TITLE

A randomised, double blind, placebo-controlled crossover trial of the influence of the HCN channel blocker ivabradine in a healthy volunteer pain model - an enriched population trial

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ABSTRACT

Preclinical studies suggest that type 2 hyperpolarization-activated cyclic nucleotide gated ion channels (HCN2) are necessary for neuropathic pain. This trial assessed the influence of ivabradine, a non-selective HCN channel blocker, on capsaicin-induced hyperalgesia and pain in healthy human subjects. An enriched population comprising subjects who developed >20cm^2 of punctate hyperalgesia from topical capsaicin (0.5% cream applied onto 9cm^2 area) was identified. These subjects then received ivabradine (15mg) or placebo one hour prior to capsaicin application in randomly allocated order in a crossover study. The forearm site for capsaicin alternated with each application of the cream. The interval of time from screening to the 1st and to the 2nd treatment visits were at least 3 and 5 weeks respectively to minimize carry-over effects. 55 participants were screened, of which 25 completed at least one treatment visit. Intention-to-treat hierarchical analysis revealed no significant effects of the drug on primary trial outcome, defined as a difference in effects of placebo and ivabradine on the area of punctate hyperalgesia (ivabradine – placebo: mean=3.22 cm^2, 95% CI: = -4.04, 10.48, p=0.37). However, ivabradine caused a slowing of heart rate (difference of 10.10 beats per min (95% CI – 6.48, – 13.73; p-value <0.0001)). We conclude that ivabradine lacks analgesic effects in the capsaicin pain model at a dose that caused appreciable slowing of heart rate, and hence is unlikely to prove a useful analgesic in humans. More selective drugs are required to establish a role of HCN2 for pain in humans.

Introduction

The HCN ion channel family comprises four isoforms, HCN1-4, which carry an inward current called I_h (also I_q or I_f) [2; 18]. Previous studies have shown that HCN1 and HCN2 are the isoforms most strongly expressed in primary sensory neurons [4; 9; 30; 40]. Large non-nociceptive sensory neurons express a fast, cAMP-insensitive I_h attributable mainly to HCN1 [7; 17; 29; 40]. HCN1 is not functionally expressed in small sensory neurons, the majority of which are nociceptors [40]. In most small sensory neurons, I_h has slower kinetics and is sensitive to intracellular cAMP, consistent with expression of HCN2 [26; 40]. Inflammatory mediators that elevate intracellular cAMP, such as prostaglandin E2, accelerate the frequency of action potential firing in these small neurons by an I_h-dependent mechanism [40], which suggests that HCN2 channels may play a role in inflammatory, and possibly neuropathic pain. Pharmacological blockade and targeted or global genetic deletion of HCN channels have since confirmed the role of the HCN2 channel as a major modulator of the excitability of nociceptors in mouse models of inflammatory and neuropathic pain [11].

To date, there is no specific blocker of HCN2 channels, licensed for use in humans, with which to translate preclinical findings. However, Ivabradine, a non-selective and peripherally restricted HCN blocker, is clinically employed for the treatment of chronic angina and mild-moderate heart failure with systolic dysfunction [36]. Ivabradine slows the heart rate (bradycardia) but this effect is well tolerated when the drug is prescribed within its licensed posology at healthy volunteers and in patients with chronic angina. Furthermore, the drug does not cross the blood brain barrier appreciably, and hence is devoid of effects on the central nervous system. Therefore, the drug can be safely employed to investigate the role of HCN channels in experimental models of pain in humans.

Several experimental models of pain are available in humans. Of those, the topical capsaicin pain model is a safe, reversible and non-invasive assay that is sensitive to several classes of clinically licensed analgesics [41], including gabapentinoids, non-steroidal anti-inflammatory drugs, local anaesthetics and opioids. In humans, topical capsaicin activates TRPV1 channels expressed by the free nerve endings nociceptors to cause neurogenic inflammation, the symptoms of which are spontaneous ‘burning’ pain and hyperalgesia on thermal and mechanical stimulation of the skin at, and adjacent to where capsaicin is applied [33]. We therefore investigated the effects of ivabradine in the topical capsaicin pain model in a randomised, double-blinded, placebo-controlled crossover trial.

The study aimed to assess the analgesic potential of peripherally acting HCN blockers, which represents a novel class of analgesics that are devoid of sedative or psychotrophic effects in humans. Since ivabradine is currently available for prescription, broadening its indications to pain management (should robust analgesic effects occur within its licensed posology) would be relatively feasible, compared to developing a selective HCN2 channel blocker for use in humans.

Methods

The Phase 2 clinical trial was conducted in compliance with the Declaration of Helsinki and all International Conference on Harmonisation Good Clinical Practice guidelines. The trial was registered with the European Union Drug Regulating Authorities Clinical Trials (EudraCT number: 2012-005627-32). The trial protocol and participant information sheet were reviewed by the National Research Ethics Service (NRES REC number: 14-EE-0132) and is on request via the corresponding author or the Cambridge Clinical Trials Unit (https://www.cuh.nhs.uk/contacts/contact-cctu). Written informed consent was obtained from every participant before initiation of protocol-specified procedures.

Study Design

This was a single-centre, randomised, double blind, placebo-controlled, 2-period cross-over trial in an enriched population of healthy volunteers who displayed a defined degree of hyperalgesia in response to topical capsaicin cream applied on the forearm (Figure 1). The washout period of 3 weeks between Visit 1 (screening) and Visit 2 ensured a minimum 4-week washout of ivabradine between Visit 1 and Visit 3 and therefore on the forearm used twice. This design ensured that there were no residual effects of topical capsaicin.

The brevity of the trial (approximately 4-8 weeks) mitigated against volunteer drop-out, and the cross-over design allowed subjects to provide their own control observations, thus increasing the accuracy of the treatment effect estimates, in comparison to a parallel-arms trial. The choice of an enriched population trial was justified by a pilot study (see Analysis) suggesting that the effect of ivabradine was greater in those who developed a large area of hyperalgesia (i.e. responded to capsaicin). This method of screening for capsaicin-responders and non-responders prior to the treatment phase of the trial has been reported previously [42]. We assumed that 40% of participants responded to capsaicin as defined by the spatial extent of mechanical punctate hyperalgesia they displayed (see section: identification of capsaicin responders).

Participants

Healthy volunteers were recruited through local advertisements. Respondents were provided with written information (see Supplemental Materials) and pre-screened by telephone or email before scheduling their first onsite visit for screening (Visit 1) at the Addenbrooke's Centre of Clinical Investigation in Cambridge. All participants provided informed consent and those who met the eligibility criteria (supplemental table 1) were enrolled.

Topical Capsaicin Pain Model

Topical capsaicin (0.5% cream; 1ml drawn in a 2ml plastic syringe; The Specials Laboratory Limited, Northumberland) was applied without occlusive dressing to a 9cm^2 area of skin that was marked on the volar aspect of the designated forearm (see supplemental figure 1). The cream was left on for 75 minutes and removed once the final assessments for areas of brush allodynia and punctate hyperalgesia were completed (Figure 2). Please refer to supplemental materials for full details.

Identification of capsaicin responders

Subjects who developed an area of punctate hyperalgesia equal to or greater than 20cm^2, rounded to the nearest cm^2, at 75 minutes post-capsaicin application during Visit 1 were identified as capsaicin responders. The method for assessing areas of hyperalgesia is described in the relevant section below.

Randomisation

The use of the dominant or non-dominant forearm was allocated at screening (Visit 1), to be followed by the other forearm in the first treatment visit (Visit 2) and returning to the forearm used at screening for the second treatment, i.e. final visit (Visit 3). Dominance of forearm was assumed based on self-reported right or left-handedness. The dominant forearm was used at screening for the first consented subject. The dominant forearm was used for screening until a subject was determined to be capsaicin-responder, after which the non-dominant arm was used to determine response to capsaicin at screening for subsequent subjects (Figure 1). The switch to the other forearm for the screening of the following subject, whenever a capsaicin responder was identified, ensured an exact balance in the subjects included in the treatment phase of the trial. Capsaicin responders who proceeded to the treatment phase were randomised 1:1 to a sequence of treatments for the two periods (Ivabradine-Placebo or Placebo-Ivabradine) using the method of blocked randomisation (block size = 4) stratified by the sequence of forearms used. The online central randomisation service, TENALEA (https://www.aleaclinical.eu/), was employed to generate treatment sequence allocation.

Ivabradine and placebo drug treatments

The active treatment consisted of a single oral 15mg dose of Ivabradine that was administered as two tablets, each containing 7.5mg of the drug. The dose chosen has been shown to slow heart rate without reduction of systemic blood pressure in both healthy volunteers and patients with chronic angina [3].

Ivabradine and placebo tablets were identical in appearance. The tablets for the treatment visits were supplied by Servier (manufacturer of Procoralan®) as blister packs. Each pack contained two placebo or two Ivabradine tablets, and was identified by a randomisation number, for a single per participant dose. The randomised allocation schedule could only be accessed by a trial pharmacist who had no role in dispensing the medication.

Tablet consumption (with still water) was witnessed at each treatment visit by the investigator performing the study assessments.

Study assessments

Each participant attended one screening and two treatment visits. The same investigator performed study assessments for all three visits. All study assessments were undertaken in the same temperature-controlled environment.

The assessments carried out at the screening (Visit 1) and treatments (Visits 2 and 3) are illustrated in Figure 2. The drug was administered 60 minutes before the application of topical capsaicin to the designated forearm. The primary and secondary endpoints were 75 minutes after capsaicin cream was applied, which was at 135 minutes (~2 hours) after administration of ivabradine/placebo, the reason being that peak plasma concentration of ivabradine is known to occur between 2 and 3 hours of a single oral dose [10; 34].

Warmth detection, heat pain, cool detection and cold heat thresholds

The sensory thresholds were determined at the forearm skin site before capsaicin cream was applied and again after the cream was removed. A computer-controlled contact thermode (3cm x 3cm, Pathway ATS, Medoc, Israel) was placed on the skin (see supplemental figure 1). The subject was allowed 3-5 minutes to acclimatise to the baseline temperature of 32^{o}C. The method of limits was then employed to determine the sensory thresholds in the following sequence: warm detection (WDT), heat pain (HPT), cool detection (CDT) and cold pain (CPT) (see supplemental materials for details). This sequence was chosen instead of the sequence CDT, WDT, CPT and HPT that is employed by established clinical quantitative sensory testing protocols, for example the DFNS protocol described Rolke et al [35]. The reason was because rewarming of the thermode, which occurs during determination of CDT and CPT was found in our pilot study to cause warm or even painful heat sensations after the skin is sensitized by application of topical capsaicin. In some cases, those sensations confused the evaluation of WDT and HPT, which follows CDT and CPT respectively in the DFNS protocol. Heat allodynia is a well-established effect of topical capsaicin application. Hence the WDT and HPT were determined first and in consecutive sequence to optimize assessment of drug effects on those thresholds.

Capsaicin-induced spontaneous 'burning' pain scores

Subjects were asked to rate the intensity of 'burning' pain sensation localised to the region where capsaicin was applied. The ratings were collected before and every 15 minutes after capsaicin application until the cream was removed. The 100mm visual analogue scale (VAS) described above was used to collect ratings of 'burning' pain sensations. The extreme left and right anchors on the VAS were 'none' and 'intolerable'.

The words 'worst imaginable pain' is commonly employed as the extreme right anchor for the VAS that is used for the assessment of pain in clinical settings. Pain in this trial is caused experimentally, is far less intense in comparison and entirely within the participant's control (with cooling of the skin by cold towel and removing the cream). Hence, the use of the words 'worst imaginable' may result in relatively low pain scores, which may reduce sensitivity of the VAS to detect an analgesic drug effect. The word 'intolerable' was used for

VAS in this trial because the word was easily understood in the context of this procedure. The participant was told that a rating of 100mm would indicate to the investigator that the intensity of pain was 'intolerable' and that the experiment must cease, and capsaicin cream removed as soon as possible. A single VAS was printed on A4 size paper and the line measured to within 1mm by a ruler before use. The participant rated pain by indicating a mark along the 100mm line. The distance from 0mm was measured and recorded by the investigator.

Capsaicin-induced area of brush allodynia and punctate hyperalgesia.

The area of brush allodynia (BA) was determined by stroking the skin with a soft Q-tip bud. The stimulus was applied starting at the outermost point of each 'spoke' starting with 'A' (see supplemental figure 1). The Q-tip bud was applied using a smooth sweeping motion as if to 'draw' a 1cm line perpendicular to the radial spoke. The stimulus was then applied to the successively inward sites marked on the skin along the radial spoke. The subject was asked to report when the sensation became uncomfortable. The stimulus was then applied at two sites further inward and along the radial spoke. If the discomfort persisted, the point on the radial spoke where the discomfort was first experienced was recorded by the name of the spoke and distance in cm to the point of line intersection. The process was repeated for spokes B, C, D, E and F in a clockwise fashion. The area of punctate hyperalgesia (PA) was determined in a similar fashion but using a 26g Von Frey monofilament. The areas of punctate hyperalgesia or brush allodynia were calculated using the formula 1/2*(A*B + B*C + C*D + D*E + E*F + F*A) * sin(60); the variables were the distances (in cm) between where the marking on the named-spokes were made, and the point where all the spokes intersected.

Blood pressure and pulse rate

Non-invasive forearm blood pressure, heart rate and pulse oximetry (Dash 3000, GE Healthcare) were obtained before treatment (ivabradine or placebo tablets) administration and prior to subject discharge home. The heart rate via finger plethysmography was recorded immediately before the treatment, and then monitored continuously till after completion of all experimental procedures. These recordings were obtained during the treatment visits (Visits 2 and 3) only and were taken by and recorded by a trial-independent nurse in order to maintain double blinding of the investigator.

Analytical Plan

Sample size estimation and interim analyses

The primary endpoint was the area of punctate hyperalgesia at 75 minutes post-application of capsaicin. Preliminary data from four capsaicin-responders provided a within-subject standard deviation of 18 cm^2, (IISNeP, EudraCT: 2011-003933-32), which implied that a sample of 24 capsaicin-responders who complete the trial protocol would detect a mean difference of −10 cm^2 (ivabradine – placebo) with 80% power at the two-sided 5% significance level.

However, this sample size estimate, based on a very limited number of observations, was likely to be imprecise. Hence, a pre-planned interim analysis was undertaken before the enrolment of the 24th capsaicin-responder. The purpose was to correct the sample size, if required, to maintain statistical power without any assessment of efficacy. A mixed effect model was fitted for the primary endpoint and adjusted for baseline covariates using the methods of Kenward and Roger [20]. The interim analysis, conducted with data from the first 14 participants who had completed both treatment visits, revealed a within-subject standard deviation (SD) of 22.1 cm^2 (95% CI: 17.5, 30), which resulted in a recommendation to increase sample size to 42 subjects to maintain power, but with high levels of uncertainty around this estimate (95% CI: 28, 74).

At the interim analysis, the conditional power was calculated for a range of possible sample sizes for further recruitment of participants. The conditional power uses the estimated treatment effect and SD from the existing data, plus assumptions regarding the mean (−10 cm^2) and SD (updated SD estimate) for future potential data to calculate the probability that the overall final test statistic would be statistically significant at a nominal 1-sided 2.5% level. The sample size needed to achieve 80% conditional power was thus to be identified. Stopping boundaries were chosen to stop early for futility or early efficacy. Futility would result, on grounds of practicality, if the future total sample size were to exceed 42. Early efficacy would result if the future total sample size were to be less than 38 (which was chosen to provide an overall 1-sided type-1 error rate of 2.5%). The calculations to identify the bounds were based on using the SD value estimated at the first interim (22.1 cm^2), and used sequential t-tests [16]. The statistical analysis plan is available upon request.

Due to logistical constraints, the decision was then taken to perform a second and final interim analysis once 24 subjects had completed the trial. The second interim analysis provided a within-subject standard deviation of 19 cm^2 (95% CI: 17.5, 42), which after incorporating the estimated treatment effect equates to an estimated conditional power at the maximum size of n=42 of only 10% (95% CI 4%-20%). The trial was stopped at this point because it was considered infeasible to achieve the large sample size would have been required to detect the effect size of interest at the intended power (80%) of the study.

Statistical analysis

The statistical software SAS (version 9.4) was employed to fit the mixed effects model. R (version 3.3.1) was used for the rest of the analyses and to generate graphs.

Continuous variables were summarised using the following descriptive statistics: n (non-missing sample size), mean, standard deviation, median, maximum and minimum. The frequency and percentages (based on the non-missing sample size) of observed levels are reported for categorical measures.

The primary endpoint (punctate hyperalgesia) was analysed using a linear mixed effects model with fixed effects for the treatment, forearm and period (visit order), and the two pre-capsaicin values from both periods [20], and a random intercept at the subject level. The value of punctate hyperalgesia observed at the screening visit was not used in any analysis to avoid biases arising from regression to the mean. The null hypothesis was that the treatment effect is zero. Estimates of the treatment effect (Ivabradine – Placebo) with 95% confidence intervals are provided with associated p-values. Summary statistics (mean, SE, median, max, min) will be provided for the within-subject difference (Placebo minus Ivabradine) and for each treatment. Intention-to-treat (IT) analyses were performed for the primary outcome for sensitivity analyses, along with per-protocol (PP) analyses and to test the robustness of the findings.

The pre-specified secondary endpoints were area (cm^2) of brush allodynia, VAS(0-100mm) scores for capsaicin-induced spontaneous burning pain and temperatures(°C) for WDT, HPT, CDT, CPT (°C)). Heart rate (beat per minute) was considered a safety end-point because ivabradine is already known to cause slowing of the heart rate at the dose prescribed for the trial. Analyses of these endpoints and mirror those for the primary endpoint and are intended to be exploratory given the power of study. Results reported are from IT analysis for all endpoints.

Results

39 subjects were eligible and were assessed for response to topical capsaicin at screening. Of those, 8 were non-responders, and 4 dropped out (un-contactable) after the screening visit.

27 subjects were randomised during a 13-month period beginning in Jan-2015 (Figure 3). 15 were allocated to receive Ivabradine in Visit 2 then Placebo in Visit 3 (Ivabradine -> Placebo group). 12 were allocated to receive the treatments in the opposite sequence (Placebo-> Ivabradine group). For the Ivabradine-Placebo group, two subjects did not receive any treatment and one subject received Ivabradine only. For the Placebo->Ivabradine group, all 12 subjects completed the study visits.

Baseline characteristics were similar between the two groups (Table 1).

Variable	Statistics	Ivabradine -> Placebo	Placebo -> Ivabradine
Age (years)	n	15	12
	Mean (SD)	32.1 (10.7)	38.3 (15.6)
	Median	31	32
	Min, Max	21, 59	22, 64
Gender	Female	60% (9/15)	66.7% (8/12)
	Male	40% (6/15)	33.3% (4/12)
Weight (kg)	n	15	12
	Mean (SD)	69.7 (12.1)	73.4 (14.6)
	Median	63.3	66.1
	Min, Max	57.3, 91.0	57.4, 96.6
Height (cm)	n	15	12
	Mean (SD)	168 (7.31)	168 (5.88)
	Median	166	170
	Min, Max	159, 182	158, 176
Body Mass Index (kg/m2)	n	15	12
	Mean (SD)	24.6 (2.62)	25.8 (3.88)
	Median	23.5	24.6
	Min, Max	20.9, 29.3	21.3, 32.4
Ethnicity	White	80% (12/15)	75% (9/12)
	Asian	20% (3/15)	16.7% (2/12)
	Hispanic	0% (0/15)	0% (0/12)
	Black	0% (0/15)	8.3% (1/12)
	Other	0% (0/15)	0% (0/12)
Dominant Arm	Left Arm	6.7% (1/15)	0% (0/12)
	Right Arm	93.3% (14/15)	100% (12/12)
Area of punctate hyperalgesia at 75 minutes induced by capsaicin	n	15	12
	Mean (SD)	46.0 (16.8)	47.2(15.2)
	Median	42.4	44.0
	Min, Max	21.1, 75.9	27.0, 83.0

Table 1 Baseline characteristics obtained at screening (Visit 1) of subjects randomised to Ivabradine->Placebo and Placebo->Ivabradine arms of the crossover trial.

Effects of Ivabradine on the topical capsaicin pain model

Descriptive statistics for the primary and secondary endpoints for ivabradine and placebo treatments are provided in Table 2.

There was no significant treatment effect on the primary end-point of the study ((ivabradine – placebo): mean=3.22 cm2, 95% CI: = -4.04, 10.48, p=0.37), which was defined as the area of punctate hyperalgesia (induced by capsaicin) that was measured 135 minutes after ivabradine or placebo administration (Figure 4, Table 3).

Nor were there any significant treatment effects on brush allodynia or VAS scores for 'burning' pain that were induced by capsaicin (Figure 4, Table 3).

Similarly, there were no significant treatment effects on temperature thresholds, or their differences, of warm detection, heat pain, cool detection or cold pain determined before and after application of topical capsaicin (Figure 5, Table 3).

Effects of ivabradine on heart rate

There was, however, a small but statistically significant difference between the effects of ivabradine and placebo on heart rate (Figure 6). Formal regression analysis, adjusting for order of treatments, and accounting for correlation of pre-post measures, estimates the mean treatment effect to be –10.10 beats per min (95% CI –6.48, –13.73; p-value <0.0001). Hence, ivabradine slowed heart rate significantly in this trial when compared to placebo.

Adverse effect of ivabradine

The dose of ivabradine was very well tolerated in all subjects. There were no reports of symptoms related to the drug or to placebo during the treatment.

Variable	Ivabradine mean (SD)	Placebo mean (SD)
VAS-100mm spontaneous 'burning' pain at 75 minutes post capsaicin [a]	40.1 (23.3)	34.5 (23.8)
Area of punctate hyperalgesia (cm^2) at 75 minutes post capsaicin [a]	34.90 (15.0)	33.45 (15.3)
Area of brush allodynia (cm^2) at 75 minutes post capsaicin [a]	23.55 (16.9)	22.02 (14.9)
Warm detection threshold (°C) pre-capsaicin [b]	34.2 (0.755)	34.3 (0.858)
Heat pain threshold (°C) pre-capsaicin [b]	41.7 (3.03)	41.8 (2.69)
Cool detection threshold (°C) pre-capsaicin [b]	30.4 (0.612)	30.4 (0.898)
Cold pain threshold (°C) pre-capsaicin [b]	20.9 (6.59)	18.9 (8.93)
Warm detection threshold (°C) post-capsaicin [c]	34.1 (0.527)	34.0 (0.477)
Heat pain threshold (°C) post-capsaicin [c]	34.7 (1.040)	34.8 (0.923)
Cool detection threshold (°C) post-capsaicin [c]	30.4 (0.612)	30.4 (0.898)
Cold pain threshold (°C) post-capsaicin [c]	8.16 (8.73)	8.34 (8.52)

Table 2 Summary of effects of ivabradine and placebo on trial variables (outcome measures)

[a] at 135 minutes after drug administration before capsaicin was removed from skin

[b] just before drug treatment was administered

[c] at 150 minutes after drug treatment was administered when capsaicin cream was removed from the skin

Variable (*ITT)	Effect		Estimate	Standard error	P-value	95% confidence interval
Area of punctate hyperalgesia (cm^2)	Treatment:	Ivabradine – Placebo	3.22	3.50	0.37	-4.04, 10.48
	Order of treatment:	1^{st} – 2^{nd}	6.70	3.46	0.07	-0.47, 13.87
	Forearm (capsaicin):	Dominant – Non-dominant	- 2.18	3.50	0.54	-9.44, 5.08
Area of brush allodynia (cm^2)	Treatment:	Ivabradine – Placebo	2.70	3.22	0.41	-3.99, 9.4
	Order of treatment:	1^{st} – 2^{nd}	2.55	3.17	0.43	-4.05, 9.15
	Forearm (capsaicin):	Dominant – Non-dominant	- 5.47	3.22	0.10	-12.16, 1.22
Burning pain VAS (0-100mm)	Treatment:	Ivabradine – Placebo	3.76	3.31	0.27	-3.11, 10.64
	Order of treatment:	1^{st} – 2^{nd}	0.17	3.27	0.96	-6.61, 6.96
	Forearm (capsaicin):	Dominant – Non-dominant	- 6.05	3.31	0.08	-12.92, 0.83
Warm detection threshold (°C)	Treatment:	Ivabradine – Placebo	0.10	0.14	0.45	-0.17, 0.38
	Order of treatment:	1^{st} – 2^{nd}	0.03	0.13	0.82	-0.24, 0.3
	Forearm (capsaicin):	Dominant – Non-dominant	- 0.09	0.14	0.51	-0.37, 0.19
Heat pain threshold (°C)	Treatment:	Ivabradine – Placebo	-0.07	0.15	0.65	-0.39, 0.25
	Order of treatment:	1^{st} – 2^{nd}	0.08	0.15	0.61	-0.23, 0.38
	Forearm (capsaicin):	Dominant – Non-dominant	0.08	0.15	0.60	-0.24, 0.4
Cool detection threshold	Treatment:	Ivabradine – Placebo	-0.04	0.33	0.91	-0.73, 0.65
	Order of treatment:	1^{st} – 2^{nd}	0.22	0.32	0.50	-0.45, 0.89
	Forearm (capsaicin):	Dominant – Non-dominant	-0.13	0.33	0.71	-0.81, 0.56
Cold pain threshold	Treatment:	Ivabradine – Placebo	-1.35	1.95	0.50	-5.4, 2.7
	Order of treatment:	1^{st} – 2^{nd}	1.08	1.92	0.58	-2.91, 5.07
	Forearm (capsaicin):	Dominant – Non-dominant	-0.15	0.33	0.71	-0.81, 0.56

Table 3 Fixed effects of treatment (ivabradine, placebo), order of treatment (1^{st} or 2^{nd}), forearm (where capsaicin was applied) for the primary and secondary outcomes at 75 minutes after capsaicin application

Discussion

Ivabradine is analgesic in behavioural and electrophysiological studies of inflammatory and neuropathic pain models in mice [46]. These models include intra-plantar formalin injection, chronic nerve constriction injury (traumatic neuropathy), systemic oxaliplatin (chemotherapy induced neuropathy) and more recently, diabetic neuropathy (neuropathic pain induced by diabetes) [39]. The analgesic effects of ivabradine result specifically from blockade of the HCN2 ion channel isoform that is expressed by nociceptors [11; 39; 46]. Hence, we sought to investigate whether the analgesic effects of ivabradine might be observed in humans.

Ivabradine blocks all HCN isoforms about equally, and therefore causes a dose-dependent slowing of heart rate caused by blockade of HCN4 in the pace-making system of the heart [2; 18; 28]. In mice, ivabradine acts as an analgesic in an inflammatory pain model with an ED50 of 2mg/kg, similar to the ED50 of 2.5mg/kg for bradycardia, a result that is not unexpected in view of the lack of selectivity of ivabradine between HCN2, which drives pain, and HCN4, which drives the heart rate [46]. The dose of 15mg used in the present human study was the maximum acceptable dose consistent with mild bradycardia, but at approximately 0.2mg/kg is 10 times lower than the ED50 for analgesia in mouse studies. The present study shows that the degree of block of HCN2 achieved by this dose was insufficient for analgesia in the capsaicin pain model. Acute application of capsaicin causes neurogenic inflammation (i.e. flare) and recapitulates some of the symptoms observed in neuropathic pain and hence was employed as an analgesic assay for neuropathic pain in humans [25]. Capsaicin may be injected intra-epidermally or applied topically as was the case in our trial. Typical amounts injected range about 10 to 250 µg in 0.1ml of solvent [15; 21], which results in the direct delivery of a concentrated but small dose to the free nerve endings of nociceptors. In contrast, topical capsaicin relies on slower diffusion across the skin barrier [43]. Consequently, topical application and intra-epidermal injection of capsaicin result in different temporal profiles of pain. Intraepidermal capsaicin cause immediate and very intense 'burning' pain, which subsides over minutes [21], whereas topical capsaicin produces prolonged but less intense 'burning' pain. Regardless, punctate hyperalgesia occurs in both models [23; 47], and the initiating mechanism (i.e. the activation of TRPV1 receptors) is the same regardless of route of capsaicin delivery.

Topical capsaicin has the key advantage over injected capsaicin of being less invasive and acceptable to healthy volunteers. However, prolonged or repeated application causes loss of epidermal free nerve endings of nociceptors, which results in desensitization of skin to heat and mechanical (punctate) stimulation. For example, Nolano and colleagues exposed skin to 0.075% capsaicin for 3 weeks continually by reapplying the cream four times daily They reported that recovery of sensory thresholds took 6 weeks [32]. In our trial, capsaicin was applied to same forearm site at the screening visit and visit 2. We used 0.5% capsaicin cream in this trial, but the cream was removed from the skin after 75 minutes. Nonetheless, to minimize effects of capsaicin desensitization, we ensured a 4-week interval between the screening visit and visit 2 and balanced the order with which ivabradine and placebo were administered. Our data revealed, on average, smaller areas of punctate hyperalgesia, reduced sensitivity to warm detection and heat pain in visit 2 compared to visit 1 (effect of

treatment order, Table 3 and Supplemental figure 2). While the differences are small in this trial and do not approach statistical significance, the findings may inform design of similar trials.

Previous studies indicate that not all subjects develop punctate hyperalgesia after capsaicin administration, even those who had intra-epidermal injection [24]. There is likely be inter individual pharmacodynamic differences in propensity for neurogenic inflammation and subsequent neural sensitization. For example, the GCH1 gene has been reported to influence extent of capsaicin induced hyperalgesia [38]. Hence, we employed an enriched design to ensure only capsaicin-responders were randomized to receive ivabradine or placebo. The key advantage was potentially an increased sensitivity of the model to detect analgesic drug effects assay [12], however, we incurred a reduced enrolled rate with the enriched design of about 1 in 4 in our trial (Figure 3).

The measures employed to assess pain and hyperalgesia generated by capsaicin rely on subjective self-reports. There is considerable within-subject variability with such measures, and which we observed in this trial. Furthermore, it remains unclear what degree of analgesic effect based these measures would predicts drug efficacy in later phase clinical trials. Nevertheless, clinically established analgesics reduce capsaicin-induced mechanical and thermal hyperalgesia significantly. Typical effect sizes can be considerable [41] and have been observed for a number of drugs that are used clinically to manage pain, including opioids and gabapentin. Our trial was powered initially to detect a reduction of 10cm^2 in the area of punctate hyperalgesia on the basis that reductions of between 25 and 40 cm^2 have been observed for gabapentinoids, which are amongst the more effective analgesics for neuropathic pain [8; 13; 42]. However, interim analyses revealed that within-subject variability was greater than anticipated, and the predicted sample size required for maintaining power was cost-prohibitive, leading to trial termination. Hence, the trial failed to detect any significant effect of ivabradine on punctate hyperalgesia, and the same was observed for secondary outcomes including spontaneous burning pain and thermal hyperalgesia that are also observed following the topical application of capsaicin.

Positive controls or comparators can be employed in clinical trials to confirm assay sensitivity and support for negative findings. Single doses of gabapentinoid and other clinically established analgesics are known to significantly reduce pain and hyperalgesia generated by capsaicin [41]. However, these analgesics are unsuitable for as comparators in this trial because their obvious sedative effects (compared to none for ivabradine) would prevent effective blinding of subjects in this trial.

We note that the area of punctate hyperalgesia was clearly greater during screening in capsaicin responders, compared to either placebo or ivabradine treatments (Figure 5). Regression to the mean may account for the reduction of punctate hyperalgesia between screening and treatment visits because participants were enrolled based on relatively large areas of punctate hyperalgesia during screening. However, similar reductions were also observed between screening and treatment visits for other outcomes (burning pain and tactile allodynia), which suggests that these differences are related to substantial placebo effects.

Although our trial did not reveal any analgesic effects of ivabradine on the capsaicin model, it does not preclude positive effects in other human experimental models. Activation of TRPV1 receptors by capsaicin increases intracellular cAMP [45], which can activate cAMP-dependent protein kinase that leads to phosphorylation of TRPV1 [1] and leading to further sensitization of TRVP1 in a feed-forward loop. Ivabradine is known to suppress the action potential firing that is induced in nociceptive neurons by elevation of intracellular cAMP [46]. Hence the drug was expected to influence capsaicin-induced sensitization and hyperalgesia [31]. However, the neurogenic inflammation induced by capsaicin is distinct from inflammation produced by the immune response triggered by tissue damage [5]. The increase in intracellular cAMP in nociceptors is caused by numerous inflammatory mediators in injured tissue, and hence may far exceed that which can be induced by TRPV1 receptor agonism (by capsaicin) alone. If that is the case, pain and hyperalgesia caused by tissue-injury, for example in experimental burn [44] or incisional [19] models, can be expected to be more amenable to HCN2 receptor blockade. It is worth bearing in mind that experimental models of pain in humans are short-lived (hours). Whilst experimental models produce symptoms similar, those found in patients who are diagnosed with inflammatory or neuropathic pain, the initiating or underlying mechanisms are clearly different. Nonetheless data from experimental pain models in healthy volunteers do inform decisions on whether or not to proceed with costly clinical trials in patients [6].

We did not quantify flare from neurogenic inflammation caused by capsaicin in this trial. However, heat hyperalgesia is known to correlate with areas of capsaicin-induced flare [37], and we found no effects of ivabradine on heat hyperalgesia compared to placebo. While flare can be measured objectively by laser doppler or thermography, the measure can be influenced via top-down modulation of sympathetic outflow to skin vasculature during by mental stress or relaxation [27]

Despite the lack of analgesic effects of a single 15mg dose of ivabradine in our trial, we observed that the same dose did slow heart rate significantly. It is likely that the analgesic dose of ivabradine exceeds that which can be safely administered because of adverse effects on heart rate in humans. More selective HCN2 blockers that are peripherally restricted and hence devoid of adverse effects on both the heart and central nervous system are required to fully address the role of HCN channels for pain in humans.

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Figure legends

Figure 1 Crossover trial design. There were 3 visits in total, for screening (Visit 1) or treatment with either Ivabradine or Placebo. Topical capsaicin was applied to the dominant forearm for the first enrolled subject and for subsequently enrolled subjects until a capsaicin responder was identified, after which the non-dominant forearm was employed. The switch **a** between the use of dominant and non-dominant forearms occurred for the subsequent subject whenever a capsaicin responder was identified. Only capsaicin responders **b** were randomized to receive either Ivabradine first (Ivabradine-Placebo trial arm) or Placebo first (Placebo-Ivabradine) trial arm. The intervals between visits ensured that the minimum period between application of capsaicin to the same forearm was at least 28 days to avoid wash-over effects.

Figure 2 Time-line and sequence of assessments (primary and secondary outcomes) conducted at the screening (Visit 1) and treatment (Visit 2 and Visit 3) visits. Topical capsaicin cream was applied to the designated forearm for 75 minutes before removal at the screening and treatment visits. Oral Ivabradine (15 mg) or placebo was administered 60 minutes before the application of capsaicin during the treatment visits. 'Burning' pain induced by capsaicin was scored using visual analogue scales (VAS), each of which is a 100mm horizontal line drawn on paper, with the words 'none' at 0mm and 'intolerable at 100mm. Temperature thresholds were determined for warm detection (WD), heat pain (HP), cool detection (CD) and cold pain (CP), after mapping of the areas of punctate hyperalgesia (PH) and brush allodynia (BA). Heart rate (HR) and blood pressure (BP) were recorded just before administration of the treatment (either ivabradine or placebo) and again between 150 and 165 minutes after determination of temperature thresholds.

Figure 3 Consort diagram for the randomised, placebo-controlled crossover drug trial. Only capsaicin responders were randomised to receive either ivabradine at the first treatment visit and placebo at the second treatment visit [ivabradine->placebo arm] or the opposite order [placebo->ivabradine arm]

Figure 4 Mean (SE) effect of topical capsaicin on area of punctate hyperalgesia (top), area of brush allodynia (middle) and spontaneous 'burning' pain VAS score (bottom) score over time. Time 0 represents the point where capsaicin cream was applied to the designated forearm during screening and treatment visits. Ivabradine (15mg) or placebo treatments were administered 60 minutes before capsaicin was applied to the skin.

Figure 5 Boxplots showing the differences in temperatures for warm detection thresholds (WDT, bottom-right), heat pain detection threshold (HPT, bottom-left), cool detection threshold (CDT, top-right) and cold pain threshold (CPT, top-left) before (Time=0 minutes) and after (Time=75 minutes) capsaicin application during the screening visit and treatment visits, when either oral placebo or ivabradine (15mg) was administered 60 minutes before

topical capsaicin. There were no significant effects of ivabradine on these differences. The lower and upper hinges of the box-plot correspond to the first and third quartiles (the 25th and 75th percentiles). The upper whisker extends from the hinge to the largest value no further than 1.5 * IQR from the hinge (where IQR is the inter-quartile range, or distance between the first and third quartiles). The lower whisker extends from the hinge to the smallest value at most 1.5 * IQR of the hinge. Data beyond the end of the whiskers are called "outlying" points and are plotted individually.

Figure 6 Box plots of the effects of Ivabradine and Placebo on Pulse Rate, recorded as beats per min (BPM) before and after administration of capsaicin. The pre-capsaicin time point was 60 minutes after administration of treatment (ivabradine or placebo) and the post-capsaicin time point at about 150-165 minutes after drug treatment. There was a significant lowering of heart rate about 2.5 hours after administration of ivabradine compared to placebo.

Study assessments

Topical Capsaicin Pain Model

Prior to the application of capsaicin, the skin was prepared as follows: (1) cleaning with alcohol swab; (2) marking with an indelible-ink the area where capsaicin would be applied; (3) marking the points on the surrounding skin where brush or punctate stimuli would be used to map the areas of brush allodynia and punctate hyperalgesia respectively. These points were marked along 3 lines, 60 degrees apart, that intersected at the centre of the 3cm by 3cm square where capsaicin cream was applied, to form 6 spokes, A, B, C, D, E and F (Figure 2). The points on each spoke were separated by 1cm. A plastic template was used to assist with the skin markings. Topical capsaicin was thoroughly removed by alcohol wipe and the skin region dried by paper towel between 75 and 100 minutes after the cream was applied.

Warmth detection, heat pain, cool detection and cold heat thresholds

For the assessment of warm detection threshold, the thermode warmed up at a rate of 1^{o}C/s until the subject indicated by clicking on a computer-mouse when the increase in temperature was just perceptible, after which the thermode cooled down at a rate of 1^{o}C/s to baseline. The maximum possible temperature was 50^{o}C. There were four up-and-down ramps, each separated by a random interval of 4-6s. The mean temperature at which warm detection occurred was recorded.

The ramps were then repeated to determine heat pain thresholds. For those ramps, the subject indicated when the increase in temperature became just about painful. There were three ramps, each separated by a random interval of 10-12s. The mean temperature at which heat pain occurred was recorded.

For the assessment of cool detection threshold, the thermode was cooled down at a rate of 1^{o}C/s until the subject indicated by clicking on a computer-mouse when the decrease in temperature was just perceptible, after which the thermode warmed up at a rate of 1^{o}C/s to baseline. The minimum possible temperature was 0^{o}C. There were four down-and-up ramps, each separated by a random interval of 4-6s. The mean temperature at which cool detection occurred was recorded.

The ramps were then repeated to determine cold pain thresholds. For those ramps, the subject indicated when the decrease in temperature became just about painful. There were three ramps, each separated by a random interval of 10-12s. The mean temperature at which heat pain occurred was recorded.

Key inclusion Criteria

To be included in the trial the participant must:

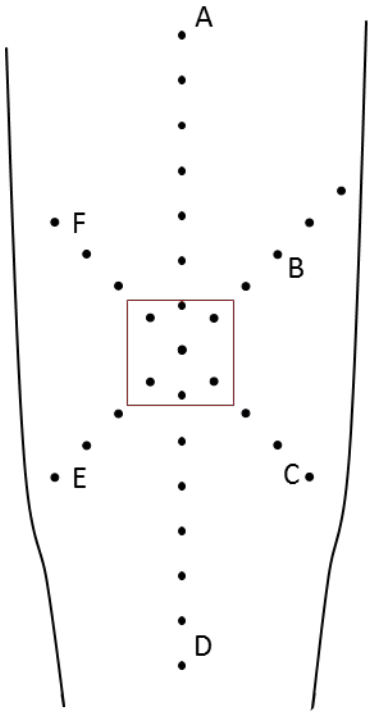
- be male or female and aged 18-64 years
- be free from chronic pain and not using any medicine for pain relief
- be in good general health with a Body Mass Index (BMI) between 19 and 35
- have a normal resting multi-lead standard ECG including (measured for 1 minute on lead D2):
    - Normal sinus rhythm;
    - 60 bpm HR or greater on resting ECG;
    - PR interval ≤ 210 ms;
    - QTcB ≤ 430 ms for men and QTcB ≤ 450 ms for women;
    - QRS duration ≤ 120 ms;

Key exclusion criteria

The presence of any of the following will preclude participant inclusion:

- Previous surgery or tattoo on either forearm
- History of disease associated with neuropathy
- Volunteers who are allergic to ivabradine or capsaicin or any of the excipients within the respective finished products
- History of personal or familial Long QT Syndrome
- History of cardiac dysrhythmia
- Use of CYP3A4 inhibitors such as ketoconazole, itraconazole, macrolide antibiotics and the anti-retrovirals nelfinavir, nefazodone and ritonavir.
- Use of QT interval prolonging medicinal products (e.g. quinidine, disopyramide or pimozide etc.)
- Volunteers with any rash or broken skin on the arm where the capsaicin will be applied
- Volunteers with lactose intolerance, as the placebo and ivabradine tablets contain lactose
- Volunteers with a resting heart rate of 59 beats per minute or less at screening
- Female volunteers of childbearing potential who refuse to use adequate contraceptive measures for the duration of the trial
- Male volunteers who refuse to use adequate contraceptive measures for the duration of the trial
- Volunteers who smoke (≥5 cigarettes/day), take recreational drugs or consume more than the recommended allowance of alcohol units per week (21 units per week for males and 14 units per week for females)
- Participants who are not willing to abstain from drinking beverages containing quinine, caffeine and/or xanthine for 24 hours prior to the trial visit
- Volunteers who produce a positive result in a urine screen for drugs or who are known or suspected to be drug-dependent (sedatives, hypnotics, tranquilizers or any other addictive agent)
- Volunteers who produce a positive result in an alcohol breath test
- Volunteers currently participating in any interventional trial, have participated in an interventional trial within 16 weeks of screening or are currently participating in a non-interventional trial which participating in this trial would impact upon
- Volunteers who, in the opinion of the PI, have a clinically relevant abnormality or medical history that is deemed to make the participant ineligible because of a safety concern.

Supplemental Table 1 Key eligibility criteria for the trial

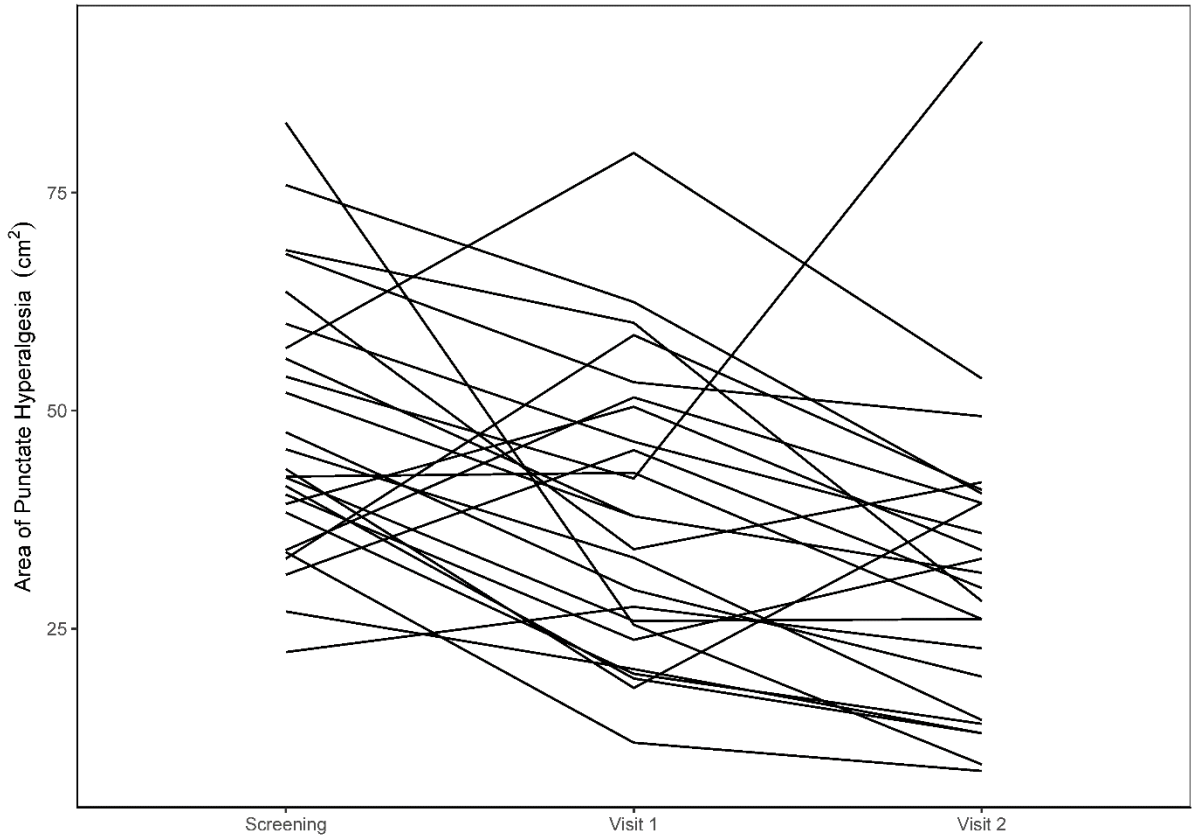


Thermal stimuli: computer-controlled skin thermode

Brush stimulus: Q-tip (cotton wisp)

Punctate stimulus 26g Von Frey filament

Supplemental Figure 1 Diagram demarcating the pain model on the volar aspect of the forearm. Topical capsaicin 0.5% cream was applied to cover a 3cm x 3 cm square (outlined in red) for 75 minutes. Warm detection, heat pain, cool detection and cold pain thresholds were determined by placing a computer-controlled thermode on the red square before capsaicin cream was applied, and again just after the cream was removed. Areas of punctate hyperalgesia and brush allodynia were determined by applying the stimulus, on the points marked on the skin. Each point was 1 cm apart and formed spokes A, B, C, D, E and F that converged in the centre of the square region where capsaicin was applied. The centre was designated 0 cm. Assessments of areas of sensitivity to the brush and punctate stimuli were performed just before and every 15 min after capsaicin cream was applied to the skin till removal of the cream. Points that fell within the square were assumed to be sensitive when capsaicin was applied to the skin.



Supplemental Figure 2 Spaghetti plots showing the area of punctate hyperalgesia induced by 75 min of capsaicin at the initial (Screening) visit and subsequent treatment visits (Visits 1 and 2). The lines represent individual subjects (n=24).