Effect in man of aspirin, standard indomethacin, and sustained release indomethacin preparations on gastric bleeding

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1 We have compared acute gastric bleeding caused by a new slow release preparation of indomethacin (indomethacin Continus) with that caused by aspirin and other indomethacin preparations.

2 In a randomized crossover study, blood loss into timed gastric aspirates was determined in 20 healthy volunteers after receiving, over 96 h, either placebo, aspirin (600 mg four times daily; 17 doses), indomethacin BP (50 mg three times daily; 13 doses), Indocid-R (75 mg twice daily; 9 doses) or indomethacin Continus (75 mg twice daily; 9 doses). A venous blood sample was also taken during each treatment period for subsequent determination of α_1 -glycoprotein, and for drug assay.

3 Gastric bleeding on placebo was 1.4 (0.7 – 2.8) $\mu\text{l } 10 \text{ min}^{-1}$ (mean, 95% confidence interval). Both aspirin and the indomethacin preparations caused significantly more bleeding ($P < 0.05$). Rates of bleeding after aspirin, indomethacin BP, Indocid-R, and indomethacin Continus were respectively 22.0 (10.7 – 47.2) $\mu\text{l } 10 \text{ min}^{-1}$, 4.4 (2.2 – 9.1) $\mu\text{l } 10 \text{ min}^{-1}$, 10.8 (5.3 – 22.3) $\mu\text{l } 10 \text{ min}^{-1}$, and 5.1 (3.0 – 10.6) $\mu\text{l } 10 \text{ min}^{-1}$.

4 Rates of bleeding after indomethacin BP and indomethacin Continus, but not Indocid-R, were significantly less than after aspirin ($P < 0.01$).

5 Salicylate or indomethacin was detectable in the plasma of all subjects after the active treatment periods, except for one instance involving a subject allocated indomethacin BP. Indomethacin levels were significantly higher 2 h after Indocid-R than with indomethacin BP or indomethacin Continus.

6 α_1 -acid glycoprotein levels were not significantly affected by prior treatment with aspirin or indomethacin.

Keywords aspirin indomethacin gastric mucosa bleeding

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are responsible for 25% of yellow card reports of suspected adverse drug reactions to the CMS (CMS Update, 1986a). The majority of these are for dyspepsia, peptic ulceration and its complications. Only recently, however, has a causal association with the more serious adverse reactions been confirmed, with elderly patients admitted to hospital with either a bleeding or perforated peptic ulcer being found to be three

or more times more likely to be taking a NSAID than matched controls (Somerville *et al.*, 1986; Collier & Pain, 1985). In those with bleeding peptic ulceration, indomethacin was the most frequently prescribed NSAID (Somerville *et al.*, 1986). Indomethacin (excluding Osmosin) is also ranked third behind piroxicam and naproxen in the number of reports of serious gastrointestinal adverse reactions from 1964 to 1985 (CMS Update, 1986b).

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In addition to case-control studies, endoscopic investigations show that patients or volunteers taking NSAIDs have a high incidence of mucosal abnormalities and a measurable increase in cellular exfoliation and mucosal bleeding. There is also growing evidence that NSAIDs, particularly indomethacin, may injure the small intestine in animals (Billingham & Tucker, 1979) and man (Bjarnason *et al.*, 1986). One indomethacin preparation, Osmosin, was withdrawn from the U.K. market because of evidence linking it with intestinal perforation (Day, 1983). Patients admitted to hospital with small or large bowel perforations are also about three times more likely than controls to be taking other NSAIDs, including currently available preparations of indomethacin (Langman *et al.*, 1985). In animal studies, levels of the acute phase protein, α_1 -acid glycoprotein, appear to correlate with the extent of indomethacin-induced intestinal damage (Billingham & Tucker, 1979): this would be a useful clinical index if the same were true in humans.

The optimal way of improving the tolerance or reducing mucosal toxicity of indomethacin is not yet clear. Co-administration of a mucosal protective or anti-secretory agent may prove to be of value, but it is likely to be prohibitively expensive. An alternative approach is to develop controlled release preparations with the aim of maintaining therapeutic potency whilst reducing adverse reactions and gastroduodenal toxicity. Indomethacin Continus consists of indomethacin incorporated in a mixture of hydrated hydroxy-alkyl cellulose and a higher aliphatic alcohol. This allows the smooth release of indomethacin over a long time scale that is independent of pH.

We have used timed measurement of bleeding into gastric washings to compare the effects of indomethacin Continus with aspirin and other indomethacin preparations in man. We have also investigated whether α_1 -acid glycoprotein levels rise following aspirin or indomethacin in humans since this might be a useful index of intestinal injury.

Methods

Twenty healthy volunteers aged 18–24 years (11 male and 9 female) participated in the study after giving written informal consent. They were non smokers and were asked to refrain from alcohol during and for 48 h prior to each dosing period. The protocol was supported by the Ethics Committee of the University of Nottingham.

Protocol

Subjects received each of the following oral treatments in randomized order (single-blind) with a 10 day washout period between each: placebo two tablets twice daily (9 doses); plain aspirin 600 mg four times daily (Evans Medical, 17 doses); indomethacin BP capsules 50 mg three times daily (13 doses); Indocid-R capsules 75 mg twice daily (Thomas Morson, 9 doses); and indomethacin Continus tablets 75 mg twice daily (Napp, 9 doses). All preparations were taken without food at 07.00 h and 22.00 h; in addition indomethacin BP was taken at 12.30 h (0.5 h before food) and aspirin at 12.30 h and 17.30 h. The drugs were swallowed whole with a small quantity of tap water.

On the morning of the fifth day of each treatment period, 2 h after the last dose, each subject underwent oro-gastric intubation with a 16 French gauge Salem sump tube (Sherwood, Belgium). Gastric contents were emptied and the stomach rinsed three times with distilled water before a timed 10 min collection period began. At 5 min a 5 ml solution of phenol red (2 mg) was instilled followed by a further 10 ml of water. This was to enable subsequent correction for fluid losses from the stomach. At 9 min 100 ml of distilled water was instilled and dispersed around the stomach by a series of standard manoeuvres by the subjects. Gastric contents were then aspirated at 10 min. This was followed immediately by two further rapid washes. A second 10 min study period then commenced. After two further rapid washes there was a third and final 10 min study period.

Venous blood (10 ml) was also collected 2 h after the last dose in each study period, the plasma separate and stored at -20°C . This was subsequently assayed for α_1 -acid glycoprotein and the drug or drug metabolite (indomethacin and salicylate) corresponding to that period.

Measurements

The pH and volume of each of the timed aspirates was measured prior to assay for phenol red and haemoglobin. Phenol red was measured spectrophotometrically after a 1:4 dilution of gastric aspirate (500 μl aliquot) with ammonium hydroxide (pH 10.5) at 560 nm.

Blood in the aspirates was quantified by the peroxidase activity of haemoglobin (Fisher & Hunt, 1976). For this assay aliquots of gastric aspirate (0.2 ml) were mixed with 1.8 ml of citrate buffer (12.6 g citrate monohydrate and 9.8 g trisodium citrate per litre, pH 3.8 at room temperature) and orthotolidine (2 g per 100 ml

of glacial acetic acid). Forty-five seconds later, 0.4 ml of hydrogen peroxide (20 volumes oxygen per 100 ml) was added and the solution gently mixed. The rate at which a blue-green colour developed was quantified by the difference in absorbance, measured at 640 nm, 30 and 60 s after the addition of hydrogen peroxide. Samples were compared with standards prepared from the subjects' own blood.

α_1 -acid glycoprotein (Boigne *et al.*, 1977) and salicylate (Brandon *et al.*, 1985) were assayed in plasma by h.p.l.c. in the Department of Clinical Chemistry, University Hospital, Nottingham. Both methods are well established for routine use. Indomethacin levels were determined by h.p.l.c. at Napp Research Centre Ltd, Cambridge.

Analysis

Gastric mucosal blood loss for each timed aspirate was calculated after correction for phenol red recovery. Data from any aspirate with a phenol red recovery of less than 25% were rejected and the median of the remaining samples used for analysis.

The values for pH, phenol red, mucosal blood loss, and α_1 -acid glycoprotein levels were subjected to nested analysis of variance with two strata, a between-subject stratum analysed for the order of treatment and within-subject analysed for the influence of treatment. Indomethacin concentrations were compared by single classification analysis of variance.

If a significant difference was present then the treatment means were compared by Duncan's test (Lentner, 1982). Phenol red and α_1 -acid glycoprotein values were analysed without transformation. pH values were converted to hydrogen activity and gastric blood loss data logarithmically transformed to approximate normal distributions for analysis. Blood losses below the level of detection (approximately $0.4 \mu\text{l } 10 \text{ min}^{-1}$) were taken to be $0.2 \mu\text{l } 10 \text{ min}^{-1}$. A value of $P < 0.05$ was considered significant.

Results

The main adverse symptoms experienced by the study group were either minor upper abdominal discomfort or lightheadiness and headache. Abdominal discomfort occurred on two occasions on indomethacin Continus and aspirin, and on three occasions on indomethacin BP and Indocid-R. Such symptomatology did not require the cessation of therapy.

The pH of the gastric aspirates did not vary

significantly from placebo when either aspirin or the indomethacin preparations were taken. The means ranged from pH of 2.0 to 2.4 (Figure 1). Phenol red recovery was also similar between treatments with the means ranging between 48% and 58% (NS) (Figure 1). Eight percent of the aspirates were rejected because of inadequate phenol red recovery.

Baseline (placebo) intra-gastric blood loss was $1.4 (0.7-2.8) \mu\text{l } 10 \text{ min}^{-1}$ (geometric mean, 95% confidence limits). All the NSAIDs caused significantly more bleeding than placebo ($P < 0.05$), with aspirin resulting in a 16-fold increase ($22.0 (10.7-45.2) \mu\text{l } 10 \text{ min}^{-1}$). Bleeding was not as marked on the indomethacin preparations with respective losses for indomethacin BP, indomethacin Continus and Indocid-R being $4.4 (2.2-9.1) \mu\text{l } 10 \text{ min}^{-1}$, $5.1 (3.0-10.6) \mu\text{l } 10 \text{ min}^{-1}$ and $10.8 (5.3-22.3) \mu\text{l } 10 \text{ min}^{-1}$. Blood loss with indomethacin BP and indomethacin Continus, but not Indocid-R ($P > 0.1$) was significantly less than with aspirin ($P < 0.01$) (Figure 1).

In all subjects, except one after taking indomethacin BP capsules, either salicylate or indomethacin was detectable in plasma 2 h after the last dose of the active medication. The mean ($\pm$ s.e. mean) salicylate concentration was $12.3 \pm 1.3 \text{ mg/dl}$.

The plasma levels for indomethacin BP and indomethacin Continus were $1.2 \pm 0.7 \mu\text{g ml}^{-1}$ and $1.6 \pm 0.8 \mu\text{g ml}^{-1}$ respectively. These were significantly less than for Indocid-R at $2.3 \pm 1.4 \mu\text{g ml}^{-1}$ ($P < 0.05$).

α_1 -acid glycoprotein levels were not significantly altered by NSAID treatment with the means ranging from $0.71-0.75 \text{ mg ml}^{-1}$ (Figure 1).

Discussion

In this study aspirin, indomethacin BP, indomethacin Continus and Indocid-R were found to cause more intra-gastric bleeding than placebo. The degree of such bleeding, however, varied between the preparations with aspirin being the most toxic. Indomethacin BP and indomethacin Continus, resulted in significantly less (4-fold) bleeding than aspirin, whilst Indocid-R was intermediate between these preparations and aspirin. All preparations were satisfactorily tolerated by the study group.

Aspirin is the usual comparator when examining toxicity of NSAIDs to the intestinal mucosa. This is because it consistently produces endoscopically visible minor injury to the gastric mucosa (Prichard & Hawkey, 1987), which has usually been greater than that of other NSAIDs in the few studies in which they have been

Figure 1 Gastric bleeding, phenol red recovery, basal intragastric pH and α_1 -acid glycoprotein (mean $\pm$ 95% confidence limits) after placebo (P), aspirin (A), indomethacin BP (I), Indocid-R (IR) and indomethacin Continus (IC).

compared (Lanza *et al.*, 1979). This is most likely because of greater topical irritancy, in addition to its ability to impair probable mucosal defence mechanisms by prostaglandin inhibition (Cohen & MacDonald, 1982).

The reasons for the relatively high rates of bleeding following Indocid-R are not clear, but may reflect temporary high levels of indomethacin. A characteristic of the Indocid-R formulation is that a third of the dose undergoes a 'burst' release upon swallowing (Yeh, 1985) and this may thus result in greater direct gastric exposure than the other two indomethacin preparations. This is supported by our finding of a high plasma concentration 2 h post-dose.

The amount of blood detected in faeces following indomethacin using radiochromate labelling of red cells is about twice that of gastric aspirate studies (Pemberton & Strand, 1979). One possible explanation is that this is due to intestinal injury. This contention is supported indirectly by the work of Bjarnason *et al.* (1986) which has shown increased small bowel mucosal permeability, bleeding and albumin loss after NSAID treatment in man. Studies in rats have shown a correlation between plasma concentrations of α_1 -acid

glycoprotein and indomethacin induced small intestinal injury (Billingham & Tucker, 1979). However, we found minimal variability of α_1 -acid glycoprotein levels between placebo and aspirin or indomethacin treatment, probably indicating that this protein is not a suitable indicator of minor intestinal injury in man.

This study has shown that measurement of blood loss into timed gastric aspirates after NSAID intake in man can discriminate between the gastric mucosal toxicity of these drugs. Indomethacin Continus, appeared to have less gastric toxicity than aspirin whereas the other sustained release preparation, Indocid-R, was not significantly different from aspirin. All indomethacin preparations caused drug related symptoms; our data do not allow us to decide whether indomethacin Continus is different to indomethacin BP or Indocid-R in this respect. These observations recommend indomethacin Continus for further clinical evaluation as an agent with relatively low rates of acute gastric mucosal bleeding.

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