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A comparison of two formulations of intradermal capsaicin as models of neuropathic pain in healthy volunteers.

Master Thesis in Pharmacy
The Pharmacy Programme

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Göteborg, Sweden 2007

Abstract

Background: Intradermal capsaicin is a putative human pain model that produces reliable pain perception, replicating the symptoms for neuropathic pain. It facilitates controlled testing of analgesic efficacy via cross-over-design in healthy volunteers. A formulation with capsaicin, which is a solution in lower concentrations and a colloidal suspension in higher concentrations, has been used extensively but it is associated with several disadvantages. A new beneficial formulation, a solution in all tried concentrations, with capsaicin dissolved in a (2-hydroxypropyl)-β-cyclodextrin-vehicle, (HP-β-CD), has been investigated. In order to have confidence in the utility of the HP-β-CD-formulation of capsaicin, a clinical validation-study comparing the two formulations was required.

Methods: 1, 10, 30 and 100 μg doses of both formulations were given as 10 μL intradermal injections in a blinded, randomized, cross-over manner to sixteen volunteers (8M/8F). Spontaneous pain (rated on VAS) and hyperalgesia (standardized von Frey hair) were assessed at intervals up to one hour post-injection.

Results: The two formulations produced pharmacodynamic comparable responses to each other, for the three lowest doses for both outcomes. The fixed effects of formulation, dose and formulation•dose were significant affecting each outcome. Gender, arm position and dominance were also significant affecting hyperalgesia.

Conclusions: The formulations can be considered to be pharmacodynamic comparable for the three lowest doses, which may be the doses most suitable for clinical use. Both formulations can be considered to be safe and tolerable but the HP-β-CD-formulation exhibits pharmaceutical benefits. These findings complemented and extended the knowledge about intradermal capsaicin as a model for neuropathic pain.

Key Words: human pain model, capsaicin, allodynia, flare, hyperalgesia, pain, neuropathic pain

Declaration

This thesis is prepared as a part of our degree of Master of Science in Pharmacy at Göteborg University.

The thesis is based on original data, obtained while we were exchange students at the Discipline of Pharmacology at the University of Adelaide, South Australia in the period July - December 2007.

The clinical trial was conducted in a team environment and the trial was dependent on contributions from:

Chai Li Lau, for the development of a HP-β-CD formulation of capsaicin and HPLC-assay after the trial.
Pharmacist Sharon Yap, for assisting us in the preparation of the actual formulations used in the trail.
Statistician Lisa Miller for creating a randomization list and conducting the statistical analysis.
Prof. Paul Rolan for assisting us in the writing and submitting of the protocol to the Research Ethics Committee.

However, we were the persons responsible for conducting the complete trial, which includes the following:

- Co-ordinate the whole study
- Write the protocol
- Write answers to the Investigational Drug Subcommittee
- Prepare and perform test-batches of both formulations
- Write the pharmacy manual and batch sheets
- Perform the first production steps of the actual formulations used in the trial
- Perform a literature study on previous capsaicin studies with similar design
- Develop the methods and writing the SOPs
- Create case report forms
- Prepare material required in the trial and write the schedule for the trial
- Execute and plan all pharmacodynamic measurements
- Assess the four outcomes during the trial
- Work-up the data and create a data file with all results
- Interpret and analyse the data and write a own personal thesis

Adelaide, December 2007

Johanna Åkesson Helena Gustafsson

1. Introduction

1.1 Background

1.1.2 Neuropathic pain

The mechanisms responsible for different types of clinical pain are only partly understood. Neuropathic pain is a type of chronic pain caused by the damage or malfunctioning of the peripheral or central nervous system and it may be unrelated to ongoing tissue damage or inflammation [1]. It often occurs in a number of clinical conditions, such as post-herpetic neuralgia, diabetic neuropathy, phantom limb pain, post stroke pain and peripheral neuropathies. Unlike physiological pain, which serve to warn and protect humans from injury, neuropathic pain serves no useful purpose. Current treatments are only partly effective (e.g. opioids, gapabentin, pregabalin), are limited by side effects (e.g. tricyclic antidepressants (amitryptylline) or carbamazepine) or are or are difficult to control (NMDA receptor antagonists). Beside these factors, a high degree of variability exists clinically between patients in their response to treatment. The prevalence of neuropathic pain is still unknown, but estimates indicate that 1 % of individuals in UK experience some form of neuropathic pain. This rate is probably an underestimate [1]. There are many potential new treatments in development for neuropathic pain.

1.1.3 Pain models

The use of the clinical pain state has evident limitations in evaluating analgesic interactions. Pain states are often multi-factorial (e.g. tissue- and nerve injury and inflammation) with treatment-regimens involving multiple medications which convey that controlled crossover interactions cannot typically be conducted on the same subject [2]. Evaluation of potential new agents treating neuropathic pain may be helped by the use of pain models in early clinical development. Volunteer models of pain permit a more well-controlled study design and are useful in the study of specific pain mechanisms. Due to these reasons, volunteer models are used in clinical drug development to demonstrate the analgesic potential of new compounds [2]. A pain model deployed in a limited number of human subjects would be clearly advantageous in reducing early development time and costs [3] as well as the number of volunteers needed. The selection of a model should be based on the mechanism of pain targeted by the compound under investigation. One putative model of neuropathic pain is the intradermal capsaicin model [3]. When capsaicin is injected into the skin it causes a transient characteristic burning and sensitivity to light touch. Such symptoms replicate the key symptoms of neuropathic pain and raise the possibility of using this tool to assess drug response.

1.1.4 Capsaicin and the TRPV1-receptor

Capsaicin is the primary active component of the heat and pain-eliciting, lipid-soluble fraction of the capsicum pepper. It is an agonist to the transient receptor potential vanilloid 1, TRPV1, a ligand gated ion channel, expressed predominantly by nociceptive afferent neurons [4]. Binding to TRPV1 causes influx of Na^+ and Ca^{2+}, hence depolarisation and initiation of action potentials [5].

Capsaicin influences the pain perception when injected into the skin. The pain sensation of capsaicin can be divided into four physiologically based categories:

1. Spontaneous pain, which is short-lived (10-30 minutes) and consist of a burning/aching sensation experienced at the site of administration.
2. Allodynia, which is pain that is evoked by a previously non-painful stimuli. The allodynia is usually short-lived (20 minutes) and appears as both primary and secondary allodynia.
3. Hyperalgesia, which is increased pain evoked by a previously painful stimulus, e.g. a pinprick. This can last between 6 and 24 hours and occurs at the site of administration (primary hyperalgesia) and in the surrounding skin area (secondary hyperalgesia).

4. Flare or neurogenic inflammation, which is the area of redness extending beyond the site of injection. The inflammation lasts between 30 and 90 minutes.

The afferent pain transmission is mediated by sensory nerves with varying anatomical dimensions. The nociceptors, sensory receptors, detect different types of stimuli and are classified afterwards. Large diameter sensory fibres have either low- (Aβ-mechanoreceptors) or high –threshold receptors (Aδ-mechanoreceptors). These finely myelinated fibres are sensitive to various mechanical stimulations. Low- threshold Aβ-mechanoreceptors do normally only convey non-painful stimuli [2]. Smaller diameter sensory fibres contain receptors sensitive to the various kinds of pain; these polymodal C-fibre receptors, are un-myelinated and hence more slowly conducting compared to the A-fibres and as the name suggests, these response to mechanical deformation, to intense heat or cold and to irritant chemicals. All three types of fibres synapse in the dorsal horn ganglion.

Capsaicin elicits burning pain and cutaneous neurogenic vasodilatation, after TRPV1-binding on the nociceptor by causing release of substance P and calcitonin gene related peptide (CGRP) from sensory C-fibres. The spontaneous burning and aching pain caused by administration of capsaicin is mediated by the C-fibre polymodal receptors, the pinprick hyperalgesia is mediated via Aδ- and C-fibres and the allodynia are mediated via Aβ-fibres. This has been suggested in several studies in both man and animal [2].
The characteristics of this capsaicin irritation vary across the body because of physical properties such as skin thickness and some differences in autonomic and sensory functions in different parts of the body [6]. The volar part of the forearm is the body part that is generally used in this model. [2,3,9,18].

Capsaicin can even be intended to function as a potential ‘biomarker’ of central sensitisation, since both allodynia and hyperalgesia are believed to be mediated by central sensitization. Central sensitisation is an altered central processing, which is thought to underlie many clinical pain states such as post herpetic neuralgia. This altered central processing of the pain input in the spinal cord explains why the normally non painful stimulation of Aβ-fibres in example allodynia is experienced as painful [2].

The response properties of nociceptors and the peripheral neural mechanisms contributing to pain and altered pain states are similar in monkeys and humans, however some species differences have been found. The flare that surrounds a local cutaneous injury in human skin is believed to be mediated by an axon reflex which not is present in monkey skin. So the conclusion is that if the hyperalgesia is caused by the axon reflex it is best seen in humans [7]. In addition to anatomical differences between animals and humans, animal pain models also have the disadvantage that the interpretation of pain usually relies on the observation of animals behaviour and the lack of possibility to measure allodynia and hyperalgesia and their underlying mechanism under standardized experimental conditions [8].

1.1.5 Capsaicin formulations

Capsaicin is insoluble in water and this causes a problem for intradermal use. It is highly soluble in ethanol but a formulation of 10 % ethanol causes significant pain on injection due to the vehicle, which confounds the scientific integrity of the model. A formulation with capsaicin, which is a solution in lower concentrations and a colloidal suspension in higher concentrations, has been used extensively but it is associated with several disadvantages [9]. A colloidal suspension may reduce the effective local concentration, result in unequal given dosages or act as a depot. The formulation is also difficult to prepare and must be made freshly for use. However, such a formulation has been used extensively [9]. An alternative formulation, which is a sterile solution within the whole investigated dose range, is easier to prepare and does not need to be made freshly, would make the use of this technique more practicable [2].

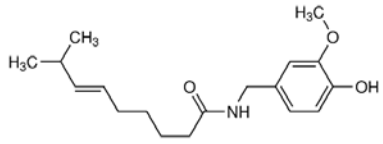

Figure 1. Structure of capsaicin molecule [10].

Because of the poor water solubility, excipients such as solvents might be methods for increasing its solubility. The use of ethanol, propylene glycol and surfactants are unsuitable because of pain and tissue damage. Adjusting pH in this case will be unsuitable because the pKa of capsaicin is 9.76 (CAS 404-86-4), which means that high pH is needed to solubilise the compound. In addition, a low pH will cause pain and tissue irritation on injection since TRPV1 is activated by acidic conditions (pH < 5.9) [11] and by elevated temperatures (~ 43^{o}C) [12,13].

Cyclodextrins (CD) are potentially useful agents for increasing the aqueous solubility of lipophilic compounds like capsaicin. They have lipophilic inner cavities and lipophobic outer surfaces, capable of interacting with a large variety of guest molecules to form non-covalent inclusion complexes [14]. See Figure 2 for chemical structures of the three cyclodextrins. Cyclodextrins are cyclic oligosaccharides, containing at least 6 D-(+) glucosapyranose units attached by α-1,4 glucosidic bonds. The three natural CDs, α-CD, β-CD and γ-CD (with 6, 7, and 8 glucose units respectively) differ in their ring size and solubility. The cavity size of α-CD is insufficient for many substances. The β-CD has been widely used in the pharmaceutical applications because of its ready availability and cavity size suitable for the widest range of substances [14]. In addition to increased solubility, cyclodextrin can also improve the stability of substances against dehydration, hydrolysis, oxidation, and photodecomposition and thus increasing the shelf life of the substances [15]. Substitution of any of the hydrogen bonds forming hydroxyl groups, even by hydrophobic moieties such as methoxy and ethoxy functions, will increase the solubility of the β-CD further, due to transformation of the crystalline cyclodextrin into more amorphous mixtures of isomeric derivatives [15]. (2-Hydroxypropyl)-β-cyclodextrin, (HP-β-CD), has been shown to both increase the solubility of capsaicin by 205 fold and increase the shelf life of capsaicin [16].

Figure 2. (a) The chemical structure of different cyclodextrins [17] and (b) the cone shape of the β-CD molecule [14].

1.2 Aim for the study

A study has been published in which the dose-response relationship of capsaicin in a HP-β-CD formulation used in volunteers was investigated [2]. However, to our knowledge; no previous study has compared the performance of such a formulation against the performance of a standard formulation on which most of the clinical validation is based. In order to have confidence in the utility of a HP-β-CD formulation of capsaicin, a clinical validation study comparing the 2 formulations was required.
The objectives were to compare the dose-response and dose-duration curves for pain, flare, hyperalgesia and allodynia of two formulations of intradermal capsaicin in healthy volunteers.

1.2.1 Hypothesis

The hypothesis was that the 2 formulations would produce similar pharmacodynamic profiles.

2. Methods and Materials

2.1 Trial design and subjects

Sixteen healthy Caucasian human volunteers, eight men and eight female, aged 19-58 (mean 25.7), participated in this randomized, blinded, cross over study comparing two different formulations of capsaicin, after given written informed consent. The study was approved by the Royal Adelaide Hospital Research Ethics Committee and the Investigational Drug Subcommittee.
The objectives of the study were to clinically compare the performance of a HP-β-CD formulation, against the performance of a standard formulation on which most of the clinical validation is based. The study took place at the Pain and Anaesthesia Research Clinic (PARC) within the Royal Adelaide Hospital. This study was carried out in accordance with Principles of International Conference on Harmonisation (ICH) Good Clinical Practice (GCP), as adopted in Australia, which build upon the ethical codes contained in the Declaration of Helsinki and The Australian National Statement on Ethical Conduct in Research Involving Humans.
The subjects were recruited to the study through flyers.

Twenty-two subjects were screened. An initial test session performed during the screening familiarized the subjects with the four assessments. To minimize withdrawals from the study and to ensure recruitment of test drug ”responders”, the screening also contained a familiarization event with the injection of the highest dose (100 μg) of the HP-β-CD formulation into the dominant forearm [3]. The screening event also contained an alcohol swab response test to confirm that the subjects did not respond with a localised flare due to swabbing of the skin with alcohol.
The screening took place no more than 7 days prior to the first scheduled dosing date. Each potential participant had to meet the following inclusion and exclusion criteria in order to qualify for admission into the study:

Inclusion criteria

A subject was eligible for inclusion in this study only if all of the following criteria applied:

1. Healthy subjects not suffering from any clinically significant painful condition
2. Agree to and be capable of signing an informed consent form.
3. Be of either gender and aged between 18 and 65.
4. Fair skin colour (so that the flare can be observed)

Exclusion criteria

A subject was excluded from the study if any of the following criteria applied:

1. Pregnant or breastfeeding
2. Allergy or intolerance to capsaicin
3. Scarring or tattoos on the forearms.
4. Regular use of analgesics

During the trial phase, the eight injections were separated into two (dosing) occasions. During each occasion the subjects received a total of four injections, each injection was separated by one hour and five minutes. The two dosing occasions were separated by a minimum of four days. Each of the sixteen subjects was scheduled to receive all eight injections.

The injections were administered according to a randomized Latin Square Design, produced by the Discipline of Public Health, University of Adelaide, assuring that injection site, formulation and session were balanced. This design assured that a formulation did not by chance always appear before the other formulation which could lead to the possibility of confounding a treatment effect with a time or carryover effect.
On each trial day, each subject came in on either mornings or afternoons to decrease the influence of time of day variability within a subject [3]. Subjects were placed in a bed and superficial skin temperature was fixed at 34 – 36^{o}C, using a 250 W heat lamp (Philips) positioned about 50 cm from

the subject's volar arm. The temperature was monitored by a thermocouple placed on the skin of the subject. A fixed temperature has been shown to decrease the variability of the model [18]. The subjects were blindfolded during the assessments. After completion of the study, the subjects were paid $200.

2.2 Capsaicin injection

Spontaneous pain and hyperalgesia were induced by intradermal injection of capsaicin, (8-methyl N-vanillyl 6-nonamid) in two different formulations. Preparation of test batches preceded the preparations of trial batches. The two formulations were given in the strengths 100 µg, 30 µg, 10 µg or 1 µg in 10 µL each, doses known to be tolerable to subjects while producing areas of allodynia, hyperalgesia, spontaneous pain and neurogenic inflammation of sufficient size to be measured accurately [2].

The conventional formulation was prepared according to Simone et. al. [9]. The dose of capsaicin used in this trial was 100, 30, 10 and 1 µg in 10 µL of vehicle (Tween 80, 7.5 % w/v in normal saline). The formulation appeared as solution for the three lowest doses and a colloidal suspension beyond this.

The new HP-β-CD formulation was prepared according to Hughes et. al. [3] with the exception of the concentration of the HP-β-CD vehicle, which was 38 % in this study, a concentration known to be equivalent to isotonic solution [16]. The HP-β-CD formulation was passed through a sterile syringe filter (Sterivex 0.22 µm) into a sterile vial before syringes were prepared. Information about the substances is given in Table I.

Table I. List of chemicals used in preparation of the two different formulations.

Chemical	Batch number	Manufactured by	Purity
Capsaicin	21 748	Fluka, Switzerland	≥ 97 %
Tween 80	073K00643	SigmaUltra	UK
(2-Hydroxypropyl)- β-cyclodextrin, (HP-β-CD)	56 332	Fluka, Switzerland	UK
Sodium Chloride Injection BP 0.9 %, (saline)	30 18 92	Astra Zeneca	UK

The formulations were produced by a licensed pharmacist or under the strictly supervision of a licensed pharmacist by the Pharmacy School of the University of South Australia and Pharmacy Department at the Royal Adelaide Hospital, according to standards appropriate for a product for human administration.

Each injection was drawn into syringes for administration no more than a maximum of one week in advance, since the adsorption of the formulation to syringes could be a problem for the HP-β-CD formulation [16]. The both formulations were used within one month of preparations due to stability data [16,18].
In each experiment, a volume a 10 µL was injected intradermally into the skin to the midline of either the dominant or non-dominant arm and to either forearm or upperarm, avoiding any veins. The syringes used were 0.3 mL sterile insulin syringes (BD Ultra-Fine II). All injection sites were marked with a blue pen.

2.3 HPLC analysis

After the last occasion in the trial, HPLC analyses were implemented to determine the concentrations of capsaicin in all given formulations and doses. The neat formulations were diluted 1:1 with mobile phase before 10 µL were injected. The isocratic mobile phase was circulated at a flow rate of 1.0 mL/min at ambient temperature of 21°C. The composition of the mobile phase is

showed in Table II and the mobile phase was degassed (500T degasser, Soniclean, SA, Australia) 30 minutes before use. The UV-detector was set to 280 nm. Under these given circumstances, capsaicin appeared as a single peak after approximately eight minutes.

Table II. HPLC conditions including column and mobile phase for detection of capsaicin.

Substance	Running time	Column	Mobile phase
capsaicin	15 minutes	Luna C18 (2) RP-column (5µm, 4.6 x 250 mm)	methanol / water /acetic acid, 75:25:0.1 v/v/v

2.4 Pain assessments

Information about the assessments and testing schedule were repeated to the subject before every occasion. The assessments were measured five minutely intervals up to 30 minutes post injection and then every 10 minutes to one hour post injection. The assessments of pain were performed in the following order: spontaneous pain, area of flare, allodynia and hyperalgesia. One staff member performed all measurements of each assessment to decrease observer bias. A parallel master thesis investigated allodynia and flare [19].

2.4.1 Determination of the spontaneous pain

Perception of spontaneous pain was assessed using a numeric visual analogue scale (VAS). The scale was 100 mm in length and was calibrated from 0 to 100, where 0 = "no pain" and 100 = "worst pain imaginable". Actual measurements were converted to a scale of 0 – 100 mm to correspond with the VAS calibration and recorded in the CRF.

2.4.2 Determination of the average radius of hyperalgesia

The average radius of pin pricked-induced hyperalgesia was assessed by applying a standard von Frey hair, number 5.46 [2,3] with microfilament bending threshold 26G, (TouchTest 800-821-9319, Semmes Weinstein, Stoelting, IL, USA). The subjects were told to report when the hair caused a greater or changed pain sensation compared to the pinprick sensation felt in the area of normal sensitivity.

The hair was applied in 8 compass point directions and assessments started in the area of normal sensitivity. The point of commencement for the procedure was the highest point in line with the glenohumeral joint (shoulder), 10 cm above the injection site. This point was referred to as north. The hair was reapplied at approximately 1 cm intervals every second, moving towards the injection site [3]. The procedure continued until the site of injection if, the subject did report any change in pain state/sensation. The procedure was repeated using the following sequence; S, E, W, NE, SW, SE and finally NW. The resulting points, demarcating transition from normal sensitivity to hyperalgesia, were traced onto acetate, using a red water resistant pen [20]. The transition was made directly after the measurement to decrease the bias. Each radius was then measured with a ruler and recorded in the CRF before an average radius for each injection was calculated. Since not all assessments resulted in eight points, an average radius was used in preference to a calculated area of hyperalgesia.

2.5 Statistical analysis

The subject number was similar to that in previous studies; hence an adequate statistical power should be provided.

All calculations were performed using Microsoft Office Excel Professional edition 2003 (Redmond, WA, USA) and SAS version 9.1 (Cary, NC, USA).

A mixed model was fit to all outcomes separately with subject as a random effect and gender, arm

dominance, arm position, formulation, dose and the interaction of the formulation and the dose each as fixed effects.
Subject, occasion, injection and time were fit as repeated measures. The outcome variables were VAS (mm), flare (mm^2), allodynia (average radius, mm) and hyperalgesia (average radius, mm). The independent variables were gender (male or female), arm dominance (dominant or non dominant), arm position (upper or lower), formulation (HP-β-CD or conventional) and dose (1 μg, 10 μg, 30 μg or 100 μg).

A P-value of less than 0.05 was required for statistical significance. P-values from the post hoc analyses were adjusted using the sidak method to account for multiple testing. A log transformation of VAS was used to obtain normally distributed residuals, resulting in a median VAS estimate.
In all outcomes, the fixed effect was dropped from the mixed model, if no significant difference was seen.

3. Results

Sixteen volunteers entered into the trial. Two subjects withdrew after the first occasion. Both of the withdrawn subjects were female but the withdrawals were not related to adverse events related to the capsaicin injections. No adverse events occurred during the study other than symptoms under assessments. The capsaicin produced a sensation of pain described primarily as "itching" or "burning".

The two formulations produced pharmacodynamic comparable responses to each other, for the three lowest doses for all four assessments, with the exceptions for the lowest dose in flare and the highest dose for all assessments. For all time points in all four outcomes, the HP-β-CD formulation generally resulted in responses with longer duration and higher magnitude compared to the conventional formulation in the unadjusted data, but no significance could be established for the three lowest doses. Summary statistics for each of the four outcomes are presented in Table III, Figures 3-4, 6-7.

The fixed effects of formulation, dose and formulation•dose was significantly different for all four assessments in the mixed effects model (Table IV). The position of arm was also significantly affecting spontaneous pain and hyperalgesia.
The magnitude of responses of spontaneous pain and hyperalgesia were significantly dose-dependent for the three lowest doses for both formulations but the only linear dose dependence seen was the HP-β-CD formulation in hyperalgesia (Figure 5, 8, Table VI, VIII).

Table III. Quantitative pain assessments by formulation and dose of capsaicin.
(n= 16 for doses 1 and 10 µg of the HP-β-CD formulation, n = 14 for doses 30 and 100 µg of the HP-β-CD formulation, n=15 for all doses of conventional formulation).

Assessment	**Formulation and dose** 1 µg		**Formulation and dose** 10 µg		**Formulation and dose** 30 µg		**Formulation and dose** 100 µg	
	HP-β-CD	Conventional	HP-β-CD	Conventional	HP-β-CD	Conventional	HP-β-CD	Conventional
Spontaneous Pain (rated on VAS (mm•hour)) $AUC_{(0\text{-}1h)}$ (mean ± SD)	541.1 ± 34.1	282.2 ± 25.0	721.9 ± 42.6	571.2 ± 31.5	915.4 ± 41.9	793.7 ± 47.1	1453.4 ± 42.5	685.5 ± 40.5
Flare (visual inspection, average area (mm^2•hour)) $AUC_{(0\text{-}1h)}$ (mean ± SD)	309.81 ± 9.29	153.75 ± 4.94	405.84 ± 12.49	323.48 ± 8.32	483.10 ± 14.42	448.15 ± 12.79	743.70 ± 28.33	454.2 ± 13.57
Allodynia (foam brush stimulation, average radius (mm•hour)) $AUC_{(0\text{-}1h)}$ (mean ± SD)	163.59 ± 5.06	69.73 ± 2.88	264.14 ± 8.47	248.83 ± 6.77	205.42 ± 3.73	191.71 ± 6.65	349.56 ± 9.72	151.35 ± 5.72
Hyperalgesia (pin-prick stimulation, average radius (mm•hour)) $AUC_{(0\text{-}1h)}$ (mean ± SD)	908.5 ± 38.3	648.0 ± 21.5	1232.0 ± 60.6	1041.3 ± 42.9	1282.0 ± 67.5	1265.0 ± 71.9	1545.3 ± 194.1	1097.0 ± 58.6

Table IV. The overall significance of fixed effects in the mixed effects model for the four outcomes. P-value less than 0.05 were required for statistical significance, marked *.

Effect	Spontaneous Pain		Flare		Allodynia		Hyperalgesia	
	FValue	ProbF	FValue	ProbF	FValue	ProbF	FValue	ProbF
Gender	0.01	0.9262	0.06	0.8013	0.91	0.3412	4.34	0.0375*
Arm dominance	0.83	0.3630	4.04	0.0447*	26.19	<.0001*	33.08	<.0001*
Position	19.39	<.0001*	0.12	0.7253	30.75	<.0001*	11.11	0.0009*
Formulation	51.54	<.0001*	75.76	<.0001*	31.84	<.0001*	38.14	<.0001*
Dose	52.99	<.0001*	89.69	<.0001*	34.97	<.0001*	59.13	<.0001*
Formulation•Dose	5.76	0.0007*	10.56	<.0001*	13.73	<.0001*	7.22	<.0001*

The covariance parameter estimates provided the estimate of the covariance between the random effect and the repeated measures. For all four assessments, the intra-individual variability was less than the inter-individual variability, the variance obtained from multiple subject, occasion, injection and time measures (Table V). Ratio column shows the ratio of each parameter estimate to that of the residual variance. For the log VAS estimate, the subject variance was about half of the variance that was seen with the repeated measures. For hyperalgesia, the ratio of subject variance to repeated measures was 0.7:1.

Table V. The covariance parameter estimates for the four assessments.

Covariance Parameter Estimates					
Covariance Parameter	Ratio	Estimate	Standard Error	Z Value	Pr Z
Spontaneous Pain (log VAS)					
subject	0.5192	0.7109	0.2686	2.65	0.0041*
subject•occasion•injection•time	1.0000	1.3693	0.05959	22.98	<.0001*
Flare					
subject	0.1324	2.7957	1.1540	2.42	0.0077*
subject•occasion•injection•time	1.0000	21.1187	0.9197	22.96	<.0001*
Allodynia (log)					
subject	1.0104	0.6595	0.2464	2.68	0.0037*
subject•occasion•injection•time	1.0000	0.6528	0.02844	22.95	<.0001*
Hyperalgesia					
subject	0.6529	69.6014	27.1675	2.56	0.0052*
subject•occasion•injection•time	1.0000	106.61	4.6444	22.95	<.0001*

3.2 Spontaneous Pain (VAS)

Capsaicin produced spontaneous pain in a dose dependent manner for both formulations but the dose dependences were not linear in either of the two formulations. For all time points and doses, the median VAS was higher for the HP-β-CD formulation compared to the conventional formulation. The dose duration curves were comparable with rapid onset for both formulations with the fall-off for the HP-β-CD formulation was not completed after 60 minutes (Figures 3, 4, 5).

The overall significance of fixed effects in the mixed effects model is presented in table IV and shows that a significant difference was seen in the estimated log VAS between the upper and lower arm, (diff = 0.38, $p<0.001$). For every one mm increase in VAS in the upper arm the lower arm

VAS increased by 1.38 mm, after adjusting for other variables in the model (Table VI). A statistical significantly difference in the estimated median VAS was also seen within the formulations, where the HP-β-CD formulation produced a higher score compared to conventional formulation (diff = 0.67, $p<0.001$) (Table VI). The mixed model also showed a significant difference in the estimated median VAS between dosages. Table VI shows a significantly smaller estimated median VAS for a dose of 1 µg compared to dosages of 10, 30 and 100 µg (diff=0.31 $p=0.0009$, diff=0.63 $p<0.001$ and diff=0.68 $p<0.001$ respectively). A 10 µg dose has a significantly lower median VAS compared to 30 and 100 µg (diff=0.46 $p<0.001$ and diff=0.53 $p<0.001$ respectively). There was no significant difference between a dose of 30 µg and a dose of 100 µg.

Table VII pointed out the difference between all combinations of formulations and doses, all highlighted (*) p-values showed a significant difference in median VAS. These can be interpreted in the same way as the previous tables, e.g. a 1 µg dose of the HP-β-CD formulation has a significantly lower median VAS compared to 30 and 100 µg doses of the HP-β-CD formulation (diff=0.64 $p<0.001$ and diff=0.77 $p<0.001$ respectively).

For VAS, there was no significant difference in spontaneous pain between the two formulations for the 1, 10 and 30 µg doses. There was a statistical significant difference between the dose 100 µg for the HP-β-CD formulation compared to the conventional formulation. Hence, the two formulations produced comparable pain scores except for the 100 µg dose.

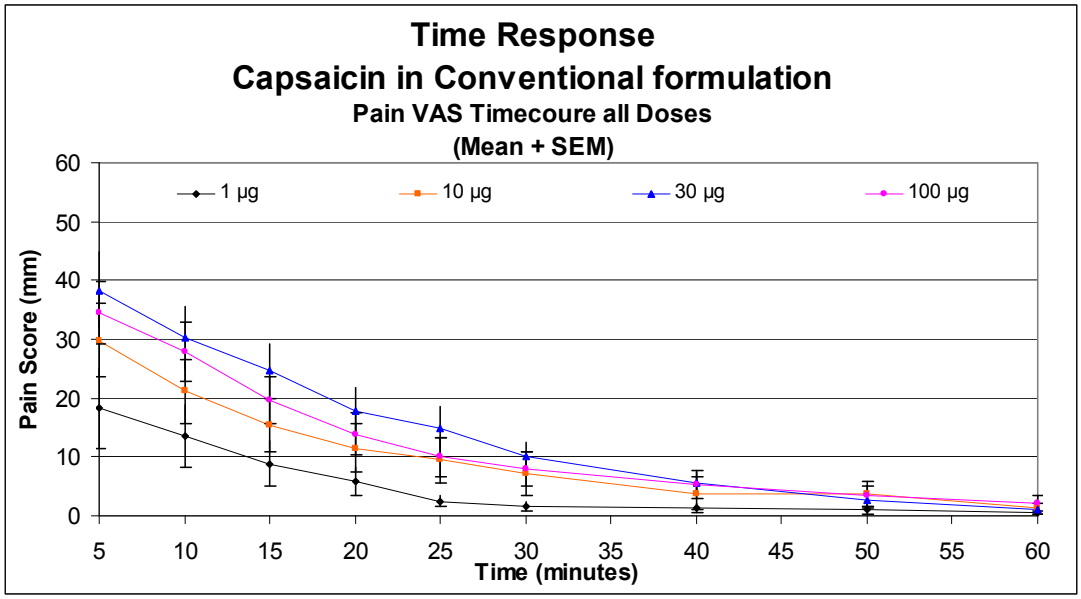

Figure 3. The mean score of spontaneous pain (rated on VAS) as a function of time. The data is based on all injections of the conventional formulation containing capsaicin dissolved in Tween 80. (n=15 for all doses)

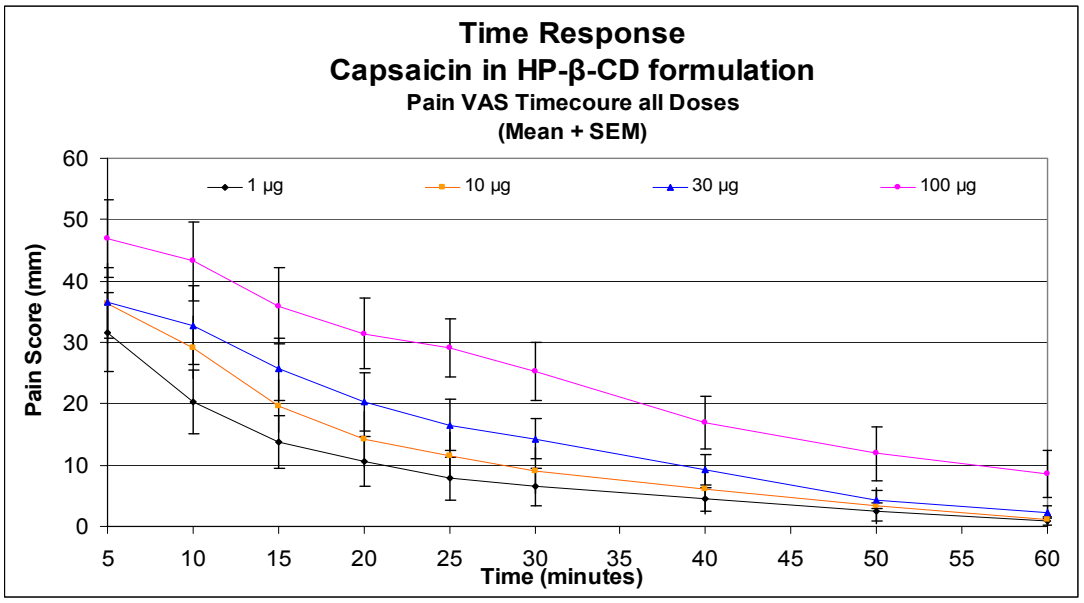

Figure 4. The mean score of spontaneous pain (rated on VAS) as a function of time. The data is based on all injections of the new formulation containing capsaicin dissolved in HP-β-CD. (n= 16 for doses 1 and 10 µg, n = 14 for doses 30 and 100 µg)

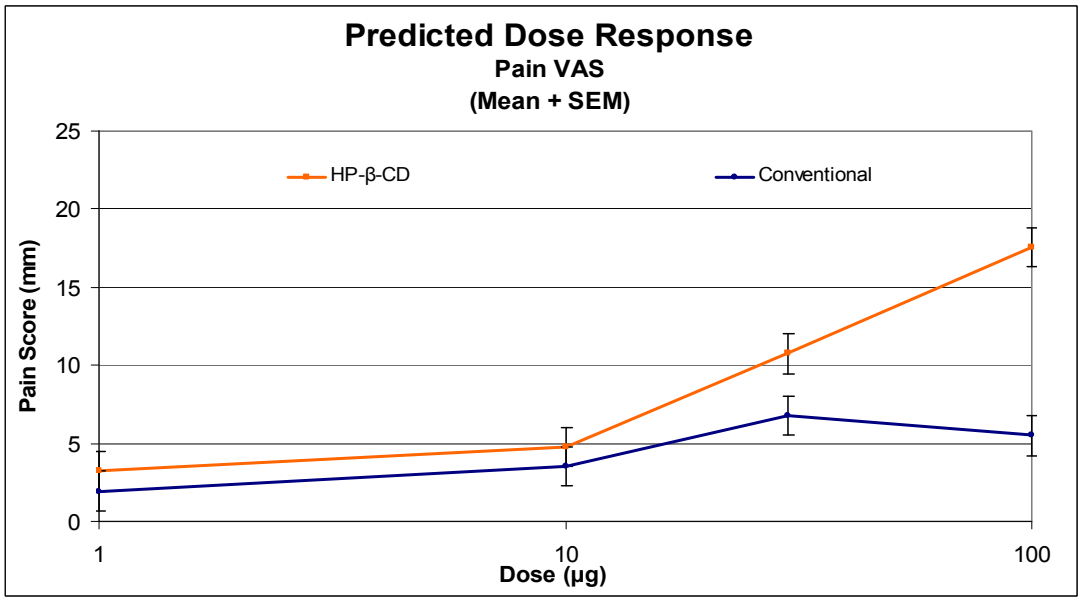

Figure 5. Plot of the predicted median VAS ± SEM for each dosage and formulation based on the mixed model, adjusted for the not significant fixed effects.

Table VI: Difference in the estimated median VAS between independent variables with statistical significant difference (position, formulation and doses). P-value less than 0.05 were required for statistical significance, marked *.

Effect	Indepedent variables		Log Estimate	Standard Error	P Value	Estimated Diff
Position	Lower	Upper	0.3208	0.07332	<.0001*	0.37819
Formulation	HP-β-CD	Conventional	0.5147	0.07214	<.0001*	0.67311
Dose	1	10	-0.3774	0.09937	0.0009*	-0.31438
Dose	1	30	-1.0022	0.1020	<.0001*	-0.63293
Dose	1	100	-1.1406	0.1021	<.0001*	-0.68038
Dose	10	30	-0.6248	0.1016	<.0001*	-0.46462
Dose	10	100	-0.7632	0.1017	<.0001*	-0.53383
Dose	30	100	-0.1384	0.1025	0.6896	-0.12926

Table VII: Difference in the estimated median VAS between all combinations of formulations and doses. P-value less than 0.05 were required for statistical significance, marked *.

Effect	Formulation	Dose	Formulation	Dose	Log Estimate	Standard Error	Adj P Value	Estimated Diff
Formulation•Dose	HP-β-CD	1	HP-β-CD	10	-0.3179	0.1382	0.4579	-0.27233
Formulation•Dose	HP-β-CD	1	HP-β-CD	30	-1.0278	0.1438	<.0001*	-0.64220
Formulation•Dose	HP-β-CD	1	HP-β-CD	100	-1.4853	0.1442	<.0001*	-0.77357
Formulation•Dose	HP-β-CD	1	Conventional	1	0.3593	0.1409	0.2647	0.43236
Formulation•Dose	HP-β-CD	1	Conventional	10	-0.07763	0.1413	1.0000	-0.07469
Formulation•Dose	HP-β-CD	1	Conventional	30	-0.6173	0.1432	0.0005*	-0.46061
Formulation•Dose	HP-β-CD	1	Conventional	100	-0.4366	0.1417	0.0575	-0.35378
Formulation•Dose	HP-β-CD	10	HP-β-CD	30	-0.7099	0.1441	<.0001*	-0.50829
Formulation•Dose	HP-β-CD	10	HP-β-CD	100	-1.1674	0.1438	<.0001*	-0.68883
Formulation•Dose	HP-β-CD	10	Conventional	1	0.6772	0.1407	<.0001*	0.96841
Formulation•Dose	HP-β-CD	10	Conventional	10	0.2403	0.1408	0.9244	0.27160
Formulation•Dose	HP-β-CD	10	Conventional	30	-0.2994	0.1418	0.6304	-0.25875
Formulation•Dose	HP-β-CD	10	Conventional	100	-0.1187	0.1409	1.0000	-0.11194
Formulation•Dose	HP-β-CD	30	HP-β-CD	100	-0.4575	0.1478	0.0549	-0.36716
Formulation•Dose	HP-β-CD	30	Conventional	1	1.3871	0.1458	<.0001*	3.00321
Formulation•Dose	HP-β-CD	30	Conventional	10	0.9501	0.1461	<.0001*	1.58608
Formulation•Dose	HP-β-CD	30	Conventional	30	0.4104	0.1480	0.1462	0.50749
Formulation•Dose	HP-β-CD	30	Conventional	100	0.5912	0.1465	0.0016*	0.80607
Formulation•Dose	HP-β-CD	100	Conventional	1	1.8446	0.1456	<.0001*	5.32575
Formulation•Dose	HP-β-CD	100	Conventional	10	1.4077	0.1456	<.0001*	3.08645
Formulation•Dose	HP-β-CD	100	Conventional	30	0.8680	0.1464	<.0001*	1.38210
Formulation•Dose	HP-β-CD	100	Conventional	100	1.0487	0.1457	<.0001*	1.85390
Formulation•Dose	Conventional	1	Conventional	10	-0.4370	0.1425	0.0604	-0.35400
Formulation•Dose	Conventional	1	Conventional	30	-0.9766	0.1448	<.0001*	-0.62343
Formulation•Dose	Conventional	1	Conventional	100	-0.7959	0.1439	<.0001*	-0.54884

Effect	Formulation	Dose	Formulation	Dose	Log Estimate	Standard Error	Adj P Value	Estimated Diff
Formulation•Dose	Conventional	10	Conventional	30	-0.5397	0.1443	0.0054*	-0.41707
Formulation•Dose	Conventional	10	Conventional	100	-0.3590	0.1436	0.2989	-0.30162
Formulation•Dose	Conventional	30	Conventional	100	0.1807	0.1428	0.9984	0.19806

3.3 Hyperalgesia (von Frey Test)

Capsaicin produced hyperalgesia in a dose dependent manner for both formulations, except for the 100 µg dose of the conventional formulation. The dose dependence was linear for the HP-β-CD formulation. For all time points, the mean average radius for every dose was noticeably lower for the conventional formulation compared to the HP-β-CD formulation (Figure 6-8, Table III).

Both formulations produced a similar average radius initially with the doses of the HP-β-CD formulation initially increasing more with time as well as declining more slowly than the corresponding doses for the conventional formulation. The effects were lasting longer than 60 minutes for all doses in both formulations (Figures 6-7).

The predicted average radius was not significantly higher for HP-β-CD formulation compared to the conventional formulation at the three lowest doses but a significant difference between the two formulations was seen in the 100 µg dosages (Figure 8, Table IX).

The overall significance of fixed effects in the mixed effects model showed a statistical significance for all independent variables; gender, arm dominance, arm position, formulation, dose and formulation•dose (Table IV, VIII). Table VIII shows a significantly larger estimated hyperalgesia (average radius) for females compared to males (difference = 8.8, $p=0.038$), for HP-β-CD formulation compared to conventional formulation (diff = 3.9, $p<0.001$), for the lower position of the arm compared to the upper arm (difference = 2.2, $p=0.0009$) and for the non dominant arm compared to the dominant arm (difference = 3.8, $p<0.001$), after adjusted for other variables in the model. The mixed model also showed a significant difference in the estimated average radius of hyperalgesia between dosages. After adjustment for other variables in the model, a dose of 1 µg had significantly lower average radius compared to dosages of 10, 30 and 100 µg (diff= 6.31 $p<0.0001$, diff=9.37 $p<0.0001$ and diff=11.29 $p<0.0001$ respectively). A 10 µg dose has a significantly lower average radius compared to 30 and 100 µg (diff=3.06 $p= 0.0043$ and diff=4.98 $p<0.0001$ respectively). There was no significant difference between a dose of 30 µg and a dose of 100 µg.

The difference between all combinations of formulation and doses are showed in Table IX, where all highlighted (*) p-values showed a significant difference in average radius. Using the same interpretation as previous tables, a 1 µg dose of HP-β-CD formulation had a significantly lower average radius compared to 10, 30 and 100 µg of the HP-β-CD formulation (diff=5.6 $p=0.0001$, diff=8.9 $p<0.0001$ and diff=14.3 $p<0.0001$ respectively) after adjustments for other variables in the model.

For hyperalgesia, the same trend was seen as for spontaneous pain, with no significant difference in average radius between the two formulations for the 1, 10 and 30 µg doses while there was as a statistical significant difference between the dose 100 µg for the HP-β-CD formulation compared to the conventional formulation. Hence were the formulations pharmacodynamic comparable with respect to the three lowest doses when adjusted for all significant parameters.

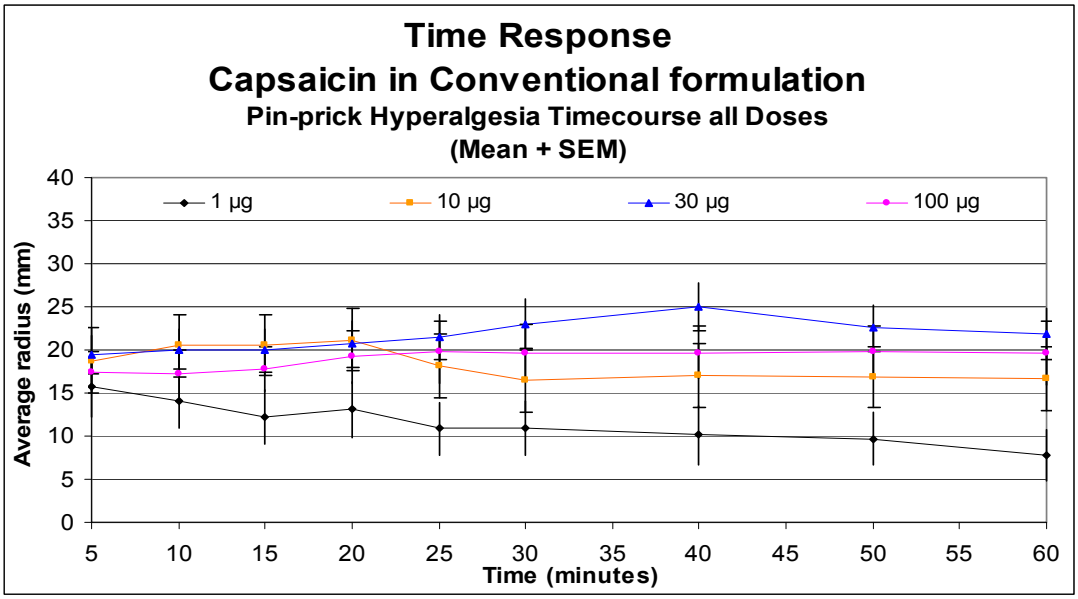

Figure 6. The mean average radius of hyperalgesia (von Frey hair) as a function of time. The data is based on all injections of the conventional formulation containing capsaicin dissolved in Tween 80. (n=15 for all doses).

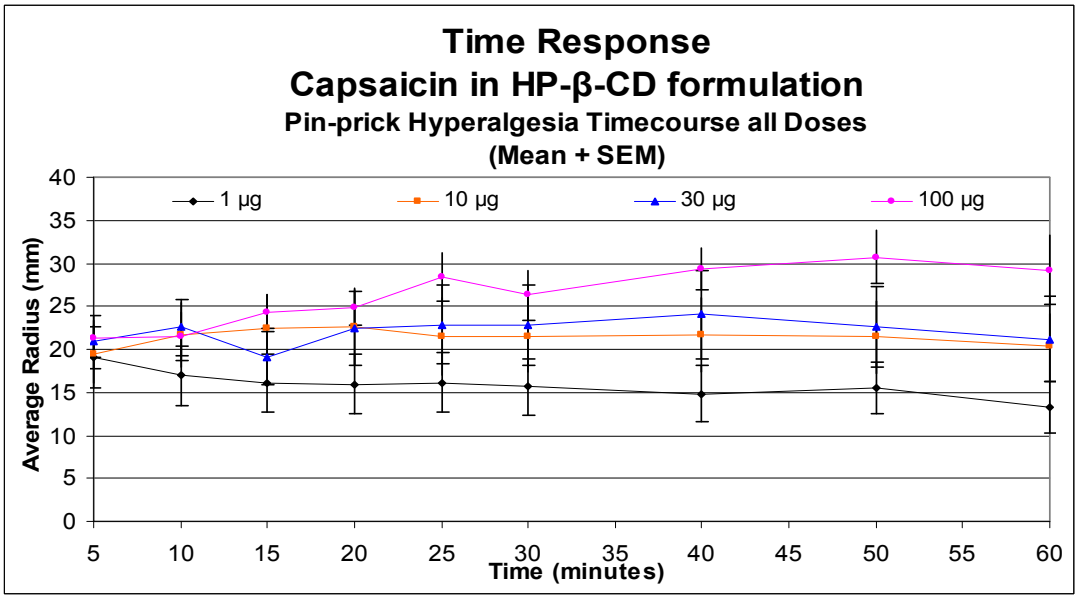

Figure 7. The mean average radius of hyperalgesia (von Frey hair) as a function of time. The data is based on all injections of the new formulation containing capsaicin dissolved in HP-β-CD. (n= 16 for doses 1 and 10 µg, n = 14 for doses 30 and 100 µg).

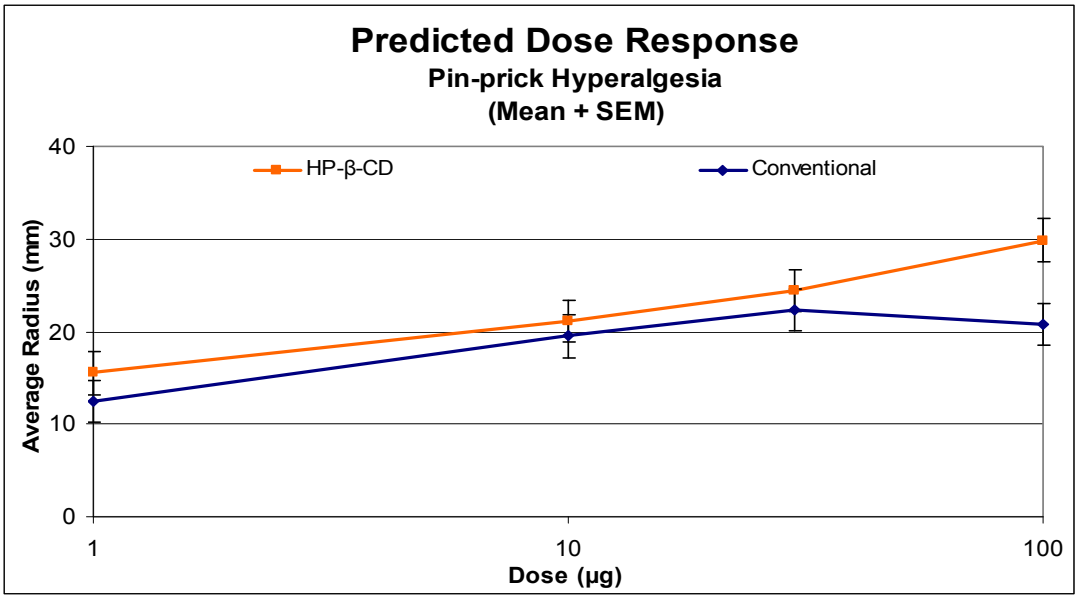

Figure 8. Plot of the predicted average radius ± SEM in hyperalgesia for each dosage and formulation based on the mixed model, adjusted for the non significant fixed effects.

Table VIII. Difference in the estimated averages radius of pin prick hyperalgesia between independent variables with statistical significant difference (gender, arm dominance, arm position, formulations and doses). A p-value less than 0.05 were required for statistical significance, marked *.

Effect	Independent variables		Estimate	Standard Error	P Value	Lower CI	Upper CI
Gender	Female	Male	8.7918	4.2214	0.0375*	0.5085	17.0750
Arm dominance	Dominant	Non-dominant	-3.7809	0.6573	<.0001*	-5.0707	-2.4911
Position	Lower	Upper	2.1596	0.6478	0.0009*	0.8885	3.4307
Formulation	HP-β-CD	Conventional	3.9427	0.6384	<.0001*	2.6900	5.1954
Dose	1	10	-6.3136	0.8793	<.0001*	-8.6313	-3.9958
Dose	1	30	-9.3733	0.9008	<.0001*	-11.7479	-6.9988
Dose	1	100	-11.2913	0.9196	<.0001*	-13.7155	-8.8671
Dose	10	30	-3.0598	0.9015	0.0043*	-5.4360	-0.6835
Dose	10	100	-4.9777	0.9054	<.0001*	-7.3644	-2.5911
Dose	30	100	-1.9180	0.9285	0.2128	-4.3654	0.5295

Table IX: Difference in the estimated hyperalgesia between all combinations of formulations and doses. P-value less than 0.05 were required for statistical significance, marked *.

Effect	Formulation	Dose	Formulation	Dose	Estimate	Standard Error	Adj. P-Value	Lower CI	Upper CI
Formulation•Dose	HP-β-CD	1	HP-β-CD	10	-5.6552	1.2225	0.0001*	-9.4747	-1.8356
Formulation•Dose	HP-β-CD	1	HP-β-CD	30	-8.9069	1.2697	<.0001*	-12.8741	-4.9397
Formulation•Dose	HP-β-CD	1	HP-β-CD	100	-14.3468	1.3139	<.0001*	-18.4521	-10.2415
Formulation•Dose	HP-β-CD	1	Conventional	1	2.9774	1.2526	0.3924	-0.9364	6.8911
Formulation•Dose	HP-β-CD	1	Conventional	10	-3.9946	1.2619	0.0437*	-7.9374	-0.05177
Formulation•Dose	HP-β-CD	1	Conventional	30	-6.8624	1.2652	<.0001*	-10.8155	-2.9094
Formulation•Dose	HP-β-CD	1	Conventional	100	-5.2584	1.2656	0.0010*	-9.2127	-1.3041

Effect	Formulation	Dose	Formulation	Dose	Estimate	Standard Error	Adj. P-Value	Lower CI	Upper CI
Formulation•Dose	HP-β-CD	10	HP-β-CD	30	-3.2517	1.2730	0.2618	-7.2292	0.7258
Formulation•Dose	HP-β-CD	10	HP-β-CD	100	-8.6916	1.2930	<.0001*	-12.7316	-4.6517
Formulation•Dose	HP-β-CD	10	Conventional	1	8.6325	1.2435	<.0001*	4.7473	12.5178
Formulation•Dose	HP-β-CD	10	Conventional	10	1.6606	1.2470	0.9965	-2.2356	5.5567
Formulation•Dose	HP-β-CD	10	Conventional	30	-1.2073	1.2511	1.0000	-5.1164	2.7018
Formulation•Dose	HP-β-CD	10	Conventional	100	0.3968	1.2484	1.0000	-3.5037	4.2972
Formulation•Dose	HP-β-CD	30	HP-β-CD	100	-5.4399	1.3371	0.0014*	-9.6175	-1.2624
Formulation•Dose	HP-β-CD	30	Conventional	1	11.8842	1.2914	<.0001*	7.8493	15.9192
Formulation•Dose	HP-β-CD	30	Conventional	10	4.9123	1.2994	0.0046*	0.8525	8.9720
Formulation•Dose	HP-β-CD	30	Conventional	30	2.0444	1.3060	0.9701	-2.0359	6.1248
Formulation•Dose	HP-β-CD	30	Conventional	100	3.6485	1.3030	0.1359	-0.4225	7.7195
Formulation•Dose	HP-β-CD	100	Conventional	1	17.3242	1.2979	<.0001*	13.2691	21.3793
Formulation•Dose	HP-β-CD	100	Conventional	10	10.3522	1.2928	<.0001*	6.3128	14.3916
Formulation•Dose	HP-β-CD	100	Conventional	30	7.4844	1.3189	<.0001*	3.3636	11.6052
Formulation•Dose	HP-β-CD	100	Conventional	100	9.0884	1.2940	<.0001*	5.0455	13.1313
Formulation•Dose	Conventional	1	Conventional	10	-6.9720	1.2583	<.0001*	-10.9034	-3.0406
Formulation•Dose	Conventional	1	Conventional	30	-9.8398	1.2805	<.0001*	-13.8406	-5.8390
Formulation•Dose	Conventional	1	Conventional	100	-8.2358	1.2703	<.0001*	-12.2049	-4.2667
Formulation•Dose	Conventional	10	Conventional	30	-2.8678	1.2794	0.5106	-6.8652	1.1295
Formulation•Dose	Conventional	10	Conventional	100	-1.2638	1.2676	1.0000	-5.2242	2.6966
Formulation•Dose	Conventional	30	Conventional	100	1.6040	1.2662	0.9984	-2.3521	5.5601

3.4 HPLC assay

After the last occasion in the trial, HPLC analyses were implemented to determine the concentrations of capsaicin in every given formulations and doses (Table X).

Table X. The assayed concentration of capsaicin in the given doses.

Labelled Concentration (µg/10 µL)	Assayed Concentration (µg/10 µL)	
	Conventional Formulation (Accuracy)	HP-β-CD Formulation (Accuracy)
1	1.01 (101 %)	1.13 (113 %)
10	10.14 (101 %)	12.05 (121 %)
30	31.04 (103 %)	28.77 (96 %)
100	38.53 (39 %)	111.12 (111 %)

4. Discussion

In this present study, the two formulations produced pharmacodynamically comparable responses to each other, for the three lowest doses for all four assessments. The exceptions were the lowest dose in flare and the highest dose for all four assessments. This study showed both differences and similarities between the two formulations within the investigated dose range. The top dose was different between the two formulations and doses equal to or less than 30 µg produced smaller difference considered to be clinically irrelevant.

Intradermal injections of capsaicin produced significant dose-dependent responses for all four outcomes; spontaneous pain, flare, allodynia and hyperalgesia within the dose range of the three lowest doses with respect to both formulations. A formulation with capsaicin dissolved in 20 % HP-β-CD vehicle has previously been shown to exhibit dose-dependent effects in human subjects measured in fixed time points [2], results totally in accordance with this study.

These findings complemented and further extended the knowledge about intradermal capsaicin as a model for neuropathic pain. Both formulations can be considered to be safe and tolerable but the HP-β-CD formulation exhibits clearly advantageous pharmaceutical and production factors in addition to the pattern of more obvious dose dependence for the examined dose range. The HP-β-CD formulation of capsaicin is suitable for use in the pharmacological profiling of putative analgesic substances both from the tolerability and variability aspects. All doses gave adequate responses in minimum 25 minutes for spontaneous pain and over 60 minutes for hyperalgesia. The HP-β-CD formulation appears to be more suitable for clinical trials since the same dose levels as conventional formulation resulted in comparable results for the three lowest doses but longer lasting responses in all four assessments. This is advantageous in clinical trials since it is desirable to have stable responses with sufficient duration to allow pharmacological intervention without having to administer intolerable amounts of capsaicin. According to the literature, doses of capsaicin up to 250 µg have previously been given [3]. The results in this study suggest that it is not necessary to take such risks since 10 µg, 30 µg and 100 µg of the HP-β-CD formulation produces satisfactory responses. Using 10 µg or 30 µg may be the most clearly suitable dose levels, since stimulus from the 100 µg dose has been reported to be too intense to detect certain analgesic drug effects [2].

Another benefit of the 38 % HP-β-CD vehicle compared to the 20 % HP-β-CD vehicle, is that the 38 % HP-β-CD vehicle is isotonic directly after production and does not require further dilution with saline. Other production factors which make the 38 % HP-β-CD formulation advantageous compared to the conventional formulation are that it is free from preservatives but still does not have do be made freshly. The stability of capsaicin in HP-β-CD formulation is also enhanced compared to conventional formulation. The HP-β-CD formulation can be stored at room temperature as well as in refrigerator (< 4^{o}C), which is clearly beneficial in a blinded design of clinical trials. Since the HP-β-CD formulation is a solution within the dose range given in this study, it is possible to sterilize by filtration with a 0.22 µm membrane filter. The conventional formulation was not sterile due to the fact that the 100 µg dose was a colloidal suspension. The sterility is an obvious problem despite the tiny volume of injection. The ability to remain as a solution at all doses used in this study was also optimizing dose accuracy for the HP-β-CD formulation compared to the conventional formulation at the highest dose level.

Introduction of new excipients are always associated with novel safety aspects.
The HP-β-CD vehicle has previously been reported to be associated with nephrotoxicity [21] but is nowadays accepted as safe. In an intravenous human toxicity dosing study, single doses of 3 g were not associated with measurable negative effect on kidney function and were well-tolerated by all volunteers [22] and substitution with hydroxypropyl is considered to be even more safe due to its

increased water-solubility [21]. The tiny injection volume in our study was associated with no adverse event or other complaints for either the HP-β-CD-, or the conventional formulation.

The HP-β-CD-formulation produced higher results in every single variable at all time points compared to the conventional formulation. There is no obvious explanation to this finding. Since both formulations are isotonic, the tonicity is probably not the explanation. As a suggestion, the explanations to these results are the pH of the formulation or the HP-β-CD-vehicle alone. Since the TRPV1 is reported to be activated by pH 5.9, the concentrations of protons from the HP-β-CD formulation with reported pH 6.0, is probably affecting and activating the TRPV1 and hence may produce higher results. A simple pH assay should be a compulsorily assignment in future studies and a study of the HP-β-CD-vehicle alone should also be implemented to determine the base line level of the HP-β-CD-vehicle for all four assessments. A clearly stated pH is also important for correct comparisons between future results within this model. In this study, the lowest dose, 1 μg, was considered to be active placebo and hence were no injection with only the HP-β-CD vehicle given.

4.1 Spontaneous Pain (VAS)

Pain scores resulting from capsaicin injection were found to be significantly dose dependent within the dose range of the three lowest doses with respect to both formulations. The 100 μg dose in HP-β-CD formulation was clearly considered to be highest with statistically significantly different pain scores compared to all other doses given and a time-response curve that did not decline to baseline after 60 minutes. This further reinforces the previous discussion about its suitability in clinical practice. The validating results were beneficial, with the two formulations producing pharmacodynamically comparable pain scores to each other, for the three lowest dose levels.

The poor dose dependent relationship seen for the 100 μg dose of the conventional formulation is doubtless explained by its actually, assayed soluble concentration of 39 μg.

Another beneficial result of this study was that gender had a smaller effect than anticipated. Gender demonstrated no statistically significant difference in the VAS measurements. This conveys that a gender-associated investigational bias does not have to be taken in to consideration, a common problem in this subjective type of assessment.

Since the capsaicin model is promising, further development of the model is essential. This may include consideration of changes in capsaicin induced pain during the menstrual cycle. Menstruating women have been showed to rate their capsaicin-evoked pain higher than males ($P < 0.001$) while no significant difference was observed in pain rating between females in luteal phase and males ($P= 0.056$), in a study performed using capsaicin administered intradermally into the forehead, a area known to be more sensitive than the arm to capsaicin [6]. Hence, it may be that our results may be affected by the hormonal status of the female healthy volunteers. To avoid this problem, the hormonal status of the female subjects should be recorded in future studies using this model.

4.2 Hyperalgesia (von Frey Test)

The hyperalgesia following capsaicin injection was also found to be significantly dose dependent within the dose range of the three lowest doses with respect to both formulations.

Even the validating results were positive with the two formulations producing pharmacodynamically comparable average radiuses to each other for the three lowest doses.
In accordance with Scanlon et. al. [2], the HP-β-CD formulation produced hyperalgesia in a linear dose dependent manner, unfortunately not significantly within the whole dose range. The dose

dependence seen in the conventional formulation is consistent with Simone et. al. but since the measured variables in that study were area rather than average radius, a comparison between the results are difficult [9].
Simone et al reported a maximum mean area within 5-7 minutes after doses of 10 and 100 µg, respectively [9]. This pattern is not seen in either of the two formulations in this study. The demonstrated lag time, with the greatest magnitude of pain not always perceived during the first minutes following the injection, can be explained by either the HP-β-CD-vehicle or the appearance of the highest dose of conventional formulation as a colloidal suspension or a combination of both. Simone et al. [9] reported dose dependence between the 10 µg and 100 µg doses of the conventional formulation but in their study, they did not include the critical 30 µg dose, a dose known to be close to the maximum solubility of capsaicin in Tween 80. Hence, it is not surprising that their study showed dose dependence since they did not cover the range of concentrations where the plateau is reached. For the same reason, they might not either discovered the formation of a colloidal suspension after passed the level of maximum solubility. Future studies with the conventional formulation have no benefit of injecting higher doses than 39 µg, since the plateau is reached at that particular concentration. Hence, higher concentrations are not associated with higher responses since the maximum solubility is reached.

Since Simone et. al. neither made a concentration determination nor stated whether the 100 µg was a colloidal suspension or not [9] it is hard to appraise the appearance of their highest dose. The anticipated pattern of HP-β-CD formulation to act as a depot with longer duration than the conventional formulation was not as obvious as predicted, since its time response pattern had no distinguishing factors worth mentioning compared to the conventional formulation. The HP-β-CDs didn't hide the capsaicin, which instead appeared as free, bio active and available with a reasonable quick diffusion constant.

We further confirm the proposal from Hughes et al. where the non dominant arm is reported to be significantly more sensitive to pain than the dominant arm for hyperalgesia [3]. Even the position of the arm had a smaller effect. To our knowledge, there is no present study which has investigated the influence of position of arm. It is demonstrated that the area of hyperalgesia is reduced when the capsaicin is injected to approximately the same area 7-10 days later [3], a phenomenon probably explained by local degeneration of epidermal nerve fibres. In this study, the approach with arm position (upper and lower arm) was both to investigate the extent of influence of arm position as well as to avoid any possible to carry-over or desensitizing effects [3]. This study confirms that it must be further research on arm position and that both these variables must be taken into consideration in forthcoming studies.

In accordance to most published studies, women were more sensitive to experimental pain than men. Enhanced central pain processing in women, as well as psychosocial factors have been suggested as the responsible mechanisms to these findings [23]. In 2006, Jensen et. al. established that there were no gender differences in capsaicin evoked von Frey hair stimulation adjusted for forearm surface area [23]. The difference between the studies, seen in gender effect can thus be explained by the fact that this study did not adjust for the forearm surface area. This information was inadvertently overlooked in our study and becomes an important approach that needs further investigation in forthcoming studies.

4.3 HPLC assay

The HPLC assay confirmed the suspicion that the 100 µg conventional formulation was a colloid suspension. Crystals were visible at visual inspection and the production pharmacist reported difficulties in the withdrawal process into syringes from this vial. The doses injected were probably suspensions with the same concentrations as the assayed one (39 µg). This is confirmed in figures 5

and 8, where the 100 µg dose of the conventional formulation is demonstrated to give a similar response in spontaneous pain and hyperalgesia as the 30 µg dose of the same formulation.

This reduced dose probably affected the statistical analyses extensively. A dose with higher accuracy could have resulted in pharmacodynamic comparable assessment results within the whole investigated dose range. This defective concentration may also principally be the explanation to why the HP-β-CD formulation is higher in both rate of spontaneous pain and average radius in hyperalgesia.

There's most likely also prevailing a large variability of this dose, since time factors, production factors (such as mixture procedures before withdrawal from vials) and crystal structure of the compound all influence the dose withdrawn into the syringe to be administered to the subjects. The HP-β-CD formulation has an enhanced stability compared to the conventional formulation which can further explain the better dose response pattern that exists within the HP-β-CD formulation.

In previous studies with the conventional formulation, an assay has not been performed [2,3,9] or has been performed, but without the concentrations clearly stated [18]. Thus, it is unclear whether the maximum solubility of capsaicin in Tween 80 is 39 µg / 10 µL or if production of the formulation was unsuccessful at this higher dose level. In our study, the drug content in this actual dose was probably prepared correct since the HPLC assay did just measure the soluble amount of the drug. Another approach for further developing the conventional formulation and this model is to implement a HPLC assay on the extracted 100 µg dose. This approach has the ability to show both the soluble content in the liquid and the insoluble drug content of the capsaicin and hence has the capability to judge whether the total drug content in both liquid and crystals was correct or not.

The HPLC assay showed an acceptable accuracy but all the doses of the HP-β-CD formulation were higher than expected. This is probably explained by the fact that the three lower doses are prepared in dilution steps from the highest dose. To develop this model further and increase the accuracy of the doses given, an approach may be to perform HPLC assay of an aliquot of the highest concentration of HP-β-CD formulation immediately after production and dilute the other doses according to the assay results.

4.4 Sources of variability

With respect to the unadjusted data for the whole population, the dose response pattern was diverse for the two formulations, with particularly the highest dose of the conventional formulation being poor and variable. Despite the fact that all critical experimental conditions for minimizing variability were followed [3], variability was seen. The discrepancy that exists is mostly explained by inter-individual variability (Table V), which is described to account for most of the variability in the intradermal capsaicin model [3]. This study is also consistent with previous studies that have found that allodynia was more short-lived and showed greater variability than hyperalgesia [2]. Since the foam brush method is considered to have very good repeatability both within and between days [24] an approach for decreasing the variability in allodynia is to further practice the method and familiarize the subjects with the foam brush to a greater extent.

Cross-over design, where the volunteers served as their own controls, and pre-screening of subjects with a test dose of capsaicin are design approaches that are recommended to be permanently included in the model. This may reduce inter-individually variability, minimizes withdrawals and ensures adequate responses. The injections given at the screening event resulted in exclusion of three abnormal responders, an observation in accordance with Liu et.al. [18], which prove the importance of this approach in further studies.

Another suggestion for minimizing variability is to just have one person administering the injections, as having more than one nurse responsible for dosing may increase the bias of injection technique due to different angles and depth of the needle. During the trial, a phenomenon with the appearance of a small blebby blister was seen after some injections. This was a small bubble resulting from the injection volume just under the skin and the skin within this small bleb is reported to be hypoalgesic to stimulation from von Frey hair [7].

5. Conclusions

In this present study, the two formulations produced pharmacodynamically comparable responses to each other, for the three lowest doses for all four assessments. The exceptions were the lowest dose in flare and the highest dose for all assessments. Hence, the formulations can be considered to be comparable and the stated hypothesis can be considered to be confirmed.

These findings complemented and further extended the knowledge about intradermal capsaicin as a model for neuropathic pain. Both formulations can be considered to be safe and tolerable but the HP-β-CD formulation exhibits clearly advantageous pharmaceutical- and production factors in addition to the pattern of more obviously dose dependence for the whole investigated dose range. The most clearly demonstrated advantage for the HP-β-CD formulation relates to its ability to remain as a solution at all doses used throughout this study. Dose accuracy is thus optimal, when compared to the conventional formulation at the highest dose level. This also enables sterilization through a filter for the HP-β-CD formulation.

The HP-β-CD formulation of intradermal capsaicin is suitable for use in the pharmacological profiling of putative analgesic substances both from the tolerability and variability aspects. The recommended doses to use are 10 µg or 30 µg since these produce comparable results with satisfactorily duration and magnitude of pain and stimulus from the 100 µg dose has been reported to be too intense to detect certain analgesic drug effects [2].

Inter- individual variability was shown to be a more frequent and extensive problem than intra-individual variability but all sources of decreasing the variability must be taken into consideration for this model for further development of this model.

When these factors are taken into consideration, the capsaicin model is a putative well-designed model. It has a proven ability, to analyse specific pharmacological effects in a targeted approach in a healthy volunteer population simultaneously as confounding variables (e.g. tissue- and nerve injury and inflammation) found in clinical pain states are minimized.

6. Acknowledgements

First and foremost I would like to thank my supervisor Prof. Paul Rolan for all the time he has spent and for excellent support and supervision during this project.

I want to thank Helena Gustafsson, who has incessantly standing by my side in prosperity as well as in misfortune, during these six months.

I also want to thank Melanie Gentgall and Jennie Williams for all assistance, encouraging talks and help with practical work at PARC during the clinical trial. Dr. Des Williams, Chai Li Lau and the staff at School of Pharmacy and Medical Sciences, University of South Australia for assisting me in the pharmaceutical component of this thesis and the RAH Pharmacy with particular thanks to Pharmacist Sharon Yap, for helping me with the writing of a pharmacy manual and preparation of the syringes used in trail.

I would like to express my gratitude to Prof. Gunnar Tobin and Michael Andersson at Göteborg University for being examiner for this thesis and for making this cherished semester possible.

Thank You, Lisa Miller, statistician at the Discipline of Public Health, University of Adelaide for the statistical analyses and interpretations and all the sixteen volunteers for providing me with data. Without the subjects this thesis would not be possible.

Finally I would like to give the warmest thanks to everyone at Discipline of Pharmacology, University of Adelaide and all the residents at Kathleen Lumley College, for making my time in Adelaide enjoyable, pleasant and unforgettable.

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