Oxidative Stress and Endothelin-1 in Atherosclerotic Renal Artery Stenosis and Effects of Renal Angioplasty

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Key Words

Endothelin-1 • Oxidative stress • Renal angioplasty • Renal artery stenosis • Renovascular hypertension

Abstract

Aims: To examine biomarkers of oxidative stress (oxs), and endothelin (ET)-1, in hypertensive patients with atherosclerotic renal artery stenosis (ARAS) and to evaluate the effect of percutaneous transluminal renal angioplasty (PTRA).

Methods: Baseline measurements were made immediately before renal angiography in patients with suspected ARAS (significant ARAS, n = 83, and non-RAS, n = 59) and in 20 healthy, matched controls. In patients with ARAS, analyses were repeated 4 weeks after PTRA. All patients were treated with statins and acetylsalicylic acid throughout. **Results:** At baseline there were no significant differences between groups in biomarkers of oxs, whereas high-sensitivity C-reactive protein and blood leukocytes were significantly elevated in group ARAS versus both healthy controls and group non-RAS. Plasma levels of ET-1 and uric acid were significantly increased in group ARAS versus healthy controls prior to angiography and were significantly reduced compared to baseline 4 weeks after PTRA. PTRA had no significant effects on biomarkers of oxs, inflammation or serum creatinine concentrations. **Conclusions:** ARAS patients on treatment with

antihypertensive agents, acetylsalicylic acid and statins showed elevated inflammatory indices but no increase in oxs. PTRA had no significant effects on inflammatory indices 4 weeks after intervention but reduced plasma ET-1 and uric acid.

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Introduction

Renal artery stenosis (RAS) is relatively common in patients with generalized atherosclerosis and may lead to aggravated hypertension, decreased glomerular filtration rate (GFR) and eventually to end-stage renal disease [1]. It is well established that atherosclerotic RAS (ARAS) is associated with increased cardiovascular morbidity and mortality [2, 3]. In addition, a more severe degree of luminal narrowing correlates to increased mortality, at least in patients undergoing coronary angiography, suggesting a direct pathogenetic role for the stenotic lesion [3]. Endovascular treatment by percutaneous transluminal renal angioplasty (PTRA), with or without stenting, is a commonly used treatment of RAS in selected patients. Despite improvement of vessel patency, it is at present uncertain if PTRA improves renal and cardiovascular outcomes in patients with ARAS [4].

Table 1. Exclusion criteria

Renal size <7.5 cm at the stenotic side
Age >80 years
Pregnancy or nursing
CKD stage 5 (eGFR <15 ml/min/1.73 m ²)
Congestive heart failure
RAS of other etiology than atherosclerosis
Bilateral RAS or RAS of a solitary kidney
Urinary albumin excretion >1 g/day
Diabetes mellitus with urinary albumin excretion >0.3 g/day
Contraindication for renal angiography/angioplasty (e.g. serious contrast allergy)
Other forms of secondary hypertension
Malignant disease
Treatment with immune modulating drugs, e.g. cyclosporine or oral steroids

CKD = Chronic kidney disease; eGFR = estimated glomerular filtration rate according to the 4-variable equation from the Modification of Diet in Renal Disease (MDRD) study; RAS = renal artery stenosis.

Elevated levels of angiotensin II (Ang II) due to activation of the renin-angiotensin-aldosterone system (RAAS) could in part contribute to the increase in cardiovascular risk. In addition to its vasoconstrictor effect, Ang II can impair endothelial function, accelerate atherosclerosis and promote cardiovascular remodeling [5, 6]. In addition, Ang II can induce oxidative stress (oxs) by stimulating the production of superoxide through nicotinamide adenine dinucleotide phosphate oxidase [7]. A large number of studies have demonstrated that Ang II increases oxs in experimental models of renovascular hypertension (RVH) [7, 8]. In addition, treatment with superoxide dismutase mimetics, and other antioxidants, has been shown to reduce blood pressure (BP) and to diminish end-organ damage in these models [9, 10]. However, the association between RVH and oxs in humans is less consistent and only a few studies have addressed this issue [11–13]. Endothelin (ET)-1 is a powerful vasoconstrictor peptide with pro-oxidant and growth-promoting effects that is produced by the vascular endothelium in response to Ang II [5, 14]. Experimental data indicate that ET-1, mainly via the ETA receptor, may be an important factor in mediating the hypertensive effects of Ang II [14].

The aim of this hypothesis-generating study was to examine biomarkers of oxs and plasma ET-1 levels in patients with ARAS, and to evaluate the impact of PTRAs on these variables during the first month after intervention.

Material and Methods

Study Participants

Between 2003 and 2008, all patients at the Nephrology Section, Sahlgrenska University Hospital, Gothenburg, and the Department of Vascular Diseases at Skåne University Hospital, Malmö, Sweden, undergoing renal angiography for suspected RAS, were considered for this study. Indications for angiography were hypertension (resistant, accelerated, malignant, or with elevation of serum creatinine during treatment with angiotensin-converting enzyme inhibitors or Ang II receptor blockers), hypertension accompanied by a progressive increase in serum creatinine levels, or recurrent pulmonary edema without overt left ventricular dysfunction, together with a positive screening test for RAS by duplex ultrasonography or by CT or MR angiography ($\geq 50\%$ diameter stenosis). To avoid pharmacological interference with the RAAS, patients in whom treatment with angiotensin-converting enzyme inhibitors, Ang II receptor blockers or aldosterone receptor antagonists were clearly indicated (e.g. patients with congestive heart failure or diabetic nephropathy) were excluded (for exclusion criteria see table 1). In the remaining patients treated with RAAS inhibitors, who were included in the study, these agents were replaced by other antihypertensive drugs 2 weeks prior to renal angiography. Hence, included patients were not on any RAAS-inhibiting drugs during the study period starting from 2 weeks prior to baseline measurements. In addition, only patients with unilateral ARAS were included and individuals with RAS of other etiology, or with either bilateral RAS or stenosis of a solitary kidney, were excluded. Twenty age-matched healthy subjects without any medications were recruited from the database of the Gothenburg MONICA study [15] and served as controls. The Ethics Committees of the Universities of Gothenburg and Lund approved the study and all participants gave written consent to participate.

Protocol and Measurements

Patients were subjected to baseline measurements 1 day before angiography (see study overview in fig. 1). A significant RAS was defined as a lesion with a trans-stenotic mean arterial pressure gradient (MAPG) of ≥ 10 mm Hg or a $\geq 50\%$ diameter stenosis on angiography in those cases in which the MAPG was not measured because of technical difficulties due to high-grade stenosis and luminal occlusion during the procedure. Accordingly, 83 patients had significant RAS and underwent PTRAs, whereas 59 individuals had no significant RAS and were therefore only subjected to the diagnostic procedure (fig. 1).

Systolic and diastolic BP (SBP and DBP) were measured after 5 min rest in the sitting position immediately before and 1 day and 4 weeks after renal angiography. Routine laboratory analyses and biomarkers were measured immediately before renal angiography in all patients. In patients that were subjected to PTRAs ($n = 83$) analyses were repeated 4 weeks after intervention. Estimated GFR (eGFR) was calculated according to the 4-variable equation from the Modification of Diet in Renal Disease (MDRD) Study [16]. Notably, all patients with suspected RAS had been on treatment with a HMG-CoA reductase inhibitor (i.e. statin) for at least 2 weeks at the time of baseline measurements. The majority of patients was already on statin therapy at the time of study inclusion and continued with the same dosage throughout. In the remaining patients, treatment with simvastatin was started at the time of

Fig. 1. Patients were subjected to baseline measurements 1 day before renal angiography. 83 patients had significant ARAS and underwent PTRAs, whereas 59 individuals had no significant RAS and were therefore only subjected to the diagnostic procedure (see Methods). In patients with ARAS, measurements were repeated 4 weeks after PTRAs.

study inclusion with a daily dose of 20 mg that was maintained throughout. In addition, most patients in both hypertensive groups (90% in group ARAS and 74% in non-RAS) were already on low-dose acetylsalicylic acid (ASA) at this time-point, while treatment with ASA was started 1–2 days before PTRAs among those patients who were not already on this treatment. Treatments with statins and ASA were maintained unaltered in patients with ARAS throughout the study period. Healthy controls ($n = 20$) were only studied at one time-point and data were compared with baseline values from hypertensive groups.

Biochemical Analyses

Standard laboratory methods at the Departments of Clinical Chemistry at Sahlgrenska University Hospital and Skåne University Hospital (SWEDAC approved according to European norm 45001) were used for routine analyses. Plasma renin activity (PRA) was measured by a radioimmunoassay kit (DiaSorin, Stillwater, Minn., USA), with inter- and intra-assay coefficients of variation (CV)s less than 10%. Plasma concentrations of Ang II (Euro-Diagnostica, Malmö, Sweden) and ET-1 (Nichols Institute Diagnostics, San Juan Capistrano, Calif., USA) were measured by radioimmunoassay kits. The detection limit for ET-1 was 0.25 pg/ml, the intra-assay CV based on pooled samples was 11.3%, and the inter-assay CV was 22%.

Biomarkers of Oxidative Stress

Plasma levels of baseline-conjugated dienes in isolated LDL-cholesterol (LDL-BDC) were estimated by the method of Ahotupa et al. [17]. In brief, serum LDL-cholesterol (C) was isolated by precipitation with buffered heparin. Lipids were extracted from LDL-C samples by chloroform-methanol, dried under nitrogen, then redissolved in cyclohexane and analyzed spectrophotometrically at 234 nm (Perkin-Elmer Lambda 2 spectrometer). Intra- and inter-assay CV were 9.6 and 10.9%, respectively. The levels of LDL-BDC were corrected for serum LDL-C (LDL-BDC:LDL-C) to express the LDL-C oxidation degree.

Plasma total antioxidant capacity (TAOC) was determined by Trolox equivalent antioxidant capacity assay according to Rice-Evans and Miller [18] with some modification. In brief, potassium peroxydisulfate was used to induce the oxidation of 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) (Aldrich, Deisen-

hofen, Germany) to form the stable radical cation (ABTS⁺). The cation ABTS⁺ was measured photometrically at 734 nm. Antioxidants present in the added plasma caused a reduction in absorption proportional to their concentration. Results are expressed in mmol/l of Trolox equivalent.

Plasma protein carbonyls were measured by a colorimetric assay as described by Reznick and Packer [19] using a commercially available kit (Cayman Chemicals, Ann Arbor, Mich., USA). The intra- and inter-assay CVs were 4.7 and 8.5% respectively.

Urinary 8-iso-PGF₂α was measured in spot urine samples by an 8-isoprostane EIA Kit (Cayman Chemicals). Urinary creatinine was measured by standard enzymatic laboratory methods and the levels of U-8-iso-PGF₂α were corrected for urinary creatinine values. Results are expressed in pg/mg of creatinine. The intra- and inter-assay CVs were 18.6 and 29.3%, respectively.

Renal Angiography and Angioplasty

Digital subtraction angiography was used for evaluating renal arteries. The procedures of renal angiography and PTRAs have been described previously [20]. A 4-Fr catheter was used for measurements of intra-arterial pressure gradients. The diameter of stenosis was estimated manually in all cases. Indications for stent placement were angioplasty failure (elastic recoil or flow-limiting dissection resulting in >30% residual luminal narrowing, absence of antegrade flow, or significant residual MAPG), or restenosis. Of patients treated with PTRAs, 39 (47%) received stents.

Statistics

Analyses were performed using one-way analysis of variance (ANOVA). If data were not normally distributed, Kruskal-Wallis one-way ANOVA on ranks was used. Unpaired t test or Mann-Whitney U test was used when appropriate. Bonferroni corrections were made for multiple comparisons. The Pearson correlation (Spearman correlation when data did not meet assumption about normality) coefficient was used to evaluate correlations. All tests were two-tailed and p values <0.05 were considered significant. Results are presented as means ± SD. Software SPSS 18.0.0 for Windows (Release 2009; SPSS Inc., Chicago, Ill., USA) was used.

Table 2. Blood pressure, kidney function and biomarkers at baseline and 4 weeks after PTR

	Healthy controls (n = 20)	Non-RAS baseline (n = 59)	ARAS baseline (n = 83)	ARAS 4 weeks post-PTRA (n = 83)
SBP, mm Hg	116 ± 10	153 ± 22*	163 ± 24*, **	159 ± 22
DBP, mm Hg	74 ± 7	89 ± 11*	87 ± 11*	86 ± 11
Antihypertensive drugs, n	0	2.5 ± 1.1*	2.6 ± 0.9*	2.4 ± 1.1
S-creatinine, µmol/l	77 ± 14	102 ± 36*	121 ± 52*	118 ± 44
eGFR, ml/min/1.73 m ²	78 ± 13	64 ± 23*	56 ± 22*	57 ± 21
S-albumin, g/l	39 ± 3	38 ± 4	37 ± 4	39 ± 3***
tU-albumin, mg/24 h	7 ± 4	63 ± 138*	91 ± 160*	115 ± 250
S-uric acid, µmol/l	267 ± 40	370 ± 122*	392 ± 101*	366 ± 92***
Fasting B-glucose, mmol/l	4.7 ± 0.9	5.9 ± 1.3*	6.7 ± 2.6*	6.5 ± 3.6
P-ET-1, pg/ml	0.54 ± 0.24	1.08 ± 0.52*	1.29 ± 0.71*	1.04 ± 0.45***
S-aldosterone, nmol/l	0.55 ± 0.21	0.51 ± 0.49	0.57 ± 0.72	0.54 ± 0.97
PRA, ng/ml/h	1.23 ± 0.65	1.23 ± 1.44	3.67 ± 6.41**	2.33 ± 2.78
P-Ang II, pg/ml	11.9 ± 4.6	11.4 ± 7.2	18.2 ± 18.3**	16.0 ± 17.9
U-8-iso-PGF _{2α} , pg/mg creatinine	884 ± 755	648 ± 511	657 ± 533	615 ± 419
P-TAOC, mmol/l	4.1 ± 0.7	4.6 ± 0.8*	4.6 ± 0.9*	4.6 ± 0.8
P-LDL-BDC:LDL-C, µmol/mmol	12.5 ± 4.0	15.4 ± 7.1	14.6 ± 5.3	16.1 ± 5.8
P-PC, nmol/mg	0.24 ± 0.06	0.27 ± 0.08	0.28 ± 0.08	0.27 ± 0.07
hs-CRP, mg/l	1.9 ± 1.6	4.2 ± 7.5	5.6 ± 6.3*, **	6.4 ± 8.5
B-WBC, × 10 ⁹ /l	6.3 ± 2.1	6.9 ± 1.8	7.8 ± 1.9*, **	7.9 ± 2.1

Data are means ± SD. * p < 0.05 vs. healthy controls; ** p < 0.05 vs. non-RAS; *** p < 0.05 ARAS post-PTRA vs. ARAS baseline.

PTRA = Percutaneous transluminal renal angioplasty; RAS = renal artery stenosis; ARAS = atherosclerotic RAS; SBP = systolic blood pressure; DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate according to the 4-variable equation from the Modification of Diet in Renal Disease (MDRD) study; tU-albumin = total urinary albumin excretion; P-ET-1 = plasma endothelin-1; PRA = plasma renin activity; P-Ang II = plasma angiotensin II; U-8-iso-PGF_{2α} = urinary 8-iso-prostaglandin F_{2α}; P-TAOC = plasma total antioxidant capacity; P-LDL-BDC:LDL-C = plasma baseline-conjugated dienes in isolated low-density lipoprotein:isolated low-density lipoprotein-cholesterol ratio; P-PC = plasma protein carbonyls; hs-CRP = high-sensitivity C-reactive protein; B-WBC = blood leukocyte count.

Results

Patient Characteristics, BP and Kidney Function at Baseline

SBP, DBP and serum creatinine levels were elevated, and eGFR reduced, in both hypertensive groups (ARAS and non-RAS) compared to healthy controls (table 2). In addition, hypertensive groups showed elevated fasting blood glucose, serum uric acid (UA) and urinary albumin excretion, and decreased plasma levels of total cholesterol and HDL-C compared to controls (tables 2, 3). There were no statistically significant differences between groups in BMI or in smoking habits (table 3).

Patients with ARAS were older, more often men, and showed higher SBP than patients without significant RAS (tables 2, 3). Baseline serum creatinine levels tended to be elevated in group ARAS compared to non-RAS (121 ± 52 vs. 102 ± 36 µmol/l, respectively, p = 0.051; table 2).

RAAS and ET-1 at Baseline

In patients with ARAS, plasma Ang II concentrations and PRA were significantly elevated compared to group non-RAS, but did not reach statistical significance versus healthy controls (table 2). There was no significant difference between groups in serum aldosterone levels (table 2). Plasma levels of ET-1 were significantly increased in groups ARAS and non-RAS versus healthy controls (table 2). However, there was no significant difference in plasma ET-1 concentrations between group ARAS and non-RAS (p = 0.15; table 2).

Oxidative Stress and Inflammation at Baseline

Plasma TAOC was significantly elevated in groups ARAS and non-RAS compared to healthy controls (table 2). However, there were no statistically significant differences between groups in oxs markers plasma protein carbonyls, LDL-BDC:LDL-C or in urinary 8-iso-PGF_{2α} (table 2). Patients with ARAS showed increased levels of

Table 3. Baseline characteristics before renal angiography

	Healthy controls (n = 20)	Non-RAS (n = 59)	ARAS (n = 83)
Age, years	63 ± 11	59 ± 12	65 ± 9**
BMI, kg/m ²	26 ± 4	27 ± 5	27 ± 3
Male gender, n (%)	9 (45)	28 (48)	56 (68)**
Current cigarette smoking, n (%)	2 (10)	14 (24)	26 (31)
Diabetes mellitus, n (%)	–	1 (2)	13 (16)**
Duration of hypertension, years	–	10.4 ± 10.5	8.3 ± 8.7
S-triglyceride, mmol/l	1.4 ± 0.6	1.5 ± 0.6	1.7 ± 0.7
S-TC, mmol/l	5.4 ± 0.9	4.4 ± 1.0*	4.5 ± 1.0*
S-LDL-C, mmol/l	3.0 ± 0.9	2.3 ± 0.8*	2.5 ± 0.9
S-HDL-C, mmol/l	1.7 ± 0.4	1.4 ± 0.4*	1.2 ± 0.4*

Data are expressed as means ± SD if not otherwise stated. * $p < 0.05$ vs. healthy controls; ** $p < 0.05$ vs. non-RAS.

RAS = Renal artery stenosis; ARAS = atherosclerotic renal artery stenosis; BMI = body mass index; S-TC = serum total cholesterol; S-LDL-C = serum low-density lipoprotein-cholesterol; S-HDL-C = serum high-density lipoprotein-cholesterol.

high-sensitivity C-reactive protein (hs-CRP) and blood leukocyte count (WBC) compared to healthy controls and group non-RAS (table 2). At baseline, hs-CRP and WBC levels showed no significant correlations to biomarkers of oxs, components of the RAAS or ET-1 in patients with ARAS. In addition, hs-CRP and WBC showed no significant correlation to SBP. Baseline hs-CRP was correlated to age ($r = 0.39$, $p = 0.001$), baseline levels of serum creatinine ($r = 0.44$, $p < 0.001$), HDL ($r = -0.29$, $p = 0.035$), and UA ($r = 0.44$, $p < 0.001$). However, in multiple regression analysis, only baseline serum UA was statistically significant, with a β value of 0.38 ($p < 0.01$).

Effects of PTR A on Functional Variables in Patients with ARAS

Four weeks after PTR A, SBP had decreased from 163 ± 24 to 159 ± 22 mm Hg ($p = 0.053$), whereas DBP remained largely unchanged (table 2). The number of antihypertensive drugs was not significantly reduced (table 2). In a stepwise multiple regression analysis, only baseline SBP and DBP significantly predicted changes in SBP following PTR A (adjusted R^2 for model 0.42, $p < 0.05$). Notably, serum creatinine and eGFR were not significantly affected by PTR A (table 2).

Effects of PTR A on Biomarkers in Patients with ARAS

Serum levels of UA were significantly reduced by PTR A (table 2). However, there was no significant correlation between changes in serum UA levels and in SBP

following PTR A. Plasma ET-1 was significantly reduced 4 weeks after PTR A, whereas there were no significant changes in PRA or in plasma levels of Ang II or aldosterone (table 2). Changes in plasma levels of ET-1 4 weeks after PTR A correlated only to changes in serum UA concentrations ($r = 0.32$, $p < 0.05$; fig. 2). There was no significant correlation between changes in plasma ET-1 and those in SBP following PTR A. There were no statistically significant effects of PTR A on any of the other biomarkers (table 2).

Discussion

In the present study, we found no difference in biomarkers of oxs between hypertensive ARAS and non-RAS patients, although inflammatory indices were elevated in individuals with ARAS. Our findings are different from those in two previous studies in which patients with ARAS showed increased levels of oxs biomarkers compared to healthy controls and to patients with essential hypertension [11, 12]. Higashi et al. [11] found that baseline urinary excretion of 8-hydroxy-2-deoxyguanosine (8-OHdG) and serum malondialdehyde-modified LDL (MDA-LDL) were elevated in 15 patients with RVH compared to matched healthy subjects. In addition, these biomarkers were significantly reduced following renal angioplasty. However, no antihypertensive agents were administered for at least 2 weeks before mea-

Fig. 2. Correlation between changes in plasma levels of ET-1 and serum UA in response to PTRa in patients with ARAS ($r = 0.32$, $p < 0.05$, $n = 83$). Measurements were performed before angiography and 4 weeks after PTRa.

measurements and no patient received ASA or statins [11]. In the study by Minuz et al. [12], urinary 8-iso-PGF_{2α} excretion was elevated in patients with RVH. As in our study, all patients were on antihypertensive treatment and drugs interfering with the RAAS were not used. However, only 5 patients were on low-dose ASA and no patient received statin treatment [12]. In contrast, all patients (ARAS and non-RAS) in our study were treated with statins and low-dose ASA throughout the study period. A growing body of evidence from both experimental [21] and human studies [22, 23] clearly indicate antioxidant effects of statins. In addition, ASA has been shown to possess antioxidant properties [24]. Hence, the discrepancy between our findings and earlier results could at least in part be explained by differences in drug use between study populations. Another possible explanation for the discrepancy between results in the present study and those by Higashi et al. [11] could be that different biomarkers of oxs were analyzed. Higashi et al. analyzed urinary 8-OHdG excretion which is a marker of oxidative DNA damage that occurs in the cell nucleus or mitochondria. Thus, it is possible, at least in theory, that patients with ARAS have increased intracellular oxs leading to oxidative DNA damage that we were unable to detect in the present study with our biomarkers. In addition, protein oxidation was not assessed by Higashi et al., whereas we

examined protein oxidation by measuring protein carbonyls. However, lipid peroxidation was evaluated both by Higashi et al., who measured serum levels of MDA-LDL [11], and by us in the present study (U-8-iso-PGF_{2α} excretion). Both U-8-iso-PGF_{2α} excretion and serum MDA-LDL are considered reliable markers of lipid peroxidation [25, 26]. For instance, treatment of patients with coronary atherosclerosis with statins has been shown to reduce both U-8-iso-PGF_{2α} [22] and serum MDA-LDL [26]. Thus, differences between these biomarkers of lipid peroxidation are less likely to explain the discrepant results in the present study compared to those by Higashi et al. [11]. It should also be acknowledged that the small sample size of the healthy control group in the present study might have reduced the power in detecting differences between groups in biomarkers. Notably, plasma TAOC was significantly elevated in both hypertensive groups compared to healthy controls. This could at least partly be explained by the antioxidant effects of ASA and statins. In addition, although several studies have shown reduced antioxidant capacity in patients with atherosclerosis [27], adaptive elevations in antioxidant activity have also been reported [21, 28].

Accumulating evidence demonstrates that statins also exert anti-inflammatory actions [29, 30]. Still, patients with ARAS who were on statin treatment showed significantly increased levels of hs-CRP and WBC in the present study compared to both healthy controls and hypertensive patients without RAS. This finding clearly indicates that in our cohort of patients, ARAS was associated with inflammation but not with increased oxs. Notably, PTRa had no significant effects on hs-CRP or WBC when analyzed 4 weeks after intervention in the present study. These results indicate that inflammation in ARAS patients was not caused by the stenotic lesion of the renal artery per se but may have reflected the burden of generalized atherosclerotic disease.

ET-1, a powerful vasoconstrictor peptide, has been implicated in Ang II-mediated hypertension [14] and treatment with ETA receptor antagonists has been shown to attenuate cardiovascular remodeling in experimental models of Ang II-dependent hypertension [5]. Consistent with these findings, plasma levels of ET-1 in the present study were significantly increased in both hypertensive groups versus healthy controls. In addition, PTRa significantly reduced plasma ET-1 concentrations in ARAS patients, suggesting a beneficial effect of PTRa on vascular integrity. In line with our findings, Higashi et al. [11] showed impaired endothelium-dependent vasodilatation in patients with RVH prior to PTRa and an improved

vasodilatory response following intervention. However, the functional consequences of reduced plasma levels of ET-1 following PTRa in the present study need to be examined further. We also observed that changes in plasma levels of ET-1 in response to PTRa only correlated to changes in serum UA during the same time period, suggesting that reductions in ET-1 might have been mediated by decreased UA levels. Interestingly, UA has been implicated in endothelial dysfunction in hypertension [31] and has been shown to stimulate ET-1 gene expression [32]. The association between plasma levels of UA and ET-1 following PTRa is a novel observation that needs to be investigated further.

Evidence from previous studies shows strong associations between serum levels of UA and biomarkers of inflammation [33]. Consistent with these findings, the present study showed that baseline hs-CRP only correlated to serum UA levels in patients with ARAS. However, the reduction in serum UA after PTRa was independent of inflammatory biomarkers as hs-CRP was not affected by intervention (table 2). In addition, the reduction in UA after PTRa did not correlate to changes in SBP or in serum creatinine levels. The mechanism by which PTRa reduced serum UA in the present study is unclear and needs to be elucidated. Interestingly, experimental studies have shown increased blood and tissue levels of UA precursors adenosine, inosine, and hypoxanthine after only a few minutes of renal ischemia [34, 35]. In addition, reperfusion for only 15 min resulted in a normalization of tissue levels of adenosine and inosine [34]. Thus,

one could speculate that elevated serum UA in patients with ARAS was caused by renal ischemia and that PTRa reduced levels of UA by restoring renal blood flow.

In conclusion, patients with ARAS showed no increase in oxs, although inflammatory indices were significantly elevated. These results indicate that despite treatment with antihypertensive agents, ASA and statins, inflammation is still elevated in ARAS patients and seems to be independent of oxs. In addition, PTRa had a differentiated effect on biomarkers and reduced plasma levels of ET-1 and UA but did not affect hs-CRP. The clinical implications of these findings need to be examined further.

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Disclosure Statement

There are no conflicts of interest to declare.

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