A Simple Contact Heat Experimental Pain Model for Evaluation of Analgesic Agents in Healthy Volunteers

Sunil Kumar Reddy Khambam, MSc, PhD; Madireddy Umanaheshwar Rao Naidu, MD; Pingali Usha Rani, MD, DM; and Takallapalli Ramesh Kumar Rao, MD

ICMR Advance Centre for Clinical Pharmacodynamics, Departments of Clinical Pharmacology & Therapeutics, Nizam’s Institute of Medical Sciences, Andhra Pradesh, India

ABSTRACT

**Background:** Human experimental pain models help to understand the mechanism of the underlying clinical pain conditions and can be adopted to test analgesic efficacy of drugs used in the management of pain. In early phases, the clinical development of new analgesic agents is severely hindered due to lack of reliable sensitive tests for the experimental pain models.

**Objective:** The aim of the present study was to standardize and validate a simple contact heat pain model that can be used for future screening of various analgesic agents.

**Methods:** The method was standardized by recording heat detection and heat pain detection threshold in degrees centigrade in 24 healthy volunteers. Reproducibility of the test procedure was evaluated by recording the thermal threshold parameters by a single observer on 2 sessions (inter-day reproducibility) and a second observer on 1 session (inter-observer reproducibility) separately. Validity of model was further tested by evaluating the analgesic effect of tramadol on 12 healthy volunteers.

**Results:** Thermal pain model using contact heat method was found to produce low variability with coefficient of variation <5%. Inter-observer and inter-day reproducibility was very good, as shown by Bland–Altman Plot; with most of the values within 2 SD. There was a significant difference in both heat detection threshold and heat pain detection threshold produced by tramadol, as compared with placebo ($P < 0.05$).

**Conclusions:** The newly developed pain model produces a type of experimental pain that is responsive to analgesic effects of tramadol at clinically relevant doses. The model might be useful in early screening of new therapeutic agents before proceeding to expensive clinical trials in acute and chronic pain sufferers. (Curr Ther Res Clin Exp. 2011;72:233–242)

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INTRODUCTION

Pain is the most prevalent health care problem, and characterization of pain is of major importance in the diagnosis and choice of treatment. Studies of analgesic efficacy in patients already suffering pain raise ethical issues. Also, in clinical practice, the different symptoms of the underlying disease or complaints relating to psychological, cognitive, and social aspects of the illness, as well as systemic reactions such as fever and general malaise, confound the characterization of pain. In contrast, experimentally induced pain avoids some ethical issues and is often advantageous in preclinical investigation of analgesics because of the close control of the environment and the intensity and nature of the noxious stimulus.

Several experimental approaches have been used in the early screening of new analgesics. Commonly, these tests measure subjective pain after pain stimuli. However, these approaches are limited mostly because of the poor standardization of subjective pain ratings. Reproducibility is an important factor in the testing of analgesics, where it is necessary to repeat the pain stimulus several times during active and placebo treatments; if reproducibility is good, the model can be useful in drug screening.

An alternative for determining the effect of analgesics is quantitative sensory testing (QST). QST has the particular advantage of being a functional test that provides a quantitative pain stimulus and assesses the subject’s individual response to the stimulus. The repeatability of the visual analogue scale has been shown to be poor in a setting of human experimental heat pain compared with thermal QST. QST also provides a reliable assessment of changes in pain thresholds.

Our objective was to standardize a simple thermal pain model using QST as a functional test, applicable in early clinical trials with groups of reduced numbers of subjects for the evaluation of a possible analgesic effect. Further validation of the experimental model was carried out by assessing the analgesic effect of tramadol.

SUBJECTS AND METHODS

Subjects

Twenty-four healthy volunteers ranging in age from 21 to 35 years were studied. The volunteers were given a short explanation of the purpose of the research and a description of the procedure to be followed. They were also given a description of any reasonably foreseeable risks and discomforts. Written consent to participate was obtained from each volunteer. Before it was initiated, the study was approved by the Institutional Ethics Committee on Research Involving Human Beings.

Contact Heat Method

Heat pain was induced using the Sensitometer HCP (Dhansai Laboratory, Mumbai, India) with a skin contact surface area of 4 cm². The probe was kept in touch with the volar aspect of the forearm of the nondominant arm, taking care to ensure that the
entire surface of the probe was in contact with the skin. The basal skin adaptation temperature of the probe was maintained at 32°C. Tests were performed using the method of limits (i.e., ascending method of limits, where some property of stimulus starts out at a level so low that the stimulus could not be detected, then this level is gradually increased at a fixed rate until the participant reports that they are aware of it), with change of 0.5°C/sec for heat detection and heat pain detection threshold testing and the cut-off temperature was set to 49°C. The more rapid temperature change used to determine pain thresholds was chosen to avoid sensitization of the skin due to thermal stimuli. All tests were performed in the same room with an ambient room temperature of 21°C (±1°C) and always at the morning session. Volunteers were blinded during the procedure and no auditory cues were given to indicate stimulus onset. Volunteers were instructed to report the time point at which they perceived heat sensation (warmth), that is, the heat detection threshold (HDT) and the time point at which they perceived heat pain sensation, that is, the heat pain detection threshold (HPDT). The sensory qualities were always tested in the same order: HDT and HPDT. The recordings were noted in degrees centigrade. Four measurements were performed for each threshold (HDT and HPDT) on the same volar aspect of the nondominant forearm and the mean was taken. There was a gap of at least 20 seconds between each measurement. In addition, to assess the reproducibility of the method, each volunteer participated in 3 experimental sessions separated by intervals of 4 to 5 days. The recordings of the thermal threshold parameters were noted by a single observer on 2 sessions (inter-day reproducibility) and the second observer on 1 session (inter-observer reproducibility) separately.

**Effect of Tramadol**

Twelve healthy male volunteers with a mean age of 31.3 (6.7) years participated in the study. Volunteers were randomly assigned to receive either placebo or the tramadol 50 mg at the morning session after a light breakfast, according to the crossover design. There was a 1-week washout period between the drug and placebo phases. Randomization of the sequence of placebo and active treatment periods was performed by a pharmacist who had no contact with the volunteers or the experimenter. On the day of experiment, the procedure, as described earlier, to detect sensory qualities was carried out on the volar aspect of the nondominant forearm and all measurements were taken from the same site. HDT and HPDT were recorded at baseline (0 minute) and then at 30 minutes, 60 minutes, 120 minutes, and 180 minutes after administration of the drug. During the application of the study drugs, a sedation score (0 = awake, 1 = tired, 2 = asleep but arousable, 3 = nonarousable) was assessed every 10 minutes. All side effects were noted.

**Statistical Analysis**

The data on HDT and HPDT were recorded in degrees centigrade and presented as mean, standard error, and coefficient of variance.

Bland–Altman plotting was performed for the assessment of method reproducibility. The relative (positive or negative) differences between each pair of measure-
ments were plotted against the mean of the pair to make sure that no obvious relationship appeared between the estimated values of mean and difference. The Bland–Altman analysis was done to compare the values of HDT and HPDT obtained by 2 observers separately. Similarly, the comparisons were also made to confirm the reproducibility by analyzing the HDT and HPDT values obtained on 2 sessions.

The paired Student t test was used to compare the difference within the group and between the 2 groups, a value of \( P < 0.05 \) was considered to indicate statistical significance. All statistical analyses were performed using Graph Pad PRISM software 4 (Graph Pad Software Inc., San Diego, California).

RESULTS

Results of the method standardized refer to a group of 24 healthy volunteers, aged 21 to 35 years, who were apparently healthy on the basis of their medical examination and laboratory investigations. In no subject was burn injury observed.

The Table lists the mean, standard error, and coefficient of variance of HDT and HPDT.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (°C)</th>
<th>SE</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDT</td>
<td>36.85</td>
<td>0.26</td>
<td>3.46</td>
</tr>
<tr>
<td>HPDT</td>
<td>44.97</td>
<td>0.24</td>
<td>2.62</td>
</tr>
</tbody>
</table>

CV = coefficient of variation; HDT = heat detection threshold; HPDT = heat pain detection threshold.

Analgesic Efficacy

To confirm the validity of the method, we have used tramadol as a reference opioid analgesic. Twelve healthy male volunteers (mean age 31.3 [6.7] years; height 167.8 [6.2] cm; weight 65.6 [6.5] kg; and body mass index 23.1 [2.3] kg/m\(^2\)) completed the study.

HDT to Contact Heat Pain Method

HDT values increased markedly with the active treatment but remained unchanged after placebo. Peak effects were observed 30 minutes after administration.

Table. Mean, SE, and coefficient of variation of heat detection threshold and heat pain detection threshold (n = 24).

Current Therapeutic Research
Tramadol 50 mg produced significant elevations of HDT at all time points compared with placebo ($P < 0.01$).

**HPDT to Contact Heat Pain Method**

HPDT values increased after administration of the active drug, but remained unchanged after placebo. Peak effects were observed 30 minutes after administration (Figure 3). Tramadol...
50 mg produced significant elevations of HPDT at 30, 60, and 120 minutes compared with placebo (P < 0.05 at 60 and 120 minutes, P < 0.01 at 30 minutes).

Mild sedation was seen in some volunteers, but never exceeded sedation score 1.

**Figure 2.** Thermal sensory threshold. The effect of placebo and tramadol on the tested heat detection threshold (HDT). Data are expressed as mean (SE) of the difference between the baseline threshold values measured before (0) and 30 minutes, 60 minutes, 120 minutes, and 180 minutes after administration. Statistical significance of change in threshold compared with placebo (*P < 0.01).

**Figure 3.** Thermal pain threshold. The effect of placebo and tramadol on the tested heat pain detection threshold (HPDT). Data are expressed as mean (SE) of the difference between the baseline threshold values measured before (0) and 30 minutes, 60 minutes, 120 minutes, and 180 minutes after administration. Statistical significance of change in threshold compared with placebo (*P < 0.01, †P < 0.05).
DISCUSSION
The present study describes an experimental pain technique in humans that is sensitive to detect changes in HDT and HPDT using tramadol at doses known to be effective in acute pain. In addition, it is simple to perform and requires few personnel to conduct the study. Although a flare response was observed in the vast majority of subjects, no burn injuries occurred in any of our subjects.

In the present study, data obtained on HDT and HPDT was highly reproducible with a coefficient of variance <5%. One well-known index of accuracy of a method is the coefficient of variation (SD/mean) and coefficient of variance <10% is considered to be the hallmark of a good assay for a subjective phenomenon.9

In addition, the data obtained for both HDT and HPDT on the relationship and Bland–Altman plot comparing inter-day and inter-observer measurements were to evaluate the reproducibility of an experimental pain model. There was no significant difference in the values for reproducibility reported between the observers and between the sessions and most of the values range within mean (2 SD) of the Bland–Altman plot. Reproducibility is an important factor in the testing of analgesics, where it is necessary to repeat the pain stimulation several times during active and placebo treatments. Earlier studies aimed to present data of heat-induced pain and assessed and reported inter-session repeatability employing methods based on standard recognized statistical technique.8

The major criticism of experimental pain techniques is short duration of exposure to the stimuli, which differs from clinical pain. The ability of this method to discriminate tramadol from placebo was attributed to the tonic nature of the stimulus. However, it is probably irrelevant whether the experimental pain stimulus is delivered as phasic or tonic. It is more important that the stimulus reaches sufficient intensity to produce pain sensation (burning quality due to activity in the unmyelinated C nociceptors) because it is the latter sensation that is reliably attenuated by both non-narcotic and narcotic analgesics.10

The tonic heat model also offers an important theoretical advantage compared to repetitive-phasic stimulation models. In the latter, the subject goes through an alternation of pain anticipation and pain-relief states. There is ample evidence from human brain mapping studies that both anticipation and termination of a painful event can activate the brain reward system,11,12 and that this process is modulated by dopaminergic mechanisms.13 This inter-relationship makes it difficult to disentangle pain-related and reward-related changes in dopamine receptor availability.

In the present study, we have utilized the crossover design to compare the analgesic effect of tramadol and placebo in healthy subjects and a single investigator performed all pain assessments. In general, parallel studies give a weaker statistical power than a crossover design, demanding larger sample sizes.14 In case of crossover-designed studies, it is important that the investigator is the same in all pain assessments because gender and appearance of the investigator can influence the pain rating of the volunteers.15

The sensitivity of a given experimental model for detecting analgesia is affected by the method used to measure this pain. Good sensitivity of a model is obtained by
using a pain assessment that is reliable and producing data with modest variance (noise). In the study by Thurauf et al, the value of objective pain assessment was shown because an effect of tramadol was found on evoked brain potentials only and not on pain ratings.\textsuperscript{16} It is, however, important to note that, although evoked potentials can be a sensitive measure of nociceptive processes, they only measure a single dimension of pain. Pain is a multidimensional sensation, and this is reflected better in the subjective pain measure. This limits the translation of analgesic effect on evoked brain potentials into effect on clinical pain measures. In the present study, we have used QST as a functional test that provides a reliable assessment of changes in pain thresholds. Also, thermal QST (heat and cold) allows a distinction between predominantly C-fiber activity and A-delta fiber activity.

The study duration of the drug was based on consideration of \( T_{\text{max}} \). Thus, tramadol, which has a \( T_{\text{max}} \) of \( \sim3 \) hours was tested for 3 hours. There is strong evidence that for most analgesics, clinical analgesia is not a direct function of drug concentration. Therefore, the time course of analgesic effect for analgesic drugs is characteristic of pharmacologic effect (analgesia), consistent with a role of an endogenous substance in the analgesic effect. In the present study, the time to peak effect after tramadol occurred at 30 minutes.

The kinetic profile is necessary to determine when it is optimal to perform the pain tests, bearing in mind that bad timing of pain testing can jeopardize an otherwise well-designed trial. For opioids, it is particularly important to remember that they often need to cross the blood–brain barrier and enter the central nervous system to have an analgesic effect. This causes a lag time to the onset of analgesia. The study design should consider these different lag times for different opioids. In the present study, considering the \( T_{\text{max}} \) of the tramadol, we performed all pain assessments at 30, 60, 120, and 180 minutes post-drug.

In the present study, subjects were given a light breakfast because oral administration of tramadol with food does not substantially affect its rate or extent of absorption. Also, fasting the subjects to increase the absorption of analgesics, we believe, can introduce additional stress. However, dietary manipulation can alter human pain sensitivity in that rapid increases in circulating glucose produce a decrease in the ability to tolerate pain.

In our study, as compared with placebo administration, tramadol produced a substantial analgesic effect. Many experimental studies in healthy volunteers have failed to determine the pharmacodynamic profile of agents with moderate analgesic activity in clinically relevant doses.\textsuperscript{17,18} There have been many attempts to develop experimental pain models in humans. Few techniques have been proven useful for evaluation of analgesics\textsuperscript{19} or for the elucidation of pain mechanisms.\textsuperscript{20}

Response to pain stimulus is highly subjective and varies from subject to subject, necessitating proper sample size estimation for evaluation of an analgesic drug on human participants. For method optimization and standardization, we have included a total of 24 healthy human volunteers in our experiment. Trials involving experimental pain often use small sample sizes because the variation of the outcome measures is less than in traditional clinical trials. Trials with fewer than 10 to 12
subjects are hard to test statistically and findings are therefore questionable. However, it has been shown that experimental models with a high reproducibility and a sample size <10 are powered to show the effect of analgesics.\textsuperscript{21}

In the method described here, tramadol induced a considerable increase in the HDT and HPDT, indicating that the study design was valid and capable of detecting an analgesic effect on heat pain. There are still major problems in the exact determination of the activated pathways and pain mechanisms in human experimental pain.\textsuperscript{22} Nevertheless, the experimental human models allow the possibility to obtain reproducible results in test-retest experiments and be useful for drug screening.\textsuperscript{23}

**CONCLUSIONS**

There is a great practical need for a dependable method of assessing the effects of analgesic drugs on experimental pain in humans. Results of the present study show that appropriate use of the contact heat method technique produces a type of experimental pain that is responsive to the analgesic effects of tramadol. Our data also indicate that the variation between different subject’s thresholds is less than what has been expected heretofore. The model might potentially allow analgesic effects of new compounds to be quantified in healthy volunteers before proceeding to expensive clinical trials in acute and chronic pain sufferers.

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**CONFLICTS OF INTEREST**

The authors have indicated that they have no conflicts of interest with regard to the content of the article.

**REFERENCES**