Clinical response to non-steroidal anti-inflammatory drugs in urate-crystal induced inflammation: a simultaneous study of intersubject and intrasubject variability

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- 1 It is well known that an individual subject often responds preferentially to a particular nonsteroidal anti-inflammatory drug (NSAID) and clinical response to these drugs is characterised by considerable variability between individuals. Variability in response has often been attributed to the episodic nature of musculoskeletal disease. Few studies have studied intrasubject variability in response to these drugs using a multiple crossover design. A major difficulty has been the lack of objective, validated measures of inflammation sensitive to NSAIDs. The primary aim of the present study was to test the utility of urate-crystal induced inflammation as a tool to predict NSAID response in humans.
- 2 An inflammatory reaction was established in twenty-five healthy subjects with intradermal injection of urate crystals on four separate occasions separated by 1 week. Each subject was randomly assigned to receive either ibuprofen on two of these occasions (800 mg four times over 36 h) or matched placebo on the other two occasions using a double-blind, cross-over design. Decrease in the area under the wheal size-time curve was used to indicate anti-inflammatory response.
- 3 Peak inflammatory response was observed at about 32 h and had dissipated by 56 h post-urate injection. The logarithmic mean wheal area was significantly lower after ibuprofen (mean $\pm$ s.e. mean; 6.74 ± 0.09) compared with placebo (6.96 ± 0.07 mm h); a difference of 20% (95% confidence interval for difference: 1 to 35%; $P < 0.05$).
- 4 There was marked intra- and intersubject variability in response to ibuprofen over the four treatment periods. Intrasubject variability was less for placebo compared with ibuprofen and intersubject variability was approximately 50% for both treatments.
- 5 Despite the use of a 'stable' inflammatory model significant variability in response to NSAIDs was noted. Thus, the episodic nature of the clinical inflammatory disease does not necessarily account for the large variability in response to NSAIDs. Future studies require a consideration of both intra- and intersubject variability. The present model could be a valuable tool to determine factors that affect response to NSAIDs.

Keywords non-steroidal anti-inflammatory drugs ibuprofen
crystal-induced inflammation pharmacodynamic variability

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed drugs but there are few studies that have assessed intrasubject variability in response to these drugs [1]. The NSAIDs act not only as analgesics but also they have anti-inflammatory actions. When considering variability in response, there is the problem of the episodic nature of many inflammatory diseases. Furthermore, in rheumatological conditions no single measure of response accurately reflects outcome and rheumatologists rely on a battery of clinical tests including pain assessments and laboratory tests.

Individuals respond differently to non-steroidal anti-inflammatory drugs (NSAIDs) such that subjects have been classified as either 'responders' or 'non-responders' to a particular treatment [2–4]. Failure to obtain relief with one drug usually leads to a rotation through a series of drugs until a satisfactory response is obtained [5]. Significant between-subject variation has been noted in several studies [4, 5, 6–11]. The basis for this is unknown but it has been suggested that this variability could be either pharmacokinetic and/or pharmacodynamic in origin. Differences in NSAID plasma pharmacokinetics between classified 'responders' and 'non-responders' have never been demonstrated although response categorization was crude in these studies [12–14]. We have developed a 'skin-blister' technique that more closely approximates tissue and joint 'effusions' with respect to drug concentration-time profiles than do plasma concentrations [15] and an experimental pain model that can distinguish the analgesic effects of NSAIDs from placebo [16]. A simple experimental method to measure the anti-inflammatory response to NSAIDs would be useful in many respects: e.g. to predict 'non-responders' before therapy is initiated to prevent the use of treatments of doubtful efficacy; research into determinants of response; utilisation of normal volunteers for study of anti-inflammatory drugs; differentiation of drug response from the variation characteristic of human inflammatory disease.

Although a wide variety of substances for producing experimental inflammation in human subjects has been investigated, including histamine, capsaicin, platelet activating factor and bradykinin, it has been difficult to demonstrate the efficacy of NSAIDs in these models [17–19]. However, experimental crystal-induced inflammation has been studied in both animals [20] and humans [21] and the acute inflammatory response can be reversed by treatment with anti-inflammatory drugs [22, 23]. Accumulating evidence implicates prostaglandins in the genesis of this type of inflammation which leads to vasodilation, increased blood flow and pain. For example, pretreatment with indomethacin and piroxicam suppressed inflammation induced by intradermal uric acid crystal injection in human subjects [23]. However, none of these previous investigations has tested the reproducibility of responses to drug and placebo. The aim of the present study was to test the value of urate-crystal induced inflammation as a tool to measure

NSAID response in humans. Ibuprofen was chosen for these studies since it has a rapid onset of effect and short duration of action.

Methods

Subjects

Twenty-eight healthy subjects (13 men and 15 women), 18 to 55 (mean $\pm$ s.d.; 24 ± 7) years of age and 65 ± 14 kg (mean $\pm$ s.d.) body weight were recruited from the Medical School and staff at St Vincents' Hospital. Subjects were free to withdraw from the study at any time. Informed and free consent was obtained from each subject in conformity with the guidelines on human experimentation laid down by St Vincent's Hospital and the University of New South Wales and subjects were compensated for the inconvenience involved. A full medical history and examination was performed and a blood sample was taken for routine haematology and biochemical screening. Similarly, a urine sample was examined. Significant abnormalities resulting from these tests led to exclusion of that subject. Other exclusion criteria were regular smoking, regular use of medication, abuse of alcohol, participation in a trial with an investigational drug in the 4 weeks preceding the study, surgery within the previous 3 months, and symptoms of a clinically important illness within 4 weeks of the study.

Inflammation model

Urate crystals were prepared by the method of Seegmiller and co-workers [24]. The crystal mass was gently ground and passed through a sieve of 150 μ m. The product was then examined by microscope to confirm the presence of classical needle like structures. The crystals were sterilized by heat at 180°C for 3 h to eliminate endotoxin contamination. A sterile suspension of the crystals was prepared in saline (25 mg ml⁻¹) immediately prior to injection.

An inflammatory reaction was established in the healthy volunteers with two intradermal injections of urate at different sites in the skin of the same forearm repeated on four separate occasions separated by 1 week. A volume of 0.2 ml was injected into the skin of the forearm at each site using a 25 gauge needle. The inflammatory response was quantitated by measuring the extent of the erythematous lesion in two diameters, longitudinal and across the line of the arm using a calibrated plastic ruler. Each experimental subject acted as their own control and was trained, by the same observer, to measure the dimension of the inflammatory reaction. The level of pain or discomfort upon gentle constant pressure exerted by the first digit was assessed using a six point scale from 0 to 5. Reproducibility in measurements was confirmed between observer and the subject for at least one wheal. Subjects were excluded from the study if they did not comprehend the measurement techniques. In a preliminary study, the time-course of

the lesions was as follows: increasing up to about 24 h; plateau 24–48 h; declining after 48 h and disappearing completely within about 1 week.

Ibuprofen dosage

Each subject received an NSAID (ibuprofen) on two occasions and a matched placebo on two other occasions using a randomized, double-blind, cross-over design (Table 1). Subjects were fasted for 8 h prior to drug administration and a single dose of ibuprofen (800 mg, Wyeth ACT 3®; 4 × 200 mg capsules) or matched placebo (4 capsules), was given 1 h prior to urate injection and thereafter at 12, 24 and 36 h. Measurements of inflammation and pain on pressure were made at 0, 8, 24, 32, 48 and 56 h after injection.

Data treatment

As the study utilised a randomised cross-over design, a generalised linear, (random effect) model was used to analyse the data. In this model the effects of sequence and subjects within sequences were considered random and the effects of treatment and visit were considered fixed. Interaction effects between treatment and visit; and treatment and sequence were analysed. Variability between wheals was found to be negligible thus the data for both wheals were averaged for a given week and individual subject. For each subject at each visit, the pooled mean wheal diameter and pain scores measured at each time were reduced to the area under the effect-time curve (AUEC_w and AUEC_p, integrated effect, respectively) using the linear trapezoidal algorithm to the last time point. The AUEC was found to be non-normally distributed using Shapiro & Wilks Test [25] therefore a logarithmic transformation of the data was used. Thus, logarithmic AUEC data were subjected to analysis of variance (ANOVA) [26]. Comparison between treatments and sequences were derived based on the ANOVA results with appropriate expected mean square error and degrees of freedom.

The magnitude of variability was assessed by using the variance component modelling approach which partitions the variance into four components: sequences, subjects, interaction between visit and sequence and between treatment and sequence. The variance components were estimated by restricted maximum likelihood method (REML) [27].

All computations were performed by means of the Statistical Analysis System [28]. Values of $P < 0.05$ were considered to be significant. Results are reported as mean $\pm$ s.e. mean and 95% confidence intervals where appropriate.

Results

There were no reports of any serious side effects by any of the subjects after ibuprofen (or placebo). Three subjects reported an aching arm after urate

injection (two subjects in week 1 and one subject in week 4 of treatment). One subject also reported a headache in week 4. All subjects that reported side effects were on placebo treatment at that time.

Twenty-eight subjects entered the study, of these three subjects were excluded due to failure to understand how to take measurements (one) and urate injection failure due to technical difficulties (two). Thus, data from 25 subjects (12 males and 13 females) were analysed. Of these 25 subjects, six patients did not complete one leg of the study due to technical difficulties ($n = 4$ for ibuprofen and $n = 2$ for placebo).

The variability between the pairs of wheals for both placebo and ibuprofen treatments was found to be negligible and non-significant, thus data for both wheals were pooled each week. The time-course of the inflammatory reaction in 25 subjects was very similar and reproducible after either placebo and ibuprofen on each occasion (Figure 1). The mean peak inflammatory response was observed by approximately 32 h but had not always returned to baseline at 56 h for both placebo and ibuprofen (Figure 1).

The overall response to either placebo or ibuprofen was independent of both the sequence (A to D) or visit number (1 to 4). However, there was marked inter-individual and intra-individual variability over the four treatment periods (Table 1, Figure 2). There were no significant differences in response between males and females. The logarithmic mean $\pm$ s.e. mean wheal area was significantly lower after ibuprofen (6.74 ± 0.09) compared with placebo (6.96 ± 0.07); a difference of 20% (95% confidence interval: 1% to 35%; $P = 0.049$) (Figure 3). However, considerable inter- and intrasubject variability, with the former being higher than the latter, was observed (Table 2). Subjects were classified as 'responders' if there was $> 10\%$ decrease in wheal area after ibuprofen compared with placebo. Using this criteria, approximately half of the subjects appeared to be 'non-responders' ($\leq 10\%$) and the other half as 'responders' ($> 10\%$) to ibuprofen. Of the thirteen subjects who experienced a favourable effect for ibuprofen (ibuprofen-placebo), the effect was around 48% in three subjects (range: 43–50%), ~23% in four subjects (range: 20–30%) and ~13% in the remaining six subjects (range: 11–16%). Eight subjects experienced no effect from ibuprofen ($< 10\%$) and four subjects showed a placebo response (~36%; range: 16–46%). Furthermore, the effects were highly variable from week to week, such that response categorisation was not necessarily sustained. In 10 subjects the response was the same on both occasions. The other 11 subjects moved from one category to another between one ibuprofen treatment and the next. Thus, a positive but only weak relationship was observed between the two treatments of either placebo ($r = 0.52$, $P < 0.01$) or ibuprofen ($r = 0.44$, $P < 0.04$). Interestingly, intrasubject variability was less for placebo (mean AUC_w ratio of treatment 1/treatment 2 = 1.00 ± 0.1) compared with active drug (mean AUC_w ratio of treatment 1/treatment 2 = 1.44 ± 0.15). The trend for the response to ibuprofen to be more pronounced upon second exposure to this

Figure 1 The time course of inflammatory response (wheal diameter) after two oral treatments with placebo (○, □) and two oral treatments with 800 mg (R,S)-ibuprofen (●, ■) in three individual subjects, one subject in whom ibuprofen reduced both the size and the time course of the inflammatory response (a), one subject where both treatments were equal (b) and one subject in whom placebo had better efficacy than ibuprofen (c).

drug was only significant for sequence C (Table 2).

The variance resulting from sequence and its interaction with treatment was negligible (Table 3). There was a small amount of variance resulting from difference between treatments and visits interaction but it was half that resulting between subjects. The major source of variance was due to random error (Table 3).

Reports of pain upon pressure, as assessed by a numerical scale were also variable between subjects, however, the majority of subjects reported pain scores from 0 to 3 after applying pressure to the injection site. The AUEC_p was not different between the two treatment periods for both ibuprofen and placebo respectively but pain reports after ibuprofen (pooled AUEC_p: 89 ± 6) were significantly lower than those after placebo (pooled AUEC_p: 105 ± 6, $P < 0.01$; 95% confidence interval for the difference = -25.4, -4.3).

Discussion

In considering variability of response to NSAIDs there are three components namely, intersubject and intrasubject variability and placebo response. There have been a number of studies that have addressed the issue of intersubject variability in response to NSAIDs and there is evidence to suggest the existence of distinct 'responders' and 'nonresponders' to a range of non-steroidal anti-inflammatory drugs [2, 3, 4, 5, 11, 12]. In the clinical setting intrasubject variability must also be taken into account. Additionally, the placebo response is involved and also needs to be considered [29]. Only one previous study has exposed subjects to the same NSAID more than once to address the issue of intrasubject variability [30]. In the present study, we attempted to investigate these issues simultaneously. We used a 'stable' experimental model of inflammation, in subjects exposed to placebo and ibuprofen twice using either a cross-over design or a consecutive design, over a period of 4 weeks. This design enabled us to assess the sources of variance more precisely.

We found that ibuprofen had a statistically significantly favourable effect in the reduction of inflammation, compared with placebo and about half of the subjects appeared to respond to ibuprofen. This finding agrees with a previous study of rheumatoid

Table 1 Mean anti-inflammatory response to ibuprofen according to sequence

Sequence	Order of treatment ^a	Ibuprofen (I) ^b	Placebo (P)	Difference (I-P)	P value	95% CI
A	I-I-P-P	6.67 ± 0.20	6.84 ± 0.14	-0.17 ± 0.17	0.45	0.17, -0.51
B	I-P-I-P	6.70 ± 0.13	7.11 ± 0.16	-0.41 ± 0.15	0.03	-0.11, -0.71
C	P-I-P-I	6.96 ± 0.09	7.17 ± 0.06	-0.21 ± 0.10	0.05	-0.01, -0.41
D	P-P-I-I	7.03 ± 0.10	6.78 ± 0.18	0.25 ± 0.15	0.23	0.05, -0.55

^aTreatment was assigned according to visit order. The pooled mean for the two visits with placebo and ibuprofen are presented. ^bLogarithm of area under the response-time data. ^cMixed effect ANOVA indicates treatment effect was statistically significant ($P = 0.05$); sequence effect ($P = 0.19$); subjects ($P = 0.05$); visits ($P = 0.33$); interaction between visit and treatment ($P = 0.25$) and interaction between sequence and treatment ($P = 0.08$).

Figure 2 Inflammatory response ($AUEC_w$, mm h) following two oral exposures with either a), placebo or b), (R,S)-ibuprofen, 3200 mg in each individual subject. Data are presented for subjects receiving either sequence A (I-I-P-P, ○), sequence B (I-P-I-P, ●), sequence C (P-I-P-I, □) or sequence D (P-P-I-I, ■).

Figure 3 Mean inflammatory response ($AUEC_w$, mm h) following either placebo (left) or 3200 mg (R,S)-ibuprofen (right). Pooled data from both exposures to either placebo or ibuprofen are presented for each individual subject. Data are presented for subjects receiving either sequence A (I-I-P-P, ○), sequence B (I-P-I-P, ●), sequence C (P-I-P-I, □) or sequence D (P-P-I-I, ■).

arthritis patients where a similar rate of response was found [31]. Our results also confirm that urate crystal induced inflammation in the skin is a safe, simple and reproducible test of inflammation in healthy human subjects [23]. The amount of effect, namely approximately 20% reduction, was small and suggests that the urate test is not particularly sensitive. Our observation that cyclo-oxygenase inhibitors, such as ibuprofen, can modify the acute wheal response supports the concept that prostaglandins mediate the vaso-

dilation effect [23]. In contrast, inflammatory models that do not involve prostaglandins such as those induced by synthetic platelet activating factor were not significantly affected by concomitant administration of indomethacin [19]. Our observations of high reproducibility in inflammatory response after placebo as manifest in the time to reach peak effect were in accordance with findings from other groups [21, 23]. We found no significant difference in response between males and females.

Despite an apparently 'stable' experimental model for inflammation in this study, we have observed a large intrasubject as well as intersubject variability during the four weeks of treatment, regardless of the order of treatment presentation, that is, consecutive ibuprofens or ibuprofen-placebo or placebo-ibuprofen sequences. Similar results have also been found in rheumatoid patients receiving flurbiprofen twice consecutively [30]. These data raise serious doubt concerning the concept of 'responder' or 'nonresponder' to a particular NSAID.

The predominant source of the variance in this study was due to random error (78%), followed by that due to 'between' and 'within' subjects variance (13% and 7%, respectively) whereas variance due to treatment sequences was negligible. The random error variance was surprisingly large and reflects factors that we have not considered in this study. Previous anecdotal reports have suggested that variability in response to NSAIDs may be an artifact related to the episodic nature of the disease process itself and to the variability and subjectivity of some of the measures

Table 2 Intrasubject variability: mean $\pm$ s.e. mean of logarithmic area under the curve of wheal size by sequence

Sequence	Placebo 1	Placebo 2	P value ^a	Ibuprofen 1	Ibuprofen 2	P value ^a
A	6.73 $\pm$ 0.09	6.90 $\pm$ 0.20	0.29	6.58 $\pm$ 0.46	6.61 $\pm$ 0.25	0.83
B	7.11 $\pm$ 0.15	7.22 $\pm$ 0.15	0.92	6.09 $\pm$ 0.52	6.84 $\pm$ 0.09	0.52
C	7.10 $\pm$ 0.11	7.20 $\pm$ 0.07	0.50	7.14 $\pm$ 0.10	6.71 $\pm$ 0.13	0.02*
D	6.67 $\pm$ 0.26	6.76 $\pm$ 0.18	0.99	7.08 $\pm$ 0.11	6.94 $\pm$ 0.14	0.26

^aP value of simple paired *t*-test for intrasubject difference between two administrations of either placebo or ibuprofen.

Table 3 Estimate of variance components using restricted maximum likelihood method

Effects	Estimated variance (%)
Sequences	0.00
Subjects	12.9
Treatments $\times$ sequences	2.0
Treatments $\times$ visits	7.2
Random errors	77.9

of pain and inflammation. For example, a subject apparently may not respond to one NSAID during an exacerbation of the disease but to another NSAID during a less severe phase of the disease [31]. The present experimental model should control for underlying disease variability and utilises an objective measure of inflammation but despite this significant response variability remains. Other pharmacokinetic/pharmacodynamic factors such as the interactions between genetic, pathophysiological, and environmental factors have been known to have effects on responses to other drugs and may also be involved [32, 33].

Interestingly, when ibuprofen was administered first (I-I-P-P and I-P-I-P), a more pronounced decrease in wheal diameter was observed. Furthermore, when both placebo exposures preceded ibuprofen, (P-P-I-I), a positive placebo response was observed. These findings support the concept of either patient expectancy [29] or a rise in endogenous opioids following placebo that may inhibit the release of pro-inflammatory neuropeptides leading to an inflammatory response [34] but both these hypotheses require further evaluation. Our results support those of Hashish and co-workers where a strong placebo effect was found not only on pain reduction but also swelling which is usually considered an expression of local tissue damage [34]. There was a trend for an increased effect on ibuprofen on the second exposure, but this was significant only for the subjects who received ibuprofen 2 weeks apart and preceded by placebo.

Analysis of variance revealed no significant carry-over effect between treatments ($P = 0.857$). An underlying assumption of this study is that ibuprofen acts reversibly. For NSAIDs, however, this may not necessarily be true. For example, the effects of aspirin or cyclo-oxygenase are irreversible and although its plasma half-life is only 1 to 2 h, its effect on platelet function persists for up to 1 week. Furthermore, the fact that the erythematous reaction was only partially inhibited by ibuprofen (20%) suggests the vascular changes induced by urate crystals are only partly due to prostaglandins via activation of cyclo-oxygenase. Our data provide indirect evidence suggesting that differences in biochemical determinants of inflam-

mation between subjects may explain variability in response to NSAIDs [11].

The lack of dose-response data in this experiment may be criticised. The aim of the present study was to investigate when a model of inflammation could be used as a tool to characterise variability in response to NSAIDs. As such, our experiment was designed to test the stability of response by exposing each subject to each treatment twice rather than a range of doses. It is also unlikely, that the small change in mean anti-inflammatory response could be attributed to inadequate doses of ibuprofen because 1600 mg/day was administered which has previously been shown to be effective in subjects with musculoskeletal diseases [35].

Urate-crystal-induced inflammation also produced touch-evoked pain probably due to the activation of low-threshold mechanoreceptors and this pain was reliably reduced by ibuprofen on both occasions. The present studies do not address the site of action of ibuprofen, i.e. peripheral vs central. It is assumed that the flare response is a peripheral event mediated by the release of vasoactive substances. However, NSAIDs can act via both central and peripheral mechanisms [16] and the relative importance of these sites of action in this model remains to be determined.

In conclusion, oral administration of ibuprofen 1 h prior to and concomitant with the development of inflammation had a modest effect on the time course of crystal-induced inflammation in healthy volunteers. Using a novel experimental design, we have found that although ibuprofen had favourable effects on wheal inflammation, only half of the subjects could be classified as 'responders'; but that 'responder' classification was not predictably sustained over 2 exposure periods to ibuprofen. Thus, considerable intersubject and intrasubject variability was observed. Formal statistical analysis revealed a large amount of variance attributable to random error. Taken together with the data of Cush and co-workers, differences in biochemical determinants of inflammation within and between subjects need to be explored to explain variable response status [11]. Our data indicate that examination of response to NSAIDs' requires consideration of not just intersubject and intrasubject variability, but also patient expectation or placebo response. We believe this model and the design methodology will be useful for further studies of variability of response to NSAID.

We would like to acknowledge the cooperation and support of Wyeth Pharmaceuticals, Australia for the supply of ibuprofen and placebo. We also wish to thank Alana K. Chandler and Suzanne I. Duffy for assisting with this work and Associate Professor John Carmody for valuable discussions. This work was supported by a grant from the National Health and Medical Research Council of Australia (900242, 927715).

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(Received 10 December 1993,
accepted 6 June 1994)