EFFECT OF CLOPIDOGREL ON NAPROXEN-INDUCED GASTROINTESTINAL BLOOD LOSS IN HEALTHY VOLUNTEERS

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SUMMARY

The effect of clopidogrel, a potent inhibitor of platelet aggregation, on naproxen-induced faecal blood loss was investigated in 30 healthy volunteers in a randomized, double-blind, placebo-controlled, two parallel treatment groups study. All subjects first received naproxen 250 mg b.i.d. during 7 days, after which they were randomly allocated to additionally receive either clopidogrel 75 mg once daily (n=15) or matching placebo (n=15) for 11 days. Faecal blood loss was measured by the ^{51}Cr-labelled erythrocyte method during the last four days of each of the four study periods, i.e. baseline, treatment with naproxen alone, combined treatment and wash-out.

Mean daily faecal blood loss was below 0.5 ml/day during the baseline period in both treatment groups and increased during treatment with naproxen alone to (mean ± SD) 1.14 ± 0.58 ml/day in the naproxen+placebo group and to 1.93 ± 1.51 ml/day in the naproxen+clopidogrel group. Addition of clopidogrel to naproxen treatment was associated with an increase of the mean daily blood loss to 6.83 ± 9.32 ml/day, which was statistically significantly higher than 1.75 ± 1.40 ml/day observed during treatment with naproxen+placebo. During the wash-out period, mean daily blood loss decreased to 0.98 ±

0.51 ml/day in the naproxen+placebo group and to 1.07 ± 0.46 ml/day in the naproxen+clopidogrel group.

Based on these results, it can be concluded that clopidogrel increases naproxen-induced gastrointestinal blood loss in healthy volunteers. Caution should therefore be called for when these drugs are coadministered.

KEY WORDS

clopidogrel, naproxen, faecal blood loss, interaction

INTRODUCTION

Clopidogrel is a thienopyridine derivative that selectively and potently blocks adenosine diphosphate (ADP)-induced platelet activation by irreversibly inhibiting the binding of ADP to its platelet receptor /1-3/. Thereby it inhibits ADP-dependent expression of the GPIIb-IIIa complex, the major receptor for fibrinogen on the platelet surface. The inhibitory effect is cumulative on repeated dosing and reaches a plateau after 4-7 days. The level of inhibition (approximately 50%) of *ex vivo* ADP-induced platelet aggregation, observed after repeated oral doses of 75 mg per day, was similar to that obtained with the therapeutic (250 mg b.i.d) doses of ticlopidine, a chemically related antiplatelet drug that is used worldwide /4/. A recently performed international trial in approximately 20,000 patients showed clopidogrel to be more effective than aspirin in reducing the combined risk of ischaemic stroke, myocardial infarction, or vascular death in patients with atherosclerotic vascular disease. Clopidogrel also appeared to be at least as safe as medium-dose aspirin with significantly less gastrointestinal bleeding and to have a more favourable safety profile than ticlopidine /5/.

Nonsteroidal anti-inflammatory drugs (NSAIDs) remain the agents of choice in the treatment of many rheumatic diseases because of their analgesic and anti-inflammatory properties. However, short term and chronic therapy with NSAIDs is associated with an increased risk of mucosal damage and bleeding in the upper gastrointestinal (GI) tract and small intestine /6-8/. Combination of NSAIDs with a potent

antiplatelet agent such as clopidogrel might further increase the risk of GI bleeding.

The present study was designed to investigate whether clopidogrel influences the occult faecal blood loss induced by naproxen and as such affects the safety of the two drugs when given in combination. The determination of faecal blood loss using the chromium-51 tagged erythrocyte method is considered a relevant model in the evaluation of GI bleeding induced by drugs /9,10/.

MATERIALS AND METHODS

Subjects

Thirty healthy men with a mean ± SD age of 22.4 ± 2.5 years and a mean ± SD body weight of 75.3 ± 10.8 kg participated in this trial.

Prestudy screening included a medical history, physical examination, ECG, urine screen for drugs of abuse, bleeding time assessment, platelet aggregation tests, blood coagulation tests and routine laboratory tests (haematology, biochemistry, serology and urinalysis). Exclusion criteria were, amongst others, a history of peptic ulcer or of any organic lesion susceptible to bleeding, bleeding gums or any sign of haemorrhagic diathesis.

The protocol was approved by the University Hospital Ethics Committee and each volunteer gave written informed consent. The study was conducted according to the guidelines of Good Clinical Practice for Trials on Medicinal Products in the European Community

Study design

This was a 32-day, randomized, placebo-controlled, double-blind study in two parallel treatment groups. The study consisted of four consecutive study periods as shown in Table 1: period 1 (day 1-4), period 2 (day 5-11), period 3 (day 12-22) and period 4 (day 23-32).

On day 1, 10 ml of blood were taken from the subjects and mixed with 1.5 ml acid-citrate dextrose (ACD) solution ($Na_3Ci.2H_2O$ 22 g, $H_3Ci.H_2O$ 8 g, dextrose 25 g, water to 1l). The erythrocytes were isolated by centrifugation of the blood/ACD mixture at 1200-1500 *g* for 5 min. The supernatant and buffy coat were discarded and to the cells was added 11.1 MBq of $Na_2{}^{51}CrO_4$. After incubation for 15 min

at room temperature, the cells were washed twice in 4-5 volumes of sterile saline and finally resuspended in 10 ml of sterile saline. The resuspended erythrocytes were then reinjected into the subject /9,10/.

In period 2, all subjects received naproxen 250 mg b.i.d. with a standard light snack. In period 3, they were randomly assigned to additionally receive either placebo (group I) or clopidogrel 75 mg once daily (group II). Clopidogrel or placebo was administered in the fasting state 30 min before intake of the morning dose of naproxen (Table 1).

TABLE 1

Study design

Treatment Group	PERIOD 1 Day 1-4	PERIOD 2 Day 5-11	PERIOD 3 Day 12-22	PERIOD 4 Day 23-32
Group I (N+P) n=15	baseline	naproxen*	naproxen* + placebo	wash-out
Group II (N+C) n=15	baseline	naproxen*	naproxen* + clopidogrel 75 mg o.d.	wash-out

* 250 mg b.i.d; N=naproxen; P=placebo; C=clopidogrel

Assessment of GI blood loss

Stools were quantitatively collected during the last 3 to 4 days of each study period for determination of GI blood loss. Venous blood samples (5 ml) were collected on the days of planned faeces collections to correlate radioactivity in the faeces with radioactivity in the blood. Stools were collected in plastic receptacles and, after homogenization, diluted to 1000 ml with water. Five ml of blood, also diluted to 1000 ml of water in a similar receptacle, served as a

standard. The radioactivity of standard and faeces was then determined using a 4-in. NaI(Tl) scintillation detector, coupled to a multichannel analyser (Canberra S 100), calibrated with the appropriate standard sources. The volume of blood in the 24-h faeces collections was calculated as

$$\frac{\text{cpm/24-h faeces collection}}{\text{cpm/ml blood}}$$

Pharmacodynamics

Blood for platelet aggregation tests was collected on 129 mM trisodium citrate, before the morning dose on day 5 (baseline), day 12 (following 7 days of intake of naproxen), day 22 (following 10 days of intake of naproxen + placebo or of naproxen + clopidogrel) and at the end of the study. *Ex vivo* aggregation in platelet-rich plasma was measured after induction by adenosine diphosphate (ADP 5 μM) and the maximum percentage increase of light transmission was recorded (maximal amplitude). Results were expressed as percentage inhibition from baseline.

Bleeding times were measured at the same time points, by the two-point Ivy-Nelson method as described by Thebault *et al.* /11,12/, using a sterile lancet (Microlance®, Becton Dickinson). The effect on bleeding time was expressed by a prolongation factor versus baseline.

Safety

To assess safety, physical examination including vital signs and routine laboratory tests were performed at regular intervals during the study. All subjective and objective side effects were recorded.

Statistical analysis

The primary endpoint was the difference in mean daily GI blood loss between the naproxen+placebo and the naproxen+clopidogrel groups during combined treatment. The variables used in the analysis of faecal blood loss were subjects' averages of daily blood loss, calculated by dividing the total blood loss over a given collection period by the actual number of collection days.

Hypothesis testing was performed to test the clinical equality in daily blood loss between the naproxen+clopidogrel and the

naproxen+placebo groups during combined treatment (period 3) using a one-tailed Student's t-test. A difference in faecal blood loss of less than 0.8 ml/day (=δ) was considered as being not clinically relevant. The null hypothesis (Ho) tested was therefore:

Ho: Mean daily blood loss N+C - Mean daily blood loss N+P > 0.8 ml/day.

One-sided 95% confidence intervals for this difference were also determined. If the upper confidence bound is smaller than δ it can be concluded that the effects of both treatments are equivalent. To assess the difference in daily faecal blood loss between the two treatment groups during treatment with naproxen alone (period 2), a two-tailed Student's t-test was performed.

The secondary objective was to assess the effect of the concomitant treatment on bleeding time and platelet aggregation. Differences in maximum intensity of platelet aggregation and in prolongation of bleeding times between the two treatment groups were assessed using a two-tailed Student's t-test at all time points. Analysis of bleeding time prolongation was performed on log transformed data.

RESULTS

Thirty subjects were enrolled in the study, 15 in each treatment group (Table 1). There were no significant differences in age, body weight or height between the two treatment groups.

Due to a significantly prolonged bleeding time, administration of naproxen and clopidogrel was discontinued in three subjects from day 19 onwards and in three subjects on day 22. For the same reason, administration of naproxen was stopped in one subject on day 22. All these subjects belonged to the naproxen+clopidogrel group and were nevertheless included in all analyses because it is assumed that they had reached steady-state for clopidogrel-induced pharmacological activity.

Mean ± SD daily faecal blood loss in ml/day during the four study periods for the two treatment groups is presented in Table 2.

Mean faecal blood loss was below 0.5 ml/day during the baseline period in both treatment groups: 0.37 ± 0.42 ml/day in the naproxen+placebo group and 0.28 ± 0.22 ml/day in the naproxen +clopidogrel group. Faecal blood loss increased during treatment with naproxen alone to 1.14 ± 0.58 ml/day in the naproxen+placebo group

TABLE 2

Mean faecal blood loss in ml/day during the four study periods for the naproxen+placebo (N+P) and naproxen+clopidogrel (N+C) group

Treatment Group		Daily faecal blood loss (ml/day)			
		Period 1 Baseline	Period 2 Naproxen alone	Period 3 Combined treatment	Period 4 Wash-out
N + P	Mean	0.37	1.14	1.75	0.98
(n=15)	SD	0.42	0.58	1.40	0.51
	Min	0.04	0.41	0.37	0.46
	Max	1.76	2.39	5.55	2.39
N +C	Mean	0.28	1.93	6.83	1.07
(n=15)	SD	0.22	1.51	9.32	0.46
	Min	0.04	0.50	0.90	0.57
	Max	1.02	5.77	28.28	2.08

and to 1.93 ± 1.51 ml/day in the naproxen+clopidogrel group (p=0.07).

Addition of clopidogrel to naproxen treatment was associated with an increase of the mean daily blood loss to 6.83 ± 9.32 ml whereas the daily blood loss during combined treatment with naproxen and placebo averaged 1.75 ± 1.40 ml. Mean daily blood loss was above 10 ml in four subjects of the naproxen+clopidogrel group, while the maximum value observed in the naproxen+placebo group was 5.6 ml/day. The mean difference between the two treatments was 5.08 ml/day with an upper 95% confidence limit of 10.06 ml/day, exceeding the predetermined clinically acceptable difference of 0.8 ml/day. When testing for equality in daily faecal blood loss between the N+C and N+P groups, Student's t-test showed indeed that it is very unlikely (p=0.955) that the effects of the treatments on faecal blood loss are equivalent.

During the wash-out period, mean daily blood loss decreased to 0.98 ± 0.51 ml in the naproxen+placebo group and to 1.07 ± 0.46 ml in the naproxen+clopidogrel group.

Table 3 summarizes the mean (SD) percent inhibition of platelet aggregation (ADP 5μM) and mean bleeding time prolongation versus baseline for the two treatment groups at various time points.

On day 22, the mean percent inhibition in ADP-induced platelet aggregation in the naproxen+clopidogrel group was 67.9 compared to a mean of 9.9 in the naproxen+placebo group. These results are in line with the known anti-aggregatory effect of clopidogrel. When testing for a difference in maximum intensity of platelet aggregation between the two groups on day 22, the results of the Student's t-test demonstrate significantly lower ($p < 0.001$) mean values for the naproxen+clopidogrel group as compared to the naproxen+placebo group. There was no significant difference between the two treatment groups on day 12.

On day 22, the prolongation of bleeding time was significantly greater ($p<0.001$) in the naproxen+clopidogrel group than in the naproxen+placebo group. Six subjects had bleeding time prolongation factors above 5: all six were in the naproxen+clopidogrel group. No significant difference existed between the two treatment groups during treatment with naproxen alone. At the end of the study, bleeding times in both treatment groups returned to baseline.

TABLE 3

Mean (SD) percent inhibition of platelet aggregation (ADP 5 µM) and mean bleeding time prolongation factor for the two treatment groups at various time points

	Platelet Aggregation (ADP)		Bleeding Time	
Study day	**% inhibition vs baseline**		**Prolongation vs baseline**	
(treatment)	**N + P group**	**N +C group**	**N + P group**	**N +C group**
day 12 (N alone)	9.4 (39.8)	15.5 (31.6)	1.22 (0.43)	1.44 (0.53)
day 22 (combined)	9.9 (48.0)	67.9 (24.2)	1.44 (0.46)	3.27 (2.65)
end study (none)	-2.2 (31.0)	10.5 (28.1)	1.02 (0.22)	1.00 (0.21)

N=naproxen P=placebo; C=clopidogrel

Treatment with naproxen alone or in combination with clopidogrel did not significantly affect laboratory parameters, vital signs or ECG.

Twenty-three subjects experienced 52 adverse events during the course of the study, of which 26 occurred in each treatment group. Most of them were mild, due to intercurrent illnesses, and not considered as treatment related. One subject, however, showed subcutaneous haemorrhages of moderate intensity at several localizations at the end of treatment with naproxen+clopidogrel. They disappeared gradually over 10 days and were considered as drug-related.

DISCUSSION AND CONCLUSIONS

This study was performed to examine a possible pharmacodynamic interaction between naproxen, a nonsteroidal anti-inflammatory drug, and clopidogrel, an antiplatelet agent, in healthy volunteers. As a primary objective, the effect of clopidogrel on occult GI blood loss induced by naproxen was assessed. The secondary objective was to assess the effect of the combined treatment on platelet aggregation and bleeding time.

The mean daily faecal blood loss observed during the baseline period was similar in both treatment groups (0.37 and 0.28 ml respectively) and in good agreement with reported data from other studies with healthy volunteers /13,14/. As expected, treatment with naproxen 250 mg b.i.d increased faecal blood loss to 1.14 ± 0.58 and 1.93 ± 1.51 ml/day in the two treatment groups, respectively, which is comparable with data from other investigators: 1.59 ml/day at 750 mg naproxen per day /15/ and 1.2 to 1.4 ml/day at 1000 mg per day /13,16/.

In comparison with placebo, clopidogrel 75 mg/day significantly increased faecal blood loss in subjects treated with naproxen.

This study was designed as an equivalence study with the null hypothesis that the excess faecal blood loss in the naproxen+clopidogrel group, as compared to the naproxen+placebo group, is greater than or equal to 0.8 ml/day, a difference estimated not to be of clinical relevance. This hypothesis could not be rejected in this study and the results therefore indicate that addition of clopidogrel to a treatment with naproxen is very likely ($p=0.955$) to increase the daily faecal blood loss by more than 0.8 ml/day.

These results are not unexpected in view of the nature of the drugs combined in this study: nonsteroidal anti-inflammatory drugs are known to produce GI erosions and bleeding, at least in part related to the inhibitory action of these drugs on cyclooxygenase (cox), resulting in decreased synthesis of cytoprotective prostaglandins in the GI mucosa. These erosions cause the increase in occult faecal blood loss observed during treatment with practically all drugs of this class. Platelet aggregation most probably limits blood loss at the site of the mucosal erosions. Although naproxen and other NSAIDs are themselves reversible inhibitors of platelet aggregation, the addition of an irreversible and potent inhibitor of platelet aggregation could be expected to increase the microbleeding caused by the anti-inflammatory drug.

Aspirin is the most commonly used agent in the prevention of arterial thrombotic events; for these indications it could eventually be replaced by clopidogrel. In the interest of patients requiring antiplatelet and anti-inflammatory therapy, it would therefore be interesting to know the effect on GI bleeding of aspirin in association with an NSAID. In this respect it should be noted that even low to moderate doses of aspirin increase occult blood loss /17/ and GI haemorrhage /18/ and that concurrent use of aspirin and NSAIDs roughly doubles the risk of GI haemorrhage /18/. In addition the recent CAPRIE study showed a significantly higher incidence of GI haemorrhage with aspirin 325 mg per day than with clopidogrel 75 mg per day /5/.

Recently two isoforms of the cox enzyme have been discovered: a "constitutive" cox-1 and an "inducible" cox-2. It is hypothesized that the anti-inflammatory and analgesic effects of NSAIDs are related to their inhibitory action on cox-2 while their antithrombotic and adverse effects are largely due to their inhibition of cox-1. Classic NSAIDs preferentially inhibit cox-1 /19,20/. Amongst NSAIDs, naproxen occupies an intermediate position with regard to the relative selectivity of cox-1 vs cox-2 inhibition /21/ and with regard to the relative risk of serious gastrointestinal complications /7/. Gut related side effects are the main problem associated with NSAIDs. As a better GI tolerance of highly selective cox-2 inhibitors can reasonably be expected, it would seem worthwhile to study the effects of combined treatment with an antiplatelet agent and this new class of NSAIDs.

Clopidogrel, at a dose of 75 mg/day, significantly prolongs bleeding time (Ivy-Nelson method) by a factor of approximately two /4/. Some subjects receiving clopidogrel and naproxen showed a bleeding time prolongation greater than expected. It would therefore appear that naproxen and clopidogrel, given in combination, have an additive or even a supra-additive effect on bleeding time. Statistical analysis of this issue could not be performed, however, as a clopidogrel control group was not included.

Based on the results of this study, it can be concluded that concomitant administration of naproxen and clopidogrel is associated with increased GI blood loss. Consequently, nonsteroidal anti-inflammatory drugs and clopidogrel should be coadministered with caution.

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