Effects of Ridogrel, a Thromboxane Synthase Inhibitor and Receptor Antagonist, on Blood Pressure in the Spontaneously Hypertensive Rat

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ABSTRACT—Ridogrel is a dual acting thromboxane synthase inhibitor/TP receptor antagonist. We examined the effects of single and multiple doses on systolic blood pressure in stroke-prone spontaneously hypertensive rats. Single doses of ridogrel (5 to 125 mg/kg) did not affect systolic blood pressure or furosemide-stimulated excretion rates of thromboxane B₂ or 6-keto-prostaglandin F_{1α}, although ex vivo serum thromboxane B₂ was dose-dependently reduced up to 95%. In contrast, repeated dosing (7 days) with ridogrel (3 to 25 mg/kg/day), had an antihypertensive effect in 12-week-old stroke-prone spontaneously hypertensive rats. At 25 mg/kg/day, ridogrel reduced systolic blood pressure from 200±6.1 to 173±6.7 mmHg (n=12, P<0.01). Ridogrel dose-dependently reduced serum thromboxane B₂ and increased plasma renin activity. Unlike single doses, repeated dosing reduced urinary thromboxane B₂ excretion (from 103±7 ng/day to 49±10 ng/day, P<0.01) while preserving 6-keto-prostaglandin F_{1α} excretion. Ketoprofen, a cyclo-oxygenase inhibitor, (10 mg/kg/day for 7 days), depressed urine 6-keto-prostaglandin F_{1α} in addition to attenuating serum and urine thromboxane B₂. Ketoprofen prevented the antihypertensive effects of ridogrel. Ridogrel did not lower systolic blood pressure in Sprague-Dawley rats. We conclude that the antihypertensive effect of ridogrel involves preserving renal prostaglandin synthesis during thromboxane attenuation.

Keywords: Blood pressure, Renal prostanoid, Ridogrel, Thromboxane

This work aimed to investigate the effects of ridogrel, a thromboxane synthase inhibitor and receptor antagonist, on blood pressure in stroke-prone spontaneously hypertensive rats (SHRSP). Thromboxane (TX) A₂ is a potent vasoconstrictor and aggregator of platelets (1). Its immediate precursor is prostaglandin H₂, which can also be metabolized to prostacyclin (PGI₂). PGI₂ has many actions that are opposite to those of TXA₂ including vasodilation and inhibition of platelet aggregation (2). Platelets metabolize arachidonate mainly to TXA₂, while vessel walls form mostly PGI₂ (3, 4). Cyclo-oxygenase inhibitors like aspirin and indomethacin reduce the synthesis of both TXA₂ and PGI₂ (4). Drugs that selectively inhibit TXA₂ have the property of reducing TXA₂ synthesis, while preserving and enhancing PGI₂ synthesis (5–11). Prostaglandin H₂ itself is active at TP receptors, and accumulates when thromboxane synthase is inhibited (12). Therefore, another class of agents that act as an-

tagonists at the TP receptors, as well as inhibiting thromboxane synthase, has been developed (13). Ridogrel (Fig. 1) is such an agent: inhibiting thromboxane synthase (ID₅₀ of 4 nM) and competitively antagonizing the thromboxane mimetic U 46619 at the TP receptor (ED₅₀ of 1 μM). In rats, at doses of up to 500 mg/kg, it does not inhibit cyclo-oxygenase; prostacyclin synthase; most cytochrome P450 reactions; or 5-, 12- or 15-lipoxygenases (14, 15).

The kidney expresses all enzymes necessary to synthesize TXA₂ and PGI₂ (16). The importance of these cyclo-oxygenase metabolites to kidney function under normal circumstances is minimal, but when the kidney is exposed to vasoconstrictors such as angiotensin II, arachidonate is released and their synthesis is increased (17). When renal TXA₂ synthesis is selectively inhibited, the vasoconstrictor effect of angiotensin II is reduced. If cyclo-oxygenase is inhibited, the vasoconstrictor effect of angiotensin II may compromise renal blood flow (18–20). This suggests that while TXA₂ may augment renal vasoconstriction, lack of the vasodilator prostaglandin effect is most

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Blood pressure measurements

Tail cuff blood pressure measurements were obtained using a 15-mm cuff and a piezo-electric sensor (Buffington Clinical Systems, Cleveland, OH, USA). Before each recording session, the occluder cuff pressure was calibrated against a mercury manometer. The output from the sensor was amplified through a Grass 7P1 Polygraph (Grass Instruments, Quincy, MA, USA). During measurements, rats were placed in a warmed Broome restrainer (Harvard Apparatus, Ville St-Laurent, QC, Canada), to which they had previously been exposed. Each blood pressure reading was the mean of 5 measurements taken at 2- to 5-min intervals.

Analyses

Urine and plasma TXB_2 and urine 6-keto-prostaglandin $F_{1\alpha}$ were quantitated by radioimmunoassays after extraction and column chromatographic purification, using our previously published methods (41). Plasma renin activity was taken as the generation rate of angiotensin I, using the method of Stockigt et al. (42). Urine sodium was ascertained by an ion-selective electrode autoanalyzer (Kodak Ektachem 700; Kodak, Rochester, NY, USA).

Experimental protocols

Excretion rates of TXA_2 and 6-keto-prostaglandin $F_{1\alpha}$: Six- and 16-week-old male SHRSP, SHR, WKY and Sprague-Dawley rats ($n=4$ of each age and strain) were placed in metabolic cages. Two days were allowed for acclimatization after which blood pressure was measured. Then two consecutive 24-hr urine collections were made (into iced, preweighed conical centrifuge tubes), after which the blood pressure was again measured. The two sets of blood pressure determinations on each animal were averaged to obtain the reported value. Urine was weighed, gently centrifuged and frozen at -80°C for later analysis.

Single-dose studies: Groups of 4 male SHRSP, aged 12 weeks, received either vehicle (methylcellulose 500 μl) or single doses of ridogrel (5, 25 or 125 mg/kg suspended in vehicle) by gavage. For each animal, a minimum of five tail-cuff measurements were made and averaged to obtain the systolic blood pressures reported. Systolic blood pressure was measured at baseline, prior to a 24-hr acclimatization period in the metabolic cages. In the first 16 rats (four groups of 4 rats), systolic blood pressure was again measured for comparison to baseline at about 2 hr after gavage dosing with ridogrel at the respective dose for each group. Two hours coincides with the time to reach maximal plasma concentrations for ridogrel in rats (36). Upon finding that no significant antihypertensive effect had occurred with respect to any single dose of ridogrel, we extended the protocol, starting with another 16 rats (four

groups of 4 rats) to assess whether single doses of ridogrel caused an expected dose-dependent enhancement of furosemide-stimulated urinary prostanoid excretion. Each animal was then given furosemide at 2 mg/kg, intravenously, using the left internal jugular technique (43). Urine was collected via metabolic cage for 40 min, weighed and frozen for later analysis. Blood pressure was again measured, following which the animals were anesthetized with ketamine (70 mg/kg) and xylazine (3 mg/kg) given as a single intramuscular injection. Blood, collected by cardiac puncture, was placed in plain glass tubes, for serum TXB_2 measurement, and EDTA-containing tubes, for plasma renin activity.

Repeated-dose studies: After baseline systolic blood pressure readings, groups of 3 to 7 male 12-week-old SHRSP received either vehicle, suspensions of ridogrel (3, 12.5 or 25 mg/kg/day), ketoprofen (10 mg/kg/day) or the combination of ridogrel (25 mg/kg/day) plus ketoprofen (10 mg/kg/day). A further group of four 12-week-old Sprague-Dawley rats received ridogrel at 25 mg/kg/day. Drugs were administered daily as two equally divided doses, spaced 12 hr apart, for 7 days. For the day prior to drug administration and throughout this seven day regimen, the animals were housed in metabolic cages, and urine was collected each 24 hr, again using iced centrifuge tubes. Aliquots of the baseline and final urine sample were analyzed for prostanoids. Two hours after the final dose of drug or vehicle, rats were anesthetized with ketamine (70 mg/kg) and xylazine (3 mg/kg) given as a single intramuscular injection, and blood was collected by cardiac puncture. For sodium balance studies, intake was calculated by weighing the food consumed each day.

Statistical analyses

When groups of animals were compared, each parameter was subjected to a one way ANOVA. When significant differences were found, pairwise comparisons were made using Duncan's multiple range test (StatMost 3.2; Datamost Corp, Sandy, UT, USA). Paired *t*-tests were used to assess changes in the same animals. Unless otherwise stated, means $\pm$ S.E.M are reported.

RESULTS

Excretion rates of TXA_2 and 6-keto-prostaglandin $F_{1\alpha}$ in untreated rats

Table 1 shows weights, systolic blood pressure values and urine excretion rates of prostanoids in 6- and 16-week-old SHRSP and WKY rats. Both normotensive strains excreted more TXB_2 than SHRSP (and SHR), and tended to excrete higher amounts of 6-keto-prostaglandin $F_{1\alpha}$ at 6 and 16 weeks. At 6 weeks, the ratio of excretion

of 6-keto-prostaglandin $F_{1\alpha}$ to TXB_2 was 4.51 ± 0.11 in SHRSP and 4.84 ± 0.22 in WKY ($P=0.76$). By 16 weeks, this ratio was higher in SHRSP, 7.06 ± 0.46 vs 4.76 ± 0.45 ($P<0.002$). Although WKY rats weighed more at 16 weeks, their water intake, corrected for body weight, was similar: 11.8 ± 1.3 ml/100 g/day for SHRSP and 11.4 ± 1.0 ml/100 g/day for WKY ($P>0.05$). Adjusted for body weight, the volume of urine collected from the 6-week-old normotensive strains (WKY and Sprague-Dawley rats) was 2–3 times greater than the hypertensive rats (SHRSP and SHR). Systolic blood pressures were clearly higher in SHRSP (and SHR) at both ages. At 16 weeks of age, the magnitude of the systolic blood pressure differences between hypertensive and normotensive rats had increased substantially, and while urine output in relation to body weight had increased along with the increment in systolic blood pressure in SHR, urine output from SHRSP remained significantly lower in comparison to that from normotensive rats.

Single-dose studies

Results are shown in Table 2. Ridogrel, at any dose, did not change systolic blood pressure at 2 hr post dose. Although furosemide tended to lower blood pressure in all groups (the mean pretreatment systolic blood pressure

was 199 ± 15 mmHg), none of the differences between post-furosemide values and pretreatment values reached statistical significance. Furthermore, there was no difference in systolic blood pressure after furosemide between the vehicle-treated group, and any ridogrel-treated group, or the ridogrel-treated group as a whole. Urine volume was markedly increased after furosemide in all groups, but was unaffected by ridogrel pretreatment. Serum TXB_2 , a measure of platelet TXA_2 synthesis, was reduced by both higher doses of ridogrel; the highest dose reduced it by 95%. In contrast, none of the doses of ridogrel had any effect on urinary TXB_2 excretion. Plasma renin activity appeared to increase in relation to the dose of ridogrel, and 6-keto-prostaglandin $F_{1\alpha}$ excretion rate tended to be higher after the 125 mg/kg dose ($0.05 < P < 0.1$).

Repeated-dose studies

Figure 2 shows the change from baseline of systolic blood pressure in each of the groups of SHRSP after treatment for 7 days. It is apparent that blood pressure increased by about 40 mmHg in control rats. Ridogrel lessened the spontaneous systolic blood pressure rise in a dose-related fashion. At the highest dose, ridogrel lowered blood pressure from 200 ± 6.1 mmHg at baseline to 173 ± 6.7 mmHg after 7 days treatment ($P<0.01$).

Table 1. Comparison of prostanoid excretion rates in hypertensive (SHRSP) and normotensive (WKY) rats at ages 6 weeks and 16 weeks[†]

Age	SHRSP						WKY					
	n	Weight g	SBP mmHg	u6-keto ng/day	uTXB ₂ ng/day	urine ml/day	n	Weight g	SBP mmHg	u6-keto ng/day	uTXB ₂ ng/day	urine ml/day
6 weeks	4	86 ± 5.6	143 ± 3	12.1 ± 2.3	2.7 ± 0.4	2.5 ± 0.8	4	$126 \pm 5.6^*$	$122 \pm 2^*$	19.5 ± 3.1	$4.0 \pm 0.2^*$	$11.1 \pm 1.0^*$
16 weeks	4	313 ± 7.2	208 ± 14	24.0 ± 1.8	3.5 ± 0.3	6.8 ± 0.4	4	$415 \pm 2.5^*$	$146 \pm 7^*$	28.3 ± 0.9	$6.0 \pm 0.2^*$	$19.1 \pm 0.9^*$

[†]Although differences between SHRSP and WKY differed most significantly, comparisons also included hypertensive (SHR) and normotensive (Sprague-Dawley) rats (not shown), which revealed qualitatively similar (but statistically less significant) differences in prostanoid excretion rates. SBP=average of 5 tail-cuff systolic blood pressure measurements, u6-keto=urine 6-keto-PGF_{1 α} excretion in ng/day, uTXB₂=urine TXB₂ excretion in ng/day, urine=volume output in ml/day. * $P<0.05$ between strains of same age.

Table 2. Single-dose studies

Treatment group	n	Weight	Post-furosemide					
		g	urine ml/40 min	SBP mmHg	seTXB ₂ ng/ml	uTXB ₂ ng/40 min	u6-keto ng/40 min	PRA ng/ml/hr
Control	4	231 ± 6	2.4 ± 0.5	170 ± 17	148 ± 53	2.9 ± 1.2	7.6 ± 1.3	1 ± 0.7
Ridogrel (5 mg/kg)	4	228 ± 5	2.5 ± 0.5	184 ± 12	125 ± 26	2.7 ± 0.4	7.7 ± 0.4	$9 \pm 3^*$
Ridogrel (25 mg/kg)	4	239 ± 6	2.6 ± 0.4	175 ± 8	$10 \pm 2^*$	2.4 ± 0.6	6.5 ± 0.4	$22 \pm 6^*$
Ridogrel (125 mg/kg)	4	226 ± 5	2.5 ± 0.4	177 ± 13	$8 \pm 3^*$	2.5 ± 0.7	10.1 ± 1	$122 \pm 25^*$

urine=volume output in ml over a 40-min period following the furosemide (2 mg/kg) injection, SBP=average of 5 tail-cuff systolic blood pressure measurements, taken 40 min after furosemide, seTXB₂=ex vivo serum thromboxane B₂ in ng/ml, uTXB₂=urine TXB₂ excretion in ng/40 min, u6-keto=urine 6-keto-PGF_{1 α} excretion in ng/40 min, PRA=plasma renin activity in ng of angiotensin I generated/ml/hr.

* $P<0.05$, compared to the control group.

Fig. 2. Treatment-related changes in systolic blood pressure in groups of 12-week-old SHRSP as well as one group of 12-week-old Sprague-Dawley rats. The variability in baseline systolic blood pressure measurements between groups did not approach statistical significance. The pooled baseline systolic blood pressure for all groups of SHRSP rats was 188 ± 12 mmHg. The mean baseline systolic blood pressure of Sprague-Dawley rats was 115 ± 2.8 mmHg. * $P < 0.05$, ** $P < 0.01$, compared to the control group of rats; ## $P < 0.01$, compared to within group baseline.

Neither ketoprofen, nor ridogrel plus ketoprofen given in combination, prevented the spontaneous rise in systolic blood pressure during the treatment week. The systolic

blood pressure of Sprague-Dawley rats was not changed by ridogrel (115 ± 2.8 mmHg at baseline and 125 ± 6.4 mmHg after 7 days, $P > 0.05$). All drug treatments reduced serum TXB_2 ; the reduction was over 95% for the higher doses of ridogrel, the ketoprofen and the combined treatment groups (Table 3). Similarly, all drug treatments reduced urinary TXB_2 , although to a much lesser extent than serum concentrations. Of particular note is the similar reduction in urine TXB_2 after treatment with ridogrel, with or without ketoprofen. Urine 6-keto-prostaglandin $F_{1\alpha}$ was not different after ridogrel at 25 mg/kg/day, but was reduced to about the same percentage of the control level as urine TXB_2 when ketoprofen was added.

The cumulative sodium balance over 7 days in the control and ridogrel (25 mg/kg/day)-treated animals indicated that both groups retained sodium to about the same extent.

DISCUSSION

Ridogrel, given for a week, prevented the increase in blood pressure in SHRSP in a dose-dependent fashion. At the highest dose employed, it lowered blood pressure compared to the baseline level. Published data has generally profiled relatively modest blood pressure responses to known antihypertensive drugs in the same model. Taking that into account, the antihypertensive response to ridogrel appears fairly substantial (27). Rather dramatic reductions in the incidence of cardiovascular complications have accompanied proportionally modest blood pressure responses to conventional antihypertensive agents (33, 44). The potential importance of thromboxane at about the same stage in the development of

Table 3. Repeated-dose studies

Treatment group	n	se TXB_2 ng/ml	u TXB_2 [†] ng/day	u6-keto [†] ng/day	PRA ng/ml/hr
Control	3	132 ± 10	102.6 ± 7.0	113.8 ± 8.7	1.8 ± 0.3
Ridogrel (3 mg/kg/day)	4	$59 \pm 8^*$	$67.7 \pm 15.2^{**}$	—	2.6 ± 0.4
Ridogrel (12.5 mg/kg/day)	4	$5 \pm 2^*$	$51.4 \pm 16.3^{**}$	—	$5.5 \pm 0.9^*$
Ridogrel (25 mg/kg/day)	7	$6 \pm 1^*$	$49.2 \pm 10.4^{**}$	99.2 ± 4.5	$13.0 \pm 1.6^*$
Ketoprofen (10 mg/kg/day)	3	$3 \pm 1^*$	$64.6 \pm 17.8^{**}$	$56.3 \pm 10.7^*$	4.0 ± 1.3
Ridogrel (25 mg/kg/day)	4	$2 \pm 0.7^*$	$50.5 \pm 8.2^{**}$	$51.6 \pm 9.0^{**}$	5.0 ± 1.1
plus ketoprofen (10 mg/kg/day)					
Ridogrel (25 mg/kg/day)	4	2 ± 0.8	$60.4 \pm 12.9^{**}$	$58.5 \pm 11.5^*$	—
in Sprague-Dawley rats					

se TXB_2 =ex vivo serum thromboxane B_2 in ng/ml, u TXB_2 =urine TXB_2 excretion in ng/day, u6-keto=urine 6-keto-PGF $_{1\alpha}$ excretion in ng/day, PRA=plasma renin activity in ng of angiotensin I generated/ml/hr. [†]percent of baseline rate. * $P < 0.05$, ** $P < 0.01$, compared to the control group. n=sample size. Due to mishaps, results were not obtained for every rat in each treatment group. Sample sizes are reported for the minimum number of rats from which successful measurements were obtained.

hypertension in SHRSP was supported by findings that repeated doses of UK-38485, a thromboxane synthase inhibitor, progressively lowered blood pressure in rats treated from 8.6 to 11 weeks of age (45). Ridogrel had no hypotensive effect in normotensive Sprague-Dawley rats. Single doses of ridogrel did not change blood pressure after furosemide. Furthermore, contrary to our hypothesis, the SHRSP does not excrete more TXA_2 than normotensive control rats, in either absolute terms or relative to 6-keto-prostaglandin $\text{F}_{1\alpha}$. Indeed, the ratio of TXB_2 to 6-keto-prostaglandin $\text{F}_{1\alpha}$ in the urine of 16-week-old hypertensive animals was lower, suggesting that this may be a compensatory response to hypertension.

In single-dose studies, ridogrel reduced platelet TXB_2 formation almost to zero, yet had no measurable effect on urinary TXB_2 excretion. This suggests that renal TXA_2 synthesis takes place in a compartment to which the drug has poorer access and which requires prolonged exposure to inhibit. This is consistent with human experience with ridogrel, where *ex vivo* thromboxane production was inhibited by 99% after the first dose, but urinary TXB_2 reached a nadir (of 71% of control) only after 7 days (46). Similar findings have been noted with the thromboxane synthase inhibitor dazoxiben (47). Because blood pressure was affected only in the chronic studies, it is tempting to speculate that renal TXA_2 synthesis must be inhibited before blood pressure is affected. On the other hand, plasma renin activity was increased after single doses of ridogrel. To the extent that this represents renal renin release, ridogrel had some effect on the juxtaglomerular apparatus. Prostacyclin is a potent renin releaser (48) and may have played a role in the increase seen after ridogrel. Increased renin may have also limited the fall in blood pressure, so that no change occurred after single doses.

After a one week treatment, ridogrel produced a definite reduction in urine TXA_2 , while 6-keto-prostaglandin $\text{F}_{1\alpha}$ was unchanged. Thus the ratio of the two was also changed. Ketoprofen also reduced urine TXB_2 , but reduced urine 6-keto-prostaglandin $\text{F}_{1\alpha}$ as well, so that the ratio of the two opposing prostanoids was unchanged. Similarly, when ketoprofen was combined with ridogrel, both urine TXB_2 and 6-keto-prostaglandin $\text{F}_{1\alpha}$ were lowered. Taken together, these observations suggest that reducing renal TXA_2 synthesis *per se* is not sufficient to reduce blood pressure. The balance of opposing prostanoids must be manipulated to favor PGI_2 over TXA_2 .

Of interest is the effect of both single and repeated doses of ridogrel to markedly increase plasma renin activity. Part of this action may be a direct effect: TXA_2 is known to reduce renin release while PGI_2 is known to enhance it (49, 50). It would be of interest to know whether inhibiting the production or action of angio-

tensin II would enhance the antihypertensive effect of ridogrel.

We found no evidence of a diuretic effect of ridogrel. Cumulative sodium balance was similar in the control and ridogrel-treated rats. However, it must be remembered that animals receiving ridogrel had lower blood pressures (and presumably renal perfusion pressures), yet were able to excrete the same proportion of the sodium load. This is consistent with a shift of the renal function curve to the left, allowing for excretion of the daily sodium intake at a lower perfusion pressure. Confirmation of this must await formal pressure natriuresis studies.

Whether ridogrel has an antihypertensive effect in humans is unknown. In the only published trial, two doses of ridogrel (each 300 mg) were given orally to hypertensives (51). There was no change in blood pressure. Our data suggest that the drug may have to be given for a longer period in order to produce an antihypertensive effect.

If ridogrel does reduce blood pressure in human, it would be an attractive antihypertensive. Its effect on platelet TXA_2 production translates into meaningful antiplatelet activity, at least as great as that of aspirin (52), but without disrupting the gastric cytoprotection that prostaglandin production affords. Indeed, in our own studies, we found that ridogrel, added to human whole blood, reduced platelet aggregation to the same extent as indomethacin, but with a 100-fold greater potency (D.W. Quest and T.W. Wilson, unpublished observations). While nonsteroidal antiinflammatory drugs such as aspirin and indomethacin will provide sufficient antiplatelet activity, they have a suspected potential to limit the efficacy of antihypertensive therapy. Given that many of the complications of hypertension involve platelet driven thrombosis, the possible utility of ridogrel is obvious.

In summary, we found that ridogrel, an agent that both selectively inhibits thromboxane synthase and is an antagonist at the TP receptor, reduced blood pressure in a genetic model of hypertension. Although excessive thromboxane production does not appear to be causative in this animal model, changing the ratio of vasoconstrictor to vasodilator prostanoids appears to ameliorate the hypertensive process.

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