib/Nilotinib-treated group compared to 2.36 ± 0.12 mm in controls ($P = .25$; Mann–Whitney U test) for the little finger (Fig. 3B).

4. Discussion

In this study, we have shown that inhibition of c-Kit activity, via prolonged treatment with the tyrosine-kinase blocker Imatinib or Nilotinib, is associated with decreased sensitivity to painful heat and cold in humans. In contrast, discrimination of non-noxious thermal stimuli and tactile acuity were not changed in patients undergoing such treatments. Provided that the underlying biology of nociceptors in mice and in humans is similar, our previous studies in mice led us to expect that c-Kit inhibition should lead to an elevation in heat pain thresholds. Mice treated with Imatinib or Nilotinib displayed longer latency withdrawal thresholds to noxious heat (Fig. 1A), and the threshold for noxious heat activation of identified C-fiber nociceptors was also elevated after Imatinib treatment [31]. The significant elevation in heat pain thresholds in Imatinib/Nilotinib treated patients was robust and thus consis-

tent with the hypothesis that SCF/c-Kit signaling also regulates heat nociception in humans. In patients undergoing Imatinib/Nilotinib treatment, we also found a significant blunting of cold pain thresholds, a behavior that was not studied in mice [31]. For heat pain threshold, the majority of studies have demonstrated lower heat pain thresholds in females than in males as well as clear age-dependent effects on this trait in humans [12,16]. However, here we were careful to ensure that the Imatinib/Nilotinib and control group were closely matched in terms of age and sex.

The most parsimonious explanation of our results is that interference with c-Kit signaling results in a lowered sensitivity to noxious thermal stimuli, and that c-Kit activity is involved in maintaining high responsiveness of thermal C-fiber nociceptors. Interestingly, the sensitizing effect of SCF in mice requires the presence of the heat-gated ion channel TRPV1 [7,31]. The TRPV1 ion channel has been very actively pursued as a potential target for novel analgesic drugs in the past 15 years, and several TRPV1 antagonists have been tested for effects on thermosensation in phase I trials [8,15,25,36,40,41]. A consistent and worrying observation in such trials is the ability of such drugs to elevate body temperature [15,36,41]. No such effects have, to our knowledge, been reported for drugs that block c-Kit activity. Interestingly, the increase in acute heat pain thresholds in individuals treated with Imatinib/Nilotinib was similar to that observed in healthy volunteers upon administration of several TRPV1 antagonists [8,25,41]. Imatinib is known to be poorly CNS penetrant [34], and thus it does seem likely that it may modulate heat pain thresholds solely through a peripheral mechanism [31]. Intriguingly, none of the potent TRPV1 antagonists so far reported have demonstrably elevated cold pain thresholds in humans [41]. Our finding here that cold pain thresholds were also elevated in Imatinib/Nilotinib-treated CML patients suggests that inhibition of c-Kit signaling also has an impact on cold nociception, independent of TRPV1-dependent mechanisms.

Several clinically used analgesics, including opioids, nonsteroidal anti-inflammatory drugs (NSAIDs), and selective cyclooxygenase-2

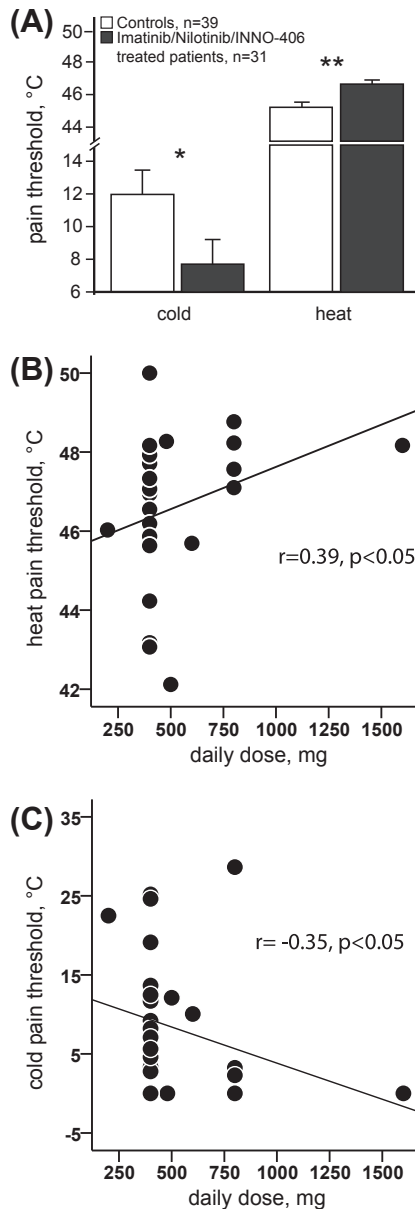


Fig. 2. Decreased pain sensitivity in subjects treated with Imatinib/Nilotinib. Subjects treated with the c-Kit inhibitor Imatinib/Nilotinib (solid bars) show decreased sensitivity to cold pain (left) and heat pain (right), compared to age- and sex-matched healthy controls (open bars). * $P < .05$, ** $P < .01$; error bars show standard error of the mean. (B) Effect of drug dose on heat pain sensitivity in subjects treated with Imatinib/Nilotinib/Inno-406. (C) Effect of drug dose on cold pain sensitivity in subjects treated with Imatinib/Nilotinib/Inno-406. (Inset) Spearman correlations values; P values are based on a 1-sided comparison.

(COX-2) inhibitors, have reduced heat sensitization in models of inflammatory pain in healthy volunteers [3]. Morphine has also been shown to increase cold pain thresholds and to lower the magnitude of pain intensity [9,21,38]. Thus, the effect of c-Kit inhibitors Imatinib and Nilotinib on acute thermal pain sensitivity shown in our study is comparable to those observed in paradigms assessing the efficacy of some clinically used analgesics on acute pain in healthy volunteers Fig. 2.

The ultimate goal of effective pain treatment is to treat hyperalgesia associated with inflammation. However, the main clinically relevant hyperalgesia is that to mechanical stimuli, so it would be interesting to see whether, in animal models, c-Kit inhibitors are effective in the context of inflammatory or neuropathic pain.

A recent study with c-Kit^{W-v} mice that carry a hypomorphic c-Kit allele, in which the kinase activity of c-Kit was reduced by 80%, indicated that c-Kit might play a role in inflammatory and neuropathic pain [54]. Thus c-Kit^{W-v} mice exhibited reduced pain behavior in the second phase of the formalin test but showed anomalous persistent, increased mechanical hypersensitivity on the contralateral side after exposure to sciatic nerve chronic constriction injury, a phenomenon not seen in control animals [54]. This murine model cannot be strictly compared to that used by us in our earlier study; those mice carried complete loss-of-function c-Kit alleles, but lethality was reversed by an erythropoietin transgene that rescued c-Kit function in blood cells [31]. It thus appears that the role of c-Kit in pathophysiological pain states may be complex; nevertheless the effects of pharmacological blockade of c-Kit receptors in neuropathic and inflammatory models remains to be studied. Our data with orally administered Nilotinib in mice indicates that this drug has robust effects on pain behavior (Fig. 1).

4.1. C-Kit inhibition and thermal sensitivity

In humans, the sensations of cooling and warming are thought to be mediated by afferent fibers that are thinly myelinated A δ -fibers and unmyelinated C-fibers, respectively [45]. In mice, the c-Kit receptor is expressed in subpopulations of A δ -fibers and C-fibers; however, the absence of changes in innocuous warming and cooling sensation in Imatinib/Nilotinib-treated patients suggest that c-Kit is not expressed in sensory afferents relevant to these sensory qualities. On the other hand, noxious cold pain sensation was significantly blunted in patients undergoing long-term Imatinib/Nilotinib treatment, which indicates that c-Kit receptors could play a significant role in cold pain. c-Kit receptors are predominantly expressed in C-fibers in mice, and there is evidence in rodents and in humans that many C-fibers start responding to cold at temperatures appropriate to signal cold pain (eg, $<15^{\circ}\text{C}$) [45,48,49].

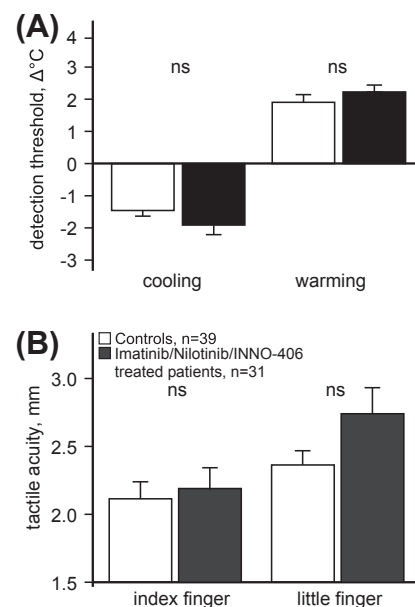


Fig. 3. Unchanged somatosensory thresholds and tactile acuity in subjects treated with Imatinib/Nilotinib. Subjects treated with c-Kit inhibitor Imatinib/Nilotinib (solid bars) have (A) normal cooling and warming thresholds, expressed as absolute change (Δ) from baseline temperature (32°C), and (B) normal tactile acuity threshold on both sites measured (index finger and little finger), when compared those in age- and sex-matched healthy controls (open bars). ns, Not significant; error bars show standard error of the mean.

4.2. c-Kit inhibition and tactile acuity

Tactile acuity, as determined using the grating orientation task, did not differ significantly between groups (Fig. 3B). Although c-Kit is expressed on a subpopulation of mechanoreceptive afferents in the adult dorsal root ganglion, and lack of SCF/c-Kit during development results in hypersensitivity of these fibers to mechanical stimulation, c-Kit activation via SCF does not alter the behavioral response of adult mice to mechanical stimuli, nor does blocking of the c-Kit receptor with Imatinib have any effect on the incidence or functional properties of mechanoreceptors in adult mice [31]. It has therefore been suggested that SCF/c-Kit signaling is required during development to set the normal function of mechanoreceptors [31]. Lack of significant difference in tactile acuity between Imatinib/Nilotinib-treated subjects and healthy controls observed in our study is consistent with the idea that the effects of c-Kit/SCF signaling occur during development rather than in the adult organism.

4.3. Off-target effects of Imatinib and Nilotinib

The drugs used to treat CML are broad-range tyrosine kinase inhibitors with significant inhibition of c-Kit, Abl1, PDGFR α , PDGFR β (platelet-derived growth factor receptors α,β), DDR1, and DDR2 (discoidin-domain receptors 1 and 2), and CSF-1R (colony stimulating factor-1) [51]. Importantly, Imatinib and Nilotinib have no described activity in inhibiting tyrosine kinase receptors or protein kinases that have been implicated in pain, for example, TrkA and protein kinase C [28,32,51]. There is no published experimental evidence that inhibition of Abl1, DDR1/2, or CSF-1R is associated with pain in any context. Two studies have implicated spinal cord PDGFR signaling in the development of tactile allodynia associated with neuropathic pain in animals [30,33], and a third study has implicated central PDGFR signaling in the development of morphine tolerance [58]. None of these studies provide evidence that PDGFR signaling is directly involved in modulating heat and cold pain sensitivity.

4.4. Conclusion

In conclusion, this is the first study to show the effect of prolonged c-Kit inhibition on acute pain perception in humans. We suggest that interfering with c-Kit signaling is associated with lowered thermal and cold pain sensitivity, without altering normal thermal and tactile sensitivity. An obvious limitation of this study is that we assessed the effect of c-Kit inhibition on pain perception in a group of patients with chronic myeloid leukemia rather than testing the effects in healthy subjects. We included only asymptomatic, chronic phase CML patients in remission in our study, as ascertained by stringent inclusion criteria equally applied to both groups, which ensured that the groups did not significantly differ on any study-relevant parameters. Imatinib/Nilotinib are known to target kinases other than c-Kit at clinically relevant levels, most notably the BCR-ABL kinase, whose proliferation it inhibits in CML treatment. It is worth considering that the effect of Imatinib/Nilotinib on thermal pain sensitivity might be exerted through one of these alternative pathways, or that these targets contribute, to some extent, to the analgesic properties of these drugs. However, Imatinib, Nilotinib, and INNO-406 targets other than c-Kit have been thoroughly described [51], and there is, at present, no evidence suggesting that they are involved in modulating pain perception. It is possible that novel analgesic medication based on the nerve growth factor sequestration enters routine clinical use [17,28,47]. Our study findings make it clear that the sensory consequences of treatment with tyrosine kinase inhibitors such as Imatinib and Nilotinib may overlap with anti-nerve growth factor

medications, and this should be taken into account when prescribing such drugs.

Conflict of interest statement

The authors declare no conflict of interest in regard to this work.

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