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

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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, PDFGFRα, and PDFGFRβ, 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.

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 GFRalpha2, 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

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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, hematopoetic 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, χ^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.

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).

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 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.

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