CLINICAL ASSESSMENT OF ANALGESICS USING ULTRASONIC STIMULATION — A NEW METHOD —

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Accepted March 7, 1977

Abstract — A quantitative method for measuring pain threshold by the use of ultrasonic stimulation in man was designed and the possibility of clinical application in assessing analgesics was investigated. Ultrasonic stimulus was given to Japanese subjects on the palmar distal part of the 2nd, 3rd and 4th fingers of both hands. The latent time between start of the stimulation and withdrawal of the hand when perceiving pain was considered the pain threshold. The ultrasonic evoked pain was a sharp pin-prick type, without sensations such as thermal and mechanical. The pain threshold lowered with increasing either stimulus intensity or water bath temperature when the hand of the subject was immersed during measurement. Normal threshold to ultrasonic stimulation measured in both 50 men and 50 women gave nearly normal distribution curves: women being more sensitive to ultrasonics than men. Analgesia with codeine phosphate (20 mg p.o.), aspirin (1.5, 1.0, 0.5 g p.o.), aminopyrine (100 mg p.o.) and mefenamic acid (500 mg p.o.) in volunteers of both sexes was demonstrated significantly using this method under double blind circumstances. Pentobarbital, diazepam, butylscopolamine, bromelain and placebo each in the usual dose used clinically failed to alter the pain threshold. Humans were at least 25 fold more sensitive than mice to the analgesics used herein.

Several methods for inducing experimental pain in man have been documented. However, a quantitative measurement of pain is difficult as pain is essentially a subjective phenomenon where perception is greatly influenced by emotion, environment, individual difference, etc. Pain has been produced experimentally in humans by use of radiant heat (1–7), conduction heat (8), chemicals (9–11), electricity (12–18), mechanical (19–21) and ischemic methods (22–29). Even the radiant heat method devised by Hardy, et al. (1–4) which has been extensively used as a relatively good method for assessing analgesics in man has however the drawback of sometimes producing a bulla-like tissue damage.

We have already reported (30) that ultrasonic waves generated by a vibrator evoked pain in the tail of mouse and such waves can be used in laboratory animals as an analgesic screening method. In the present work, we applied this ultrasonic stimulation to human volunteers in an attempt to assess the degree of analgesia of certain drugs.

MATERIALS AND METHODS

The apparatus for measuring pain threshold is presented schematically in Fig. 1. The ultrasonic stimulus was provided by an ultrasonic stimulator (UR-200P, Tomi Seiko, Co., Ltd.). The stimulating parameters were as follows: frequency of ultrasonic wave, 20 KHz;
voltage, 100 V; stimulus intensity, 46 W/cm² (supplied output). The tip, that is, the peripheral end from which ultrasonic wave irradiates, has a diameter of 0.3 cm. Each palmer distal part of the 2nd, 3rd and 4th fingers of both hands immersed under a water bath at 35°C was gently pressed on the tip of the apparatus and the peripheral apex of the finger was in contact with a microswitch which was also in the water bath and connected with a digital stop-watch having 0.01 sec scaling (Takei Kiki Kogyo, Co., Ltd.). When pain was felt, the subject pulled back his hand which left the microswitch, thereby stopping the digital stop-watch. Each finger was separately stimulated at about 10 sec intervals. The latent time (sec) between start of the stimulation and withdrawal of the hand when perceiving pain was considered the pain threshold. Subjects were told to withdraw their hand when feeling a pain, either bearable or unbearable. In assessing analgesics, the two extremes among six fingers were eliminated and the average latency value of the remaining four fingers was taken as the threshold at a given time in a subject. There was almost no difference in threshold among the six fingers.

Healthy male and female Japanese students of Hoshi College of Pharmacy at ages 18 to 25 acted as volunteers. All subjects had no apparent history of hypersensitivity to drugs, and all were cooperative. Analgesic effects of drugs were investigated in men and women separately using a double blind test. The code on drug administrations was kept by a controller and opened only after terminating the experiment. Subjects swallowed the given compound together with a glass of water 30 min after having a light lunch.

The drugs administered were as follows: aspirin (Bayer), aminopyrine (Sankyo), mefenamic acid (Pontal, Sankyo), codeine phosphate (Sankyo), pentobarbital calcium (Ravona, Tanabe) and bromelain (Kimotab, Mochida). Each drug was given in the dose used clinically, while lactose served as the placebo. Drugs were pulverized, and lactose was added as required to adjust the bulk. The pulverized powder was enclosed in 2 microthin sheets of digestable ricepaper so as not to be distinguished by external appearance, odor, taste, color and bulk. Untoward effects of the drugs were not experienced except for one male who complained of a slight stomach ache after ingesting aspirin.
Tests were also carried out in mice following the method previously described (30) except that in the present work the stimulus intensity was 46 W/cm² instead of 52 W/cm².

Statistical significance was determined using the Student’s t-test, the level of significance being P = 0.05.

RESULTS

Pain threshold at various temperatures and stimulus intensities

Pain threshold lowered when temperature of the bath water was increased (Fig. 2a). With stimulus intensity of 46 W/cm², the end point of pain perception was unclear at below 25°C, while pain evoked was unbearable at over 40°C. At a physiological temperature of 35°C subjects perceived a usual sensation. The pain that was felt when the threshold was exceeded was a sharp pin-prick type, not accompanied by thermosensation unless the latent time was exceeded by about 10 sec. With the stimulus condition of 46 W/cm² and 35°C, the pain sensation evoked disappeared just after cessation of the stimulation.

With increasing stimulus intensity, pain threshold lowered (Fig. 2b). At the intensity of 90 W/cm² the stimulated site rarely and only slightly reddened.

The stimulus condition of 46 W/cm² and 35°C was employed in all further experiments.

![Fig. 2. Normal pain threshold at various temperatures and stimulus intensities in men and women. a) Pain threshold at various temperatures ranging from 20°C to 45°C under the stimulus intensity of 46 W/cm². b) Pain threshold at various stimulus intensities under the water bath temperature of 35°C.](image)

Frequency distribution of normal threshold

The frequency distributions in a normal threshold are shown in Fig. 3. The distribution curves were almost normal. Females were found to be more sensitive to the stimulation. The mode values of threshold were 3.20–3.99 and 2.40–3.19 sec, and the mean values were 3.90 ± 0.18 (S.E.M.) and 3.16 ± 0.15 sec in men and women, respectively.

Analgesic effect of codeine phosphate

The time-action curves for codeine in a dose of 20 mg p.o. in 5 men and 5 women are shown in Fig. 4. Codeine was administered at zero time just after the first measurement.
FIG. 3. Frequency distribution curves of normal pain thresholds to ultrasonic stimulation in 50 men and 50 women.

FIG. 4. Analgesic effect of codeine phosphate in men and women with application of the ultrasonic method. Each point represents the mean with standard error for 5 men and 5 women. *Significant at $P=0.05$.

FIG. 5. Dose-response relationships of aspirin.

Analgesic effects of aspirin, aminopyrine and mefenamic acid

Aspirin caused a dose-dependent increase in pain threshold in 5 subjects (Fig. 5) and the threshold dose was less than 0.5 g. The time-action curves for aspirin, 1.0 g, aminopyrine, 100 mg, and mefenamic acid, 500 mg, in 5 men and 5 women are given in Fig. 6. All three drugs caused significant analgesia, which reached a maximum at 60 to 90 min and lasted for at least 150 min. Analgesic effects of these drugs appeared to be stronger in the females.
Effect of drugs other than analgesics

Pentobarbital, 50 mg, diazepam, 5 mg, (sedatives), butylscopolamine, 20 mg, (an anti-spasmodic) and bromelain, 400 mg, (an anti-inflammatory drug) did not alter the pain threshold up to 150 min after administration in 5 men and 5 women.

Comparison of analgesic effects between humans and mice

Fig. 7 is a comparison of the effects of codeine, aspirin, aminopyrine and mefenamic acid between humans and mice under almost the same experimental condition as described in Materials and Methods. Data from humans include the peak changes in the threshold obtained with each drug and which are shown in Figs. 4 and 6. Data from mice were obtained after oral dosing of each drug in values 25 fold higher doses per kg body weight than in humans (the human body weight was assessed to be 50 kg); namely, codeine, 10 mg/kg, aspirin, 500 mg/kg, aminopyrine, 50 mg/kg, and mefenamic acid, 250 mg/kg, in mice.

In mice, all dosings raised the threshold significantly. The effect of each drug, par-
particularly of codeine, was higher in humans than in mice, even though humans were given much less of the dose per kg.

**DISCUSSION**

There is a paucity in literature regarding pain and analgesics as obtained in experiments on humans. Thermal, mechanical, chemical and electrical stimulations have all been used to produce painful sensations. The radiant heat method of Hardy, et al. (2), in which a 1000 W lamp was focussed on the forehead for 3 sec to produce superficial burn-like pain, has often been used and increased our knowledge of the effects of analgesics in man. Burn (21) devised a controlled compression apparatus with which pain was produced on the soft tissue near the Achilles tendon. There are at least two drawbacks of this method: first, the painful application of the device elicited a pressure sensation, sometimes with bruising, and secondly, no rise in pain threshold was seen with ingestion of aspirin. Armstrong, et al. (9) formed a blister on the skin by applying cantharidin. The method is useful for detecting algiesic activities of chemicals and tissue fluid components, but has not been applied as an analgesic testing method since it premises tissue damage on the skin. Goetzl, et al. (17) used electrical stimulation to the teeth to produce pain. Thereafter an electrical method involving stimulation of the earlobe was devised by Siker, et al. (15), but has been little used since electrical stimulus produces an electrical sensation. Benjamin (23) put a sphygmomanometer cuff around the subject’s arm to occlude the blood flow and made the subject tap with his index finger. The technique is called the ischemic pain method and is considered to be a model for determination of a deep pain. We found that ultrasonic stimulation can be applied both to laboratory animals (30) and humans. Character of the pain produced is a sharp pricking type, without thermal, electrical or mechanical sensations.

The analgesic activity of codeine phosphate has been detected in experimental analgesic testing methods in humans using a dose of 15 mg i.m. by the radiant heat method (3), 64 mg s.c. by the mechanical method using stimulation to tooth pulp (18). Using the ultrasonic method we demonstrated the prominent threshold-raising effect of codeine with a dose of 20 mg p.o. The analgesic activity of aspirin was detected in a dose of 1.8 g p.o. by the radiant heat method (2), 1.8 g p.o. by the electrical method using stimulation to tooth pulp (18), 0.3 g p.o. by the mechanical method by Burn (21) and 0.6 g p.o. by the ischemic pain method (28). We have demonstrated with our method a threshold-raising effect of aspirin in doses of 0.5, 1.0 and 1.5 g p.o. Experimental appraisal of antipyretic analgesics other than aspirin in humans has not been reported. Aminopyrine, 100 mg p.o., and mefenamic acid, 500 mg p.o., (each in the usual dose used clinically) were found to be equipotential to aspirin, 1.0 g p.o. when the ultrasonic method was used. Thus this method is useful to evaluate both strong and mild analgesics.

It is most necessary that we know the species differences regarding analgesic effects of drugs between humans and laboratory animals, yet there is apparently no fundamental data on the subject. We have applied the same stimulation to man and mice, and found that humans appear to be more than 25 fold more sensitive to the analgesics used (parti-
TABLE 1. Comparison of ultrasonic method with Hardy's method

|                        | Ultrasonic method | Hardy's method (1-4) |
|------------------------|-------------------|----------------------|
| Noxious stimulus       | ultrasonic        | thermal              |
| Sensation              | pain              | pain and heat        |
| Character of the pain  | a prick type of pain | a prick type of pain |
| Site of stimulation    | fingers           | forehead             |
| Injury to the tissue   | none              | sometimes bulla      |
| Operating procedure    | simple            | more complicated     |
| Mild analgesics        | can be detected   | can be detected (?)  |
| Application            | humans and animals| humans and animals   |
| Necessity for selection of subjects | little            | more                 |

ularly to codeine). Codeine, 10 mg/kg p.o., and aminopyrine, 50 mg/kg p.o., in mice used in our work were lower doses than the ED50 of codeine (97 mg/kg p.o.) reported by Kissel, et al. (31) who used the tail-pinching method, and the ED50 of aminopyrine (98 mg/kg p.o.) reported by Siegmund, et al. (32) who used the phenylquinone writhing method, respectively. Aspirin, 500 mg/kg p.o., was a higher dose than the ED50 of aspirin (165 mg/kg p.o.) reported by Siegmund, et al. (32).

Finally, our method is compared with Hardy's radiant heat method (Table 1). Hardy's method has at least two serious drawbacks: first, both thermal and painful sensations are involved, and secondly, twice the intense stimulus required to induce pain sometimes makes a bulla on the forehead. On the other hand, ultrasonic stimulation causes a genuine pain sensation, and produces no tissue damage. The operating procedure is simple and many subjects can be tested within a given time. Use of the ultrasonic method also enables detection of small changes in pain sensitivity because the end point of the response is clear. The ultrasonic method we have devised is considered to be useful as an analgesic test in phase I of clinical evaluation of drugs.

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