J. Pharm. Pharmacol. 1982, 34: 576–579
Received March 8, 1982

0022–3573/82/090576–04 $02.50/0

Effect of nabumetone (BRL 14777), a new anti-inflammatory drug, on human platelet reactivity ex vivo: comparison with naproxen

BARBARA NUNN* AND P. D. CHAMBERLAIN

Beecham Pharmaceuticals Research Division, Biosciences Research Centre, Great Burgh, Yew Tree Bottom Road, Epsom, Surrey, KT18 5XQ, U.K.

The effect of nabumetone (BRL 14777) on human platelet reactivity ex vivo was compared with that of naproxen at equitherapeutic doses in the same six subjects. Nabumetone had only a weak and equivocal effect on collagen-induced and second phase aggregation in response to adenosine diphosphate and adrenaline. After nabumetone, platelets fully aggregated in response to sodium arachidonate, though approximately twice as much was needed as on control occasions. Sodium arachidonate was unable to elicit a full aggregation response after naproxen. These results suggest that nabumetone may cause less interference with haemostasis than other non-steroidal anti-inflammatory drugs.

Nabumetone, (4-[6-methoxy-2-naphthyl]-butan-2-one) is a novel non-acidic compound which displays anti-inflammatory activity in a range of laboratory tests. It is particularly well tolerated by the gastrointestinal tract possibly because it is non-acidic as presented to the gut and is only a weak inhibitor of prostaglandin synthesis (Boyle et al 1982). Nabumetone is however extensively metabolized in man and laboratory animals, the major metabolite in plasma being 6-methoxy-2-naphthyl acetic acid (Langley et al, to be published). The acidic, non-steroidal anti-inflammatory drugs are all inhibitors of prostaglandin synthesis (Flower 1974) and this property appears to explain their common capacity to depress platelet aggregation (Zucker & Peterson 1970; Smith & Willis 1971; Hamberg et al 1974; McIntyre & Philp 1977). The effect on platelet function probably accounts at least in part for the prolongation of bleeding time that follows administration of drugs such as aspirin (Mielke et al 1969), indomethacin (Buchanan et al 1977) and naproxen (Nadell et al 1974). Such interference with haemostasis precludes their use in many patients, for example those taking anticoagulant therapy or those with coagulation disorders (Quick 1970), and hence a well-tolerated anti-inflammatory agent without this property should present a considerable safety advantage. It is in this context that the effect of nabumetone on human platelet reactivity ex vivo has been studied and compared with naproxen.

* Correspondence.

METHODS

Subjects

Subjects (4 male and 2 female, age range 21–35 years) were healthy and had received no medication, including the contraceptive pill, for at least ten days before the study. The aim of each experiment was explained to the subjects and informed consent obtained. All experiments were carried out under medical supervision. Alcohol was forbidden throughout each study and no smoking was allowed on each study day until after the blood sample had been drawn.

Study of nabumetone

Each subject was studied seven days before, one day before and seven days after beginning to take nabumetone, 1 g daily, which is an effective anti-inflammatory dose (Verbroggen et al; Famaye et al; Fostiropoulos et al; studies to be published). Although it is proposed to give nabumetone at night, the drug was taken with breakfast in the current study so that blood for platelet studies could conveniently be taken 4 h later when peak blood levels occur (to be published). On each study day, two or four subjects presented at 09.00 h having fasted from 23.00 h the previous night. They were given a standard breakfast of one piece of lightly buttered toast and one cup of coffee. No other food or fluid was permitted until 13.00 h when blood (18 ml) was drawn from the antecubital vein for platelet studies. The final dose of nabumetone was taken with the standard breakfast at the study unit and the final blood sample taken 4 h later. The presence of food

does not adversely affect the absorption of nabumetone (to be published).

Study of naproxen

Three weeks after the final dose of nabumetone, and with no other drugs being ingested in the intervening period, each subject presented for study following the protocol already described except blood samples were taken 2 h rather than 4 h after the standard breakfast. The first dose of naproxen (Naprosyn, Syntex) was taken the following morning and continued for the next seven days (250 mg twice daily). The final dose was taken with the standard breakfast at the study unit and blood drawn for platelet studies 2 h later. According to Runkel et al (1972), blood levels of naproxen are maximal at approximately 2 h in fasted subjects, and absorption is slightly retarded by food. Final blood samples were taken 3 days (2 subjects) or 4 days (4 subjects) later. Two subjects forgot to take the second of the thirteen doses of naproxen, but continued with the study. It was completed in 12 days.

Platelet studies

Preparation of platelet-rich plasma (PRP) from citrated blood, measurement of aggregation in response to adenosine 5'-diphosphate (ADP), adrenaline and collagen, release of [^{14}C]5-hydroxytryptamine (5-HT) and platelet adhesion to collagen were carried out as described in detail by Nunn & James (1980). In addition, responsiveness to sodium arachidonate was assessed. Arachidonic acid, Grade 1 (approx. 99% pure, Sigma Chemical Company, London) was dissolved in 100mM Na_2CO_3 to give a stock solution of 20mM sodium arachidonate. This was stored at −20 °C and thawed and diluted with 154mM NaCl as required. Log_{10} concentration-response curves were constructed for each aggregating agent and the concentration (EC50) giving a response of 50% maximum rate (ADP) and 50% maximum increase in light transmission (collagen and arachidonate) read by interpolation. Dose-ratios for each aggregating agent and each subject were then calculated by dividing the EC50 obtained after the drug by the EC50 obtained on the occasion immediately before the drug. In addition, all responses to each concentration of aggregating agent on corresponding occasions were averaged so that group concentration-response curves could be constructed. All results were analysed using the Mann-Whitney U test (Mann & Whitney 1947).

RESULTS

Effect on collagen-induced aggregation

Nabumetone reduced aggregation in response to collagen but the effect failed to reach statistical significance (see Fig. 1 and EC50 values in Table 1). Individual dose-ratios ranged from 1·0 to 2·4 (mean ± s.e. = 1·5 ± 0·2). This figure is similar to that which can be calculated from the two sets of control data (1·3 ± 0·2) and which represents the week to week variation. In contrast, naproxen had a highly significant ($P < 0·001$) inhibitory effect on collagen-induced aggregation (see EC50 values in Table 1). Individual dose-ratios ranged from 2·4 to 5·1 (mean ± s.e. = 4·0 ± 0·4). The group dose-response curve for collagen-induced aggregation after naproxen was well to the right of that after nabumetone (Fig. 1).

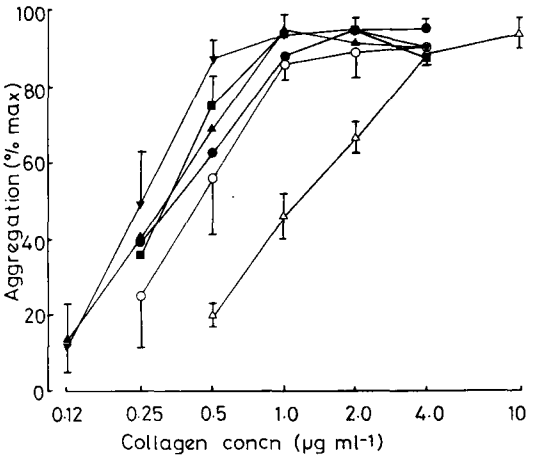

FIG. 1. Dose-response curves for collagen-induced aggregation of human platelets. Platelet-rich plasma was prepared from blood samples drawn from six subjects 7 days before (▼), 1 day before (▲) the first dose of nabumetone; 4 h after (○) the last of 7 doses (1 g daily) of nabumetone; 1 day before (■) the first dose of naproxen; 2 h after (△) and 3 or 4 days after (●) the last of 13 doses (250 mg twice daily) of naproxen. Some standard error bars are omitted for the sake of clarity.

The release of [^{14}C]5-HT associated with collagen-induced aggregation was depressed significantly by both drugs though naproxen had the greater effect (Fig. 2).

Effect on adhesion to collagen

Both drugs significantly reduced platelet adhesion to collagen (Table 1) and to a similar extent. However, naproxen had a greater effect than nabumetone on adhesion-induced release of [^{14}C]5-HT (Table 1).

Table 1. Effect of nabumetone and naproxen on human platelet reactivity ex vivo.

	Before nabumetone		4 h after 7th dose nabumetone	One day before naproxen	2 h after 13th dose naproxen	Three or four days after naproxen
	One week	One day				
Collagen EC50 (μg ml^{-1})	0·27 ± 0·04	0·37 ± 0·09	0·48 ± 0·09	0·33 ± 0·06	1·22 ± 0·15***	0·42 ± 0·11
ADP EC50 (μM)	0·63 ± 0·07	0·71 ± 0·05	0·76 ± 0·09	0·54 ± 0·05	0·49 ± 0·06	†
Na arachidonate EC50 (μM)	†	†	850 ± 146	368 ± 87	>2000***	383 ± 47
First phase aggregation in response to adrenaline (2 μM) (mm min^{-1})	82 ± 6	69 ± 6	75 ± 10	74 ± 4	69 ± 8	76 ± 5
Adhesion to collagen (% maximum ΔT)	39 ± 2	43 ± 2	31 ± 3**	47 ± 2	32 ± 2**	39 ± 6
Adhesion-induced release of [^{14}C]5-HT (%)	46 ± 4	50 ± 3	35 ± 5*	49 ± 5	18 ± 3**	44 ± 8

Figures are mean ± s.e. of values obtained from six subjects taking nabumetone (1 g daily) or naproxen (250 mg twice daily).
* $P < 0·013$, ** $P < 0·002$, *** $P < 0·001$ compared with previous control occasion.
† Not measured.

Effect on arachidonate-induced aggregation

Sodium arachidonate, at the maximum concentration tested (2 mM), was unable to induce full aggregation in any of the six PRP samples obtained 2 h after the final dose of naproxen (Table 1). In contrast, the post-nabumetone samples responded to arachidonate, but higher concentrations were required than on control occasions (see EC50 values, Table 1). Responses to arachidonate over the course of the study are typified in Fig. 3.

Effect on ADP- and adrenaline-induced aggregation

Neither drug had any effect on first phase aggregation in response to ADP or adrenaline (Table 1). Naproxen had a greater effect than nabumetone on second phase aggregation measured as release of [^{14}C]5-HT (Fig. 2).

DISCUSSION

The present study has shown that a new non-acidic, anti-inflammatory drug, nabumetone had significantly weaker inhibitory properties than naproxen on platelet reactivity ex vivo when the two drugs were given to the same six healthy subjects at equitherapeutic doses. Thus, although nabumetone is converted largely to an acidic metabolite in vivo (Langley et al, to be published) its effect on collagen-induced and second phase aggregation was weak and equivocal, whereas naproxen profoundly depressed both facets of platelet function.

The effect of naproxen on collagen-induced aggregation was similar to that reported in a previous study (Nunn & James 1980) using only a single dose (250 mg) of the drug. Hence repeated administration of naproxen does not appreciably alter its anti-aggregant activity. As would be expected of drugs in this class, neither naproxen nor nabumetone affected first phase aggregation.

It is still not clear whether the capacity of the acidic non-steroidal anti-inflammatory drugs to impair platelet responsiveness is entirely explicable in terms of inhibition of prostaglandin synthesis. However, the results of the present study, which used platelet responsiveness to arachidonate as a measure of the integrity of cyclo-oxygenase, are consistent with the two properties being causally related. Thus, naproxen virtually abolished responsiveness to arachi-

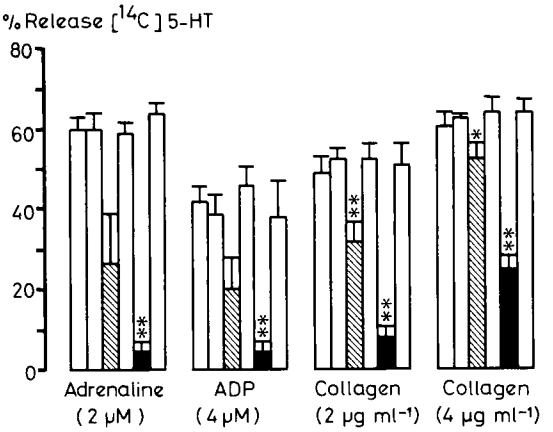

FIG. 2. Effect of nabumetone and naproxen on aggregation-associated release of [^{14}C]5-HT from human platelets. Blood samples were drawn from six subjects 4 h after the last of seven doses (1 g daily) of nabumetone (hatched) or 2 h after the last of 13 doses (250 mg twice daily) of naproxen (solid). Platelet-rich plasma was incubated with [^{14}C]5-HT and aliquots stirred for 4 min at 37 °C with the aggregating agent stated. Open columns show results obtained on control occasions. * $P < 0·008$, ** $P < 0·001$, compared with previous control occasion.

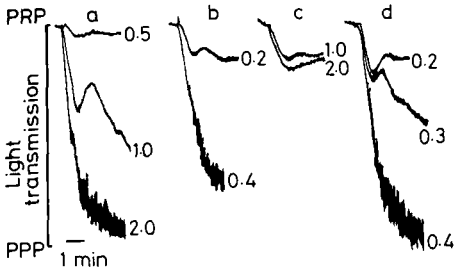

FIG. 3 Aggregation responses to sodium arachidonate in platelet-rich plasma (PRP) prepared from blood samples drawn from one subject (RH) 4 h after the last of 7 doses (1 g daily) of nabumetone (a); 1 day before the first dose of naproxen (b); 2 h after (c) and 3 days after (d) the last of 13 doses (250 mg twice daily) of naproxen. The figure at the end of each aggregation curve gives the concentration (mM) of sodium arachidonate stirred with aliquots of PRP at 37 °C.

donate and caused a four-fold depression of responsiveness to collagen whereas nabumetone only halved responsiveness to arachidonate and caused a weak, non-significant depression of collagen-induced aggregation.

Both drugs reduced adhesion to collagen and to a similar extent. This property can also be demonstrated for aspirin ex vivo (Nunn & Lindsay 1980) and could theoretically contribute to the mechanism by which aspirin and naproxen prolong bleeding time. If so, nabumetone might also be expected to interfere with haemostasis despite having only a weak effect on platelet aggregation. However, a separate study has shown that nabumetone, in contrast to naproxen, did not prolong bleeding time (von Schrader et al to be published). The effect on platelet adhesion to collagen seems therefore to have no physiological consequences. The reason for this may lie in the demonstration that aspirin inhibits platelet adhesion to collagen only in the absence of erythrocytes (Cazenave et al 1978).

In summary, the present study has shown that nabumetone, a new non-acidic, anti-inflammatory drug has only weak anti-platelet activity in human PRP ex vivo. These results are consistent with the lack of effect of nabumetone on haemostasis (von Schrader et al to be published).

Acknowledgement

We would like to thank Sister P. Warren for taking blood samples and helping to organize these studies.

REFERENCES

Boyle, E. A., Freeman, P. C., Mangan, F. R., Thomson, M. J. (1982) J. Pharm. Pharmacol. 34: 562–569

Buchanan, G. R., Martin, V., Levine, P. H., Scoon, K., Handin, R. I. (1977) Am. J. Clin. Pathol. 68: 355–359

Cazenave, J-P., Kinlough-Rathbone, R. L., Packham, M. A., Mustard, J. F. (1978) Thromb. Res. 13: 971–981

Flower, R. J. (1974) Pharmacol. Rev. 26: 33–67

Hamberg, M., Svensson, J., Wakabayashi, T., Samuelsson, B. (1974) Proc. Nat. Acad. Sci. USA 71: 345–349

McIntyre, B. A., Philp, B. (1977) Thromb. Res. 12: 67–77

Mann, H. B., Whitney, D. R. (1947) Ann. Math. Statist. 18: 52–60

Mielke, C. H., Kaneshiro, M. M., Maher, I. A., Weiner, J. M., Rapaport, S. I. (1969) Blood 34: 204–215

Nadell, J., Bruno, J., Varady, J., Segre, E. J. (1974) J. Clin. Pharmacol. 14: 176–182

Nunn, B., James, F. J. (1980) Br. J. Clin. Pharmacol. 9: 239–245

Nunn, B., Lindsay, R. (1980) Thromb. Res. 18: 807–813

Quick, A. J. (1970) Bleeding Problems in Clinical Medicine. Saunders, Philadelphia

Runkel, R. A., Kraft, K. S., Boost, G., Sevelius, H., Forchielli, E., Hill, R., Mogoun, R., Szakacs, J. B., Segre, E. (1972) Chem. Pharm. Bull. 20: 1457–1466

Smith, J. B., Willis, A. L. (1971) Nature New Biol. 231: 235–237

Zucker, M. B., Peterson, J. (1970) J. Lab. Clin. Med. 76: 66–75