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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

Michael C. Lee a,*, Simon Bond b, Daniel Wheeler a, Ingrid Scholtes a, Graham Armstrong b, Peter McNaughton c, David Menon a

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 nonselective HCN channel blocker, on capsaicin-induced hyperalgesia and pain in healthy human subjects. An enriched population comprising subjects who developed >20 cm² of punctate hyperalgesia from topical capsaicin (0.5% cream applied onto 9 cm² area) was identified. These subjects then received ivabradine (15 mg) or placebo 1 hour before 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 first and to the second treatment visits was at least 3 and 5 weeks, respectively, to minimize carryover effects. Fifty-five participants were screened, of which 25 completed at least 1 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 cm², 95% confidence interval: = −4.04 to 10.48, P = 0.37). However, ivabradine caused a slowing of heart rate (difference of 10.10 beats per minute [95% confidence interval −6.48 to −13.73; P-value <0.001]). 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.

Keywords: Clinical trial, HCN channels, Ivabradine, Capsaicin, Hyperalgesia, Pain

1. Introduction
The hyperpolarization-activated cyclic nucleotide gated (HCN) ion channel family comprises 4 isoforms, HCN1-4, which carry an inward current called I h (also I q or I f). Previous studies have shown that HCN1 and HCN2 are the isoforms most strongly expressed in primary sensory neurons. Large nonnociceptive sensory neurons express a fast, cyclic adenosine monophosphate (cAMP)-insensitive I h, attributable mainly to HCN1. HCN1 is not functionally expressed in small sensory neurons, the majority of which are nociceptors. In most small sensory neurons, I h has slower kinetics and is sensitive to intracellular cAMP, consistent with expression of HCN2.

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, 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.

To date, there is no specific blocker of HCN2 channels, licensed for use in humans, with which to translate preclinical findings. However, ivabradine, a nonselective and peripherally restricted HCN blocker, is clinically used for the treatment of chronic angina and mild-moderate heart failure with systolic dysfunction. 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 used 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 noninvasive assay that is sensitive to several classes of
clinically licensed analgesics, including gabapentinoids, non-steroidal anti-inflammatory drugs, local anaesthetics, and opioids. In humans, topical capsaicin activates TRPV1 channels expressed by the free nerve ending 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. 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 with developing a selective HCN2 channel blocker for use in humans.

2. 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 are available on request through 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.

2.1. Study design

This was a single-centre, randomised, double-blind, placebo-controlled, 2-period crossover 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 (Fig. 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 dropout, and the crossover design allowed subjects to provide their own control observations, thus increasing the accuracy of the treatment effect estimates, in comparison with a parallel-arm 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 (ie, responded to capsaicin). This method of screening for capsaicin responders and nonresponders before the treatment phase of the trial has been reported previously.11 We assumed that 40% of participants responded to capsaicin as defined by the spatial extent of mechanical punctate hyperalgesia (PH) they displayed (see section "Identification of capsaicin responders").

2.2. Participants

Healthy volunteers were recruited through local advertisements. Respondents were provided with written information (see Supplemental Materials, available at http://links.lww.com/PAIN/A835) and prescreened 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, available at http://links.lww.com/PAIN/A835) were enrolled.

2.3. Topical capsaicin pain model

Topical capsaicin (0.5% cream; 1 mL drawn in a 2-mL plastic syringe; The Specials Laboratory Limited, Northumberland, United Kingdom) was applied without occlusive dressing to a 9 cm² area of skin that was marked on the volar aspect of the designated forearm (see supplemental Fig. 1, available at http://links.lww.com/PAIN/A835). The cream was left on for 75 minutes and removed once the final assessments for areas of brush allodynia (BA) and PH were completed (Fig. 2). Please refer to supplemental materials for full details (available at http://links.lww.com/PAIN/A835).

2.4. Identification of capsaicin responders

Subjects who developed an area of PH equal to or greater than 20 cm², rounded to the nearest cm², at 75-minute postcapsaicin application during visit 1 were identified as capsaicin responders. The method for assessing areas of hyperalgesia is described in the relevant section below.

2.5. Randomisation

The use of the dominant or nondominant 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, ie, 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 nondominant arm was used to determine response to capsaicin at screening for subsequent subjects (Fig. 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 2 periods (ivabradine-placebo or placebo-ivabradine) using the method of blocked randomisation (block size = 4) stratified by the sequence of forearm used. The online central randomisation service, TENABLEA (https://www.aleaclinical.eu/), was used to generate treatment sequence allocation.

2.6. Ivabradine and placebo drug treatments

The active treatment consisted of a single oral 15-mg dose of ivabradine that was administered as 2 tablets, each containing 7.5 mg 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 Procoralain) as blister packs. Each pack contained 2 placebo or 2 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.

2.7. Study assessments

Each participant attended 1 screening and 2 treatment visits. The same investigator performed study assessments for all 3 visits. All study assessments were undertaken in the same temperature-controlled environment.

The assessments performed 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.10,33

2.8. 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 (3 x 3 cm, Pathway ATS; Medoc, Ramat Yishai, Israel) was placed on the skin (see supplemental Fig. 1, available at http://links.lww.com/PAIN/A835). The subject was allowed 3 to 5 minutes to acclimatise to the baseline temperature of 32°C. The method of limits was then used to determine the sensory thresholds in the following sequence: warm detection threshold (WDT), heat pain threshold (HPT), cool detection threshold (CDT), and cold pain threshold (CPT) (see supplemental materials for details, available at http://links.lww.com/PAIN/A835). This sequence was chosen instead of the sequence CDT, WDT, CPT, and HPT that is used by established clinical quantitative sensory testing protocols, eg, the Deutscher Forschungsverbund Neuropathischer Schmerz (DFNS) protocol described by Rolke et al.34 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.

2.9. 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 100-mm 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” are commonly used as the extreme right anchor for the VAS to assess pain in clinical settings. Pain in this trial is caused experimentally, is far less intense in comparison, and is 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 100 mm 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 1 mm by a ruler before use. The participant rated pain by indicating a mark along the 100-mm line. The distance from 0 mm was measured and recorded by the investigator.

2.10. Capsaicin-induced area of brush allodynia and punctate hyperalgesia

The area of 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 Fig. 1, available at http://links.lww.com/PAIN/A835). The Q-tip bud was applied using a smooth sweeping motion as if to “draw” a 1-cm line perpendicular to the radial spoke. The stimulus was then applied at 2 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 PH was determined in a similar fashion but using a 26-g von Frey monofilament. The areas of PH or BA were calculated using the formula 1/2 \( x_1 x_2 x_3 x_4 \sin(60) \); 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.

Figure 2. Timeline and sequence of assessments (primary and secondary outcomes) conducted at the screening (visit 1) and treatment (visits 2 and 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 the visual analogue scales (VAS), each of which is a 100-mm horizontal line drawn on paper, with the words “none” at 0 mm and “intolerable” at 100 mm. 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.
2.11. Blood pressure and pulse rate

Noninvasive forearm blood pressure, heart rate, and pulse oximetry (Dash 3000; GE Healthcare, Buckinghamshire, United Kingdom) were obtained before treatment (ivabradine or placebo tablets) administration and before subject discharge home. The heart rate through 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 to maintain double blinding of the investigator.

2.12. Analytical plan

2.12.1. Sample size estimation and interim analyses

The primary endpoint was the area of PH at 75 minutes after application of capsaicin. Preliminary data from 4 capsaicin responders provided a within-subject SD of 18 cm² (ISSNeP, 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 cm² (ivabradine – placebo) with 80% power at the 2-sided 5% significance level.

However, this sample size estimate, based on a very limited number of observations, was likely to be imprecise. Hence, a preplanned 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-effects model was fitted for the primary endpoint and adjusted for baseline covariates using the methods of Kenward and Roger.19 The interim analysis, conducted with data from the first 14 participants who had completed both treatment visits, revealed a within-subject SD of 22.1 cm² (95% confidence interval [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).

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].
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 (\(\mu\)) 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 was 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.\(^{15}\) 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 SD 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.

### 2.13. Statistical analysis

The statistical software SAS (version 9.4) was used 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: \(n\) (nonmissing sample size), mean, SD, median, maximum, and minimum. The frequency and percentages (based on the nonmissing sample size) of observed levels are reported for categorical measures.

The primary endpoint (PH) was analysed using a linear mixed-effects model with fixed effects for the treatment, forearm and period (visit order), and the 2 precapsaicin values from both periods,\(^{19}\) and a random intercept at the subject level. The value of PH 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% CIs are provided with associated \(P\)-values. Summary statistics (mean, SE, median, max, and min) will be provided for the within-subject difference (placebo minus ivabradine) and for each treatment. Intention-to-treat analyses were performed for the primary outcome for sensitivity analyses, along with per-protocol analyses and to test the robustness of the findings.

| Variable                                        | Statistics | Ivabradine -> placebo | Placebo -> ivabradine |
|-------------------------------------------------|------------|------------------------|-----------------------|
| **Age (y)**                                     | \(n\)      | 15                     | 12                    |
|                                                | Mean (SD)  | 32.1 (10.7)            | 38.3 (15.6)           |
|                                                | Median     | 31                     | 32                    |
|                                                | Min-max    | 21-59                  | 22-64                 |
| **Sex**                                         |            |                        |                       |
| Female                                          | \(n\)      | 60% (9/15)             | 66.7% (8/12)          |
| Male                                            | \(n\)      | 40% (6/15)             | 33.3% (4/12)          |
| **Weight (kg)**                                 | \(n\)      | 15                     | 12                    |
|                                                | Mean (SD)  | 69.7 (12.1)            | 73.4 (14.6)           |
|                                                | Median     | 66.1                   | 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/m\(^2\))**                | \(n\)      | 15                     | 12                    |
|                                                | Mean (SD)  | 24.6 (2.62)            | 25.8 (3.88)           |
|                                                | Median     | 24.6                   | 24.6                  |
|                                                | Min-max    | 20.9-29.3              | 21.3-32.4             |
| **Ethnicity**                                   |            |                        |                       |
| White                                           | \(n\)      | 80% (12/15)            | 75% (9/12)            |
| Asian                                           | \(n\)      | 20% (3/15)             | 16.7% (2/12)          |
| Hispanic                                        | \(n\)      | 0% (0/15)              | 0% (0/12)             |
| Black                                           | \(n\)      | 0% (0/15)              | 8.3% (1/12)           |
| Other                                           | \(n\)      | 0% (0/15)              | 0% (0/12)             |
| **Dominant arm**                                |            |                        |                       |
| Left arm                                        | \(n\)      | 6.7% (1/15)            | 0% (0/12)             |
|                                                | Median     | 93.3% (14/15)          | 100% (12/12)          |
| Right arm                                       | \(n\)      | 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.

| Variable                                        | Statistics | Ivabradine -> placebo | Placebo -> ivabradine |
|-------------------------------------------------|------------|------------------------|-----------------------|
| **Area of punctate hyperalgesia at 75 minutes**  | \(n\)      | 15                     | 12                    |
| induced by capsaicin                             | 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             |

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 was 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.\(^{15}\) The statistical analysis plan is available upon request.

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

The primary endpoint (PH) was analysed using a linear mixed-effects model with fixed effects for the treatment, forearm and period (visit order), and the 2 precapsaicin values from both periods,\(^{19}\) and a random intercept at the subject level. The value of PH 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% CIs are provided with associated \(P\)-values. Summary statistics (mean, SE, median, max, and min) will be provided for the within-subject difference (placebo minus ivabradine) and for each treatment. Intention-to-treat analyses were performed for the primary outcome for sensitivity analyses, along with per-protocol analyses and to test the robustness of the findings.

The prespecified secondary endpoints were area (cm\(^2\)) of BA, VAS (0-100 mm) scores for capsaicin-induced spontaneous burning pain, and temperatures (\(^\circ\)C) for WDT, HPT, CDT, and CPT (\(^\circ\)C). Heart rate (beats per minute) was considered a safety
3. Results

Thirty-nine subjects were eligible and were assessed for response to topical capsaicin at screening. Of those, 8 were nonresponders, and 4 dropped out (uncontactable) after the screening visit.

Twenty-seven subjects were randomised during a 13-month period beginning in January 2015 (Fig. 3). Fifteen were allocated to receive ivabradine in visit 2 then placebo in visit 3 (ivabradine-placebo group). Twelve were allocated to receive the treatments in the opposite sequence (placebo-ivabradine group). For the ivabradine-placebo group, 2 subjects did not receive any treatment and 1 subject received ivabradine only. For the placebo-ivabradine group, all 12 subjects completed the study visits.

Baseline characteristics were similar between the 2 groups (Table 1).

### 3.1. 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.

| Variable                              | Ivabradine mean (SD) | Placebo mean (SD) |
|---------------------------------------|----------------------|------------------|
| VAS 100 mm spontaneous “burning” pain at 75 minutes after capsaicin* | 40.1 (23.3) | 34.5 (23.8) |
| Area of punctate hyperalgesia (cm²) at 75 minutes after capsaicin* | 34.90 (15.0) | 33.45 (15.3) |
| Area of brush allodynia (cm²) at 75 minutes after capsaicin* | 23.55 (16.9) | 22.02 (14.9) |
| Warm detection threshold (°C) before capsaicin† | 34.2 (0.755) | 34.3 (0.858) |
| Heat pain threshold (°C) before capsaicin† | 41.7 (3.03) | 41.8 (2.69) |
| Cool detection threshold (°C) before capsaicin† | 30.4 (0.612) | 30.4 (0.898) |
| Cold pain threshold (°C) before capsaicin† | 20.9 (6.59) | 18.9 (8.93) |
| Warm detection threshold (°C) after capsaicin‡ | 34.1 (0.527) | 34.0 (0.477) |
| Heat pain threshold (°C) after capsaicin‡ | 34.7 (1.040) | 34.8 (0.923) |
| Cool detection threshold (°C) after capsaicin‡ | 30.4 (0.612) | 30.4 (0.898) |
| Cold pain threshold (°C) after capsaicin‡ | 8.16 (8.73) | 8.34 (8.52) |

*At 135 minutes after drug administration before capsaicin was removed from skin.
†Just before drug treatment was administered.
‡At 150 minutes after drug treatment was administered when capsaicin cream was removed from the skin.

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 (Fig. 5 and Table 3).

### 3.2. 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 (Fig. 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 minute (95% CI −6.48 to −13.73; P-value < 0.0001). Hence, ivabradine slowed heart rate significantly in this trial when compared with placebo.

### 3.3. 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.

### 4. Discussion

Ivabradine is analgesic in behavioural and electrophysiological studies of inflammatory and neuropathic pain models in mice. These models include intraplantar formalin injection, chronic nerve constriction injury (traumatic neuropathy), systemic oxaliplatin (chemotherapy-induced neuropathy), and more recently, diabetic neuropathy (neuropathic pain induced by diabetes). The analgesic effects of ivabradine result specifically from blockade of the HCN2 ion channel isoform that is expressed by nociceptors. 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. In mice, ivabradine acts as an analgesic in an inflammatory pain model with an ED50 of 2 mg/kg, similar to the ED50 of 2.5 mg/kg.
for bradycardia, a result which is not unexpected in view of the lack of selectivity of ivabradine between HCN2, which drives pain, and HCN4, which drives the heart rate. The dose of 15 mg used in the present human study was the maximum acceptable dose consistent with mild bradycardia, but at approximately 0.2 mg/kg is 10 times lower than the ED50 for analgesia in mouse studies.

This 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 (ie, flare) and recapitulates some of the symptoms observed in neuropathic pain and hence was used as an analgesic assay for neuropathic pain in humans. Capsaicin


Previous studies indicate that not all subjects develop PH after capsaicin administration, even those who had intraepidermal injection.23 There is likely be interindividual 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.32 Hence, we used 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 effect assay12; however, we incurred a reduced enrolled rate with the enriched design of about 1 in 4 in our trial (Fig. 3).

The measures used 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 considerable41 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 10 cm² in the area of PH on the basis that reductions of between 25 and 40 cm² have been observed for gabapentinoids, which are amongst the more effective analgesics for neuropathic pain.8,13,41 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 PH, and the same was observed for secondary outcomes including spontaneous burning pain and thermal hyperalgesia that are also observed after the topical application of capsaicin.

### Table 3

| Variable (ITT) | Effect | Estimate | SE  | P     | 95% confidence interval |
|---------------|--------|----------|-----|-------|------------------------|
| Area of punctate hyperalgesia (cm²) | Treatment: | Ivabradine — placebo | 3.22 | 3.50 | 0.37 | −4.04 to 10.48 |
|                | Order of treatment: | First — second | 6.70 | 3.46 | 0.07 | −0.47 to 13.87 |
|                | Forearm (capsaicin): | Dominant — nondominant | −2.18 | 3.50 | 0.54 | −9.44 to 5.08 |
| Area of brush allodynia (cm²) | Treatment: | Ivabradine — placebo | 2.70 | 3.22 | 0.41 | −3.99 to 9.4 |
|                | Order of treatment: | First — second | 2.55 | 3.17 | 0.43 | −4.05 to 9.15 |
|                | Forearm (capsaicin): | Dominant — nondominant | −5.47 | 3.22 | 0.10 | −12.16 to 1.22 |
| Buring pain VAS (0-100 mm) | Treatment: | Ivabradine — placebo | 3.76 | 3.31 | 0.27 | −3.11 to 10.64 |
|                | Order of treatment: | First — second | 0.17 | 3.27 | 0.96 | −6.61 to 6.96 |
|                | Forearm (capsaicin): | Dominant — nondominant | −0.08 | 3.31 | 0.08 | −12.92 to 0.83 |
| Warm detection threshold (°C) | Treatment: | Ivabradine — placebo | 0.10 | 0.14 | 0.45 | −0.17 to 0.38 |
|                | Order of treatment: | First — second | 0.03 | 0.13 | 0.82 | −0.24 to 0.3 |
|                | Forearm (capsaicin): | Dominant — nondominant | −0.09 | 0.14 | 0.51 | −0.37 to 0.19 |
| Heat pain threshold (°C) | Treatment: | Ivabradine — placebo | −0.07 | 0.15 | 0.65 | −0.39 to 0.25 |
|                | Order of treatment: | First — second | 0.08 | 0.15 | 0.61 | −0.23 to 0.38 |
|                | Forearm (capsaicin): | Dominant — nondominant | 0.08 | 0.15 | 0.60 | −0.24 to 0.4 |
| Cool detection threshold | Treatment: | Ivabradine — placebo | −0.04 | 0.33 | 0.91 | −0.73 to 0.65 |
|                | Order of treatment: | First — second | 0.22 | 0.32 | 0.50 | −0.45 to 0.89 |
|                | Forearm (capsaicin): | Dominant — nondominant | −0.13 | 0.33 | 0.71 | −0.81 to 0.56 |
| Cold pain threshold | Treatment: | Ivabradine — placebo | −1.35 | 1.96 | 0.50 | −5.4 to 2.7 |
|                | Order of treatment: | First — second | 1.08 | 1.92 | 0.58 | −2.91 to 5.07 |
|                | Forearm (capsaicin): | Dominant — nondominant | −0.15 | 0.33 | 0.71 | −0.81 to 0.56 |

**ITT, results reported are from intention-to-treat (ITT) analysis; VAS, visual analogue scale.**
Positive controls or comparators can be used 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. However, these analgesics are unsuitable for as comparators in this trial because their obvious sedative effects (compared with none for ivabradine) would prevent effective blinding of subjects in this trial.

We note that the area of PH was clearly greater during screening in capsaicin responders, compared with either placebo or ivabradine treatments (Fig. 4). Regression to the mean may account for the reduction of PH between screening and treatment visits because participants were enrolled based on relatively large areas of PH 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, which can activate cAMP-dependent protein kinase that leads to phosphorylation of TRPV1 and leading to further sensitization of TRPV1 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. Hence, the drug was expected to influence capsaicin-induced sensitization and hyperalgesia. However, the neurogenic inflammation induced by capsaicin is distinct from inflammation produced by the immune response triggered by tissue damage. 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, eg, in experimental burn or incisional 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). Although 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.

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, and we found no effects of ivabradine on heat hyperalgesia compared to placebo. Although flare can be measured objectively by laser Doppler or thermography, the measure can be influenced through top-down modulation of sympathetic outflow to skin vasculature during by mental stress or relaxation.

Despite the lack of analgesic effects of a single 15-mg 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.

**Conflict of interest statement**

P. McNaughton is involved in a drug discovery program, funded by the Wellcome Trust, to develop HCN2-selective molecules as analgesics. The remaining authors have no conflicts of interests to declare. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR, or the Department of Health and Social Care.

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**Appendix A. Supplemental digital content**

Supplemental digital content associated with this article can be found online at http://links.lww.com/PAIN/A835.

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