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Comparison of the effects of nabumetone with indomethacin on rat gastric mucosal 6-keto-$PGF_{1\alpha}$ production and on bile salt-induced changes in gastric mucosal function

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The effect of nabumetone on rat gastric mucosal cyclooxygenase activity ex-vivo and in-vitro has been compared with that of indomethacin. Nabumetone was less potent and less active in inhibiting the production of gastric mucosal 6-keto-$PGF_{1\alpha}$ compared with indomethacin either ex-vivo or in-vitro. Anti-inflammatory doses of nabumetone failed to enhance bile salt-induced gastric erosion or mucosal permeability to dextran whereas indomethacin significantly enhanced gastric erosion and increased dextran permeability. These results suggest that nabumetone fails to promote gastric damage or increase permeability because of minimal effects on gastric mucosal cyclooxygenase.

Nabumetone, (4-(6-methoxy-2-naphthyl)-butan-2-one) was reported to be an effective anti-inflammatory agent in a variety of animal models and, unlike most non-steroidal anti-inflammatory agents (NSAIA), lacked gastric mucosal irritancy in the rat (Boyle et al 1982). It was further suggested that the lack of gastric damage was due, in part, to the non-acidic nature of nabumetone combined with low cyclooxygenase inhibitory activity as demonstrated in a bovine seminal vesicle (BSV) preparation. In the present study, the actions of nabumetone on rat gastric mucosal cyclooxygenase activity ex-vivo and in-vitro have been investigated and compared with results obtained with the potent cyclooxygenase inhibitor, indomethacin. A preliminary account of this work has been published (Melarange & Rashbrook 1984).

Bile reflux into the gastric lumen has been implicated in the pathogenesis of NSAIA-induced gastric damage and the presence of bile constituents, such as sodium taurocholate, has been shown to enhance damage due to topical or parenteral NSAIA (Semple & Russell 1975; Abtahi & Djahanguiri 1975; Whittle 1977, 1983). It was therefore desirable to know whether nabumetone, unlike indomethacin, lacked the ability to enhance bile salt-induced damage in the rat. To assess changes in gastric mucosal function, a rat gastric chamber preparation was employed where mucosal permeability was measured by the appearance of [^{3}H]dextran in the mucosal solution and morphological damage was estimated by observing the number and size of haemorrhagic erosions.

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MATERIALS AND METHODS

Materials

The 6-keto-$PGF_{1\alpha}$ radio-immunoassay kits were purchased from American Biomedical Technologies (ABT) West Berlin, FRG and the [^{3}H]dextran (3·7–18·5 GBq g^{-1}; 70 000 mol wt) from Amersham. Mannitol, dimethyl sulphoxide (DMSO), urethane and citric acid were purchased from BDH and Trizma base (Sigma) was prepared to give a Tris-HCl buffer (50 mM; pH 7·4). Sodium taurocholate (>98% purity) was obtained from Maybridge Chemical Company.

Estimation of rat gastric mucosal 6-keto $PGF_{1\alpha}$ production

The measurement of 6-keto-$PGF_{1\alpha}$, the stable breakdown product of PGI_2, from the rat gastric mucosa has been described previously (Melarange & Rashbrook 1986). Briefly, for ex-vivo studies, drugs were administered orally (in 0·5% methylcellulose) to fasted (18 h) male Wistar rats (150–200 g) which were then killed after 2 h. Following removal of their stomachs, mucosal sections were prepared by separating the overlying muscle by blistering (Forte et al 1975). This procedure involved inserting the tip of a fine needle (26 gauge) between the muscle and mucosa and injecting Tris buffer. The raised muscle layer was then carefully cut away. Uniform sections

of corpus mucosa (area 8 mm^2), removed using a cork borer, were placed onto the sides of polypropylene tubes before vortexing for 5 s in 1 mL Tris buffer. Each section was incubated at 37 °C for 5 min after which, cyclooxygenase activity was stopped by addition of 25 μL 2 M citric acid. For in-vitro experiments, drugs were dissolved in 1% DMSO before incubation. Aliquots were then removed and assessed for the presence of 6-keto-$PGF_{1\alpha}$ using radio-immunoassay. Results were expressed as ng/section.

Gastric mucosal damage in the rat

An in-vivo gastric chamber preparation (Mersereau & Hinchey 1973) was used which allows direct observation of the mucosa. Male Wistar rats, 250–300 g, were fasted for 18 h but allowed water. Anaesthesia was induced with urethane (1·6 g kg^{-1} as a 25% solution given i.p.). After setting up the preparation, the mucosal solution (4 mL containing 80 mM HCl iso-osmotic with mannitol) was replaced every 20 min. The presence of a relatively high concentration of acid was necessary for the production of taurocholate-induced gastric erosions (Main & Melarange 1977). There were seven 20 min periods, the first of which was designated a control period and where [^{3}H]dextran (0·37 MBq kg^{-1}) was given as an intravenous bolus injection. Results were expressed as total d min^{-1} of [^{3}H]dextran appearing in the mucosal solution. Preliminary studies showed that 57·5 ± 9·3% (n = 4) of the total radioactivity was still present in the plasma at the end of the experiment. Following the control period, sodium taurocholate (10 mM dissolved in the mucosal solution) was administered topically to the mucosa and then removed after 20 min to be replaced in the subsequent five periods by the mucosal solution. In further experiments, either indomethacin or nabumetone was administered into the duodenum, via a catheter placed approximately 3 cm from the pylorus, for effects on (a) gastric erosion formation and [^{3}H]dextran permeability and (b) taurocholate-induced damage. Haemorrhagic erosions, which formed only in the gastric glandular mucosa, were counted and each rated on a 1–4 scale (1 = less than 1 mm; 2 = up to 2 mm; 3 up to 3 mm; 4 = greater than 3 mm). The total was designated the 'erosion index' for a particular stomach.

Statistical analysis

The results are expressed as the mean ± s.e.m. where (n) is the number of observations in the group. Student's *t*-tests along with the Mann-Whitney U test, for non-parametric data, were used to evaluate significance levels. $P < 0.05$ was taken as significant.

RESULTS

Gastric mucosal 6-keto-$PGF_{1\alpha}$ production

Ex-vivo. Control mucosae produced 4·7 ± 0·2 ng/section of 6-keto-$PGF_{1\alpha}$ over the course of a 5 min incubation. This value was significantly reduced by increasing doses of indomethacin (Fig. 1). A maximum inhibition of 79% ($P < 0.001$)

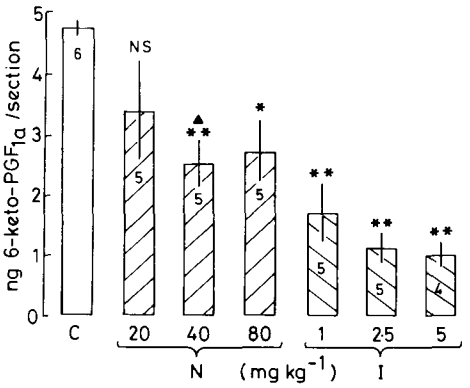

FIG. 1. The effect of nabumetone (N) or indomethacin (I) on the ex-vivo production of 6-keto-$PGF_{1\alpha}$ from the rat gastric mucosa. Values are shown as the mean ± s.e.m. where (n) = number in the group. ▲ $P = 0.02$ when compared with indomethacin (5 mg kg^{-1} p.o.). *$P < 0.01$, **$P < 0.001$ compared with control value.

occurred with the 5 mg kg^{-1} dose. In contrast, nabumetone produced a shallow dose-response curve with a maximum inhibition of only 47% ($P < 0.001$) being obtained with 40 mg kg^{-1}. Furthermore, a dose of 80 mg kg^{-1} produced no further inhibition. Nabumetone was found therefore to be less active ($P = 0.02$) than indomethacin in inhibiting gastric mucosal cyclooxygenase.

In-vitro. Control experiments showed that the mucosa generated 7·2 ± 0·42 ng/section with 1% DMSO in the incubate and 6·03 ± 0·9 ng/section in the absence of DMSO ($P > 0.05$). Fig. 2 shows that indomethacin produced a graded inhibition of cyclooxygenase activity yielding an IC50 value of approximately 0·5 μM. Nabumetone produced a pattern of inhibition in-vitro which was comparable with that demonstrated ex-vivo with a maximum inhibition occurring between 50 and 200 μM. A concentration of 400 μM was found to precipitate gradually over the course of a 5 min incubation which would

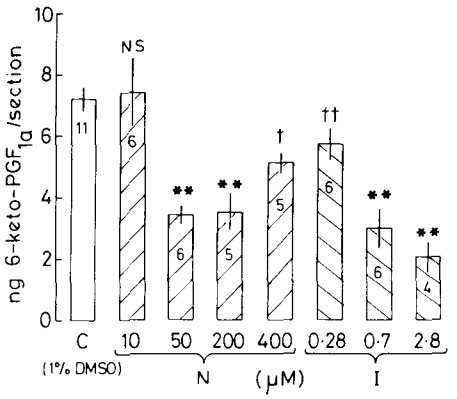

FIG. 2. The effect of nabumetone (N) or indomethacin (I) on the in-vitro production of 6-keto-$PGF_{1\alpha}$ from the rat gastric mucosa. Values are shown as the mean ± s.e.m. where (n) = number in the group. $^{**}P < 0{\cdot}001$, $^{\dagger}P < 0{\cdot}02$, $^{\dagger\dagger}P < 0{\cdot}05$ compared with control value.

explain why less inhibition was observed compared with the value obtained with the 200 μM concentration.

Gastric damage

Sodium taurocholate induced a time-related increase in erosion formation over the course of 2 h (Fig. 3). Concurrent dosing of indomethacin (5 mg kg^{-1} i.duod.) significantly enhanced damage ($P < 0{\cdot}009$) compared with sodium taurocholate alone. However, nabumetone (40 mg kg^{-1} i.duod.), when given concurrently with taurocholate, failed to alter damage significantly ($P > 0{\cdot}05$) when compared with the corresponding bile salt alone value (Fig. 3). In

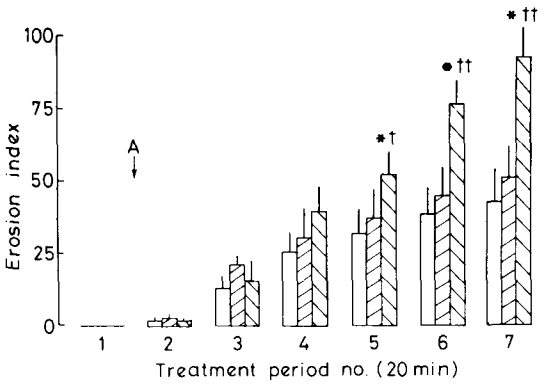

FIG. 3. The effect of nabumetone or indomethacin (i.duod.) on the formation of sodium taurocholate-induced gastric erosions in the anaesthetized rat. Values are shown as the mean ± s.e.m. (n = 7). Key: blank, control; from right to left hatched, nabumetone; from left to right hatched, indomethacin. $^{\dagger}P < 0{\cdot}036$, $^{\dagger\dagger}P < 0{\cdot}019$ compared with the corresponding nabumetone value. $^{*}P < 0{\cdot}009$ compared with the corresponding control value. At A, topical sodium taurocholate and intraduodenal drug was administered.

separate experiments, dosing with either indomethacin (5 mg kg^{-1} i.duod; n = 6) or nabumetone (40 mg kg^{-1} i.duod; n = 4) alone failed to induce any erosion formation or to cause any significant changes ($P > 0{\cdot}05$) in [^{3}H]dextran permeability over the course of the experiments (data not shown). However only indomethacin produced morphological changes such as blanching of the mucosa and the appearance of petechiae.

The mucosal permeability to [^{3}H]dextran was significantly increased ($P < 0{\cdot}001$) both during and after application of sodium taurocholate (Fig. 4)

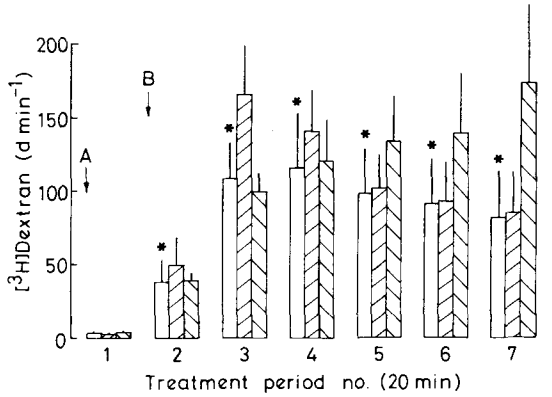

FIG. 4. The effect of nabumetone or indomethacin (i.duod.) on sodium taurocholate-induced dextran permeability in the anaesthetized rat. Values are shown as the mean ± s.e.m. (n = 7). $^{*}P < 0{\cdot}001$ compared with the corresponding value in period 1. There were no statistically significant differences within any treatment period. Key: blank, control; from right to left hatched, nabumetone; from left to right hatched, indomethacin. At A, intravenous dextran was administered; at B, topical sodium taurocholate and intraduodenal drug were administered.

compared with control period 1 and was significantly correlated with the degree of erosion formation (r = 0·52, $P < 0{\cdot}001$; n = 50). Concurrent administration of indomethacin or nabumetone with the bile salt failed to alter the permeability to [^{3}H]dextran compared with the corresponding values for sodium taurocholate alone, although with indomethacin there was a non-significant increase over the course of the experiment.

DISCUSSION

The present study confirms that sodium taurocholate in the presence of acid can induce gastric erosion formation in the rat (Main & Melarange 1977), and alter mucosal permeability as indicated by an elevation of [^{3}H]dextran in the mucosal solution. Normally dextran is confined to the plasma and lymph (Bollman 1953), therefore the small degree of

permeability to this substance observed in the control period may reflect damage produced during the setting up of the preparations. It should be noted, however, that the amount of dextran administered never exceeded 20 $\mu g\ kg^{-1}$. It is unlikely, therefore, to have altered the osmotic pressure between intravascular and extracellular fluid or to have promoted histamine release (Halpen 1956). Since there was a significant correlation between the degree of erosion formation and dextran permeability, the latter may be used objectively as an index of damage.

Concurrent administration of indomethacin and sodium taurocholate enhanced erosion formation significantly and tended to elevate the dextran permeability although this just failed to achieve statistical significance when compared with the corresponding values for taurocholate alone. In contrast, nabumetone did not enhance taurocholate-induced damage over a 2 h time-course and likewise failed to alter dextran permeability when compared with the corresponding value for the bile salt alone. Nabumetone's reduced effects on gastric mucosal cyclooxygenase activity may therefore explain why this compound fails to induce damage in the mucosa or fails to enhance taurocholate-induced damage. It is unlikely to be the result of poor absorption because acute anti-inflammatory activity is detected within the time-course of the present experiments (Boyle et al 1982). In the present work, experiments conducted either ex-vivo or in-vitro showed that nabumetone was less potent and less active than indomethacin on cyclooxygenase activity and compares with results obtained in a BSV preparation (Boyle et al 1982). Hence by failing to reduce cyclooxygenase activity by greater than 50%, nabumetone may not impair prostacyclin-mediated protection of the mucosa. In keeping with this idea are the present results where indomethacin, which enhanced damage, inhibited 6-keto-$PGF_{1\alpha}$ production by 79% and the results of Whittle (1983) where aspirin or ketoprofen enhanced bile salt damage only when mucosal prostacyclin production was inhibited by greater than 60%.

The present results support not only the idea that endogenous prostaglandins protect the gastric mucosa from damage by noxious agents such as bile salts (Whittle 1977) but also show that it is possible to develop NSAIAs like nabumetone which lack gastric irritancy when administered in anti-inflammatory doses (Boyle et al 1982). Indeed, a recent clinical study with nabumetone in rheumatoid arthritic patients has shown it to be well-tolerated with fewer gastrointestinal side effects compared with naproxen (Bianchi Porro et al 1985). The above results might suggest that patients who also present with bile reflux would be less susceptible to gastric bleeding with nabumetone compared with other NSAIA.

In summary, nabumetone, a new non-acidic anti-inflammatory agent, has been shown not to cause changes in gastric mucosal permeability and morphology or to enhance bile salt-induced damage. Lack of effects on these variables was attributable to nabumetone's limited effects on cyclooxygenase, the enzyme responsible for mucosal protective prostaglandins.

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