The influence of selective thromboxane synthetase inhibition with a novel imidazole derivative, UK-38,485, on prostanoid formation in man

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ABSTRACT UK-38,485, a novel imidazole derivative, was used in two clinical trials with healthy male subjects to study the influence of thromboxane synthetase inhibition on prostanoid formation. In an open pharmacokinetic study, UK-38,485 administered orally in doses of 10, 20, 40, 60, and 100 mg significantly reduced serum thromboxane (TXB2) concentrations. With lower doses (10 and 20 mg) peak inhibition of serum thromboxane occurred 2 hr after dosing, with a mean percentage inhibition of 78% and 91%, respectively. For the higher doses (40, 60, and 100 mg) peak inhibition exceeded 99% 1 hr after dosing. After 8 hr the inhibition was dose related, ranging between 59% and 75%, and after 24 hr between 0 and 35%. In a second multiple-dose, double-blind, placebo-controlled, cross-over study, 50 mg UK-38,485 given twice daily for 1 week selectively inhibited thromboxane synthetase. The excretion of 2,3-dinor-TXB2, the major urinary metabolite of endogenously formed thromboxane, was significantly reduced, whereas the urinary excretion of 2,3-dinor-6-keto-PGF1α, the main metabolite of endogenous prostacyclin, and the plasma concentrations of 6-keto-PGF1α showed no significant increases compared with levels in the placebo period. In platelet suspensions stimulated ex vivo with arachidonic acid and in serum of incubated whole blood, TXB2 concentrations were reduced and a significant redirection of endoperoxide metabolism to antiaggregatory and vasodilatory prostaglandins I2, E2, and D2 was demonstrated after the influence of UK-38,485. Platelet lipoxygenase metabolites were not measurably altered. The drug was well tolerated. In both studies, no clinically relevant changes in laboratory safety and hemodynamic parameters, bleeding, or clotting time were observed. From the time course of the plasma drug concentrations, the inhibition of thromboxane synthetase, and the redirection of endoperoxide metabolism it can be concluded that UK-38,485 is rapidly absorbed and has a long-lasting effect on prostanoid formation.

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THROMBOXANE A2 (TXA2) and prostaglandin (PG) I2 exert opposite effects on platelet aggregation and vascular resistance, and the balance between these compounds has been proposed to be one of the factors that determine platelet reactivity, endothelial thrombogenesis, and vascular tone.1 Increased thromboxane formation has been found to be associated with various conditions such as vasospasm and platelet aggregation in coronary heart disease,2-4 vasoconstriction in hydronephrotic kidneys,5 incipient renal graft rejection,6 severe septic shock associated with acute respiratory distress syndrome,7 pulmonary embolism,8 and allergic bronchospasm.9 Selective inhibition of thromboxane synthetase and the redirection of endoperoxide metabolism toward PGI2, PGE2, and PGD2, will possibly offer a new therapeutic approach to those disease states in which increased thromboxane formation is critically involved.

Ex vivo and in vitro studies with thromboxane synthetase inhibitors5,10-12 have demonstrated that these compounds selectively inhibited thromboxane formation and partially redirected endoperoxide metabolism.13,14 However, almost no data are available to determine whether thromboxane synthetase inhibition also redirects endoperoxide metabolism under basal in vivo conditions in man. This study reports on the pharmacokinetics and the biochemical effects ex vivo and in vivo of a novel imidazole derivative, UK-38,485,15 in healthy male subjects.

The potency and specificity of action of this drug were examined by measuring formation of TXB2 and other cyclooxygenase products in serum after incubation of whole blood, by monitoring cyclooxygenase and lipoxygenase products in platelet suspensions after...
stimulation with exogenous arachidonic acid, and by analyzing the major metabolites of endogenously formed TXA₂ and PGI₂ in urine.

Subjects and methods

All subjects gave their written informed consent according to the German Drug Law.

Single-dose study. The pharmacokinetic profile and the potency of orally administered UK-38,485 were determined in an open single-dose study in 10 healthy male subjects, ages 18 to 50 years and weighing between 60 and 90 kg. Subjects were studied in pairs receiving doses of 10, 20, 40, 60, and 100 mg of the compound. Subjects with abnormalities of pre-study laboratory data (including complete blood count, clinical chemistry, kidney and liver parameters, and electrocardiograms) were excluded from the study. Hospitalized subjects fasted for 10 hr before the beginning of the trial. Heart rate and blood pressure were measured at 30 min intervals and venous blood was drawn just before and at 0.5, 1, 2, 3, 4, 6, 8, and 24 hr after ingestion of the drug for determination of serum TXB₂ and plasma drug concentrations.

Multiple-dose study. UK-38,485 (50 mg) or identical placebo capsules were administered twice daily (8 A.M., 8 P.M.) in a double-blind, randomized cross-over design to 12 hospitalized healthy male subjects, ages 18 to 30 years and weighing between 60 and 90 kg, for 18 days. Each treatment period lasted for 6 days and was interrupted by a washout period of 8 days. Laboratory safety parameters, including a complete blood count, liver and kidney function, and postprandial blood glucose concentrations, were determined before and every other day during the treatment period. For the determination of TXB₂, PGE₂, and 6-keto-PGF₁α in serum, blood samples were taken before and on each day of the treatment periods 1 hr after drug administration. Suspensions of washed platelets were prepared in Tris buffer (50 mM, pH 7.4) before drug administration, on the sixth day of each treatment period 1 hr after dosing, and 12 hr after the last dose, and conversion products of arachidonic acid via the cyclooxygenase and lipooxygenase pathways were determined. Urinary excretion rates of 2,3-dinor-TXB₂ and of 2,3-dinor-6-keto-PGF₁α and plasma concentrations of 6-keto-PGF₁α were determined before treatment and on the sixth day of the treatment periods; plasma 6-keto-PGF₁α was also measured 12 hr after the last dose.

Materials and analytical methods. UK-38,485, 3-(1H-imidazol-1-yl-methyl)-2-methyl-1H-indole-1-propanoic acid, was synthesized by Pfizer Central Research (Sandwich, Kent, England). The drug was provided as capsules containing 5, 20, and 50 mg of the substance. Unlabeled prostaglandin standards and 19, 19', 20, 20'-H,2,3-dinor-6-keto-PGF₁α, 20'-H,2,3-dinor-6-keto-PGF₁α, and 2,3-dinor-6-keto-PGF₁α were kindly supplied by Dr. J. Pike Upjohn Co. (Kalamazoo, MI); 1H-6-keto-PGF₁α, 3H-TXB₂, 3H-PGF₁α, 1H-PGF₁α, and 14-C-C20:4 were obtained from New England Nuclear (Boston); Sep-pak C₁₈ cartridges and μ-Bondapak C₁₈ reverse-phase high-performance liquid chromatography (HPLC) columns were obtained from Waters Associates; XAD-2 Amberlite was obtained from Serva (Heidelberg, F.R.G.); and lorglone was obtained from Eastman Kodak (Rochester, NY). All other solvents and thin-layer chromatographic silica gel plates were purchased from Merck (Darmstadt, F.R.G.).

Radioimmunologic determination of TXB₂, 6-keto-PGF₁α, PGE₂, and PGF₂α in serum. For determination of serum TXB₂, 4 ml of blood was transferred to a glass tube, incubated for 60 min at 37°C, and centrifuged at 2000 g for 10 min; the serum was stored at −20°C until assayed. TXB₂ concentrations were measured by radioimmunoassay (RIA) with a specific and sensitive TXB₂ antiserum (a gift of Dr. L. Levine, Brandeis University, Waltham, MA) as described previously. In brief, the TXB₂ antiserum was used at a final dilution of 1:190,000. The binding of 3H-TXB₂ tracer was 29 ± 4% (n = 21), the 50% intercept of standard curves was 9 ± 1 pg (n = 21), and the lower limit of detection was 0.8 ± 0.15 pg. The relative cross reactions of the TXB₂ antiserum to 6-keto-PGF₁α, PGE₂, PGE₁, HHT, and 12-HETE were below 0.01%, 0.5% to PGD₂, and 50% to 60% to 2,3-dinor-TXB₂.

Cyclooxygenase metabolites were monitored in pooled serum from incubated blood. For this purpose, 1 ml of serum was extracted with Sep-pak C₁₈ cartridges and the extract was fractionated on reverse-phase HPLC columns with H₂O:acetonitrile/acidic acid (740:60:2; v:v) as the solvent system. The fractions were screened for immunoreactivity with antiserum against TXB₂, 6-keto-PGF₁α, PGE₂, and PGF₂α.

Determination of urinary 2,3-dinor-TXB₂ and 2,3-dinor-6-keto-PGF₁α and of plasma 6-keto-PGF₁α. The 24 hr excretion of 2,3-dinor-TXB₂ was measured by RIA with 3H-TXB₂ as a tracer and TXB₂ antiserum, which showed a 50% to 60% cross reaction with authentic 2,3-dinor-TXB₂. After extraction of 30 ml of acidified urine with Sep-pak C₁₈ cartridges, 2,3-dinor-TXB₂ and TXB₂ were separated on reverse-phase HPLC columns with a solvent system described previously. At a HPLC flow rate of 2 ml/min, 2,3-dinor-TXB₂ eluted between 7 and 11 min and TXB₂ between 16 and 24 min from the column. The 24 hr excretion of 2,3-dinor-6-keto-PGF₁α was determined by combined gas chromatography–mass spectrometry with 60 ml of urine according to the method of Falaradeau et al. 19, 19', 20, 20'-H,2,3-dinor-6-keto-PGF₁α was used as internal standard. Ion pairs m/z 570/574 (M⁺-31) and m/z 480/484 (M⁺-90-31) were monitored. The quantitative analysis was based on standard curves, which were shown to be linear in the observed range.

For determination of plasma 6-keto-PGF₁α levels, venous blood (50 ml) was drawn on EDTA/meclofenamate (4 mM/40 µM final concentration), chilled on ice, and centrifuged at 3000 g for 20 min. The obtained plasma (20 ml) was equilibrated with 3H-6-keto-PGF₁α, acidified with formic acid to pH 3.2, applied to a XAD-2 Amberlite column, and washed with distilled water and n-heptane. Prostanoids were then eluted with methanol and separated by thin-layer chromatography (TLC) with ethylcetate-isooctane/acidic acid:H₂O (110:50:20:100; v:v; organic phase) as solvent system. The zone corresponding to authentic 6-keto-PGF₁α was further purified on reverse-phase HPLC columns. Fractions containing the tracer were analyzed with a specific 6-keto-PGF₁α RIA. In brief, the 6-keto-PGF₁α antiserum was used at a final dilution of 1:21,000, the binding of 3H-6-keto-PGF₁α tracer was 30 ± 3% (n = 21), the 50% intercept of standard curves was 54 ± 8 pg (n = 21), and the lower limit of detection was 7 ± 4 pg. The relative cross reactions of the 6-keto-PGF₁α antiserum to TXB₂, PGE₂, and 6, 15-diketo-13, 14-dihydro-PGF₁α were below 0.05% and were between 1.5% and 7% to PGD₂, 6-keto-PGF₁α, 2,3-dinor-6-keto-PGF₁α-lactone, and PGE₂.

Measurement of cyclooxygenase and lipooxygenase products in platelets. A 100 µl volume of platelet suspension (3 × 10⁸ platelets) were incubated with 0.5 µCi [14C]-C20:4 to give a final volume of 0.5 ml. After 6 min at 37°C the sample was acidified to pH 3.2 (1N HCl) and extracted with chloroform-methanol (2:2, v:v). The cyclooxygenase metabolites were separated by TLC in chloroform-methanol-acidic acid:H₂O (90:80:1:0.8, v:v) and the lipooxygenase metabolites in lorglone:diethylether:acidic acid (40:60:1, v:v). The metabolites were located by radio-scanning and identified with authentic standards. Respective zones were eluted and an aliquot of 1/6 was quantified by liquid scintillation counting.

Plasma drug concentrations. UK-38,485 plasma concentra-
Serum Concentration detectable. At half-life was 8 hr after absorption of the dose. The limit of detection of unchanged drug was 3 ng/ml.

FIGURE 1. Plasma drug concentrations after single doses of UK-38,485. Mean values from two subjects at each dose level. The asterisks at 8 hr after dosing are means of pairs in which one value of each was not detectable. At 24 hr no drug concentration was detectable. The dotted lines below the detection limit indicate that these data are extrapolated.

Results

The plasma drug concentrations after single doses of UK-38,485 are shown in figure 1. The maximal concentration was obtained 0.5 to 1 hr, indicating a rapid absorption for all dosages. Mean plasma elimination half-life was calculated to be 0.76 hr and was independent of the dose. Mean percentage urinary recovery of unchanged drug was 52.8% and was not dose dependent.

The dose-dependent reduction of serum TXB₂ concentrations is shown in figure 2. For the 10 and 20 mg doses, peak inhibition of TXB₂ occurred 2 hr after dosing, with a mean inhibition of 78% (from 180 to 40 ng/ml TXB₂) and 91% (from 142 to 12.5 ng/ml TXB₂), respectively. For 40, 60, and 100 mg, peak inhibition exceeded 99% (from 180 to 305 ng/ml to 1.5 to 2.5 ng/ml TXB₂) 1 hr after dosing. After 8 hr the inhibition was dose-related, ranging between 59% and 75%. By 24 hr TXB₂ concentrations had returned to 65% to 100% of control.

Extensive screening of laboratory parameters, including complete blood counts, liver and kidney function tests, bleeding and clotting time, and monitoring of hemodynamic parameters, showed no clinically relevant changes in comparison to predose data. Two subjects reported side effects. One suffered mild headache and dizziness after receiving 20 mg and one reported headache and tiredness after a 60 mg dose. It was uncertain whether the symptoms were drug related. Otherwise the drug was well tolerated.

In the subsequent multiple-dose study, inhibition of TXB₂ formation was about 65% 1 hr after the first dose (with a scattering between 0% and almost 100%), whereas at 1 hr after dosing on the following days of treatment, the inhibition of TXB₂ formation was almost complete (figure 3). Twelve hours after the last dose, TXB₂ serum concentrations had returned to about 50% of the baseline.

The selectivity of inhibition of TXB₂ formation and
concomitant redirection of platelet endoperoxide metabolism into PGE$_2$ and PGD$_2$, was demonstrated during treatment with UK-38,485 after ex vivo incubation of platelet suspensions with $^{14}$C-C20:4. No change in concentration of the platelet lipoxygenase product 12-HETE was observed (figure 4, table 1). On treatment with UK-38,485, formation of 6-keto-PGF$_{1α}$, PGE$_2$, and PGF$_{2α}$ was demonstrated in serum of incubated whole blood (table 2).

Urinary excretion of 2,3-dinor-TXB$_2$ was significantly reduced during treatment with UK-38,485, indicating effective inhibition of basal, endogenous thromboxane formation by this regimen (figure 5). The excretion of 2,3-dinor-6-keto-PGF$_{1α}$, the major urinary metabolite of endogenously formed PGI$_2$ increased slightly but not significantly. Plasma levels of 6-keto-PGF$_{1α}$ remained unchanged during treatment with UK-38,485 (figure 5).

Physical examination and extensive laboratory screening during the active and placebo treatment periods and thereafter revealed no drug-related side effects. One subject complained of tiredness and dizziness on 3 days of the active treatment period; another subject reported mild dizziness on 6 days of the active treatment period, 15 min after dosing. Otherwise, the drug was well tolerated.

### Discussion

In the single-dose study, single oral doses of UK-38,485 were rapidly absorbed. The plasma drug concentrations and the inhibition of TXB$_2$ formation were dose dependent. During the control stage, serum concentrations of TXB$_2$ ranged from 142 to 305 ng/ml and were in the same range as reported recently in normal subjects. With doses of 40 mg of UK-38,485 and higher, the TXB$_2$ concentrations in serum were reduced to 1.5 to 2.5 ng/ml 1 hr after dosing, a 95% to 99% reduction of TXB$_2$ formation. Compared with another imidazole derivative, dazoxiben, the time course of TXB$_2$ inhibition was prolonged and the effective doses were considerably lower (by a factor of 3 to 5). Although the plasma half-life is short (0.76 hr) and in most of the subjects plasma concentrations of UK-38,485 were barely detectable 8 hr after ingestion of the single doses, the simultaneously measured TXB$_2$ concentrations were still reduced by 60% to 70% of control (from 142 to 305 ng/ml to 55 to 98 ng/ml TXB$_2$; see figure 2) for doses higher than 40 mg. A possible explanation for this finding could be the accumulation of UK-38,485 in or on platelets or other blood cells.

After the first dose of the multiple-dose study (figure 3) the degree of inhibition of thromboxane synthesis showed a broad scattering (from 0 to almost 100% inhibition), suggesting interindividual variations of absorption of the drug. The almost complete inhibition of
TXB₂ formation in serum (1 to 3 ng/ml TXB₂) found 1 hr after dosing on the second day of treatment and thereafter probably results from the residual inhibition of thromboxane synthesis after the preceding dose.

The selectivity of inhibition of thromboxane synthetase by UK-38,485 and redirection of the metabolism of endoperoxides toward PGE₃ and PGD₂ were evident from experiments with platelet suspensions incubated ex vivo with ¹⁴C-C₂₀:₄ (figure 4, table 1). The unchanged 12-HETE concentrations found in platelet incubations showed that no overflow toward the 12-lipoxygenase pathway occurred (table 1). The concentrations of 6-keto-PGF₁α increased about 100-fold in serum obtained from whole blood incubated ex vivo after oral treatment with UK-38,485 (table 2). A similar increase has been previously reported in dogs.¹⁵ This finding suggests that during selective inhibition of thromboxane synthesis, white blood cells can effectively utilize accumulated platelet-derived endoperoxides. Indeed, previous in vitro experiments with selective thromboxane synthetase inhibitors have demonstrated diversion of endoperoxide metabolism to PGF₂α and PGE₂ in lung, spleen, and platelets and to PGI₃ in platelet-endothelial cell mixtures and in incubated whole blood.¹³ This increased formation of 6-keto-PGF₁α in whole blood could occur in monocytes, which have been shown to possess a considerable capacity to synthesize PGI₃ from endoperoxides.²⁵ Furthermore the obtained data are comparable with the results of a recent study demonstrating increased ex vivo formation of 6-keto-PGF₁α after selective inhibition of thromboxane synthetase with dazoxiben in patients with angina pectoris and in healthy subjects.

In addition to these ex vivo experiments, we investigated whether selective inhibition of thromboxane synthetase by UK-38,485 induces a measurable reorientation of basal, endogenous endoperoxide metabolism. The plasma concentrations of 6-keto-PGF₁α were very low under basal conditions and comparable to values reported recently.¹⁸ The basal urinary excretion of 2,3-dinor-6-keto-PGF₁α was in the same range as reported recently.²⁰ Both 6-keto-PGF₁α in plasma and 2,3-dinor-6-keto-PGF₁α in urine were found to be unchanged or only slightly increased during the treatment with UK-38,485. However, the excretion of 2,3-dinor-TXB₂ was significantly reduced, indicating effective inhibition of basal endogenous thromboxane formation. Thus, under this regimen a measurable redirection of endogenously formed (probably platelet-derived) endoperoxides into PGI₃ does not occur in vivo in healthy subjects, presumably devoid of increased platelet aggregation and elevated thromboxane formation. In support of this, alterations of hemodynamic parameters, indicating enhanced systemic production of PGI₃, were not observed.

In conclusion, the data obtained in this study show that UK-38,485 is an orally active and selective inhibitor of thromboxane synthetase. The pharmacokinetic profile and the biochemical effects indicate that this novel imidazole derivative has a greater potency and longer duration than a similar compound, dazoxiben, currently being evaluated in man.¹²,¹⁴,²⁴ The absence of any clinically relevant side effects of this imidazole derivative in conjunction with its attractive therapeutic potential in those clinical conditions that may result from elevated intrinsic thromboxane formation should emerge further study of this compound.

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