Pharmacokinetics and pharmacodynamics of sibraxiban alone or in combination with ticlopidine and aspirin

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Aims The purpose of this clinical study was to evaluate the effects of a ticlopidine/aspirin combination on the pharmacokinetics and pharmacodynamics of sibraxiban and the tolerability of the combination therapy

Methods Thirty-eight healthy male volunteers were randomized to receive one of the following treatments for 7 days: sibraxiban ($n = 12$), ticlopidine/aspirin ($n = 12$), or the combination treatment sibraxiban/ticlopidine/aspirin ($n = 14$). Concentrations of the active metabolite of sibraxiban, Ro 44-3888, in plasma and urine were determined by column-switching liquid chromatography combined with tandem mass spectrometry. The pharmacodynamics of sibraxiban and ticlopidine/aspirin were examined by measuring the inhibition of ADP- or collagen-induced platelet aggregation.

Results The addition of ticlopidine/aspirin to sibraxiban did not significantly alter the pharmacokinetic parameters of Ro 44-3888. the geometric mean ratio for AUC(0,12h) was 110 (95% CI 0.82, 1.22). Separately, sibraxiban and ticlopidine/aspirin inhibited ADP- and collagen-induced platelet aggregation and the effects of the two treatments were additive. For example, the average inhibition of ADP-induced platelet aggregation over 12 h was 42% in the sibraxiban treated group, 55% in the ticlopidine/aspirin group and 69% in the sibraxiban/ticlopidine group. The bleeding time was prolonged in the treatments with ticlopidine/aspirin (8.1 min) and sibraxiban/ticlopidine/aspirin (8.6 min) compared with sibraxiban alone (3.5 min).

Conclusions This study shows a significant pharmacodynamic interaction between sibraxiban and ticlopidine/aspirin. Consequently, the simultaneous administration of sibraxiban and ticlopidine/aspirin should be carefully monitored to ensure the patient's coverage with an antiplatelet drug without exposure to an excessive bleeding risk.

Keywords: aspirin, GPIIb/IIIa antagonist, platelets, sibraxiban, ticlopidine

Introduction

Platelet activation and aggregation play an important role in the development of ischaemic complications after percutaneous coronary interventions as well as in the

pathogenesis of cardiovascular disorders including unstable angina, acute myocardial infarction, transient ischaemic attacks and stroke [1–4]. There are more than 100 agonists of platelet activation, which act through several different biochemical pathways. Aspirin, the most widely used antiplatelet agent, reduces the risk of arterial thrombosis [5] but is a weak platelet inhibitor that only affects the cyclooxygenase pathway to platelet aggregation [6]. Indeed, one third of stroke patients fail to respond to aspirin and these patients are 10 times more likely to develop an additional vascular event [7]. Other antiplatelet

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agents, such as ticlopidine [8] and clopidogrel [9], also have a limited activity, which primarily involves inhibition of ADP-mediated platelet aggregation. To optimize pharmacological antiplatelet treatments, drug development has recently focused on inhibiting a protein common to all aggregation pathways, the platelet glycoprotein (GP) IIb/IIIa receptor.

The efficacy of intravenous GPIIb/IIIa antagonists has been demonstrated in numerous clinical trials involving patients undergoing coronary interventions and patients with acute coronary syndromes (reviewed in [10, 11]). The first GPIIb/IIIa antagonist to undergo large-scale clinical investigation was the chimeric antiplatelet antibody c7E3 (abciximab), which proved superior to aspirin in high-risk angioplasty [12, 13]. Potent and selective nonantibody GPIIb/IIIa antagonists include snake venom polypeptides, linear or cyclic peptides, and synthetic nonpeptides. Among these agents, the cyclic peptide integrilin and the nonpeptides lamifiban and tirofiban are currently undergoing clinical trials for short-term intravenous treatment [14–16]. For long-term use, a number of orally active GPIIb/IIIa inhibitors [17–19] are currently under investigation. These agents may expand the therapeutic potential of GPIIb/IIIa antagonists to the secondary prevention of recurrent ischemic events in outpatients with acute coronary syndromes. Finally, combination therapies involving the coadministration of a GPIIb/IIIa antagonist and thrombolytic agents, antithrombotics or other antiplatelet agents could be very effective [14, 18]. With this approach, however, attention must be given to potential pharmacokinetic and/or pharmacodynamic interactions [18].

Sibrafiban (Ro 48-3657: [Z]-(S)-[[1-[2-[[4-(amino-hydroxyiminomethyl)-benzoyl]amino]-1-oxopropyl]-4-piperidinyl]oxyl]-acetic ethyl ester) (Figure 1) is a double prodrug. It is metabolized in man into the inactive prodrug Ro 48-3656 (amidoxime free acid), which is

further transformed into the active compound Ro 44-3888 (amidine free acid) [20]. Ro 44-3888 is a nonpeptide, selective and reversible antagonist of the platelet GPIIb/IIIa receptor.

Sibrafiban is being developed for the secondary prevention of arterial thrombosis following unstable angina and acute myocardial infarction. The present study is part of the initial clinical investigation of sibrafiban. It was designed to explore potential interactions between sibrafiban and a ticlopidine/aspirin combination. Because ticlopidine/aspirin produces a long-lasting effect on platelet function, there could be a therapeutic overlap in patients who receive ticlopidine/aspirin first and then begin a sibrafiban treatment. Thus, the primary objectives were to evaluate the effects of a ticlopidine/aspirin combination on the pharmacokinetics and pharmacodynamics of sibrafiban and the tolerability of the combination therapy.

Methods

Study population and design

The study was conducted in a single medical centre as an open-label, randomized parallel group trial involving 38 healthy male volunteers, aged 19–44 years. Written informed consent was obtained from each subject prior to enrolment. The planned sample size of the study was 12 volunteers per treatment group ($n=36$). Two volunteers had abnormal platelet aggregation before study initiation. They received only one dose of their medication and were replaced. The pharmacokinetic/pharmacodynamic data of one volunteer from the combination treatment group had to be excluded from the evaluation for technical reasons. The volunteers were randomized to receive one of the following treatments for 7 days: sibrafiban ($n=12$), ticlopidine/aspirin ($n=12$), or the combination treatment

Figure 1 Chemical structures of the double protected prodrug sibrafiban and derivatives.

sibraxifiban/ticlopidine/aspirin ($n = 14$, planned sample size of 12 plus 2 replacements). An oral dose of 2 mg twice daily sibraxifiban (equivalent to 1.7904 mg of Ro 44-3888) was chosen to yield an intermediate pharmacodynamic effect to allow the observation of a potential interaction and to provide sufficiently high plasma levels for the investigation of pharmacokinetic interactions. This dose was expected to inhibit ADP-induced platelet aggregation by 45% at trough and 70% at peak (unpublished data). Doses of 250 mg twice daily ticlopidine and 80 mg twice daily aspirin were chosen for this study because they are commonly used in stent placement. The average inhibition of ADP-induced platelet aggregation induced by this dose of ticlopidine is about 30% [21, 22] and the bleeding time is prolonged about 4-fold [21], while aspirin has no additional effect [23].

Pharmacokinetic study

Plasma samples for pharmacokinetic analysis were drawn predose on day 1 (baseline), predose on day 7, and at 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, 48, 60 and 72 h after drug administration on day 7. Urine samples were collected predose on day 1 (baseline), predose on day 7, and in the following intervals: 0–2, 2–4, 4–8, 8–12, 12–24, 24–48 and 48–72 h after drug administration on day 7. For the sibraxifiban/ticlopidine/aspirin treatment, the urine collection was continued at 24 h intervals until platelet aggregation and IVY bleeding time returned to baseline levels.

Concentrations of Ro 44-3888 in plasma and urine were determined by column-switching liquid chromatography combined with tandem mass spectrometry (LC-MS/MS). The assay for plasma has been described elsewhere [24]. It was adapted and validated for urine (data on file). Briefly, it involves protein precipitation (plasma) or dilution in buffer (urine), enrichment of the analytes on a standard bore trapping column (i.d. 4.6 mm), separation on a narrow bore analytical column (i.d. 2 mm), and detection by ion spray tandem mass spectrometry. The lower limits of quantification were 0.500 ng ml^{-1} for Ro 44-3888 in plasma and 1 ng ml^{-1} in urine.

The concentration-time data were analysed by non-compartmental methods. Peak concentration (C_{max}) and time of maximum concentration (t_{max}) were derived directly from the concentration-time curve. The area under the free plasma concentration-time curve over one dosing interval (12 h, $\text{AUC}(0,12 \text{ h})$), was calculated using the linear trapezoidal rule. The apparent systemic clearance after oral administration at steady state (CL_{ss}) was calculated as $\text{Dose}/\text{AUC}(0,12 \text{ h})$ and the average steady state concentrations (C_{ssav}) as $\text{AUC}(0,12 \text{ h})/12$. In addition, the amount of Ro 44-3888 excreted in urine was calculated and given as percent of the administered dose.

Pharmacodynamic study

Platelet aggregation was induced by two agonists: ADP or collagen. It was measured predose on day 1 (baseline), predose on day 7, and at 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, 48, 60 and 72 h after drug administration on day 7. For the sibraxifiban/ticlopidine/aspirin and ticlopidine/aspirin treatments, the measurement of platelet aggregation was continued at 24 h intervals until platelet aggregation returned to baseline levels. The blood sample was collected in citrate (1 part of 3.8% sodium citrate/9 parts of blood, v/v). The sample was centrifuged at room temperature for 10 min at 180g to produce the platelet-rich plasma (PRP). The platelet poor plasma (PPP) was obtained by centrifugation of the supernatant at 2000g for 15 min. The platelets in PRP were counted and the PRP was diluted with PPP to a concentration of 200×10^9 platelets l^{-1} . One PPP and one PRP sample (270 μl each) were aliquoted into two microcuvettes and incubated at 37°C for 3 min in a turbidimetric aggregometer (PAP-4 Aggregometer®, Alpha Laboratories Ltd, Hampshire, UK). After equilibration, a bar magnet was introduced into the PRP cuvette and set to a speed of 1000 rev min^{-1} . The calibration was set to 0% transmission with the reference (PRP) cuvette and to 100% with the blank (PPP) cuvette. The aggregation reaction was started by adding 30 μl of either agonist to a final concentration of 10 μM ADP (Sigma-Aldrich Chemical Co Ltd, Poole, UK) or 2 $\mu\text{g/ml}$ collagen (equine tendon, Horm collagen, Nycomed Ltd, Birmingham, UK). The aggregation reaction was followed for 10 min and the maximum transmission after ADP- or collagen-induced aggregation was measured. The percentage inhibition was obtained by subtracting from 100% the maximum transmission corrected for baseline after ADP- or collagen-induced aggregation.

Peak inhibition effect (E_{max}) and time of peak ($t_{E_{\text{max}}}$) were derived from the inhibition-time curve. The truncated area under the inhibition-time curve at time t_x ($\text{AUE}(0, t_x)$) was calculated using the linear trapezoidal method. The average inhibition over one dosing interval was calculated as $\text{AUE}(0,12 \text{ h})/12$.

IVY bleeding time

IVY bleeding time was measured predose on day 1 (baseline), predose on day 7, and at 6, 12, 24, 48 and 72 h after drug administration on day 7. For the sibraxifiban/ticlopidine/aspirin and ticlopidine/aspirin treatments, the measurement of IVY bleeding time was continued at 24 h intervals until it returned to baseline levels. A sphygmomanometer cuff was placed around the arm of the volunteer and inflated to a pressure of 40 mmHg. Three punctures were then made using the Autoclix® device

Figure 2 Mean free plasma concentration-time profiles of Ro 44-3888 after oral administration of sibrifiban (2 mg twice daily) alone (◆) or in combination (●) with ticlopidine (250 mg twice daily) and aspirin (80 mg twice daily) for 7 days. Data are expressed as mean $\pm$ standard deviation of the mean.

(Boehringer Mannheim UK Ltd, Diagnostics and Biochemicals, Bell Lane, Lewes, East Sussex, BN7 1LG) in the flexor aspect of the forearm and the blood was absorbed every 10–15 s with blotting paper without touching the wound, until bleeding ceased. The IVY bleeding time was measured as the median time to stop the blood flow from the three punctures. The maximum value of the IVY bleeding time and the sampling time when it occurred were noted. The prolongation from baseline was expressed as the ratio of the post dose bleeding time and the pre dose bleeding time.

Safety and tolerability

Safety parameters including vital signs, laboratory variables and any adverse events were monitored during the study and until platelet aggregation and bleeding time returned to baseline. Adverse events were ranked as mild, moderate, or severe and reported in the patient's case report form. A 12-lead ECG was recorded with the subject in the supine position at screening, predose on day 1 (baseline), predose on day 7, and at 6 h after drug administration on day 7.

Statistical analysis

The pharmacokinetic and pharmacodynamic parameters ($AUC(0,12\text{ h})$ for Ro 44-3888 and AUE for the inhibition of ADP- and collagen-induced platelet aggregation) were compared statistically for the various treatment groups using a one-way analysis of variance (ANOVA) to identify potential drug interactions between sibrifiban and ticlopidine/aspirin. The one-way ANOVA with the factor treatment was applied to $\ln(AUC(0,12\text{ h}))$ and $\ln(AUE)$ to estimate the contrasts $\tau_c - \tau_{\text{reference}}$, the between-subject

Figure 3 Inhibition of ADP-induced platelet aggregation after oral administration for 7 days of sibrifiban (2 mg twice daily) (◆), ticlopidine (250 mg twice daily)/aspirin (80 mg twice daily) (△), or the combination (●). Sibrifiban enhances the effect of ticlopidine/aspirin for the duration of a dosing interval (12 h). Data are expressed as mean $\pm$ standard deviation of the mean.

variance σ^2 and the 95% confidence intervals for these contrasts. The exponentiated estimates and confidence intervals were used to estimate the treatment effect ratios and the confidence intervals for the corresponding ratios of the untransformed values.

Results

Pharmacokinetics

Figure 2 shows the mean free plasma concentration-time profiles of Ro 44-3888 after oral administration of sibrifiban (2 mg twice daily) alone or in combination with ticlopidine (250 mg twice daily) and aspirin (80 mg twice daily) for 7 days. The pharmacokinetic results are presented in Table 1. The coadministration of ticlopidine/aspirin with sibrifiban did not appear to have an impact on the pharmacokinetic parameters derived from the free concentrations of Ro 44-3888. The geometric mean ratio for $AUC(0,12\text{ h})$ was 1.0 and the 95% confidence interval 0.82–1.22. The only difference was a higher intersubject variability in the sibrifiban group when compared to the combination treatment. The coefficient of variation (CV) corresponding to the mean of the $AUC(0,12\text{ h})$ of Ro 44-3888 was 27% for the sibrifiban treatment and 17% for the combination treatment. This difference could be due to a more variable body weight in the sibrifiban group (CV = 16%) than in other groups (CV = 10% in both ticlopidine/aspirin and sibrifiban/ticlopidine/aspirin groups), although differences in renal function may also induce variability [25].

Table 1 Pharmacokinetic parameters of Ro 44-3888 after oral administration of sibrafiban (2 mg twice daily) alone or in combination with ticlopidine (250 mg twice daily) and aspirin (80 mg twice daily) for 7 days. Parameters are derived from free plasma concentrations.

Parameter	Treatment	
	Sibrafiban (n = 12)	Sibrafiban + ticlopidine/aspirin (n = 11)
$C_{\max}$ (ng ml ⁻¹)	9.05 ± 2.35	8.97 ± 2.15
$t_{\max}$ (h)	5.4 ± 2.6	5.5 ± 3.0
AUC(0,12 h) (ng ml ⁻¹ h)	74 ± 20	72 ± 12
CL _{ss} (l h ⁻¹)	25.9 ± 7.0	25.4 ± 4.3
C_{ssav} (ng ml ⁻¹)	6.1 ± 1.6	6.0 ± 1.0
Urinary excretion*	57.0 ± 8.5 (n = 11)	63.1 ± 9.5

Values are expressed as mean ± standard deviation of the mean.

$C_{\max}$: maximal plasma concentration (mean of the maximal values observed for each volunteer).

$t_{\max}$: time of maximal plasma concentration.

AUC(0,12 h): area under the concentration-time curve from time 0 to 12 h.

CL_{ss}: apparent systemic clearance after oral administration at steady state.

C_{ssav} : average steady state concentration.

*as a percentage of the dose of RO 44-3888.

Pharmacodynamics

The pharmacodynamic effects of sibrafiban were investigated following the last of 7 twice daily administrations of sibrafiban (2 mg twice daily) alone or in combination with ticlopidine (250 mg twice daily) and aspirin (80 mg twice daily). Figures 3 and 4 show the inhibition of the platelet aggregation responses to ADP or collagen after oral administration of sibrafiban, ticlopidine/aspirin or both treatments in combination. The pharmacodynamic results are presented in Tables 2 and 4. The results of the statistical analysis for treatment comparison are shown in Table 3.

The average inhibition of ADP-induced platelet aggregation over 12 h was 42% in the sibrafiban treated

group, 55% in the ticlopidine/aspirin group and 69% in the sibrafiban/ticlopidine/aspirin group. Similar maximum inhibitions were observed for both the sibrafiban ($E_{\max} = 65\% \pm 18\%$) and the ticlopidine/aspirin ($E_{\max} = 68\% \pm 7.5\%$) groups. Therefore, the large geometric mean ratios of AUE(0,12 h) and AUE(0,24 h) comparing sibrafiban mono treatment with sibrafiban/ticlopidine/aspirin treatment (see Table 3) reflect mainly the longer duration of the effect of the triple drug combination, which was due to ticlopidine/aspirin.

The comparison of ticlopidine/aspirin with sibrafiban/ticlopidine/aspirin reveals the additive effect of sibrafiban on the inhibition of ADP-induced platelet aggregation for the duration of approximately 12 h. The geometric mean ratio for AUE(0,12 h) was 1.26 (95% CI 0.86–1.85), while

Table 2 Pharmacodynamic parameters derived from the curves of inhibition of ADP- and collagen-induced platelet aggregation *vs* time obtained after oral administration for 7 days of sibrafiban (2 mg twice daily) or ticlopidine (250 mg twice daily)/aspirin (80 mg twice daily) alone or in combination.

Parameter	Treatment		
	Sibrafiban (n = 12)	Ticlopidine + aspirin (n = 12)	Sibrafiban + ticlopidine + aspirin (n = 11)
<i>ADP-induced platelet aggregation</i>			
AUE(0,12 h) (% inhibition h)	503 ± 206	660 ± 119	833 ± 158
AUE(0,24 h) (% inhibition h)	825 ± 412	1252 ± 263	1292 ± 336
Average inhibition over 12 h (%)	42 ± 17	55 ± 10	69 ± 13
$E_{\max}$ (%)	65 ± 18	68 ± 7.5	89 ± 53
$tE_{\max}$ (h)	5.5 ± 4.1	37 ± 60	5.7 ± 1.3
<i>Collagen-induced platelet aggregation</i>			
AUE(0,12 h) (% inhibition h)	396 ± 293	962 ± 135	996 ± 159
AUE (0,24 h) (% inhibition h)	677 ± 521	1852 ± 370	1594 ± 430
Average inhibition over 12 h (%)	33 ± 24	80 ± 11	83 ± 13
$E_{\max}$ (%)	63 ± 33	90 ± 9	100 ± 0
$tE_{\max}$ (h)	10.3 ± 9.6	7.5 ± 5.3	3.6 ± 2.6

Table 3 Geometric mean ratios and 95% confidence intervals for the comparison of the pharmacodynamic parameters AUE(0,12 h) and AUE(0,24 h) derived from the inhibition of ADP- and collagen-induced platelet aggregation *vs* time curves for treatment C (sibrafiban + ticlopidine + aspirin) versus treatment A (sibrafiban) and treatment C (sibrafiban + ticlopidine + aspirin) versus treatment B (ticlopidine + aspirin).

	Geometric mean ratio C/A	Geometric mean ratio C/B
<i>ADP-induced platelet aggregation</i>		
AUE(0,12 h) ratio	1.90 [1.29–2.79]	1.26 [0.86–1.85]
AUE(0,24 h) ratio	1.88 [1.15–3.06]	1.01 [0.62–1.65]
<i>Collagen-induced platelet aggregation</i>		
AUE(0,12 h) ratio	4.07 [2.14–7.76]	1.03 [0.54–1.96]
AUE(0,24 h) ratio	3.51 [1.91–6.46]	0.84 [0.46–1.54]

it was only 1.01 for AUC(0,24 h) (95% CI 0.62–1.65, see also Table 3).

When platelet aggregation was induced by collagen, the average inhibitions over 12 h were 33% in the sibrafiban group, 80% in the ticlopidine/aspirin group and 83% in the sibrafiban/ticlopidine/aspirin group. Because the effect of ticlopidine/aspirin alone on the collagen-induced platelet aggregation was already relatively large, the addition of sibrafiban had little supplementary effect. The geometric mean ratios obtained from the comparison of the sibrafiban/ticlopidine/aspirin treatment to the ticlopidine/aspirin treatment indicate 'equality' of the treatments (Table 3). The very large geometric mean ratios resulting from the comparison of sibrafiban mono treatment with the triple drug combination reflect the lower effect and the shorter duration of the effect of sibrafiban mono therapy on collagen-induced inhibition of platelet aggregation.

The bleeding time (Table 4) was prolonged by ticlopidine/aspirin (8.1 min) and sibrafiban/ticlopidine/aspirin (8.6 min) but remained in the normal range (0.5–4 min) with sibrafiban (3.5 min). As before, the inter-subject variability of the pharmacodynamic parameters was higher in the sibrafiban group than in the ticlopidine/aspirin or combination groups.

Safety and tolerability

The number of subjects reporting adverse events was similar in each treatment group (sibrafiban: 10/12; ticlopidine/aspirin: 9/12; sibrafiban/ticlopidine/aspirin:

8/11), and there was a similar number of adverse events in each group (sibrafiban: 16 events; ticlopidine/aspirin: 18 events; sibrafiban/ticlopidine/aspirin: 19 events). However, the incidence of bleeding events (bruising, epistaxis, and other events, like bleeding while shaving) was higher in the ticlopidine/aspirin (8 events) and sibrafiban/ticlopidine/aspirin (4 events) groups than with sibrafiban alone (2 events). The adverse events reported in all treatments were considered as mild (94%) or moderate (6%). There were no clinically relevant changes in vital signs, body weight or ECG during this study.

Discussion

This study was conducted as an open-label, randomized parallel group trial involving 38 healthy male volunteers with the aim to detect potential interactions between the oral GPIIb/IIIa antagonist sibrafiban and the ticlopidine/aspirin combination. There were two reasons to suspect a pharmacokinetic interaction between sibrafiban and ticlopidine/aspirin. First, renal excretion is the main route of elimination for both Ro 44–3888 (unpublished data) and 60% of the ticlopidine metabolites [8, 26]. However, the lack of impact of the coadministration of ticlopidine/aspirin on the pharmacokinetics of Ro 44–3888 suggests that the ticlopidine metabolites and Ro 44–3888 are eliminated by different pathways in the kidney or that the capacity of a common elimination pathway is sufficient for both drugs. The potential influence of sibrafiban and its metabolites on the pharmacokinetics of ticlopidine was not assessed in this study because there is

Table 4 IVY bleeding time after oral administration of sibrafiban, ticlopidine/aspirin, or the combination for 7 days.

Parameter	Treatment		
	Sibrafiban (n = 12)	Ticlopidine + aspirin (n = 12)	Sibrafiban + ticlopidine + aspirin (n = 11)
Max IVY bleeding time (min)	3.5 ± 0.6	8.1 ± 6.6	8.6 ± 0.2
Max bleeding time prolongation from base line	1.8 ± 0.4	4.6 ± 4.4	4.8 ± 1.4

Values are expressed as mean ± standard deviation of the mean.

Figure 4 Inhibition of collagen-induced platelet aggregation after oral administration for 7 days of sibrافiban (2 mg twice daily) (◆), ticlopidine (250 mg twice daily)/aspirin (80 mg twice daily)(△), or the combination (●). Because of the large effect of ticlopidine/aspirin on collagen-induced platelet aggregation, the interaction with sibrافiban is less noticeable than for ADP-induced platelet aggregation. Data are expressed as mean $\pm$ standard deviation of the mean.

no direct correlation between the antiplatelet effect and the plasma concentrations of ticlopidine [8]. The active principle of ticlopidine is not known, but the slow onset and long duration of action point to a compound with a long half-life. The additive effect of sibrافiban on the inhibition of platelet aggregation was restricted to approximately one dosing interval and there was no obvious prolongation of the effect when sibrافiban was coadministered to ticlopidine/aspirin. Therefore, a pharmacokinetic interaction leading to higher concentrations of a ticlopidine metabolite as reason for the pharmacodynamic interaction is unlikely.

The second potential source of interaction was the inhibition by aspirin of the reduction of Ro 48-3656 to Ro 44-3888, a phenomenon observed *in vitro* at very high aspirin concentrations (unpublished data). However, *in vivo*, the similarity in the pharmacokinetic parameters of Ro 44-3888 for both groups suggests that the coadministration of aspirin and/or ticlopidine does not affect the biotransformation of sibrافiban to the active drug at the administered aspirin dose of 80 mg twice daily. At the subtherapeutic doses of sibrافiban used in this study, the coadministration of ticlopidine and aspirin has no effect on the pharmacokinetics of Ro 44-3888, but the effect of higher doses on the mechanisms presented above is not known yet.

Pharmacodynamic interaction was expected between aspirin, ticlopidine and Ro 44-3888 because all three drugs inhibit platelet aggregation with different mechanisms. Aspirin inhibits the synthesis of prostaglandins [6], which are the main agonists of collagen-induced platelet

aggregation. Consequently, aspirin inhibits collagen-induced platelet aggregation potently but it has only a modest effect on ADP-induced platelet aggregation. In contrast, the main effect of ticlopidine is a potent inhibition of ADP-induced platelet aggregation. Although the mechanism of action of ticlopidine is not completely understood, it may involve interaction with signal transduction pathways specific to ADP [27]. In this study, ticlopidine and aspirin were administered together to reproduce the clinical situation of stent patients and the effects of the single compounds could not be directly distinguished. However, since ticlopidine has a longer duration of action than aspirin and the inhibition-time curves of ADP-induced aggregation declined more slowly than the inhibition-time curves of collagen-induced aggregation, it can be concluded that ticlopidine provided the main effect on the inhibition of ADP-induced aggregation whereas the main effect of aspirin was the inhibition of collagen-induced aggregation.

Ro 44-3888, the active metabolite of sibrافiban, is a blocker of the final common mediator of platelet aggregation, the GPIIb/IIIa receptor. As such, it is expected to inhibit platelet aggregation induced by all agonists. In this study, the subtherapeutic dose of sibrافiban (2 mg twice daily) inhibited both ADP- and collagen-induced aggregation. Similar levels of maximum inhibition (E_{max}) of ADP-induced platelet aggregation were achieved with this low dose of sibrافiban and with a therapeutic dose of ticlopidine/aspirin. This finding suggests that therapeutic doses of sibrافiban (3–6 mg twice daily) may result in a higher antiplatelet effect than therapeutic doses of ticlopidine/aspirin. The average inhibition of ADP-induced platelet aggregation over 12 and 24 h as well as the AUE parameters were higher for the ticlopidine/aspirin treatment than for the sibrافiban mono-treatment. This was most likely due to the prolonged effect of the ticlopidine/aspirin treatment.

When used in combination with ticlopidine and aspirin, sibrافiban enhanced the effect of ticlopidine/aspirin on the inhibition of ADP- and to a lesser extent collagen-induced platelet aggregation for the duration of a dosing interval (12 h). This additive effect was evident for E_{max} , the average inhibition over 12 h, and AUE(0,12 h), but it was less pronounced for AUE(0,24 h). These observations suggest that sibrافiban does not lead to a prolonged effect on aggregation in the presence of ticlopidine/aspirin and are in agreement with the pharmacokinetic results.

The bleeding time remained in the normal range during sibrافiban monotherapy but it more than doubled during the ticlopidine/aspirin and sibrافiban/ticlopidine/aspirin treatments. Consequently, the incidence of minor bleeding events (bruising or epistaxis) was higher in the ticlopidine/aspirin and sibrافiban/ticlopidine/aspirin treatments than with sibrافiban alone. This finding

supports the concept that there is a correlation between the incidence of nuisance bleeding and the prolongation of the bleeding time. The bleeding risk associated with sibrifiban, which is low at the subtherapeutic dose used in this study, is likely to increase at therapeutic doses, particularly if administered in combination with ticlopidine/aspirin.

Conclusion

The availability of GPIIb/IIIa antagonists has opened a new therapeutic approach to the treatment of cardiovascular disease. However, the safety and efficacy of long-term GPIIb/IIIa inhibition remains to be proven. In this study, the oral GPIIb/IIIa antagonist sibrifiban, administered at a subtherapeutic dose in healthy men, substantially inhibited ADP- and collagen-induced platelet aggregation. Sibrifiban was well tolerated and no severe adverse events were reported. Co-administration of ticlopidine/aspirin with sibrifiban did not affect the pharmacokinetics of the active compound Ro 44-3888. However, this study shows a significant pharmacodynamic interaction between sibrifiban and ticlopidine/aspirin. Consequently, the simultaneous administration of sibrifiban and ticlopidine/aspirin should be carefully monitored to ensure the patient's coverage with an antiplatelet drug without exposure to an excessive bleeding risk.

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