Increased Oxidative Stress and Platelet Activation in Patients With Hypertension and Renovascular Disease

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Methods and Results—We measured the urinary excretion of 8-iso-prostaglandin (PG) F2α and 11-dehydro-thromboxane (TX) B2 as indexes of in vivo lipid peroxidation and platelet activation, respectively, in 25 patients with RVD, 25 patients with essential hypertension, and 25 healthy subjects. Plasma renin activity in peripheral and renal veins, angiotensin II in renal veins, cholesterol, glucose, triglycerides, homocysteine, and antioxidant vitamins A, C, and E were also determined. Patients were also studied 6 months after a technically successful angioplasty of the stenotic renal arteries. Urinary 8-iso-PGF2α was significantly higher in patients with RVD (median, 305 pg/mg creatinine; range, 124 to 1224 pg/mg creatinine) than in patients with essential hypertension (median, 176 pg/mg creatinine; range, 48 to 384 pg/mg creatinine) or in healthy subjects (median, 123 pg/mg creatinine; range, 58 to 385 pg/mg creatinine). Urinary 11-dehydro-TXB2 was also significantly higher in RVD patients compared with healthy subjects. In RVD patients, urinary 8-iso-PGF2α correlated with 11-dehydro-TXB2 (r = 0.48; P < 0.05) and renal vein renin (r = 0.67; P < 0.005) and angiotensin II (r = 0.65; P = 0.005) ratios. A reduction in 8-iso-PGF2α after angioplasty was observed in RVD patients with high baseline levels of lipid peroxidation. Changes in 8-iso-PGF2α were related to baseline lipid peroxidation (r = −0.73; P < 0.001), renal vein angiotensin II (r = −0.70; P < 0.01) and renin (r = −0.63; P < 0.05) ratios.

Conclusions—Lipid peroxidation is markedly enhanced in hypertensive patients with RVD and is related to activation of the renin-angiotensin system. Moreover, persistent platelet activation triggered or amplified by bioactive isoprostanes may contribute to the progression of cardiovascular and renal damage in this setting. (Circulation. 2002;106:2800-2805.)

Key Words: thromboxane ■ stenosis ■ hypertension ■ renin ■ angiotensin

Renovascular disease (RVD) represents a relatively rare form of secondary hypertension, which in most cases is associated with activation of the renin-angiotensin system as a result of the fall in renal blood flow and perfusion pressure due to renal artery stenosis.1 RVD is associated with increased cardiovascular mortality and is a major and increasingly prevalent cause of end-stage renal failure.1–3 This may be related to increased angiotensin II activity, resulting in vasoconstriction, increased endothelin release, vascular remodeling, extracellular matrix deposition, and accelerated atherogenesis and glomerulosclerosis.4 These effects may contribute to the progression of cardiovascular and renal damage well beyond the effects of high blood pressure per se.

Increased oxidative stress may occur as a consequence of renal artery stenosis and may play a role in the progression of RVD. Experimental evidence supports this hypothesis and suggests that increased oxidative stress may be related to activation of the renin-angiotensin system.5,6 In patients with hypertension and RVD, the pro-oxidant effects of angiotensin II may amplify those due to coexisting metabolic disorders or long-standing hypertension.1,7

In the present study, we investigated whether oxidative stress is enhanced in RVD by measuring the urinary excretion of 8-iso-prostaglandin (PG) F2α, a noninvasive index of in vivo lipid peroxidation; 8-iso-PGF2α, which is also referred to as iP9F2α-III, is a member of the F2-isoprostanes, PG isomers that are formed nonenzymatically through free radical–catalyzed peroxidation of esterified arachidonate in cell membranes and circulating lipoproteins.8 Enhanced formation of F2-isoprostanes has been reported in association with several

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cardiovascular risk factors and has been found to correlate with in vivo thromboxane (TX) A2 biosynthesis. 10–13 8-Iso-PGF2α is a vasoconstrictor and modulates platelet activation in response to other agonists. 3 These activities are mediated by the interaction with receptors that are distinct from but closely related to TXA2/PGF2 receptors. 14–16 Thus, we tested the hypothesis that angiotensin II–dependent oxidative stress is responsible for enhanced formation of bioactive F2-isoprostanes, resulting in persistent platelet activation in RVD hypertensives.

**Methods**

**Subjects**

We studied 25 patients with RVD, 25 subjects with essential hypertension, and 25 healthy subjects (Table 1). Patients were recruited among those admitted to the departments of Internal Medicine of our Institutions. Diagnosis of hypertension associated with RVD was made on the basis of angiographic evidence of renal artery stenosis to oxidative stress and platelet activation was further investigated by comparing the urinary excretion of 8-iso-PGF2α and 11-dehydro-TXB2 in hypertensive patients with RVD and control subjects. The contribution of renal artery stenosis to oxidative stress and platelet activation was further investigated by comparing the urinary excretion of 8-iso-PGF2α and 11-dehydro-TXB2 before and 6 months after PTRA.

Angiographic studies revealed the presence of significant stenosis (exceeding 65% of the lumen diameter) of a renal artery that was due to fibromuscular dysplasia in 4 subjects (aged 17 to 28 years; 3 men) and atherosclerotic lesions in the other 21 patients (aged 52 to 77 years). Four patients had bilateral stenosis. All patients were on antihypertensive treatment at the time of study. Angiotensin-converting enzyme (ACE) inhibitors and antagonists of the angiotensin receptor were replaced with other antihypertensive drugs at least 15 days before sampling to avoid interference with angiotensin II release or activity. Five patients receiving low-dose aspirin continued their antplatelet treatment. Five patients were cigarette smokers (10 to 30 cigarettes per day) and 2 had type 2 diabetes mellitus (one treated).

All RVD patients underwent a revascularization procedure of the ischemic kidney. Percutaneous transluminal angioplasty of the stenotic renal artery (PTRA) was performed in 24 patients. Stents were also implanted in patients with atherosclerotic lesions, and both renal arteries were dilated in patients with bilateral stenosis. Because of the presence of an aneurysm of the abdominal aorta, a prosthetic graft was applied surgically to the remaining patient.

Essential hypertension was diagnosed on the basis of negative results of investigation for secondary hypertension that included renal scintigraphy and/or echo-Doppler scan of the renal arteries. To match the RVD patients for potentially confounding factors, 2 of the 25 patients with essential hypertension had type 2 diabetes mellitus and 5 were cigarette smokers (10 to 30 cigarettes per day). Essential hypertensive patients were treated with calcium antagonists, α-receptor blockers, or diuretics (n = 15) or were untreated (n = 10) at the time of study.

Atherosclerotic lesions of carotid arteries (>30% reduction in lumen diameter) were detected in 15 RVD patients and 13 patients with essential hypertension.

Twenty-five healthy subjects were also recruited among members of the medical and nursing staff and their relatives in Verona and were matched with hypertensive patients for sex and age.

**Study Protocol**

A cross-sectional study was performed to compare the urinary excretion rates of 8-iso-PGF2α and 11-dehydro-TXB2 in hypertensive patients with RVD and control subjects. The contribution of renal artery stenosis to oxidative stress and platelet activation was further investigated by comparing the urinary excretion of 8-iso-PGF2α and 11-dehydro-TXB2 before and 6 months after PTRA.

In the cross-sectional study, all subjects were studied using the same protocol, which consisted of clinical evaluation, blood pressure measurement, and blood and urine sampling. Both RVD patients and those with essential hypertension were hospitalized during the study and received a standard diet containing ~150 mmol of sodium per day. They also underwent an echo-Doppler evaluation of the carotid arteries.

The urinary excretion of 8-iso-PGF2α and 11-dehydro-TXB2 was evaluated from overnight urine collections (from 8 PM to 8 AM). Urine sampling was repeated twice, 2 days apart. The timing and total volume were recorded, and two 50-mL aliquots were stored at...
Blood samples were taken to determine plasma glucose, total and HDL cholesterol, triglycerides, and creatinine. Plasma concentrations of homocysteine and the antioxidant vitamins A, C, and E were also determined to assess the oxidant-antioxidant balance. Blood was collected from a peripheral vein to measure plasma renin activity (PRA) in patients with RVD and essential hypertension. Before blood sampling, patients were kept in the supine position for at least 1 hour.

Blood pressure was recorded (the mean of 3 readings) between 10 AM and noon using a mercury column sphygmomanometer with the subjects in the supine position for 30 minutes. Body mass index was also calculated.

The effects of revascularization of the ischemic kidney on lipid peroxidation were evaluated in hypertensive patients with RVD. Blood samples were collected by catheterization from renal veins and the inferior vena cava distal to the ostium of the renal veins (for technical reasons, blood sampling was not done in 4 patients). The renal vein renin ratio (ie, the ratio of PRA in blood draining the stenotic kidney to PRA in venous blood from the contralateral kidney) was used as an index of the activation of the renin-angiotensin system and renal perfusion in patients with unilateral stenosis.17 Plasma concentration of angiotensin II was also measured in blood from the renal veins and vena cava.

Urine samples were obtained from RVD hypertensives 6 months after revascularization (7 patients were lost to follow-up). Two urine collections and blood sampling were performed 2 days apart according to the previously described procedure (ACE inhibitors and antagonists of the angiotensin receptor were replaced with other antihypertensive drugs at least 15 days in advance). Patency of the treated vessels was evaluated and confirmed by echo-Doppler investigation of the renal arteries 6 months after PTRA.

The study protocol was approved by the ethics committees of the medical centers involved, and informed consent was obtained from all subjects.

**Biochemical Analyses**

PRA was measured using a radioimmunoassay to quantify the amount of angiotensin-I generated during 1 to 3 hours of incubation of plasma at 37°C and pH 5.7. The sensitivity of the assay was 0.25 ng/mL per hour, and its interassay variability was <11%.18 Angiotensin II was measured by radioimmunoassay after extraction of the peptide from plasma, as previously described.18 Immunoactive 8-iso-PGF$_{2\alpha}$ and 11-dehydro-TXB$_2$ were extracted from 20-mL urine aliquots and analyzed by previously validated radioimmunoassay techniques.10,11 Total homocysteine concentration in plasma was determined by high-performance liquid chromatography after derivatization using fluorescence detection. Plasma vitamins A, C, and E were also measured by high-performance liquid chromatography using a fluorescence detection system. The additional biochemical parameters were determined using a Technicon DAX 96 automated analyzer (Technicon Instruments).

**Statistical Analysis**

The Kruskall-Wallis test followed by the Mann-Whitney test was used in the cross-sectional study to compare variables in the 3 groups of subjects. Bonferroni’s correction was applied to pairwise comparisons. The 2-tailed Wilcoxon signed-rank test was used to compare variables in patients with RVD studied before and after renal revascularization. The Spearman coefficient ($r_s$) was calculated to quantify correlation between variables. Stepwise multivariate regression analysis was used to explore relationships among studied variables. $P<0.05$ was considered statistically significant. Data are reported as median and range, unless otherwise indicated.

**Results**

**Cross-Sectional Study**

The baseline characteristics of the 3 groups of subjects are detailed in Table 1. The 2 groups of hypertensive patients had comparable levels of blood pressure and metabolic parameters but differed significantly in terms of creatinine clearance and peripheral PRA.

Lipid peroxidation, as reflected by urinary 8-iso-PGF$_{2\alpha}$ excretion, was significantly enhanced in hypertensive patients with RVD compared with both control groups (Figure 1A). Platelet activation, as reflected by urinary 11-dehydro-TXB$_2$ excretion, was also significantly higher in RVD patients not taking aspirin than in pair-matched healthy subjects (Figure 2).
1B). As shown in Figure 2, ≈35% of the variability in TX metabolite excretion could be accounted for by the variability in the level of lipid peroxidation in the whole study population. A relationship between 8-iso-PGF$_{2\alpha}$ and 11-dehydro-TXB$_{2}$ was also observed in hypertensive patients with RVD ($r_s=0.48; n=20; P=0.032$).

In RVD patients with unilateral renal artery stenosis, the level of lipid peroxidation correlated significantly with the renal vein renin ratio (Figure 3A) and with the ratio of angiotensin II in blood draining the hypoperfused versus contralateral kidney (Figure 3B). No correlation was found between F$_2$-isoprostane excretion and any of the other studied parameters. Stepwise linear regression analysis identified the renal vein renin ratio as the only variable independently correlated with urinary 8-iso-PGF$_{2\alpha}$ in hypertensive patients with RVD (coefficient $\beta=0.86; P<0.001$). In addition, no difference was found in urinary 8-iso-PGF$_{2\alpha}$ excretion among patients with RVD due to fibromuscular dysplasia of the renal arteries, patients with renal artery stenosis due to atherosclerosis, and patients with monolateral or bilateral stenosis (data not shown).

**Effects of Revascularization of the Ischemic Kidneys**

The revascularization procedure had limited effects on blood pressure and renal function (Table 2), and all but 5 patients (3 with fibromuscular dysplasia of renal arteries) required long-term antihypertensive therapy after the procedure, although the number of administered drugs was reduced.

A trend toward reduction in the urinary excretion of 8-iso-PGF$_{2\alpha}$ and 11-dehydro-TXB$_{2}$ was observed after angioplasty (8-iso-PGF$_{2\alpha}$: median, $-66.6$ pg/mg creatinine; range, $-889.3$ to $226.5$ pg/mg creatinine; 11-dehydro-TXB$_{2}$: median, $-284.0$ pg/mg creatinine; range, $-4628$ to $518.0$ pg/mg creatinine). We observed that the variability in individual changes in urinary 8-iso-PGF$_{2\alpha}$ excretion could be largely accounted for by the baseline level of lipid peroxidation (Figure 4). Moreover, the reduction in urinary 8-iso-PGF$_{2\alpha}$ after PTRA was detected in the patients who had baseline values $>300$ pg/mg creatinine (median, $-190.5$ pg/mg creatinine; range, $-889.3$ to $-29.0$ pg/mg creatinine; $n=8; P=0.008$); no significant change was observed in subjects with lower baseline F$_2$-isoprostane excretion (median, $-24.7$ pg/mg creatinine; range, $-87.5$ to $226.5$ pg/mg creatinine; $n=10; P=0.37$). Changes in 8-iso-PGF$_{2\alpha}$ excretion were also related to baseline values of renal vein renin ratio (Figure 5A), renal vein angiotensin II ratio (Figure 5B), and peripheral PRA ($r_s=0.61; n=18; P=0.007$). Stepwise multivariate regression analysis identified baseline 8-iso-PGF$_{2\alpha}$ as the only variable independently related to changes in urinary 8-iso-PGF$_{2\alpha}$ after revascularization (coefficient $\beta=-0.81; P=0.002$).

**Discussion**

Measurement of the urinary excretion of 8-iso-PGF$_{2\alpha}$ has been characterized as a reliable method of investigating lipid peroxidation in vivo and has been shown to reflect a
status of enhanced oxidative stress, regardless of the underlying pathophysiological triggers. A number of clinical conditions in which increased oxidative stress was postulated on the basis of experimental data or ex vivo tests have been reported to be associated with increased urinary excretion of 8-iso-PGF₂α, 9–11,19–21 In the present study, we demonstrated that this noninvasive index of lipid peroxidation is markedly increased in hypertensive patients with RVD compared with patients with essential hypertension with comparable levels of blood pressure and healthy normotensive subjects. A number of coexisting conditions may independently affect the oxidant/antioxidant balance and the level of lipid peroxidation. In our group of RVD hypertensives, the urinary excretion of 8-iso-PGF₂α does not seem to be related to the presence of conventional cardiovascular risk factors that may independently be associated with increased oxidative stress: none of them had hypercholesterolemia; moreover, low HDL cholesterol, diabetes mellitus, cigarette smoking, and overt atherosclerosis were equally represented in essential and RVD hypertensives (Table 1). Additional pro-oxidant factors may favor increased lipid peroxidation in hypertensive patients with RVD, such as increased homocysteine and reduced vitamin E plasma concentrations. 20–22 However, plasma homocysteine and vitamin E levels were comparable in the 2 groups of hypertensives. Moreover, no correlation existed between urinary 8-iso-PGF₂α and either plasma homocysteine or vitamin E.

Increased oxidative stress seemed to be related to renal artery stenosis and activation of the renin-angiotensin system. Our data are consistent with the recent observation that experimental unilateral stenosis of the renal artery in pigs is associated with the development of hypertension and with increased plasma levels of 8-iso-PGF₂α. 6 In that experimental setting, the increase in plasma 8-iso-PGF₂α correlated with the increase in blood pressure and in PRA after the surgical procedure. The increase in 8-iso-PGF₂α was also detected 10 weeks after the induction of renal artery stenosis. 6

We found a significant positive correlation between 8-iso-PGF₂α and renal vein renin ratio. This functional test is a highly specific tool for the detection of renal artery stenosis, renal hypoperfusion, and activation of the renin-angiotensin system 17; it is also a predictor of the improvement in blood pressure and renal function after revascularization. 23,24 We also observed that the rate of excreted 8-iso-PGF₂α was related to the difference in angiotensin II released from the hypoperfused kidney compared with the contralateral kidney.

The observed relation between the reduction in F₂-isoprostane excretion after successful angioplasty in RVD hypertensives with baseline 8-iso-PGF₂α, angiotensin II ratio, renal vein renin ratio, and peripheral PRA strongly suggests that reversible events associated with renal artery stenosis are responsible for enhanced oxidative stress in this setting. It also provides additional evidence for a causal link between renal activation and lipid peroxidation. These findings may suggest that angiotensin II is the stimulus for isoprostane generation. In vitro and in vivo studies provide different lines of evidence that the hypertensive effect of angiotensin II is associated with the generation of oxygen radical species. 25–27

In addition, the infusion of angiotensin II, even at subpressor doses, directly increases superoxide anion and 8-iso-PGF₂α generation through a metabolic pathway that involves membrane NADH/NADPH oxidase activity. 28–30 However, our data do not allow us to assess whether angiotensin II directly stimulates 8-iso-PGF₂α generation and whether the source of urinary F₂-isoprostanes is located within the ischemic kidney or in the systemic circulation, although the observed relations between urinary 8-iso-PGF₂α and both renin ratio and angiotensin II ratio in renal veins suggest that lipid peroxidation may occur primarily in the hypoperfused kidney.

We also investigated the relationship between lipid peroxidation and platelet activation in RVD. Davì et al have described a consistent correlation between urinary excretion rates of 8-iso-PGF₂α and 11-dehydro-TXB₂ in patients with hypercholesterolemia, 10 diabetes mellitus, 11 and homozygous homocystinuria. 21 In addition, further supportive evidence was obtained for a biochemical link between lipid peroxidation and platelet activation in vivo by showing that vitamin E dose-dependently reduced both 8-iso-PGF₂α and 11-dehydro-TXB₂ excretion in these diverse clinical settings. 10,11,21 Consistent with these observations, we found that the rate of TXA₂ biosynthesis was markedly enhanced in RVD patients and closely correlated with the rate of F₂-isoprostane formation.

It is tempting to speculate that increased generation of bioactive F₂-isoprostanes and other isoeicosanoids may be responsible, at least in part, for increased platelet activation, the development of hypertension, and ischemic nephropathy in this setting. 8-iso-PGF₂α promotes platelet aggregation and induces platelet adhesion. 13 Moreover, 8-iso-PGF₂α is a powerful constrictor of the renal vasculature 12 and increases blood pressure. 14 Besides being suggestive of a direct involvement in these functional responses, the increased rate of 8-iso-PGF₂α may reflect the presence of more abundant biologically active species formed by similar mechanisms. 8 The hypothesis of a pathophysiological role of increased isoprostane generation is also supported by the observation that TXA₂/PGH₂ receptor blockers, but not cyclooxygenase inhibitors, reduced blood pressure in experimental renovascular hypertension. 31

In conclusion, we have identified RVD with hypertension as a clinical condition characterized by increased lipid peroxidation and platelet activation, presumably as a consequence of renin-angiotensin system activation. The use of ACE inhibitors or angiotensin-receptor antagonists may represent a suitable strategy to probe the pathophysiological role of angiotensin II–dependent lipid peroxidation in this setting.

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