A Randomized, Double-Blind, Crossover Study to Evaluate the Depth Response Relationship of Intradermal Capsaicin-Induced Pain and Hyperalgesia in Healthy Adult Volunteers

Alan Silberberg, MD,* Tobias Moeller-Bertram, MD, MS,*†‡ and Mark S. Wallace, MD*

Departments of *Anesthesiology and †Psychiatry, University of California San Diego; ‡Center of Excellence for Stress and Mental Health, San Diego, California, USA

Reprint requests to: Mark S. Wallace, MD, Department of Anesthesiology, University of California San Diego, 9300 Campus Point Drive, #7651, La Jolla, CA 92037, USA. Tel: 8586577030; Fax: 8586577035; E-mail: mswallace@ucsd.edu.

Conflict of interest: There are no conflict of interests to report.

Funding sources: Funding for this project was provided by funds from the Department of Anesthesia, UCSD.

Abstract

Objective. The purpose of this study was to evaluate pain and hyperalgesia in response to different depths of intradermal (ID) capsaicin injections in healthy volunteers.

Design. Double-blind, cross-over study.

Setting. Clinical Research Laboratory.

Subjects. Fifteen healthy male subjects received ID capsaicin injections into the volar aspect of each forearm at depths of 1 mm, 3 mm, 5 mm, and 7 mm. After injection, spontaneous pain, elicited pain, flare response, heat thresholds, and area of hyperalgesia were measured at various time points.

Outcomes Measure. Spontaneous pain, elicited pain (pinprick, stroking, and hot pain), hyperalgesia area, and allodynia area.

Results. No significant difference was found between any depths in spontaneous pain, elicited pain (pinprick, stroking, hot pain), hyperalgesia area, or allodynia area. A significant difference was found in the change in heat threshold between 5 mm and 1 mm, 7 mm and 1 mm, 5 mm and 3 mm, 7 mm and 3 mm depths. A significant difference was found in flare area between 5 mm and 3 mm depths. A significant difference was found in systolic blood pressure area under the curve (AUC) between 7 mm and 1 mm depths, and for both systolic and diastolic pressures for 5 mm and 1 mm depths, and 5 mm and 3 mm depths. A significant difference was found in pulse AUC between 5 mm and 1 mm depths and 5 mm and 3 mm depths.

Conclusions. Injection of capsaicin at different depths in the skin had different effects on heart rate and blood pressure but no effect on pain. These results may have implications on the pharmacology and analgesic predictive value of the model of ID capsaicin.

Key Words. Allodynia; Capsaicin; Hyperalgesia

Introduction

The skin is a highly heterogenous organ that integrates elements of integument, nervous, vascular, immune, and endocrine functions. The extreme sensitivity to multimodal stimulus detection makes it a useful organ for experimental pain. As the use of the clinical pain state has evident limitations in evaluating analgesic interactions, human experimental models have been developed. Topical applications, intramuscular injection, and intradermal (ID)
acute pain is elicited by activation of fast A
defense mechanisms. An area of mechanical
hypersensitivity ensues as pain thresholds decrease for central
surrounding flare [16]. Mechanical hyperalgesia and
thresholds decreases below 37°C or physiologic body temperature
45°C, but when bound by capsaicin, the threshold
platelets. These channels normally open between 37 and
4°C, but when bound by capsaicin, the threshold
decreases below 37°C or physiologic body temperature
resulting in heat hyperalgesia at the site of injection and
surrounding flare [16]. Mechanical hyperalgesia and
allodynia ensue as pain thresholds decrease for central
and peripheral nociceptors. An area of mechanical
allodynia and secondary hyperalgesia occurs due to
neighboring dermatome activation. When capsaicin is ID,
acute pain is elicited by activation of fast Aδ fibers
through a direct effect of capsaicin. A flare and
weal response occurs rapidly, whereas a poorly localized,
protracted dull pain fingers from slow conducting C
fiber activation. These pain pathways
are naturally activated by tissue abrasion, burns, and
incisions.

Indeed, there have been many studies using ID capsaicin
as a model to test analgesic effects [8–10,22]. Although
the dose and volume of capsaicin injected are documented
in these studies, the rate and depth of injection
are not [8–10,22]. As the distribution of nerve fibers
sensitive to capsaicin within the skin is not homogeneous
throughout its dermal layers [23], differences in the depth
of ID capsaicin injection might affect the magnitude and
time course of the resulting pain and hyperalgesia. There
have been no studies evaluating the response to capsaicin
injected at different depths. This is important as the skin
layers range from the epidermis to the alveolar connective
tissue overlying the muscle with each layer having different
innervation.

We previously have studied the dose effects of ID capsaicin and the effects of intramuscular capsaicin [7,24]. The
primary objectives of this study was to determine the
degree of pain, hyperalgesia, allodynia, flare response,
and hemodynamic changes after injecting capsaicin at
different depths in healthy human volunteers. This study
adds to the body of literature that describes the response
of capsaicin at various doses and depths in the skin.

Methods

Participants and Setting

This randomized, double-blind, crossover study was
approved by the local Institutional Review Board (Univer-
sity of California San Diego [UCSD], San Diego, CA, USA),
and all subjects provided written informed consent. All
subjects were tested at the UCSD pain center in a quite
room at ambient temperature after the subjects gained
familiarity with the testing environment. A total of 15 males
(age range of 21–33; seven white, seven Hispanic, and 1
Asian) were enrolled (Table 1).

Screening assessments were conducted on the day 1
visit. These included a complete medical history and
focused physical exam, demographic information, prior
and concomitant medications and baseline vital signs.
Inclusion criteria were 1) male between the ages of 18 and
60 years; 2) no significant diseases in the medical history
or evidence of clinically significant findings on physical
examination, or history of abnormal clinical laboratory
evaluations (hematology, serum chemistries, and urinaly-
sis); 3) willing to refrain from using any local topical prepara-
tions including medications, lotions, creams, ointments
on the volar aspect of the forearms for 24 hours prior to
experimental visits; 4) normal, intact skin bilaterally at the
antecubital area (free of scars, scratches, bruises, and
tattoos); 5) able to communicate effectively with the study
personnel; and 6) willing to be blindfolded during the
study. Exclusion criteria were 1) over-the-counter or pre-
scription analgesics in any form within 24 hours prior to
study; 2) a known allergy to capsaicin; 3) currently partici-
ating or plan to participate in another clinical trial while
participating in this study; 4) any concomitant condition
which, in the investigator’s opinion, may interfere with the
subject’s safe participation; 5) a history of or actively using
abusing illicit drug substances or alcohol; 6) an active
dermatological disease of any origin that may interfere
with the subject’s ability to participate; 7) denuded or

Table 1  Subject demographics

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Range</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>27.7 (4.5)</td>
<td>21–33</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178 (8.7)</td>
<td>165–190</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.4 (14.9)</td>
<td>55–103</td>
</tr>
<tr>
<td>BMI</td>
<td>25.7 (3.8)</td>
<td>19.5–32.2</td>
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BMI = body mass index; SD = standard deviation.
broken skin on either forearm; 8) a history of unstable peripheral/vascular disease and/or hypertensive vascular disease; and 9) receipt of an investigational drug or product within 30 days prior to testing in this study.

Procedures

The order of injection assignments of 1 mm, 3 mm, 5 mm, and 7 mm injections was based on a computer-generated randomization schedule. Injections were randomized so that there are equal numbers of injections per group split between the right and left forearms. At the beginning of each testing session, the respective depth was looked up and recorded for that session.

After completing the screening assessments, subjects who met the eligibility criteria were randomized to receive the four injections (one for each depth) in random order. Injections were made at depths of 1 mm, 3 mm, 5 mm, and 7 mm. Two injections were made on testing session one followed by two more injections on a second testing session separated by at least 4 days from the first. The injection was applied at the volar aspect of the forearm (5 cm distal to the antecubital crease). The depth of needle insertion was measured using a Starrett Electronic Height Gauge (Starrett Company, Athol, MA, USA). The device consists of a thick metal stand with a metal base allowing for steady positioning on a table. An electronic depth measuring device with a small metal sidearm can slide on the stand and precisely move in fractions of a millimeter at a time. A 30-gauge needle was attached to the tip of the metal sidearm and connected to a 1-mL microsyringe via a three-way connector. For each injection, the subject was comfortably seated with the treatment arm secured to an intravenous board and placed next to the device. At this point, the subjects were blindfolded until the injection was performed to keep them blinded to the injection depth. The skin at the site of injection was prepared with alcohol wipes. The needle was slowly lowered toward the subjects skin and zeroed at the point the needle just touched the skin. The needle was then advanced by a technician (blinding the investigator) to the depth specified by the previous randomization order. Once the precise depth was reached, the investigator injected a volume of 10 μL (100 mcg of capsaicin) ID through the needle using the microsyringe. One hundred milligrams of capsaicin (8-methyl N-vanillyl 6-nonamide) dissolved in 10 mL of a 20% cyclodextran vehicle to achieve a concentration of 10 mg/mL was prepared and utilized using sterile technique.

Outcome Measures

The following outcome measures were assessed: 1) spontaneous pain; 2) elicited pain to three stimuli (pinprick [hyperalgesia], stroking [allodynia], hot 45°C [hot pain]); 3) heat pain threshold (°C); 4) area of hyperalgesia (cm²); 5) area of allodynia (cm²); 6) area of flare (cm²); 7) blood pressure; and 8) pulse. The following measurements were taken prior to the injection (baseline): 1) pulse (using a standard readout from the electrocardiogram); 2) blood pressure (using a standard blood pressure cuff); and 3) heat pain threshold. Immediately after the capsaicin injection and at 5 minute intervals thereafter (up to a final measurement at 20 minutes), the following measurements were taken: 1) pulse; 2) blood pressure; 3) spontaneous pain; and 4) elicited pain to three stimuli (pinprick, stroking, hot 45 degree). Twenty minutes after the capsaicin injection, the following measurements were taken: 1) heat pain threshold; 2) flare (cm²); 3) hyperalgesia to pinprick (cm²); and 4) allodynia to stroking (cm²).

The effects of the different ID injection depths on the heat pain threshold were assessed using a Thermal Sensory Analyzer (Medoc Advanced Medical System, Minneapolis, MN, USA). The heat pain threshold was determined preinjection and 20 minutes post-ID capsaicin. Briefly, a standard thermode (3 × 3 cm surface) was placed on the volar aspect of the forearm to be injected. The standard program using the “method of limits” [25] was applied and the subjects instructed to push a button to stop the temperature change once it was just felt as painful heat and the heat thresholds were recorded as the mean of three runs.

Pain scores were measured at the time of injection and every 5 minutes for 20 minutes using a visual analog scale (VAS). Briefly, the VAS consists of a 10-cm line with “no pain” written at one end and the “worst imaginable pain” written at the other end. The patient was then asked to place a mark along the line that corresponds with their pain sensation intensity. The distance, in centimeter, from the no pain end to the location of the mark gives a measurement of the pain. For each time point, four different pain intensity scores were obtained: 1) spontaneous pain; 2) elicited pain to a 45°C heat stimulus (thermal hyperalgesia) applied for 5 seconds; 3) elicited pain to a 5.18 von Frey hair bounced five times adjacent to the injection site (mechanical hyperalgesia); and 3) elicited pain from stroking with a 1 inch foam brush stroked five times adjacent to the injection site (allodynia). At 20 minutes postinjection, mapping of the area flare response, hyperalgesia to pin prick, and allodynia to stroking with a foam brush was performed and transferred to a transparency. The stimulus began away from the injection site in an area of skin that did not produce pain. The stimulus was repeated tangentially to the injection site at a progressively closer radius until the subject reported pain or tenderness. That site was marked on the skin with a felt tip pen, and a new series started from the periphery at a different angle, until four determinations of the borders of secondary hyperalgesia and allodynia were outlined on the skin. These borders, as well as the flare response, were outlined onto a transparency for area determination (cm²).

Analysis

Fifteen subjects were randomized into different injection depth sequences. This sample size is based upon power calculations done by the primary investigator for other capsaicin studies [3–6,8–10,22].
Outcome measures are expressed as the mean ± standard deviation. Areas under the curve were calculated for spontaneous pain, elicited pain (to pinprick, stroking, and 45°C), and pulse using the trapezoid rule. In general, the AUC measure has a reduced level of error than repeated measures. In addition, the AUC provides a single data point per subject; however, this single data point reflects the data provided by the full time-course postcapsaicin, rather than selection of a single (e.g., peak effect) time-point. Linear models were built to fit by the generalized least squares method of the following form:

\[ \text{Outcome Measurement} = \beta_0 + \beta_1 \times \text{Depth} + \epsilon \]

where \( \epsilon \) is a normally distributed error term with nonzero correlation allowed for observations within each subject to account for repeated measures. The correlation and variance structure was chosen by sequential likelihood ratio tests, with \( \alpha = 0.10 \). We fit the above model for each outcome measurement and report the fitted coefficient of each model as well as all linear contrasts by depth.

**Results**

After ID injection of capsaicin at different depths, all subjects reported pain, hyperalgesia, and allodynia. However, there was no significant difference found between any depths in spontaneous pain AUC, hyperalgesia AUC, allodynia AUC, hot pain AUC, hyperalgesia area (cm²), or allodynia area (cm²).

A significant difference was found in the change in heat pain threshold between 5 mm and 1 mm (estimated difference: 3.849°C, \( P < 0.001 \)), 7 mm and 1 mm (estimated difference: 2.535°C, \( P < 0.001 \)), 5 mm and 3 mm (estimated difference: 3.900°C, \( P = 0.001 \)), and 7 mm and 3 mm (estimated difference: 2.586°C, \( P = 0.009 \)) depths (Figure 1).

A significant difference was found in flare area between 5 mm and 3 mm depths, with an estimated difference of −15.92 cm² (\( P \) value: 0.021).

A significant difference was found in systolic blood pressure AUC between 7 mm and 1 mm depths, 5 mm and 1 mm depths, and 5 mm and 3 mm depths with an estimated difference of 78.50 (\( P \) value: 0.009), 94.00 (\( P \) value: 0.078), and 84.00 (\( P \) value: 0.036), respectively. A significant difference was found in diastolic blood pressure AUC between 5 mm and 1 mm depths and 5 mm and 3 mm depths, with an estimated difference of 104.33 (\( P \) value: 0.009) and 94.17 (\( P \) value: 0.017), respectively. A significant difference was found in pulse AUC between 5 mm and 1 mm depths and 5 mm and 3 mm depths, with an estimated difference of −65.33 (\( P \) value: 0.014) and −60.67 (\( P \) value: 0.049), respectively (Figure 2).

Table 2 summarizes all outcome measures.

**Discussion**

The thickness of the skin has been described using punch biopsies and echographic evaluation [26]. The average thickness of the forearm epidermis, dermis, and subcutaneous fat have been estimated to be 74.9 μm, 1.07–1.25 mm, and 4.88–5.75 mm, respectively.

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**Figure 1** Heat pain thresholds measured at the site before and after capsaicin injection. Data shown for different depths of intradermal capsaicin. * Significantly different than 1 mm (\( P = 0.001 \)) and 3 mm (\( P = 0.001 \)) depths. # Significantly different than 1 mm (\( P = 0.001 \)) and 3 mm (\( P = 0.009 \)) depths.
Therefore, any depth greater than 6 mm is likely in the muscle. This was the basis of using the 7 mm depth in order to inject the capsaicin into the muscle as a comparator. The 1 mm depth was likely in the dermis, whereas the 3 mm and 5 mm depths were likely in the superficial and deep layer of the subcutaneous fat. We considered a more superficial depth of 0.5 mm to ensure a dermal injection; however, there was some indentation of the skin prior to needle penetration, and this risked no capsaicin injection into the skin.

Until the late 1980s, it was widely believed that the epidermis contained few sensory endings and that most of the small caliber innervation implicated in cutaneous sensations of pain were located in the dermis. However, the development of an antibody against a pan-neuronal enzyme called PGP 9.5 revealed extensive, previously unknown innervation with the epidermis being the primary site of small fiber terminals [27–32]. In addition, using a three-layer heat-transfer model on monkey hairy skin, it was determined that C-fiber mechano-heat nociceptors (CMHs) depth estimates from response to ramped heat stimuli ranged from 20 to 570 microns (mean 201 microns). The average receptor depth from responses to stepped heat stimuli averaged 150 microns. They concluded that CMHs occur in both the dermis and epidermis and that there is a tight distribution of CMHs heat thresholds [33]. Our study demonstrated that the more superficial injections at 1 mm and 3 mm depths produced a reduction in heat pain thresholds but did not increase the elicited pain intensity to a 45°C stimulus. In addition, there was a trend for more flare response with more superficial injections as there was significantly more area of flare with the 3 mm depth as compared with the 5 mm depth. However, this significance was not reached between the 1 mm, 5 mm, and 7 mm depths due to some outliers. Therefore, the study may have been underpowered to detect the difference. Calcitonin gene related peptide/substance P-containing afferents have long been implicated in an axon-reflex mediated increase in dilatation and permeability of capillaries and precapillary arterioles in the upper dermis as well as pain sensation in response to intense heat [23]. These findings suggest that capsaicin activation of the epidermal TRPV1 channels are responsible for the reduction in heat pain threshold. The heat/capsaicin sensitization model involves the application of a 45°C stimulus for 5 minutes followed by the application of topical capsaicin. This results in a low intensity pain during treatment followed by a long-lasting secondary hyperalgesia [1]. This model likely results in the activation of epidermal fibers unlike the injection technique used in our study that activated the dermal, subcutaneous, and muscle fibers. The heat/capsaicin sensitization model has not described the heat hyperalgesia as results from intradermal

![Figure 2](https://academic.oup.com/painmedicine/article-abstract/16/4/745/2460697)
capsaicin. Although the pharmacology of the ID capsaicin and heat/capsaicin sensitization model appear to be similar, our finding suggest that there may be more of a sympathetic response with the ID injection. The clinical implications, if any, are yet to be determined.

There were no differences in the spontaneous or elicited pain measures at any depth. However, there was a significant trend for an increase in blood pressure and decrease in pulse with deeper injections. This suggests that the capsaicin receptors are evenly distributed throughout the skin, subcutaneous fat, and muscle. However, there seems to be more of a sympathetic response to more deeper injections. Most of the sympathetic innervation to the skin has focused on the deeper vessels that are primarily noradrenergic fibers [34]. The convergence of the sensory and sympathetic innervation may be the site of sudomotor disorders and sympathetic involvement in pain regulation implicated in such chronic pain conditions such as complex regional pain syndrome and fibromyalgia, where profound pathologies of this innervation have been observed in the skin [35,36]. Our results suggest that the sensory sympathetic convergence is more robust in the deeper dermis or epidermis. However, this conclusion is entirely being made on the findings of heart rate and blood pressure alone. More solid evidence would include the comparison of swelling and edema at the injection site with different injection depths.

A weakness of our study is that it may not have been powered enough to detect differences in all of the outcome measures. This was apparent in the trend for an increase in the flare response with more superficial injections that failed to show a statistical significance as depth of injection increased. Although human experimental models have more control over variables than in clinical studies, it is still not immune to intersubject variability and outliers that reduce power. Our study adds another variable, controlled depth of injection, that may improve power. Also, there was no control over the body mass index and the thickness of the dermis and subcutaneous fat may have been too variable as the range was 19.5–32.2. Future studies may consider the use of ultrasound to assess thickness.

In summary, our study is the first to establish and describe the depth different effects of capsaicin representing different capsaicin sensitive nerve fiber distributions throughout the dermal layers. These results may have implications on the pharmacology and analgesic predictive value of the model of ID capsaicin.

References


