Short-term effects of ridogrel, a combined thromboxane synthase inhibitor and receptor antagonist, were investigated in 16 patients with uncomplicated essential hypertension. After a 2-week placebo period without antihypertensive medication, patients were admitted to the hospital overnight on two occasions 3 weeks apart. On each occasion, they received two doses of either placebo or ridogrel (300 mg) 12 hours apart according to a double-blind crossover protocol. Renal and systemic thromboxane A₂ and prostacyclin biosynthesis were investigated by measuring urinary excretion of thromboxane B₂, 6-oxo-prostaglandin F₁α, and their respective 2,3-dinor metabolites using gas chromatography/mass spectrometry. Responses of platelets to a thromboxane A₂ mimetic and to adenosine diphosphate were studied turbidometrically. Blood pressure was measured automatically at 20-minute intervals. Ridogrel reduced excretion of 2,3-dinor-thromboxane B₂ and thromboxane B₂ compared with placebo (21±6 versus 279±28 and 14±4 versus 39±9 ng/g creatinine, respectively; P<.0001 and P<.05). Excretion of 2,3-dinor-6-oxo-prostaglandin F₁α and 6-oxo-prostaglandin F₁α was increased by ridogrel compared with placebo (184±20 versus 146±11 and 86±9 versus 58±6 ng/g creatinine, respectively; P<.05). Ridogrel selectively antagonized platelet aggregation to the thromboxane mimetic (P<.0001). Blood pressure did not differ significantly between ridogrel and placebo treatment periods. Thus, in patients with essential hypertension, acute administration of ridogrel reduces renal and extrarenal thromboxane A₂ biosynthesis, increases renal and extrarenal prostacyclin biosynthesis, inhibits thromboxane receptor-activated platelet aggregation, but has no effect on systemic arterial pressure. (Hypertension 1993;22:197-203)

**KEY WORDS** • receptors, thromboxane • thromboxanes • prostacyclin • hypertension, essential • blood platelets • spectrum analysis, mass

and is a principal cause of excess mortality in patients with mild to moderate hypertension.⁶ TXA₂ is produced by activated platelets, macrophages, and endothelial cells in renal glomeruli. It is a potent vasconstrictor and stimulates platelet aggregation.⁶ Conversely, PGI₂ is natriuretic, a vasodilator, and a potent inhibitor of platelet aggregation.⁹ It is synthesized by endothelial and other cells and has been implicated in protection from thrombosis.⁹-¹¹ An imbalance between PGI₂ and TXA₂ biosynthesis in patients with hypertension could increase peripheral vascular resistance and blood pressure through effects on both vascular smooth muscle and the kidney and, furthermore, predispose such patients to arterial thrombosis.

The least invasive and most reliable method of assessing PGI₂ and TXA₂ biosynthesis in vivo is to measure urinary excretion of their hydrolys products, 6-oxo-PGF₁α and TXB₂, and 2,3-dinor metabolites. 6-Oxo-PGF₁α and TXB₂ are thought to be derived primarily from PGI₂ and TXA₂ biosynthesis in the kidney, whereas 2,3-dinor-6-oxo-PGF₁α and 2,3-dinor-TXB₂ reflect extrarenal systemic biosynthesis.¹² Direct evidence for a role for PGI₂, TXA₂, or both in the pathogenesis of hypertension has been sought by measuring these products. Urinary excretion of 2,3-dinor-6-oxo-PGF₁α by the salt-sensitive Dahl rat is reduced compared with normotensive control rats, and this abnormality precedes the development of hypertension.¹³ However, any abnormality in PGI₂ and TXA₂ biosynthesis in human
hypertension has proved to be more difficult to assess. Whereas increased TXB2 excretion in patients with essential hypertension has been reported,14 other studies have found no such difference between hypertensive and normotensive individuals.1516 We found that mean excretion of 2,3-dinor-6-oxo-PGF1α and 6-oxo-PGF1α is similar in patients with mild essential hypertension and in normotensive subjects but that in hypertensive patients there is a negative correlation between blood pressure and these products of PG1 biosynthesis. This suggests that in hypertensive patients there is an impairment in PGI1 production that determines, in part, the extent to which blood pressure becomes elevated.15

Drug treatment that increases endogenous PGI1 biosynthesis could therefore lower blood pressure in hypertensive patients, especially if this increase were accompanied by a reduction in endogenous TXA2 production, action, or both. Such treatment could also influence blood pressure in normotensive subjects. However, the magnitude of the effect of hypotensive drug treatment on blood pressure is directly related to the initial pressure before drug administration.17 In particular, although intravenous administration of PGI1 lowers arterial pressure in patients with hypertension, it has no significant effect on mean arterial blood pressure of normotensive subjects.18 In the present investigation, we therefore studied hypertensive patients in whom a hypotensive effect would be easier to detect than in normotensive subjects. Furthermore, it is possible that modification of the balance between PGI1 and TXA2 in hypertensive patients could reduce the excess risk of coronary thrombosis that such patients experience. The purpose of the present study therefore was to investigate the short-term effects of ridogrel, a combined TX synthase inhibitor and TXA2 receptor antagonist,1920 in patients with mild essential hypertension to determine whether an alteration in the balance between PGI1 and TXA2 biosynthesis is accompanied by a change in blood pressure in these patients.

Methods

Subjects and Protocol

Patients with uncomplicated essential hypertension attending the Guy's Hospital hypertension clinic were invited to take part in the study, which had the approval of the Lewisham and North Southwark Ethics Committee. Each patient had been diagnosed as hypertensive after three or more office readings of diastolic pressure greater than or equal to 100 mm Hg. Secondary causes of hypertension and evidence of end-organ damage were sought by history, physical examination, electrocardiogram, and laboratory testing including urinalysis, plasma creatinine, and electrolytes and, when clinically relevant, determination of urinary vanillylmandelic acid excretion and renal imaging. Patients with a history of peptic ulceration or bleeding disorder or of serious renal or hepatic disease and those requiring regular medication other than antihypertensive drugs were excluded. Each patient gave written informed consent. After routine screening tests, placebo tablets were prescribed on a single-blind basis to be taken at 8:30 AM and 8:30 PM for 2 weeks, and antihypertensive medication was discontinued. Patients were given specific advice to avoid aspirin-containing products or other medications; this advice was reinforced at each subsequent visit. Blood pressure and heart rate were measured weekly using a Dinamap (Critikon, Ascot, Berkshire, UK) automatic recorder, after patients had been seated 5 minutes in a quiet room. If the diastolic arterial pressure (mean of three readings) was greater than 120 mm Hg, the patient was withdrawn from the study and antihypertensive medication begun again.

After 2 weeks of placebo treatment, 16 patients with diastolic pressures between 90 and 120 mm Hg inclusive were studied further. Their physical characteristics are summarized in the Table. Each patient was admitted to a single-bedded hospital room for 2 days. They were asked to void urine immediately before dosing with placebo at 8:30 AM, and all urine passed during the subsequent 12-hour baseline period was collected. Urine was voided on inclination and at the end of the collection period. An ambulatory monitor (Accutrack II, Suntek, New Brunswick, NJ)21 was used to measure blood pressure automatically throughout each 12-hour period. Subjects sat quietly in their rooms from 8:30 AM to 8:30 PM. They were instructed to sit with their arm relaxed and flexed at the elbow, with the forearm supported on the arm of the chair during cuff inflation and deflation. They remained seated except for visits to the toilet. The ambulatory monitor was programmed to record every 20 minutes during the 12-hour period and to repeat the measurement once if an error code indicated possible artifacts due to arm motion or weak electrocardiographic or Korotkoff signals. Error-coded readings were edited if pulse pressure was 10 mm Hg or lower, if systolic blood pressure was 40 mm Hg or lower or 255 mm Hg or higher, or if diastolic blood pressure was less than or equal to 40 or greater than or equal to 140 mm Hg without any other readings in these ranges. At 8:30 PM, immediately after completing the 12-hour baseline urine collection and removing the ambulatory monitor, patients received an oral dose of either placebo or ridogrel (300 mg) according to a randomized double-blind protocol. Patients spent the night in the hospital room and at 8:30 AM the following morning received a second identical dose of either placebo or ridogrel. The blood pressure recorder was reapplied, and a second 12-hour period of urine collection and blood pressure monitoring from 8:30 AM to 8:30 PM was begun. Patients were then discharged from the hospital. They continued to take placebo on a single-blind basis at 8:30 AM and 8:30 PM each day for a 3-week washout period. They were then readmitted to the hospital for 2 days as before and received either ridogrel or placebo.
on a crossover basis. The randomization code was prepared and held by the Janssen Research Foundation until the study was completed in all 16 subjects. Anti-hypertensive medication was begun again on final discharge.

On each baseline and experimental day, blood was obtained at 10:30 AM from an antecubital vein using a 19-gauge needle for platelet aggregation studies and determination of plasma ridogrel and serum TXB₂ concentrations in addition to routine biochemical and hematological determinations.

Patients were questioned each day in the hospital about symptoms and any possible adverse effects. They were seen for a final assessment (history, physical examination, hematology, and biochemistry screens and urinalysis) 2 to 3 weeks after admission.

Analysis
A well-mixed sample of urine (50 mL) was stored at −20°C until analyzed. 6-Oxo-PGF₁α, TXB₂, 2,3-dinor-6-oxo-PGF₁α, and 2,3-dinor-TXB₂ were assayed with immunoaffinity chromatography and gas chromatography/electron-capture mass spectrometry as described elsewhere." Briefly, urine samples (10 mL) were diluted 1:1 by volume with Tris buffer at pH 8.0, and [³H]6-oxo-PGF₁α, [³H]TXB₂, [³H]2,3-dinor-6-oxo-PGF₁α, and [³H]2,3-dinor-TXB₂ (5 ng each) were added. PGs and TXs were extracted using cyanogen bromide-activated Sepharose columns containing immobilized antibodies that had been raised against 6-oxo-PGF₁α and TXB₂ and that cross-reacted with 2,3-dinor-6-oxo-PGF₁α and 2,3-dinor-TXB₂. Urine samples were applied under vacuum to the columns, which were washed with water (10 mL). Eicosanoids were eluted by addition of acetone/water (95:5, 0.5 mL) and rotation of the columns for 15 minutes. Samples were dried in a stream of N₂ and converted to 3,5-bis-trifluoromethylbenzyl ester and trimethylsilyl ether derivatives. They were analyzed using a VG 70-SEQ gas chromatograph/mass spectrometer in the electron-capture mode with methane as reagent gas. Carboxylate anions at mass/charge ratio (m/z) 585 were monitored for 6-oxo-PGF₁α and TXB₂ and for their deuterated internal standards. Ions at m/z 557 and 561 were monitored simultaneously for the 2,3-dinor metabolites and their deuterated standards, respectively. The detection limit for each eicosanoid was 5 pg/mL when 10-mL urine samples were assayed.

Serum was prepared by allowing whole blood to clot in plain glass tubes at 37°C for 1 hour to determine the capacity of platelets to generate TXA₂ ex vivo.²³ TXB₂ was measured with immunoaffinity chromatography and gas chromatography/mass spectrometry as described above.

Routine blood and urine electrolyte determinations were performed by Simbek Ltd, Cardiff, Wales, UK. Urinary creatinine was measured in the Chemical Pathology Laboratory, Hammersmith Hospital, London, UK. Ridogrel was determined in heparinized plasma using high-performance liquid chromatography (detection limit, 1 µg/mL) by the Janssen Research Foundation, Beerse, Belgium.

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**Platelet Aggregation**
Venous blood (60 mL) was anticoagulated with trisodium citrate (final concentration, 0.13%, wt/vol). Platelet-rich and platelet-poor plasma were prepared by differential centrifugation at room temperature and kept in tightly stoppered plastic containers. Portions (500 µL) were transferred to stirred cuvettes in a two-channel Payton 600B aggregometer (Instrument Sales and Marketing Services, Dorking, Surrey, UK), and aggregation was studied turbidometrically as described by Born and Cross.²⁴ Platelet-rich plasma was allowed to equilibrate at 37°C in the aggregometer for 2 minutes before addition of a PG endoperoxide H₂ analogue, 15S-hydroxy-11a, 9α-epoxymethanoprost-5Z, 13E-dienoic acid (U46619), or adenosine diphosphate (ADP) (Sigma Chemical Co, Poole, Dorset, UK). Platelet aggregation was followed as the change in light transmission over the ensuing 5 minutes and expressed as percentage of transmission in platelet-poor plasma. Peak responses were plotted as a function of the concentration of each agonist.

**Statistical Analysis**
Data are summarized as mean±SEM. Differences on the 4 study days between blood pressure, eicosanoid production, platelet aggregation, or creatinine or electrolyte measurements were sought using two-way analysis of variance with repeated measures followed by Fisher's test. Eicosanoid excretion rates were transformed logarithmically to obtain normally distributed data before analysis. Differences were considered significant at a value of P<.05.

**Results**

**Urinary Eicosanoid Excretion**
Urinary excretion of 2,3-dinor-TXB₂, TXB₂, 2,3-dinor-6-oxo-PGF₁α, and 6-oxo-PGF₁α is summarized in Fig 1. One patient started menstruation during her second admission, and both the baseline and experimental day urine were visibly contaminated with blood. Urine TXB₂ excretion data from this patient was not included in the analysis of results. There was a 92% reduction in mean 2,3-dinor-TXB₂ excretion and a 46%
reduction in TXB₂ excretion during treatment with ridogrel compared with placebo. Mean 2,3-dinor-TXB₂ excretion fell from 279±28 to 21±6 ng/g creatinine, and TXB₂ excretion fell from 39±3 to 14±4 ng/g creatinine. There was a 21% increase in excretion of 2,3-dinor-6-oxo-PGF₁α during treatment with ridogrel compared with placebo. Mean 2,3-dinor-6-oxo-PGF₁α excretion increased from 146±11 to 184±20 ng/g creatinine, and 6-oxo-PGF₁α excretion increased from 58±6 to 86±9 ng/g creatinine. There were no significant differences between eicosanoid excretion on placebo and baseline days. Creatinine and electrolyte excretion (Na⁺ and K⁺) did not differ significantly on any of the 4 days (data not shown).

**Serum Thromboxane**

Serum TXB₂ concentrations were profoundly reduced in all patients after treatment with ridogrel. Mean serum TXB₂ concentrations were 146±16 ng/mL on the placebo day and 0.72±0.07 ng/mL after ridogrel (P<.0001). Serum TXB₂ concentrations on both baseline days were similar to placebo day values (144±25 and 110±18 ng/mL).

**Platelet Aggregation and Plasma Ridogrel Concentration**

Platelet aggregation responses to U46619 and ADP after ridogrel and placebo are plotted in Fig 2. Responses to U46619 (0.3 to 3 μM) were reduced after ridogrel, with an approximately parallel shift to the right (dose ratio of approximately 4) of a semilogarithmic plot of the U46619 dose-response curve (P<.0001). There was no significant reduction in the maximum response.
sufficiently in the presence of a TX synthase inhibitor to influence experimental coronary thrombosis, and addition of a TXA2 receptor antagonist synergizes with a TX synthase inhibitor in this canine model. Simultaneous use of two drugs has disadvantages, including the need to match pharmacokinetic profiles of each drug. TX synthase inhibitors that also possess TXA2 receptor antagonist properties represent an attractive combination that could confer unique antithrombotic activity and also may favorably influence the component of elevated blood pressure that is PG dependent.

Ridogrel has been administered to healthy normotensive subjects, but its effects on blood pressure and on TXA2 and PGI2 biosynthesis in vivo have not been described previously in hypertensive patients. Urinary excretion rates of each eicosanoid measured were similar to values obtained using the same methods in patients with essential hypertension in whom, as in the present study, medication had been withheld for 2 weeks. In this earlier study, similar excretion rates were also observed in patients during antihypertensive treatment and in a control group of normotensive subjects.

In our present study, we found that short-term administration of ridogrel causes a profound alteration in the balance between basal PGI2 and TXA2 biosynthesis. We also found that the capacity of platelets to synthesize TXA2 in blood allowed to clot ex vivo is substantially reduced by ridogrel in these patients, consistent with previous studies of normotensive subjects. Urinary excretion of hydrolysis products and metabolites of TXA2 and PGI2 can be used to determine the effect of TX synthase inhibitors. However, it is important to note that eicosanoid metabolites present in the body at the time of dosing continue to be eliminated during the first few hours after dosing even if eicosanoid biosynthesis is completely inhibited by the drug. To obtain an accurate estimate of the extent of TX synthase inhibition, we therefore had to administer two doses of ridogrel 12 hours apart and determine eicosanoid excretion after the second dose. We found that inhibition of 2,3-dinor-TXB2 excretion by ridogrel is similar to that caused by 300 mg aspirin. Excretion of TXB2 is reduced to a lesser extent than that of 2,3-dinor-TXB2, which may suggest that ridogrel is a less effective inhibitor of renal than of extrarenal TXA2 biosynthesis. A qualitatively similar disparity between inhibition of 2,3-dinor-TXB2 and TXB2 excretion has previously been reported for dazoxiben and the cyclooxygenase inhibitor sulindac. The reason why TXB2 excretion is reduced to a lesser extent than 2,3-dinor-TXB2 is not clear. Assuming that TXB2 derives primarily from the kidney, it is possible that renal TX synthase and cyclooxygenase activity are located at less accessible sites than their nonrenal counterparts or that there are distinct renal and extrarenal isoenzymes.

There is conflicting evidence in the literature concerning effects of TX synthase inhibitors on PGI2 biosynthesis. Several studies of CGS 13080 and dazoxiben in healthy subjects have reported increased 2,3-dinor-6-oxo-PGF1α excretion, However, this has been contested by Henriksson et al. in a further study of CGS 13080 in six subjects. Physical activity increases PGI2 biosynthesis in healthy humans, and Henriksson et al. suggested that failure to take this into account in earlier studies could account for the observed increase in 2,3-dinor-6-oxo-PGF1α excretion. However, we carefully controlled physical activity in the present study, not only on experimental days but also for baseline periods of 24 hours. We found that ridogrel increases excretion of 2,3-dinor-6-oxo-PGF1α under these conditions, consistent with diversion of PG endoperoxides from platelet to blood vessel wall, with a consequent increase in PGI2 production. Furthermore, there is a similar increase in 6-oxo-PGF1α excretion, suggesting that PG endoperoxides in the kidney are also diverted from TXA2 to PGI2 production. Previous studies have shown that the TX synthase inhibitor FCE 22178 increases 6-oxo-PGF1α excretion and lowers systolic blood pressure in rats after subtotal renal ablation, but increased 6-oxo-PGF1α excretion that may reflect PGI2 biosynthesis in the kidney associated with inhibition of TXA2 biosynthesis has not been documented in humans previously.

We found that ridogrel causes a selective inhibition of U46619-induced platelet aggregation consistent with antagonism of platelet TXA2 receptors. Assuming a unimolecular reversible reaction between ridogrel and TXA2 receptors, the observed dose ratio of 4 associated with a mean plasma ridogrel concentration of 2.95 X 10^-5 M is consistent with an apparent dissociation equilibrium constant of 9.8 X 10^-5 M, in fair agreement with IC50 values of 2.08 X 10^-4 to 2.66 X 10^-4 M for the effect of ridogrel on U46619-induced aggregation in platelet-rich plasma in vitro.

Despite evidence of increased PGI2 biosynthesis and reduced TXA2 biosynthesis, and of TXA2 receptor antagonism, acute administration of ridogrel did not significantly alter systolic or diastolic arterial pressure in our patients. We tried to reduce influences such as anxiety and stress on blood pressure by using carefully standardized conditions that included admission to a quiet hospital room and a baseline period of 24 hours to familiarize patients with their environment and the procedures. We also used repeated automatic blood pressure recording during both the baseline and study periods. Blood pressure recordings were analyzed both by comparing mean data for each of the four study periods and by comparing hourly average blood pressures at each time point to ensure that any transient changes (eg, at peak drug concentration) would not be overlooked. Studies of rodent models of genetic hypertension that have demonstrated a hypertensive effect of TX synthase inhibitors have generally involved long-term dosing. It is possible that more prolonged treatment of human subjects with essential hypertension with a TX synthase inhibitor receptor antagonist such as ridogrel would lower blood pressure, as it did in these rodent models. Such a slowly developing effect could occur if TX biosynthesis in the kidney (which was less completely inhibited than was systemic TX biosynthesis in our short-term study) is of particular importance in essential hypertension. Investigation of possible effects of long-term dosing with ridogrel on blood pressure was beyond the scope of the present study, but as with this drug in humans increases it may become possible to obtain information on this issue. Other studies have demonstrated a short-term PG-dependent synergy between TX synthase inhibition and angiotensin converting enzyme inhibition. Such a synergy has also
been described in human essential hypertension. These findings suggest that blood pressure in patients treated with angiotensin converting enzyme inhibitors may be acutely dependent on TXA₂, PGI₂, or both. By contrast, the present findings indicate that in untreated patients with essential hypertension, a short-term profound alteration of TXA₂/PGI₂ biosynthesis and action has no immediate effect on blood pressure. We conclude that, although these eicosanoids may be important in the long-term regulation of blood pressure, they do not participate directly in the short-term control of arterial pressure in untreated patients with essential hypertension.

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