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Acute gastric microbleeding after aspirin ingestion, compared with a similar dose of fenbufen by measurement of haemoglobin in gastric aspirates

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Gastrointestinal blood loss associated with ingestion of non-steroidal anti-inflammatory drugs (NSAID) including aspirin is common and well recognized (Faivre et al 1979).

Upper gastrointestinal endoscopy has demonstrated that a high proportion of regular users of NSAID have gastric ulcers (Caruso & Bianchi Porro 1980).

Two mechanisms by which NSAID cause gastric damage have been proposed. One is a systemic effect via inhibition of the protective mucosal cyclo-oxygenase. The other is a local action in breaking down the mucosal barrier (Whittle 1980).

Blood loss into the length of the gastrointestinal tract may be measured by labelling circulating red cells with ^{51}Cr and counting radioactivity in the faeces (Yeung Laiwak et al 1981). However in this study we have modified the 'aspirin test meal' developed by one of us to estimate the concentration of haemoglobin in gastric aspirates at intervals up to 180 min after ingestion of an NSAID or a placebo preparation as a measure of blood shed from the upper gastrointestinal tract.

In order to validate this method we have compared the effects of aspirin with those of 3-(4-biphenylylcarbonyl) propionic acid, fenbufen (Lederfen, Lederle Laboratories Ltd.) given as a capsule or tablet. Fenbufen is a pro-drug, metabolized in the liver to an active anti-inflammatory agent (Sloboda & Osterberg 1976).

Materials and Methods

Subjects. Six male and four female healthy students of pharmacology aged between 20–22 years volunteered to take part. None had a history of gastrointestinal disease, or of interolerance to aspirin or NSAID. None of the volunteers had taken aspirin or NSAID for at least two weeks before the study.

Drugs and randomization Fenbufen was prepared either as a 300 mg capsule, or a 300 mg tablet. A placebo consisted of a 300 mg capsule. Aspirin was prepared as a 300 mg capsule. The capsules were all of a similar design, but because of the tablet formulation, no attempt was made to disguise the drugs from either the volunteers or the experimenters.

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Aspiration of stomach contents. Stomach aspiration was performed via a naso-gastric tube (Unoplast size 10, 125 cm). After insertion, the tubes remained in-situ for the duration of the experiment, 180 min.

Estimation of haemoglobin concentration in gastric aspirates. Haemoglobin was measured in gastric aspirates by a variation of the method described by Fisher & Hunt (1976).

An aliquot of the total gastric aspirate was homogenized using an Ultra Turrax homogenizer. A 0·25 ml aliquot of the homogenate was added to 2·25 ml of a citric acid monohydrate/trisodium citrate buffer, pH 3·78 and 1·0 ml of freshly made orthotolidine buffer was added. A blue/green colour developed at a rate which followed first order kinetics. The rate of this reaction was proportional to the concentration of haemoglobin and was followed using a spectrophotometer at 640 nm, at a constant temperature of 20 °C. After 30–45 s, recordings of optical density were taken every 30 s from the digital display and the reaction was monitored. From results obtained with fresh blood, a standard curve was constructed which was linear over the range 0–13·9 μg ml^{-1} haemoglobin).

Procedure followed The volunteers were starved for 12 h before the start of the experiment, but were allowed to take water until 3 h before the start. The volunteers were seated and a nasogastric tube inserted. Residual stomach fluid was aspirated via a 60 ml syringe and discarded. Then, 50 ml of water was taken by mouth, aspirated via the tube and collected as a zero time sample. Two capsules or two tablets of one of the four preparations were randomly allocated to each volunteer and these were swallowed with the minimum of water. After 30 min, 50 ml of water was taken by

Table 1. The concentrate of haemoglobin (mean ± s.d.; μg ml^{-1}) found in stomach aspirates over 3 h from 10 volunteers after oral treatment with aspirin, placebo and fenbufen as described in the text.

Treatment		Hb (μg ml^{-1}
Aspirin 600 mg capsule	(A)	7·92 ± 5·37
Placebo capsule	(B)	3·64 ± 2·07
Fenbufen 600 mg capsule	(C)	1·82 ± 1·11
Fenbufen 600 mg tablet	(D)	2·54 ± 1·67

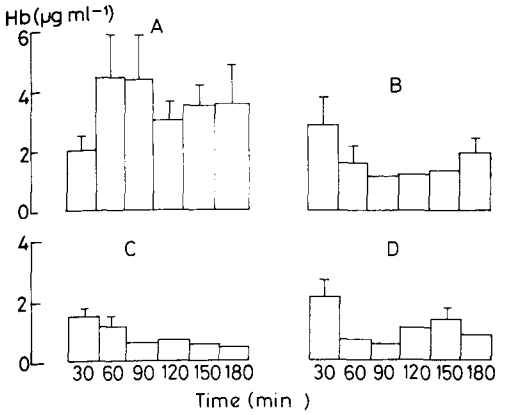

FIG. 1. Haemoglobin concentration in stomach aspirates from 10 volunteers after taking A, aspirin 600 mg in capsule form, B fenbufen 600 mg in tablet form, C fenbufen 600 mg in capsule form, and D control capsules. The histograms represent the mean haemoglobin concentration in μg ml^{-1} from 10 volunteers' gastric aspirations taken at 30 min intervals for 180 min after ingestion of drug. Time 0 sample was taken immediately after insertion of the nasogastric tube and before drug ingestion. Because of obvious contamination by blood caused by the trauma of insertion it was not included in the analysis. Vertical bars are standard error of the mean, when absent s.e.m. = <1.

mouth and after 0·5 min the stomach was aspirated as completely as possible. The volume of the aspirate was noted and an aliquot stored in a glass screw topped bottle. The sample was either assayed for haemoglobin immediately, or stored at −20 °C for assay.

Stomach aspiration continued as described for 180 min. Two weeks were allowed between each cross-over phase of the experiment.

Results

All the volunteers completed the cross-over of the formulations of 600 mg of fenbufen, aspirin and placebo during an eight week period. Some discomfort was caused by the nasogastric tube, but there were no other ill effects.

The mean concentration of haemoglobin per ml found in aliquots of the stomach aspirates over 180 min after taking the drugs in shown in Table 1, for the combination of the four drugs taken. The haemoglobin measured in the zero time aspiration samples was the greatest of the time series. We assumed that this sample was contaminated by blood from the trauma caused by the insertion of the nasogastric tube. Thus, the reading was not included in the statistical analysis of the data. By inspection of Fig. 1, the relative mean concentration of haemoglobin in aspirates after the four treatments can be seen, the zero time samples were not included.

To examine the difference between subjects and treatments, an analysis of variance was performed Table 2), which showed variation between subjects was not significant, but that the concentration of haemoglobin found in gastric aliquots did vary according to the

Table 2. Analysis of variance between subjects and treatments comparing the concentration of haemoglobin (μg ml^{-1}) found in gastric aspirates from 10 volunteers after taking aspirin, placebo and fenbufen, a single 600 mg dose by mouth. The Table demonstrates that subject variation was not significant. The effect of the drug treatments was significant (P <0·01).

	Sum of squares	d.f.	Mean square	Variance ratio	
Subjects	299·88	9	33·32	1·69	N/S
Treatments	1530·46	3	510·15	25·83	P <0·01
Interaction	1667·00	27	61·74	3·13	P <0·01
Residual	3081·64	156	19·75	1·00	
Total	6578·98	196			

treatment (P <0·01). The analysis (paired t-test) showed that preparation A (aspirin 600 mg) produced highly significantly more gastric bleeding that the other preparations. Preparations C and D (fenbufen 600 mg in tablet and capsule respectively) produced bleeding not significantly different from each other, while the placebo, B produced significantly more bleeding than did treatment C, but not D. However, C and D did not differ significantly from one another. The bleeding associated with the placebo and the two formulations of fenbufen was, in fact, very slight. The results are summarized in Table 3.

Discussion

Measuring blood loss from the gastrointestinal tract by the detection of radioactivity from ^{51}Cr labelled red cells in the faeces is sensitive, but gives no indication of the source of the bleeding or the timing of the event. The aspiration of stomach contents and direct measurement of haemoglobin by chemical means has advantages over radiolabelled red cell measurement from faeces. The technique circumvents the need to inject radioactivity and is specific for blood loss from the stomach rather than the whole of the gastrointestinal tract.

Fenbufen is a pro-drug and in clinical and animal trials has been shown to be relatively free from

Table 3. The paired t-test of the significance between treatment in gastric bleeding, A = 600 mg aspirin, B = placebo, C = fenbufen capsule 600 mg, D = fenbufen tablet 600 mg. The Table shows the significant differences to emerge between various treatments, and the P values ascribed to each comparison. The paired sign test was also used as a comparison to allow for the non normality of the data. From these data the two formulations of fenbufen did not differ in their effect on the gastric mucosa; neither did they differ significantly from the effect of placebo at the P <0·01 level. Aspirin, however, produced a highly significantly greater amount of gastric microbleeding than fenbufen and placebo.

Treatments	t	d.f.	Significance	Paired sign test	Significance
A/B	3·84	45	P <0·001	2·97	P <0·01
B/C	3·73	45	P <0·001	4·95	P <0·001
B/D	1·97	45	N/S	2·40	P <0·05
C/D	1·57	45	N/S	1·27	N/S

gastrointestinal side effects (Uthgenannt & Letzel 1980; Sloboda et al 1980). Fenbufen does not inhibit the synthesis of prostaglandins (PG), but its active anti-inflammatory metabolite biphenylacetic acid does (Tolman & Partridge 1975). In the short time we allowed the drugs to be in contact with the gastric mucosa, it is reasonable to suppose that they cause gastric irritation by a direct 'barrier breaking' action, rather than by a systemic effect on the gastric mucosa via inhibition of enzyme PG cyclo-oxygenase. Thus we were able to distinguish between the relative effect on the stomach of a local as opposed to a systemic action. In the case of the pro-drug fenbufen, the local gastric irritation as measured by blood loss was minimal, unlike that caused by aspirin. It may be postulated that when fenbufen is metabolized by the liver, only the active metabolite will be available to act via a systemic action to cause gastric irritation, via PG cyclo-oxygenase; however a primary NSAID such as aspirin will act both locally and systemically in this respect. The systemic effect will presumably depend not only on the specificity of the drug to inhibit PG cyclo-oxygenase but will also depend on the consistency of the blood levels maintained during the course of long-term treatment. Thus, a slow release preparation of a drug which has a low specificity for the inhibition of PG cyclo-oxygenase may cause more damage to the gastric mucosa than a drug given at a dose that produces high but interrupted blood levels which are allowed to fall to a low value between doses.

With gastric aspiration after taking NSAID by mouth over a short period coupled with a sensitive assay for haemoglobin in stomach contents, it is possible to distinguish between the immediate local and long-term systemic effects of drugs upon the gastric mucosa.

REFERENCES

Caruso, I., Bianchi Porro, G. (1980) Br. Med. J. 1: 75–78

Faivre, J., Faivre, M., Lery, N., Ucluzeau, D., Moulinier, B., Paliard, P. (1979) Digestion 19: 218–220

Fisher, M. A., Hunt, J. N. (1976) Ibid. 14: 409–414

Sloboda, A. E., Tolman, E. L., Osterberg, A. C., Panagides, J. (1980) Arzneim-Forsch. (Drug Res) 30: 716–720

Sloboda, A. E., Osterberg, A. C. (1976) Inflammation 1: 415–438

Tolman, E. L., Partridge, R. (1975) Prostaglandins 9: 349–353

Uthgenannt, H., Letzel, H. (1980) Aktuelle Rheumatologie 5: 99–100

Whittle, B. J. R. (1980) Brain Res. Bull. 5: suppl 1; 7–14. ANKHO International Inc

Yeung Laiwak, A. C., Hilditch, T. E., Hortin, P. W., Hunter, J. A. (1981) Ann. Rheum. Dis. 40: 455–461

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Some cardiovascular effects of LG 13979: comparison with nicotinic acid and other nicotinic acid derivatives

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2-(3-Pyridinecarbonylamino)-2-deoxy-1,3,4,6-dihydrogen-D-glucose tetrapyridinecarboxylate LG 13979 (Murmann & Ponchiroli 1982), is a new nicotinic acid derivative having more intense and longer lasting lipid-lowering activity than nicotinic acid and its derivatives in rats (Subissi et al 1983). One of the most common and troublesome side effects of nicotinic acid in man is flushing of the face and upper part of the body (Altschul 1964). As this effect is reproducible in the guinea-pig (Andersson et al 1977), we wished to find out whether LG 13979 induces flush and, if so, to what extent. Its effects in the guinea-pig were therefore compared with those of an equidose of nicotinic acid and of its derivatives niceritrol and sorbinicate. As the cholesterol-lowering and antiatherogenic effect of a drug might be secondary to a hypotensive effect (Carrier et al 1968), we also assessed the effects of LG 13979 on arterial pressure and heart rate of the conscious rabbit, in comparison with an equidose of nicotinic acid and sorbinicate.

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Materials and methods

Male Dunkin-Hartley guinea-pigs (n = 10), 265–570 g, from the Rodentia breeding station, Torre Pallavicina (BG) and female New Zealand White rabbits (n = 20), aged 95–115 days, 2·0–3·1 kg from our own breeding quarters were used. All the animals were fed on Altromin MS/K (A. Rieper, Vandoies, BZ) and had free access to water. They were kept in constant environmental conditions (temperature 18–19 °C, relative humidity 50–60%, 12 h light and 12 h dark). At the beginning of each experiment the animals had been fasted for 16 h.

The drugs used were: nicotinic acid (Merck, Darmstadt, GFR), niceritrol (Bofors Nobel Kemi, Sweden),