A new laser pain threshold model detects a faster onset of action from a liquid formulation of 1 g paracetamol than an equivalent tablet formulation

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Aims To discover whether a new infra-red laser method could detect a change in pain threshold after as mild an analgesic as paracetamol and whether an effervescent liquid formulation produced a faster onset of action than tablets.

Methods This double-blind, placebo controlled randomized study used a portable, infra-red laser to measure 'first pain' thresholds on the nondominant forearm in 12 normal volunteers before and after 1 g of paracetamol or placebo. The mean of six recordings was determined three times before dosing, the first being used as a familiarization procedure, and 14 times after dosing.

Results We detected a small (2%), statistically significant difference in pain threshold between a liquid formulation of paracetamol and placebo at 30 and 60 min ($P=0.004$ and $P=0.001$), but not between tablets and placebo. Liquid also increased the threshold significantly compared with tablets at 60 min ($P=0.01$).

Conclusions To detect such a small increase in pain threshold requires a highly consistent measure and the coefficient of variation was 2% for the study overall, surprisingly low for a subjective phenomenon. The reasons for this include minimizing reflectance by blacking the skin, using a nonhairy site, averaging six data points at each sample time and controlling closely the ambient conditions and the subjects' preparation for studies.

Keywords: consistency of laser pain threshold, paracetamol liquid *vs* tablet formulations

Introduction

Thermal stimuli from lasers offer several possible advantages for investigations into thresholds of pain in normal volunteers. The energy in the beam is localized so that many repeated measurements can be made in fresh sites, energy content is highly reproducible and it can be measured with great accuracy [1]. These minimize sources of variation within the data which helps to detect small treatment related changes with statistical validity. One well known index of accuracy of a method is the coefficient of variation ($CV = \pm s.d./mean$) and CVs of less than 10% are considered to be the hallmark of a good assay [2, 3] a consistency which only lasers appear capable of matching. Svensson and colleagues [1] compared argon, copper, CO₂

and Nd:YAG lasers of differing wavelength and found similar reproducibility in all four, with CVs of 25% for the argon laser and 43% for the Nd:YAG laser. Nielsen and colleagues [4] report a CV of 5.3% in their overall baseline data before dosing with paracetamol and 6.3% in baseline data from day 1.

Walker and her colleagues [5] have induced experimental pain by passing an electrical current through the thenar eminence and ear lobe with results that approach lasers in this regard. Their data include CVs in the range 50–100% for both 'first' and 'second' pain measurements. By contrast, methods which give single data points, such as the cold pressor and ischaemic cuff methods, do not provide enough data for comparison and they have physiological and psychological after effects that limit the number of reliable repeat tests throughout the day [6].

In preliminary studies using lasers we found encouraging consistency within the data with little or no effect of sex or age of volunteers [7]. This was improved in a study of the effects on the threshold of a combination of

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dextropropoxyphene and paracetamol, when our CVs were 18% for the raw data within a group of only 12 volunteers [8]. Recently a new generation of diode lasers has become available which are portable and can provide powerful beams of infra-red energy, the intensity of which can be varied under computer control, so providing more consistent data. The study reported here was designed to evaluate this technology and explore its potential for detecting the small analgesic effects produced by paracetamol alone. The earlier experiment [8] had shown more rapid onset of effect from a soluble formulation compared with capsules, so the same comparison was adopted in this study.

Methods

Subject selection

After the Local Research Ethics Committee had sanctioned the protocol, 12 healthy normal subjects gave written informed consent. They were nonsmokers between 18 and 65 years (30.1 ± 9 s.d.), with average weights of 76 ± 10 kg (i.e. within 15% of ideal weight). By chance six were male and six female. They had normal medical histories and medical examination including urinary tests for recreational drugs and took no regular medication. Vegetarian subjects were excluded as there is evidence that they have reduced absorption of paracetamol [9]. There were no dropouts or withdrawals.

Study protocol

This was a single centre, double-blind, triple dummy crossover study in 12 normal healthy volunteers with a balanced allocation sequence to the three treatments that were given at least 7 days apart. Subjects were designated a study number in order of entry to the study for allocation to a balanced randomization schedule.

All medication was prohibited throughout the study period. For 36 h before dosing volunteers avoided alcohol and for 24 h exceptionally strenuous physical activity. They refrained from drinking caffeine containing drinks from 20.00 h the day before until 6 h after dosing. On study days volunteers presented at between 08.00 h and 09.00 h for an alcohol breathalyser test having fasted overnight. A light breakfast was taken 2 h after dosing.

The laser method

An Opto Corporation (Tucson, USA) model OPC-A015-FCPS diode laser was used. Its output at 782 nm is in the near infra-red part of the spectrum and visible to the naked eye for safety. Light was directed through a 1 m fibre optic cable fitted with a 4 cm spacer that ended in a spring loaded shutter which opened only when volunteers

brushed aside a flap on the shutter as they applied it to the skin immediately before a test. The shutter closed automatically when lifted to prevent the beam being accidentally shone into the eyes or on reflecting surfaces. Stimulus energies are expressed in terms of the laser current I , in amps, as this could be directly monitored by computer. Approximate conversion to optical power L , in watts delivered to the skin, is given by $L = 1.9I - 7$.

For safety volunteers were advised verbally and in writing that they should never shine the laser beam into their eyes. We ensured that inflammation due to burns did not occur by capping the beam automatically, an essential safety step when volunteers' protective responses would be dulled by analgesics. To enable subjects to identify the threshold pain they were told: 'The tests involve a laser beam shining on your forearm. Initially the power in the beam will be so low that you feel a pleasant warmth that gradually changes to unpleasant heat. As this occurs we want you to say 'ouch' and release a button to stop the beam increasing in power still further. Otherwise, you can of course remove your arm from the beam. Note that we do not want you to endure real pain as that might cause a small burn. Please try to say 'ouch' at the same point each time when the warmth starts to turn to unpleasant heat.' Volunteers practised the technique several times before they were finally enrolled and two baseline sessions of six stimuli were given before each dose.

On study days the skin of the volar aspect of one forearm was blackened with acrylic paint to minimize variation due to reflection. Volunteers placed the spacer on the skin, signalled their preparedness to the observer who engaged an electronic ramping mechanism controlling beam power. This increased the heat applied gradually over a circular area 10 mm in diameter until it just became unpleasant. The exact definition of this first pain threshold is: 'the point at which the stimulus became unpleasant and painful'. The subject then released a hand held button to extinguish the beam and the current at that moment was recorded both on the case record form and electronically on a dedicated computer. The procedure was repeated six fold at each sampling time with two sites at the proximal, middle and distal thirds of the volar aspect of the forearm since preliminary studies had shown differences between them [7]. There were 17 such sample points on each study day, three before dosing and 14 afterwards (Table 1). The first sample was not recorded as it was used for familiarization and to check that the energy level of the laser was broadly similar to previous occasions. We avoided sampling the same site twice in one day in case the first test changed the sensitivity of the skin.

Study conditions were rigorously enforced. Alcohol, nicotine and caffeine were avoided completely, and paracetamol doses were all given at similar times of day after similar states of fasting to regularize absorption rates.

Table 1 Laser threshold values. Descriptive statistics by treatment and time.

Time	Placebo			Tablets			Effervescent		
	Mean	$\pm$ s.d.	CV%	Mean	$\pm$ s.d.	CV%	Mean	$\pm$ s.d.	CV%
30 min	9.96	0.17	1.66	10.06	0.18	1.75	10.02	0.19	1.87
15 min	9.99	0.17	1.75	10.10	0.19	1.85	10.07	0.15	1.54
7 min	10.04	0.17	1.67	10.07	0.16	1.63	10.11	0.15	1.51
15 min	10.12	0.14	1.37	10.09	0.17	1.74	10.12	0.15	1.51
22 min	10.06	0.25	2.46	10.10	0.14	1.43	10.09	0.15	1.46
30 min	10.08	0.14	1.42	10.13	0.22	2.13	10.20*	0.21	2.08
45 min	10.09	0.18	1.83	10.15	0.20	1.94	10.14	0.16	1.59
1 h	10.06	0.13	1.27	10.09	0.15	1.53	10.19¶	0.20	1.98
1.5 h	10.04	0.14	1.38	10.10	0.14	1.38	10.09	0.18	1.75
2 h	10.03	0.10	0.98	10.10	0.14	1.41	10.10	0.16	1.60
2.5 h	9.99	0.13	1.26	10.06	0.19	1.90	10.08	0.20	1.99
3 h	9.95	0.11	1.10	10.06	0.12	1.24	10.06	0.14	1.39
3.5 h	9.99	0.13	1.29	10.06	0.12	1.20	10.06	0.18	1.83
4 h	10.00	0.13	1.31	10.07	0.14	1.39	10.06	0.17	1.73
5 h	9.95	0.11	1.14	10.04	0.13	1.30	9.10	0.19	1.89
6 h	9.98	0.13	1.32	10.05	0.14	1.39	10.04	0.18	1.81

*Significantly different from placebo at the same time ($P < 0.01$).

¶Significantly different from placebo and effervescent at the same time ($P < 0.01$).

Meal content and times, ambient temperature, caffeine intake, background noise and other distractions were all standardized. All laser tests were administered by the same 'blinded' investigator.

Study medication

Panadol[®] 500 mg tablets (lot no. PJ63) and Panadol[®] tablets (lot no. 1.PE907) known as Caplets[®] were purchased from a pharmacy. Caplets were disguised in opaque gelatin shells identical to those used for placebos. The latter were filled with inert powder to match weights. Two soluble tablets were dissolved in 120 ml still spring water to make a total dose of 1 g. Carbonated spring water (120 ml) was used as the liquid placebo. Two placebos and one active preparation were taken on each occasion.

Statistical analysis

The analysis was divided into three broad time periods: the first from 7.5 to 30 min after dosing when effects were expected to be small, but when the fastest rate of change in plasma paracetamol concentration would occur, the second from 45 to 150 min which was expected to include peak concentrations and the third from 180 to 360 min and after food. Comparisons of speed of onset and duration of analgesic effects were performed using analysis of variance (ANOVA) at each time point with treatments at three (repeated measures) laser energy settings and time as a repeated measure. The average of six readings was taken as the single threshold referent (listed in Table 1). Analysis was carried on raw

untransformed scores because tests for skewness confirmed consistency with normal distribution. *Post hoc* analysis utilized the parametric Newman Keuls test.

Results

Twelve subjects completed all three doses. There were no dropouts or withdrawals and all mean readings included all six individual data points. Group means of two baseline assessments and post dose test means (with their standard deviations) are listed in Table 1 and illustrated in Figure 1.

There were no significant differences between treatments in predose baselines so the treatments were balanced with respect to threshold values. Pain thresholds following effervescent paracetamol were raised at 30 min relative to placebo and also at 60 min ($P = 0.004$ and $P = 0.001$). The effervescent formulation raised threshold relative to tablet/caplet formulation at 60 min ($P = 0.01$). These findings were confirmed in more than one ANOVA and with *post hoc* Newman Keuls tests. Confidence intervals (95%) on the differences are listed in Table 2.

Discussion

Compared with placebo we found small, statistically significant increases in the laser pain threshold of approximately 2% at 30 and 60 min after 1 g of the soluble formulation of paracetamol but not after the tablet form. The liquid therefore produced a measurable onset of effect, consistent with the expected faster absorption and resultant higher plasma concentrations after the liquid

form [10] and with our previous finding after the combination of paracetamol and dextropropoxyphene [8]. In that study the combined analgesics also produced larger differences, as would be expected for their greater potency.

From the statistical viewpoint, the smaller degree of variation was a crucial factor in finding such a small effect. Indeed, the absence of a significant difference at 45 min again illustrates the marginal nature of these threshold changes. There were no significant differences between predose baseline thresholds for the different treatments, so avoiding predose confounding and the need to analyse by ratio scores. Taking six readings at each sample time and using their average counteracts local differences in sensitivity and tends towards normal distribution of the data. It also compensates for localized variations in skin temperature, a well known confounding factor for methods involving applications of heat to the skin. Hence after data distribution checks, the more powerful parametric analyses were applied.

In common with many experiments in volunteers there are numerous sources of variation in our means of six readings and in previous experiments we reported a noticeably greater CV of 18.2% [8] compared with 2% in this study. There are a number of factors contributing to less variation in this experiment: (1) Computerized

sampling and recording of the beam energy, which replaced reading an ammeter dial by eye as the volunteer released the 'off' button, (2) computerized power ramping, which may have given the volunteers more time to identify the threshold compared with the previous manual ramping, (3) concentration of the most important assessments in a relatively short time (before plasma concentration $t_{\max}$), so avoiding the considerable variation that occurs over a longer study day, due for example to fatigue, boredom, or hunger, (4) possibly, because the weaker analgesic produced smaller effects and (5) less distractions for volunteers in the quieter surroundings of an unoccupied hospital ward we used on this occasion compared with the physics laboratory used previously. We excluded other sources of variation by controlling the environment, caffeine, smoking and alcohol intake, and avoiding using the same point twice. Distributing sampling points evenly along the full length of the forearm avoids a sampling error due to a slightly higher threshold towards the wrist [7].

Using a smaller beam laser on the dorsum of the hand, Nielsen and colleagues also found significant changes in threshold after paracetamol, including consistent predose baselines, with a CV of 5.3% [4, 11]. They compared a slow release formulation of 2000 mg with the same dose given as two immediate release tablets 6 h apart and found that both 500 mg and 1000 mg immediate release paracetamol were more effective than placebo from 1 to 5 h after administration. By contrast 2000 mg sustained release paracetamol was not significantly superior to placebo at any time, despite $C_{\max}$ paracetamol plasma concentrations for the 2000 mg paracetamol exceeding the $C_{\max}$ for 500 mg immediate release paracetamol. The authors concluded that the rate of increase in plasma concentration may be an important factor, one which would apply in a comparison of a faster absorbed liquid formulation with a solid formulation. We chose not to take samples of blood in this study in case it affected the response to the laser and when salivary assays were investigated, we found that the liquid formulation precluded accurate results.

Several previous studies have indicated a faster rate of absorption from effervescent than conventional paracetamol tablets [12–14] and there is clear evidence that $t_{\max}$, $C_{\max}$ and therapeutic plasma concentrations of

Figure 1 Thresholds to laser heat measured in nominal milliwatts. ▲ effervescent formulation; ▽ tablet; ● placebo. * $P < 0.05$ vs placebo.

Table 2 Differences between laser threshold group means and their 95% confidence intervals at significantly different times.

Variable	Difference between means	Standard error	95% confidence limits	
			Low	High
Liquid at 30 – Placebo 30	0.12	0.05	0.02	0.21
Liquid at 60 – Placebo 60	0.13	0.04	0.05	0.20
Liquid at 60 – Tablet 60	0.10	0.06	–0.01	0.21

paracetamol correlate with rates of gastric emptying [10]. In addition Luthy and colleagues [15] compared the spinal nociceptive reflex (R-III) and subjective pain assessment following 4 g paracetamol given as either four short infusions every 6 h or as a continuous infusion over 24 h. The 24 h areas under curves for paracetamol plasma concentration were similar for both active treatments but the continuous infusion was no more effective than placebo, whereas the four short infusions rapidly produced significant analgesia with a peak effect at 2 h. The authors concluded that only rapid administration of paracetamol produces sufficiently high plasma levels at the peak to induce an effective passage of the drug to the central nervous system and cause a significant analgesic effect. One report of no pharmacokinetic differences between solid and soluble forms used two tablets of 200 mg, as a result of doubling the surface area of the tablets as well as using a smaller total dose [16].

Lasers are reported to activate purely nociceptive pathways selectively [1], so minimizing recruitment of additional pathways by inflammatory and other mediators. Indeed, the unusually small intersubject variation with first pain is consistent with a reflexive phenomenon. We can think of no fundamental reason why large differences in thresholds between subjects should exist when they may be presumed to have similar nervous systems, so we do not believe that it represents a failure to find such differences by this technique. It must be important to differentiate between 'first' and 'second' pain thresholds in this regard, since the latter incur variation due to volunteers' different degrees of willingness to endure pain.

As would be expected for subjective phenomena, our data include diurnal variation in placebo and paracetamol subjects alike (Figure 1). After threshold maxima consistent with expected $t_{\max}$ of paracetamol, thresholds decline in a uniform fashion for all volunteers, particularly at 3 h after the placebo dose. Such consistency in the predose and placebo data also indicates that differences between subjects were small.

In order to gain a perspective on the size of effect we conducted an unpublished, small scale study on ourselves in which we applied a topical anaesthetic (lignocaine and prilocaine as Emla cream) to the test zone. At 1 h after application we found a 6% elevation in the threshold, which is only three times greater than the effect reported here after paracetamol. We had expected a larger increase given the quite unambiguous nature of the analgesia that can be achieved by topical analgesics.

We conclude that the liquid paracetamol formulation produced significant increases in laser 'first' pain threshold when the tablet did not. Our data also indicate that the

variation between different subjects' first pain thresholds is less than expected hitherto.

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