Thromboxane A₂ Receptor Antagonism and Synthase Inhibition in Essential Hypertension

James M. Ritter, Susan E. Barrow, Hilary S. Doktor, Paula D. Stratton, Jacqueline S. Edwards, John A. Henry, Susan Gould

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 adenosine diphosphate were studied turbidimetrically. 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-oxoprostaglandin 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, 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

McGiff and Vane suggested that prostaglandins (PGs) participate in blood pressure regulation via local actions within the kidney and systemic resistance vessels, and abnormal eicosanoid biosynthesis has subsequently been implicated in the pathogenesis of essential hypertension. Indirect evidence arises from observations that nonsteroidal anti-inflammatory drugs, which are inhibitors of cyclooxygenase, blunt the response to antihypertensive therapy. Furthermore, blood pressure is lowered by dietary supplementation with ω3 polyunsaturated fatty acids that increase vasodilator PG biosynthesis. Recent attention has focused on the involvement of thromboxane (TX) A₂ and prostacyclin (PGI₂) in hypertension, because these eicosanoids rapidly and potently affect not only vascular tone and renal function but may also influence thrombosis by their actions on platelets. Myocardial infarction is precipitated by arterial thrombosis and is a principal cause of excess mortality in patients with mild to moderate hypertension. TXA₂ is produced by activated platelets, macrophages, and endothelial cells, and in renal glomeruli. It is a potent vasoconstrictor and stimulates platelet aggregation. Conversely, PGI₂ is natriuretic, a vasodilator, and a potent inhibitor of platelet aggregation. It is synthesized by endothelial and other cells and has been implicated in protection from thrombosis. 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 hydrolysis 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. 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. 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, other studies have found no such difference between hypertensive and normotensive individuals. 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 PG₁ production that determines, in part, the extent to which blood pressure becomes elevated.

Drug treatment that increases endogenous PG₁ 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 hypnotic drug treatment on blood pressure is directly related to the initial pressure before drug administration. In particular, although intravenous administration of PG₁ lowers arterial pressure in patients with hypertension, it has no significant effect on mean arterial blood pressure of normotensive subjects. 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 PG₁ 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, in patients with mild essential hypertension to determine whether an alteration in the balance between PG₁ 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 began 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 (Accutracker II, Suntek, New Brunswick, NJ) 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 AM, 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 immunooaffinity 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 carrier gas. Ions at m/z 557 and 561 were monitored simultaneously for 6-oxo-PGF₁α and TXB₂, and m/z 589 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. 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.

Results

Urinary Eicosanoid Excretion

Urinary excretion of 2,3-dinor-TXB₂, TXB₂, 2,3-dinor-6-oxo-PGF₁α, and 6-oxo-PGF₁α are 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%
Line graphs show effect of ridogrel on U46619-induced and adenosine diphosphate (ADP)-induced platelet aggregation. Platelets were studied 2 hours after administration of ridogrel (○) or placebo (●). Aggregation is expressed as maximum percent light transmission in platelet-rich plasma at 37°C, 100% transmission being taken as that in platelet-poor plasma.

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±9 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.

Mean plasma ridogrel concentration at the time of platelet studies was 10.5±0.90 µg/mL (2.95±0.25×10−5 M). There were no significant differences between platelet aggregation responses to U46619 on placebo or baseline days, nor were there significant differences between ADP-induced aggregation dose-response curves on ridogrel, placebo, or baseline days.

**Blood Pressure**

Blood pressures (mean of the 12 hourly averages) were 150.1±4.6/91.4±3.1 and 148.8±5.0/89.3±3.1 mm Hg on ridogrel and placebo days, respectively. Blood pressures were also similar on baseline days (148.7±4.3/90.2±2.8 and 151.6±4.4/93.4±3.2 mm Hg on baseline placebo and baseline ridogrel days, respectively). Mean systolic and diastolic blood pressures did not differ significantly between any of the 4 study days, and hourly average blood pressures were similar throughout the recording period on each occasion (Fig 3).

**Discussion**

Inhibitors of TX synthase selectively reduce TXA2 production but can also increase PG12 biosynthesis by diverting PGG2, PGH2 (common endoperoxide precursors of TXA2 and PG12), or both from platelet TXA2 synthesis to endothelial cell PG12 synthesis. Such inhibitors lower blood pressure in some but not all rat models of hypertension. Effects of TX synthase inhibition have not been studied extensively in human hypertension. One study has shown that OKY-046 has no direct effect on blood pressure but does augment the hypotensive effect of captopril.33 A fundamental limitation of TX synthase inhibition in altering the balance of effects of PG12 and TXA2 in vivo is that PG endoperoxides are potent TXA2 agonists. Any effects caused by reduction in TXA2 biosynthesis may be offset by the direct action of PG endoperoxides on TXA2 receptors. Combined administration of a TX synthase inhibitor with a TXA2 receptor antagonist results in greater inhibition of platelet aggregation and prolongation of bleeding time than when either drug is administered alone. PG endoperoxides accumulate...
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 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 indicates that with a mean plasma ridogrel concentration of 2.95 x 10⁻⁶ 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 10⁻⁶ to 2.66 x 10⁻⁵ 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 experience 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.

Acknowledgments
This study was supported by the Janssen Research Foundation. We thank Mr Jeremy Beacham for creatinine determinations. Sally Todd, RGN, and Louise Binns, RGN, provided expert nursing assistance. We thank Mr Jeremy Beacham for creatinine determinations. Sally Todd, RGN, and Louise Binns, RGN, provided expert nursing assistance.

References
20. Minuz P, Barrow SE, Cockcroft JR, Ritter JM. Effects of non-steroidal anti-inflammatory drugs on prostacyclin and thrombox-


Thromboxane A2 receptor antagonism and synthase inhibition in essential hypertension.

Hypertension. 1993;22:197-203
doi: 10.1161/01.HYP.22.2.197

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/22/2/197