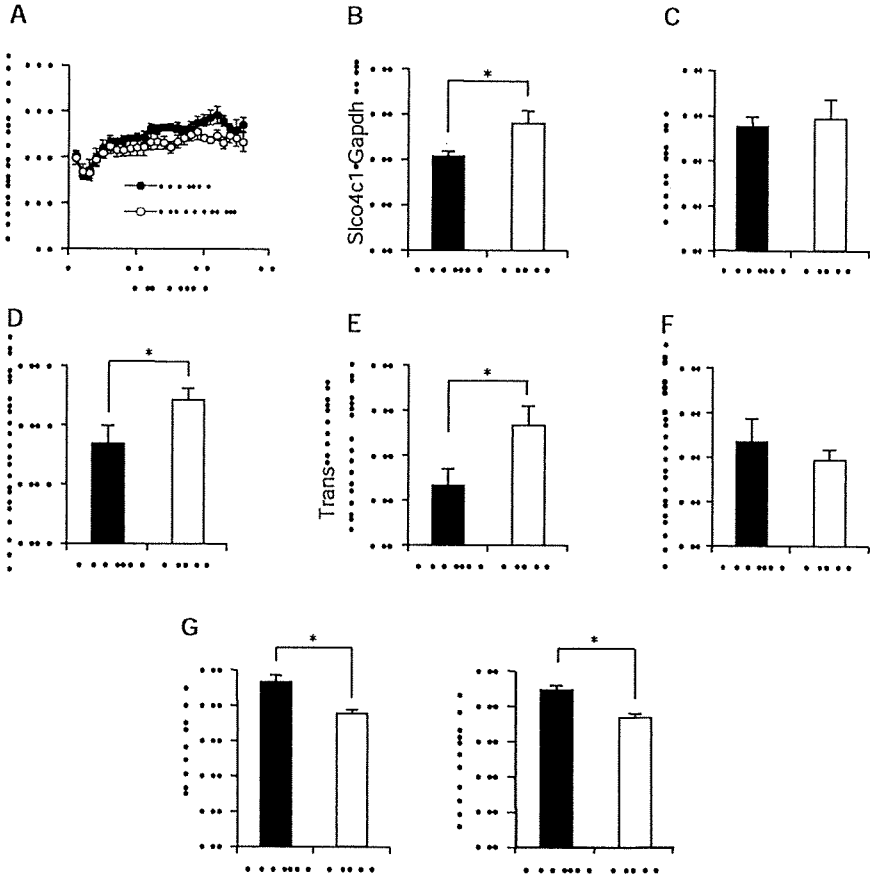

Figure 7. Effects of pravastatin in vivo. (A) BP in control and pravastatin-treated (0.1 mg/ml drinking water) rats after five-sixths Nx (n • 6 to 7 per group). (B) The mRNA expression of rat slco4c1 in the kidney after pravastatin administration (n • 11 per group). (C through F) Renal clearance of creatinine (C), ADMA (D), trans-aconitate (E), and GSA (F) 3 wk after five-sixths Nx (n • 5 to 7 per group). (G) Thickness of the interventricular septum (IVSTd) and left ventricular posterior wall at end-diastole (LVPWTd) before and after five-sixths Nx (n • 6 to 7 per group). *P • 0.05.

moter.[11] The linear purified plasmid was injected into the pronuclei of fertilized oocytes of Wistar rats. Pups were analyzed for the genomic integration by Southern blotting and by PCR amplification of tail DNA using the following primers: Forward (mouse sglt2) 5•-tccccccacttctgtt-tcccagtctatgt-3• and reverse (human SLCO4C1) 5•-acgcgatctgcagaatt-agcttgggctc-3•. Reverse transcriptase–PCR was carried out using the same primers that can amplify the full length of human SLCO4C1 cDNA. Resultant TG(•) rats showed normal breeding and development with no obvious phenotypic abnormalities in body weight, water and food intake, and renal functions compared with TG(•) littermates, whose genetic background is the same as that of TG(•) rats except for expression of human SLCO4C1 (Supplemental Figure 1A). All animal experiments were approved by the Tohoku University Animal Care Committee.

Immunohistochemistry

The rabbit antiserum against 107 peptides of the N-terminus of human SLCO4C1 was raised and immunopurified. Western blotting and immunohistochemistry were performed as described previously,[39] and the quality was confirmed by peptide absorption (Supplemental Figure 1, B and D). The mouse mAb against CD68 was purchased from Serotec (Martinstried, Germany).

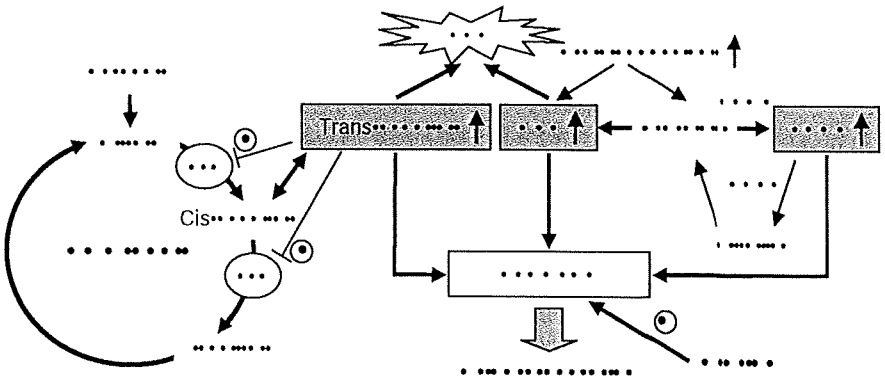

Figure 8. Uremic toxins and SLCO4C1 transporter in renal failure. ADMA is formed by protein arginine N-methyltransferase (PRMT) from arginine and degrades to citrulline by DDAH. Note that SLCO4C1 facilitates the excretion of GSA, ADMA, and trans-aconitate and that statins increase the expression and the function of SLCO4C1, resulting in reductions of the uremic toxins and BP. Trans-aconitase inhibits aconitase activity and induces reactive oxygen species (ROS). Aco, aconitase.

Nephrectomized Rat Model and BP Measurement

Five-sixths nephrectomized rats were generated as previously reported.[10] Briefly, male TG rats were intraperitoneally anesthetized with ketamine (30 mg/kg) and xylazine (2 mg/kg) and subjected to five-sixths renal ablation. At the time of surgery, rats were prepared for telemetric monitoring of BP (Data Sciences Int., St. Paul, MN).[40]

Echocardiogram

Rats were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) and studied with Doppler imaging by echocardiogram. The thickness of the interventricular septum and the left ventricular posterior wall at end-diastole were measured as described previously.[41]

CE-MS Method for Metabolome Analysis

A comprehensive and quantitative analysis of charged metabolites by CE-MS was performed.[13] Metabolites were first separated by CE on the basis of charge and size and then selectively detected using MS by monitoring over a large range of m/z values. Plasma and urine ADMA were measured by HPLC. Anionic and cationic compounds that were increased or decreased after Nx in both of the generated rat lines were nominated as statistically significant and are summarized in Supplemental Figure 2 (all analyzed CE-MS data are in Supplemental Tables 1 through 4). In the human plasma analysis, the protocols conformed to the ethical guidelines and approvals of both Tohoku University and Nagasaki University. Informed consent was obtained from each participant. The eGFR was calculated with the formula[42] eGFR (ml/min per 1.73 m^2) • 175 • creatinine$^{-1.154}$ • age$^{-0.203}$ • 0.742 (if female) • 0.741.

Measurement of Reactive Oxygen Species

The free radical formation within the human kidney proximal cell line HK-2 evoked by trans-aconitate (100 • M) was monitored by measurement of the changes in fluorescence resulting from the oxidation of dihydroethidium to ethidium as the increase of ethidium production (U/s)[43] using a 505-nm dichroic mirror with the 605/55-nm band-pass filter of an IX71 microscope (Olympus, Tokyo, Japan).

Transcriptional Assay

The human SLCO4C1 promoter DNA fragments were amplified by PCR, and the amplified fragments were inserted into the pGL3 basic luciferase expression vector (Promega, Madison, WI). The point mutation of two XREs was generated by PCR. Two micrograms of plasmid construct was transfected with 0.1 • g of Renilla Luciferase Reporter Vector PhRL-TK (Promega) as well as co-transfection with AhR and AhR nuclear translocator expression vector.[18] Forty-eight hours after ligand treatment, reporter assay was performed using Dual Luciferase Reporter Assay System (Promega). Incubation with activators of constitutive androstane receptor (clotrimazole and TCPOBOP), pregnane X receptor (rifampicin), and peroxisome proliferator–activated receptor • (bezafibrate, fenofibrate, clofibrate, and LTB_4) did not affect the SLCO4C1 transcription (data not shown).

ChIP Assay

ChIP assays were performed as described previously.[44] Briefly, cells either untreated or exposed to 3-MC (mouse HepaC1C7 cells) or fluvastatin (HEK293T cells) were crossed-linked with 1% formaldehyde, and protein-DNA complexes were immunoprecipitated using rabbit polyclonal antibody against AhR (BIOMOL, Plymouth, PA) or nonspecific anti-rabbit IgG. The recovered DNA was then subjected to PCR using primers that amplify regions containing the XRE elements of the human SLCO4C1 gene (forward primer 5•-AAGGGGAGCTTATGGCCA-GAGACTC-3• and reverse primer 5•-TCGCCTCAAGGACCAACCG-GAAG-3•) or mouse cyp1a1 gene (forward primer 5•-CTATCTCTTA-AACCCCACCCCAA-3• and reverse primer 5•-CTAAGTATGGT-GGAGGAAAGGGTG-3•). Nuclear and cytoplasmic fraction extracts were prepared and Western blotting was performed as described previously[39] using antibodies against AhR, Lamin B (Santa Cruz Biotechnology, Santa Cruz, CA), and • -tubulin (Sigma-Aldrich, St. Louis, MO).

Real-Time PCR Analysis

We performed real-time PCR analysis with probe sets from Applied Biosystems (Foster City, CA).

Statistical Analysis

The data are means • SEM. We used an unpaired t test for comparisons between two groups. For multiple comparisons, we used two-way ANOVA with repeated measures in Figures 2A, 3H, and 7A and Supplemental Figure 1D and ANOVA on rank in Supplemental Figure 3, A through C. We derived P values for Supplemental Figure 1C using log-rank test. In Figure 4, Spearman rank correlation was calculated. P • 0.05 was considered to be significant.

ACKNOWLEDGMENTS

This work was supported in part by research grants from the Miyagi Kidney Foundation; the Ministry of Education, Science and Culture of Japan; the Yokoyama Clinical Pharmacology Foundation; and Japan Science and Technology Agency.

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DISCLOSURES

None.

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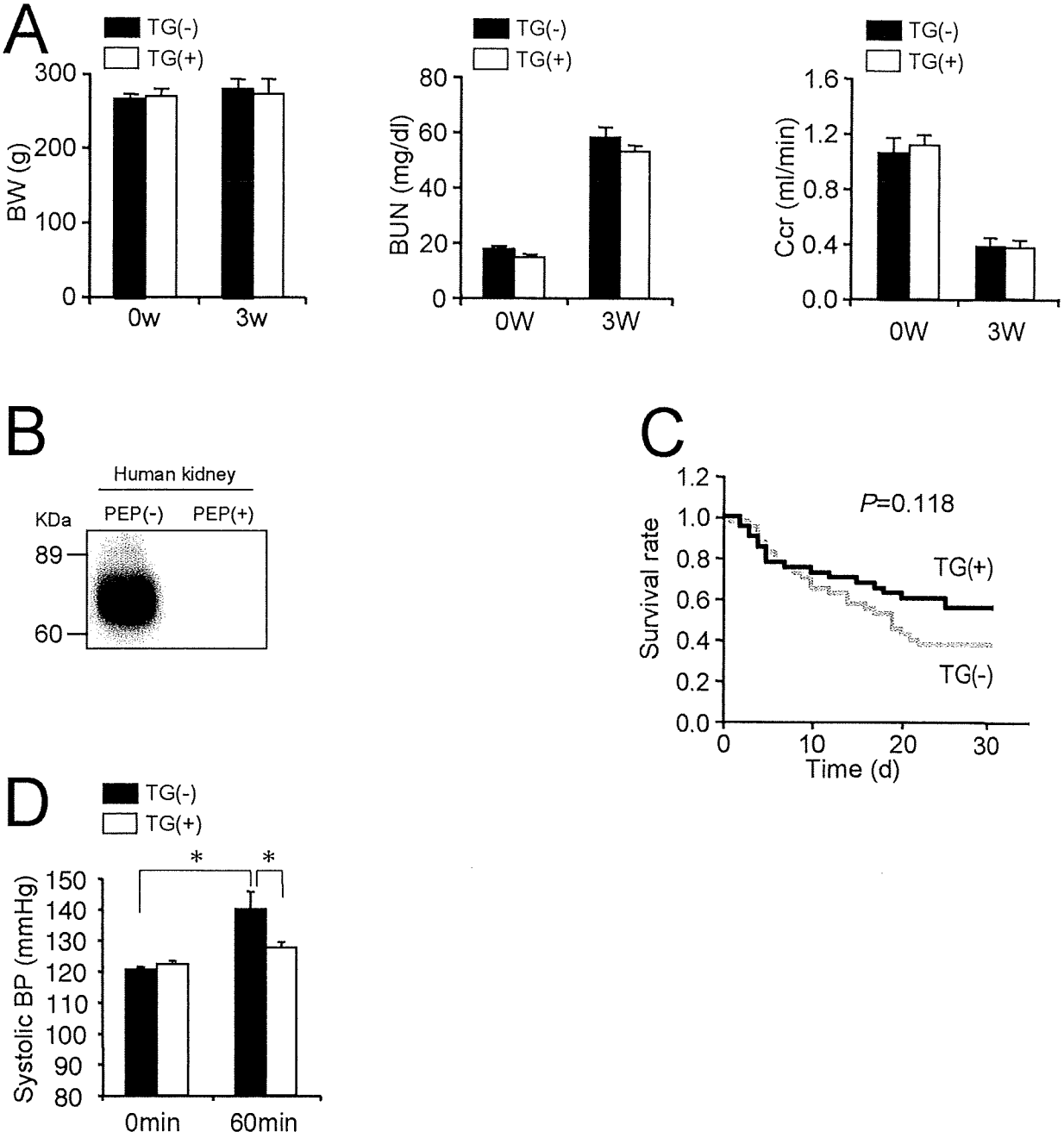
See related editorial, "Harnessing Transporters to Clear Uremic Toxins," on pages 2483–2484.

Supplemental information for this article is available online at http://www.jasn.org/.

Supplementary Figure 1

Toyohara T. et al.

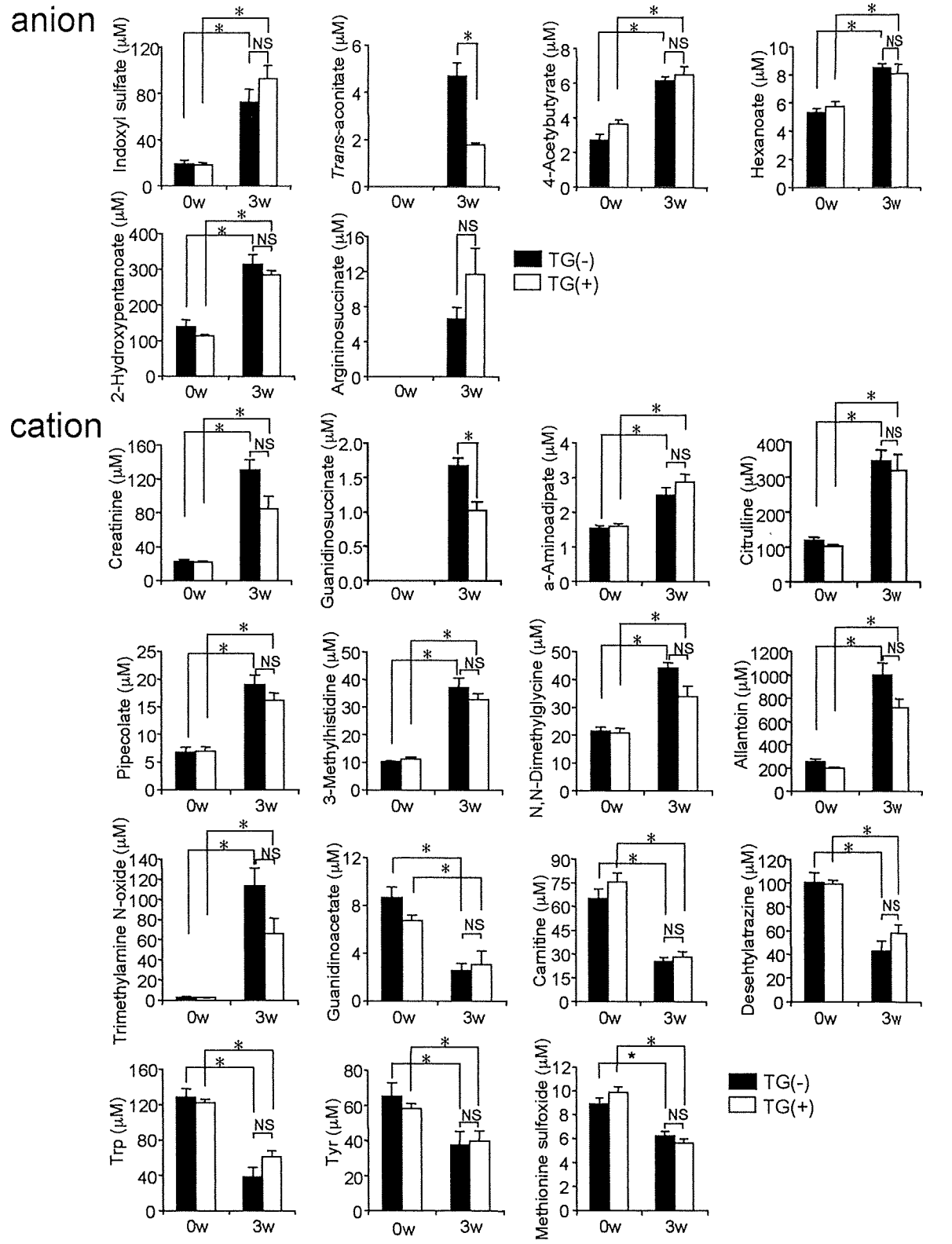


Supplementary Figure 1 Characterization of TG rats. (**A**) Body weight and biochemical examinations of nephrectomized TG rats (n=16-20 per group). (**B**) Western blotting with whole human kidney protein using antibody against human SLCO4C1. A single band was abolished by peptide absorption, suggesting the specificity of the antibody. (**C**) Survival rate of TG(-) rat (n=41) and TG(+) rat (n=40) after 5/6 nephrectomy. Kaplan-Meier curves and log-rank test were used to analyze the mortality rates. P=0.118 by log-rank test.

(**D**) Blood pressure after intraperitoneal-injection of *trans*-aconitate (400mg/kg) to TG(-) rat and TG(+) rat (n=3 per group).

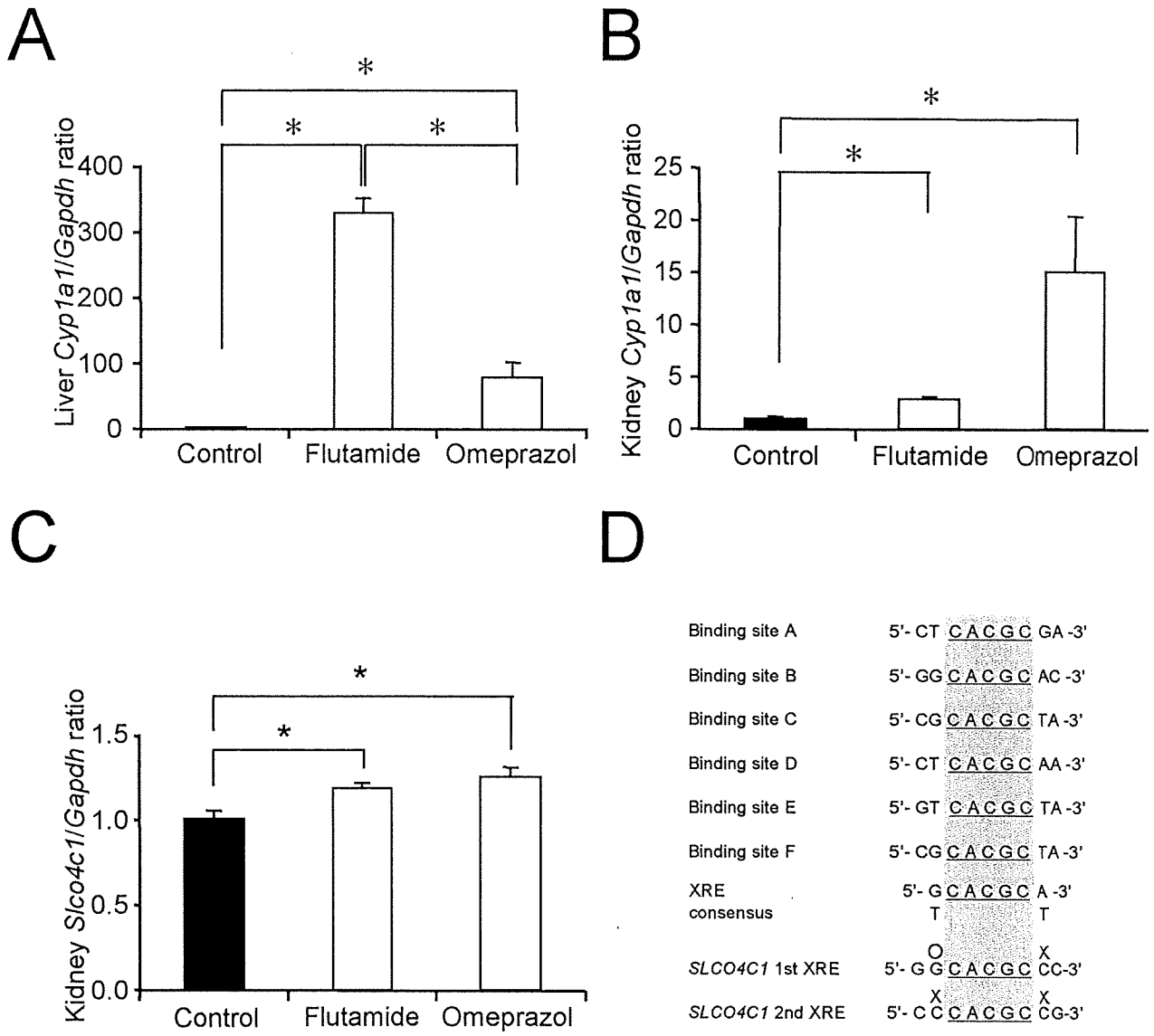
Supplementary Figure 2

Toyohara T. et al.



Supplementary Figure 2 Metabolome analysis of uremic toxins measured by CE-MS. Anionic and cationic compounds that were significantly increased or decreased after nephrectomy were nominated (all analyzed data are in Supplementary Tables 1 - 4). Note that, among these, the plasma levels of GSA and *trans*-aconitate were significantly decreased in TG(+)Nx rats 3 weeks after nephrectomy. Closed column; TG(-) rats, open column; TG(+) rats. *P<0.05. (n=4-5 per group).

Supplementary Figure 3 Toyohara T. et al.



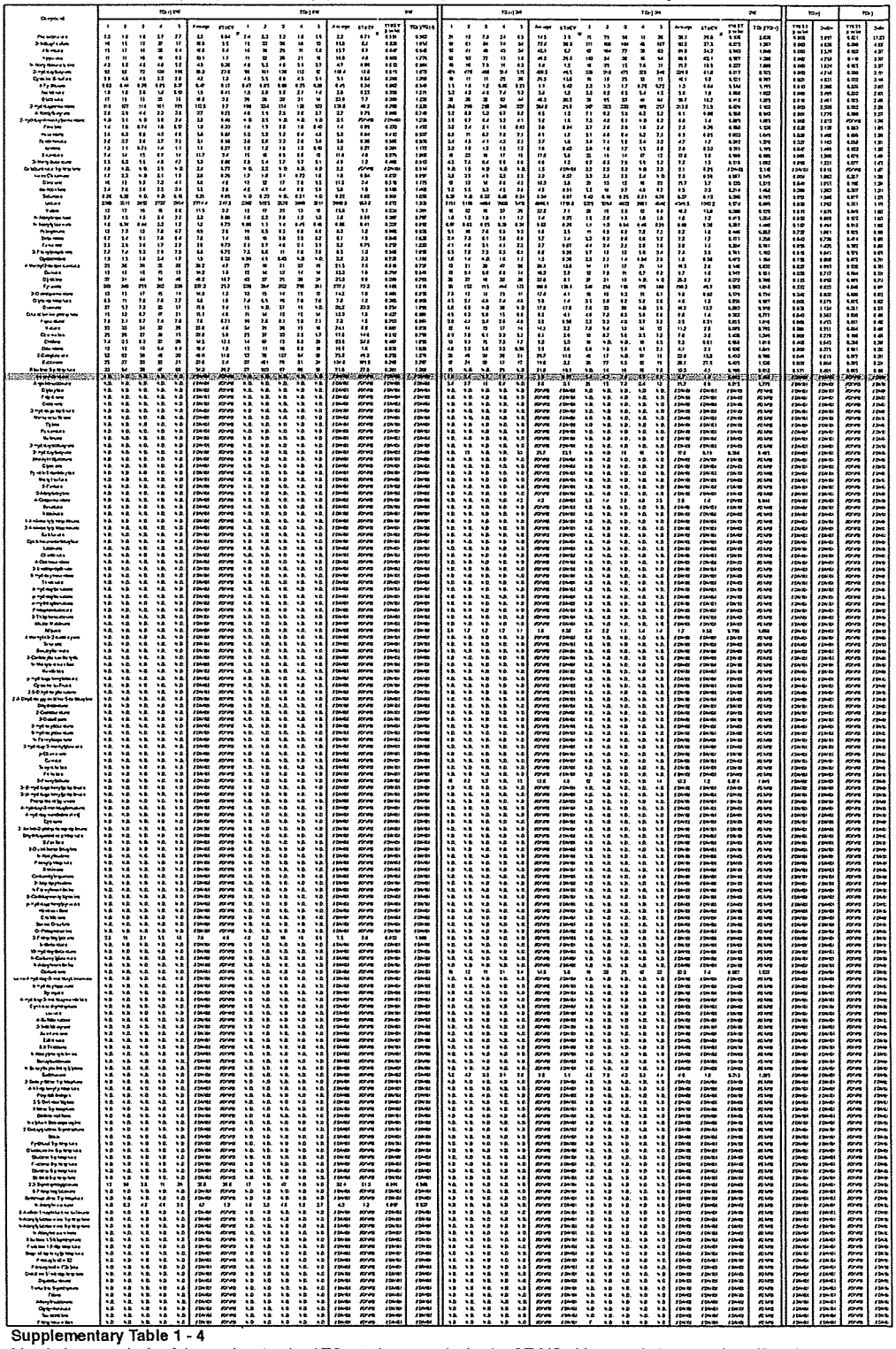
Supplementary Figure 3 Different Induction of cyp1a1 transcription by atypical AhR ligands. QT-PCR analysis of rat liver cyp1a1 (**A**), kidney cyp1a1 (**B**) and kidney slco4c1 (**C**) after administration of flutamide (15.6 mg/kg/day) or omeprazole (125 mg/kg/day). Compounds were administered to Sprague-Dawley rats in corn oil by oral gavage once daily in the morning (Coe et al, 2006). Three days after administration, the mRNA level was measured by QT-PCR. *P<0.05 (n=3 per group). (**D**) Alignment of representative XRE motifs (Wu & Whitlock, 1993) and putative consensus sequence (Nioi & Hayes, 2004). Note that, although the XRE core sequence is conserved in human SLCO4C1, its surrounding 5'- and 3'-nucreotide sequences were not identical to the other XRE consensus motifs. O, consensus pattern; X, non-consensus pattern.

Coe KJ, Nelson SD, Ulrich RG, He Y, Dai X, Cheng O, Caguyong M, Roberts CJ, Slatter JG (2006) Profiling the hepatic effects of flutamide in rats: a microarray comparison with classical aryl hydrocarbon receptor ligands and atypical CYP1A inducers. *Drug Metab Dispos* **34:** 1266-1275

Nioi P, Hayes JD (2004) Contribution of NAD(P)H:quinone oxidoreductase 1 to protection against carcinogenesis, and regulation of its gene by the Nrf2 basic-region leucine zipper and the arylhydrocarbon receptor basic helix-loop-helix transcription factors. *Mutat Res* **555:** 149-171

Wu L, Whitlock JP, Jr. (1993) Mechanism of dioxin action: receptor-enhancer interactions in intact cells. *Nucleic Acids Res* **21:** 119-125

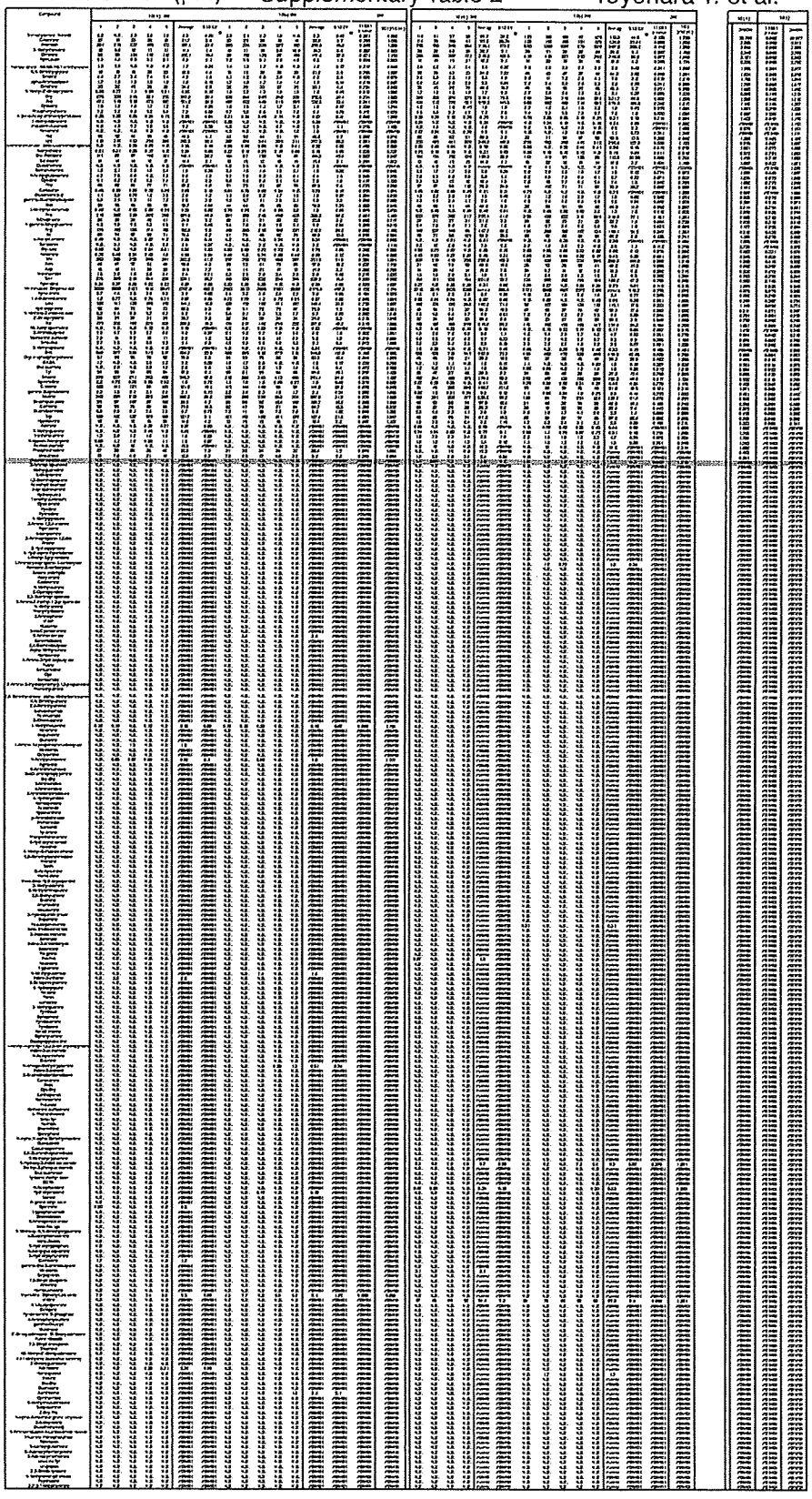
Plasma Anion(μM)　　Supplementary Table 1　　Toyohara T. et al.



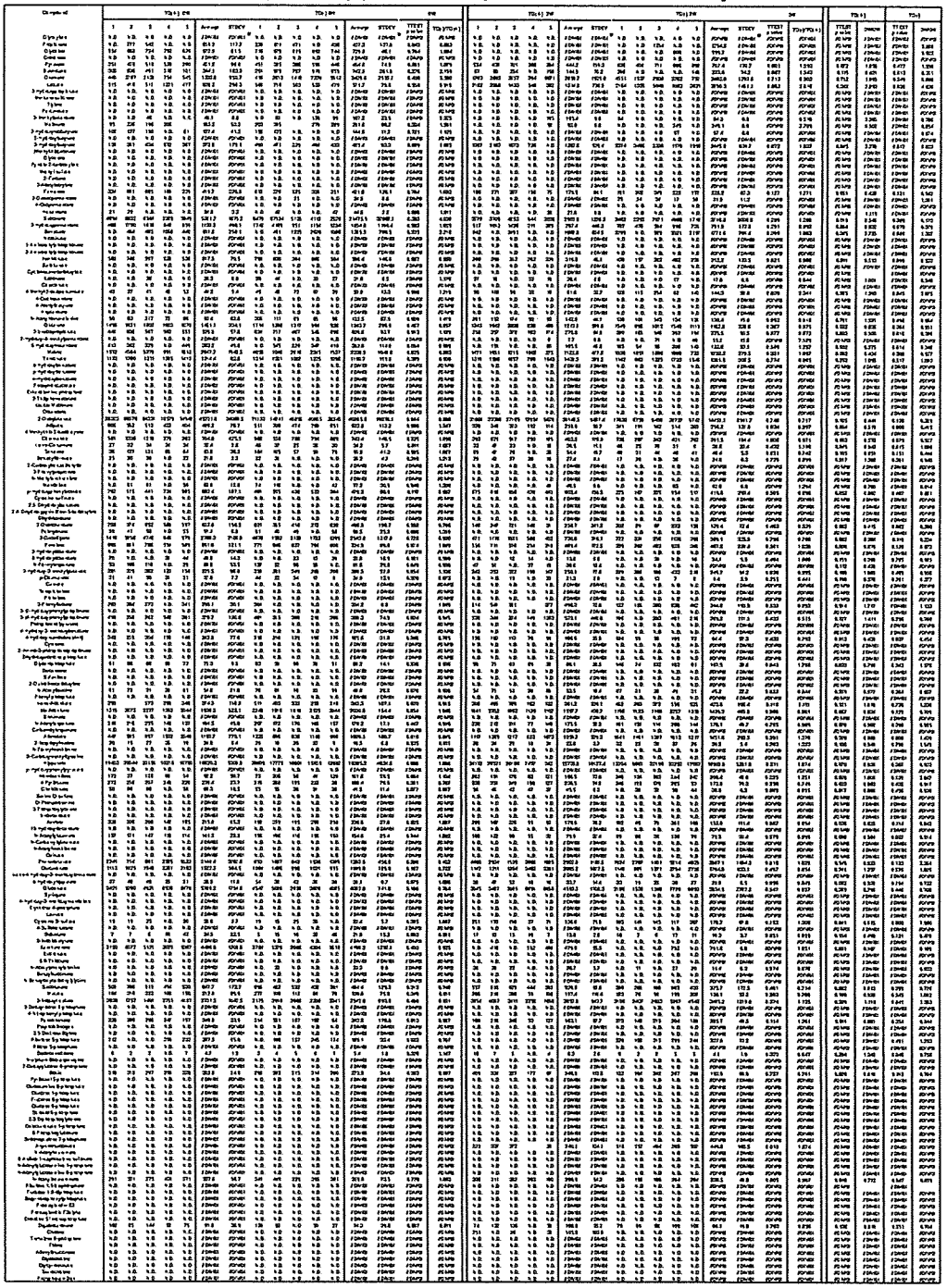
Supplementary Table 1 - 4

Metabolome analysis of the nephrectomized TG rat plasma and urine by CE-MS. Measured plasma anions (Supplementary Table 1), plasma cations (Supplementary Table 2), urine anions (Supplementary Table 3), urine cations (Supplementary Table 4) are listed. Plasma compounds are listed in the order of magnitude of 3w/0w ratio of TG(-) rat. A higher 3w/0w ratio means a high concentration of the compound at 3 weeks after nephrectomy compared with the value before nephrectomy. Yellow indicate the compounds that show a statistically significant difference in the plasma concentration before and after nephrectomy. We only nominated compounds that showed differences between both TG(-) and TG(+) rats as well as two different TG rat lines. Furthermore, among the compounds depicted in yellow, we further chose compounds that showed significantly lower concentrations in TG(+) rats than in TG(-) rats 3 weeks after nephrectomy, which are depicted in red. Note that under these criteria, we could not find a compound whose plasma concentration was increased in TG(+) rats compared to TG(-) rats 3 weeks after nephrectomy. Statistical difference was determined by Student's t-test.

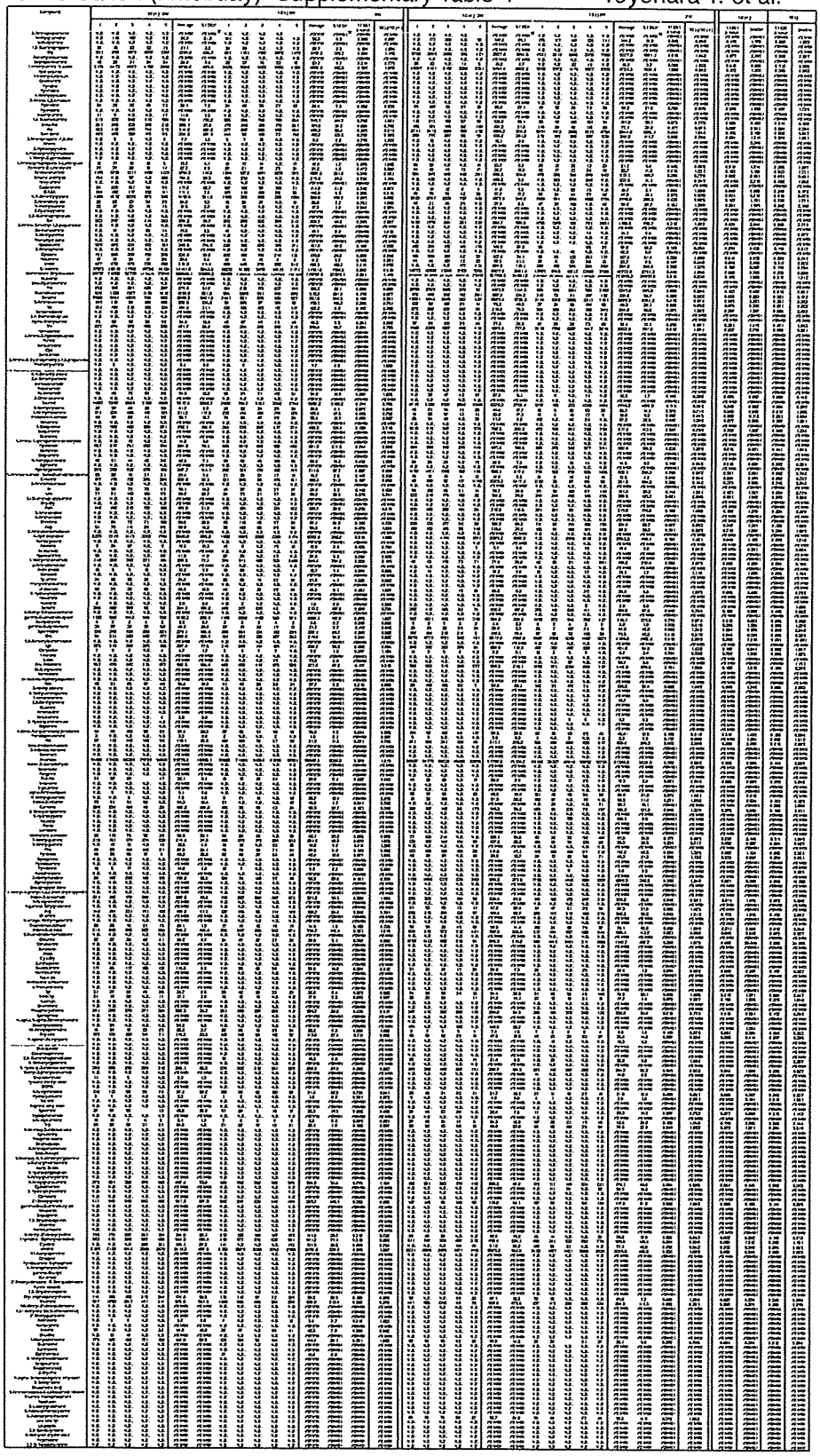
Plasma Cation (μM) Supplementary Table 2 Toyohara T. et al.



Urine Anion (nmol/day) Supplementary Table 3 Toyohara T. et al.



Urine Cation (nmol/day) Supplementary Table 4 Toyohara T. et al.



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CLINICAL STUDY

Physiologic variance of corticotropin affects diagnosis in adrenal vein sampling

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Abstract

Objective: Differentiating unilateral form from bilateral is a critical diagnostic step in primary aldosteronism (PA), for which adrenal vein sampling (AVS) is accepted to be the most reliable. However, variance of corticotropin could affect the diagnosis in AVS.

Design and methods: We conducted simultaneous bilateral AVS on ten biochemically diagnosed PA cases, and used the aldosterone-to-cortisol ratio (A/C) of the samples for the diagnosis. The diagnosis by AVS after a low-dose (0.1 μg) ACTH stimulation, which can provoke maximum-physiologic corticotropic response, was compared with those before the stimulation and after the standard-dose (250 μg) ACTH stimulation.

Results: In half of the cases, the low-dose pre-stimulation affected the diagnosis. In four out of ten cases, the side-to-side ratios of A/C were changed in the basal/low-dose/standard-dose AVS as 6.62/2.46/0.63, 2.13/0.41/0.14, 1.88/2.38/2.40, and 1.96/2.27/1.90 respectively. In three out of ten cases, the adrenal vein to the matching inferior vena cava ratio of A/C was also changed across 1, the cut-off to indicate suppression of aldosterone secretion. Additionally, the confirmation of successful sampling was difficult in five out of ten and two out of ten cases of the basal and low-dose AVS respectively, whereas it was easy in all the cases of the standard-dose AVS.

Conclusions: The diagnosis in the basal AVS could be affected by the physiologic fluctuation of ACTH at relatively high prevalence. The basal AVS would be unreliable to differentiate two forms of PA.

European Journal of Endocrinology 160 459–463

Introduction

Primary aldosteronism (PA) is recognized as a common cause of secondary hypertension, and is generally caused by either aldosterone-producing adenoma (commonly unilateral) or idiopathic hyperaldosteronism (commonly bilateral) (1–3). Differentiation of unilateral form from bilateral is a critical step in the diagnosis of PA, because the unilateral form is potentially curable by unilateral adrenalectomy and the bilateral form is generally treated with the administration of mineralocorticoid receptor antagonists.

Adrenal vein sampling (AVS) is the widely accepted way for differentiation, but the methodology used for AVS is different among different centers (3). Independent of the methodological difference, the following points are critical to perform adrenalectomy: i) confirmation of successful sampling and ii) certainty of the laterality of aldosterone hypersecretion. Adrenocortical hormones are under the effect of ACTH, and AVS without sufficient pre-stimulation could be affected by its fluctuation (4–6). Therefore, some investigators advocated pre-stimulation with synthetic corticotropin to ensure active adrenal secretion at the time of AVS (7–10). However, others expressed concern that the pre-stimulation could abolish the laterality of aldosterone secretion (11); the dose of synthetic corticotropin generally used for the pre-stimulation is extra-physiologically high (12, 13), and the exaggerated corticotropic response by it could conceal the laterality.

In this study, we performed AVS in three different conditions: before any artificial stimulation, after stimulation by a low-dose (0.1 μg) cosyntropin, which can provoke maximum-physiologic corticotropic response, and after stimulation by the standard-dose (250 μg) cosyntropin. We found that confirmation of successful sampling is difficult without sufficient pre-stimulation and that physiologically attainable corticotropin could affect the diagnosis in the AVS.

Subjects and methods

Subjects

The hypertensive patients with plasma renin activity (PRA; normal range: 0.2–2.7 ng/ml per h) ! 1.0 ng/ml per h and plasma aldosterone concentration (PAC;

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normal range: 3.6–24 ng/dl) O12 ng/dl with the PAC-to-PRA ratio (PAC/PRA) O40 were further evaluated (14, 15). After the withdrawal of b-blockers and diuretics for at least 4 weeks, a captopril test was performed; PRA and PAC were measured at 60 or 90 min after an oral administration of 50 mg of captopril, and PAC/PRA was calculated (15). The patients with PAC/PRA O30 in the captopril test were given the biochemical diagnosis of PA (3). We enrolled ten of these cases in the study. The study protocol was approved by the ethics committee of our hospital, and informed consent was obtained from all the patients.

Simultaneous bilateral AVS

Catheterization of both femoral veins was performed in all the cases in the early afternoon (1300–1400 h). Blood samples of AVS for the measurement of PAC and plasma cortisol concentration were simultaneously obtained from both the AV and the infrarenal inferior vena cava (IVC; basal AVS). A low-dose cosyntropin (0.1 mg) was administered as an i.v. bolus, and the sampling was repeated 15 min later (low-dose AVS). After the low-dose AVS, the standard-dose cosyntropin (250 mg) was administered and the sampling was repeated 15 min later (standard-dose AVS). The patients were kept supine throughout the AVS procedure.

Chemical and hormonal assays

The serum creatinine concentration (sCr) and PRA were enzymatically measured. Plasma cortisol concentration was measured with a commercially available kit (Fluorescence Polarization Immunoassay, TDX/TDXFLX Cortisol; Abbott Japan Co. Ltd). The intra-assay coefficients of variation were 7.54–7.56, 2.94–3.20, and 1.98–2.30% for its low, medium, and high levels respectively. PAC was measured using commercial laboratory test services (Mitsubishi Chemical Medience, Tokyo, Japan). The reported intra-assay coefficients of variation were 8.3, 3.9, and 1.8% for PAC of 10.3, 33.6, and 73.2 ng/dl respectively.

Diagnosis in AVS

We used the PAC-to-plasma cortisol concentration ratio (A/C) of AVS samples for the diagnosis in AVS. The ratio of A/C in one AV to the other (the side-to-side ratio) was used to judge the laterality of aldosterone secretion; the ratios O4 and O2 were taken as conservative and aggressive cut-offs respectively (3, 7–9, 16). The AV-to-IVC ratio (AV/IVC) of A/C ! 1 was taken as the suppression of aldosterone secretion for the side (7, 8, 10).

Results

Baseline characteristics

The baseline characteristics of the patients are summarized in Table 1. Except for one case who had an sCr of 106.1 mmol/l, all the other cases had preserved renal function (sCr %88.4 mmol/l, normal range: 35.4–88.4 mmol/l). Half of the cases (5/10) had hypokalemia (serum potassium concentration %3.4 mmol/l, normal range: 3.5–4.8 mmol/l), and four out of five of them had PAC O24 ng/dl. All the cases who did not have hypokalemia had PAC ! 24 ng/dl. The plasma corticotropin was less than its upper normal limit (normal range: 9–52 pg/ml).

Aldosterone and cortisol concentration in AVS

The results of AVS are shown in Table 2. The low-dose pre-stimulation increased PAC and plasma cortisol concentration in the AV samples except for one sample, and the standard-dose pre-stimulation increased them in all the AV samples. The pre-stimulation increased the AV/IVC of plasma cortisol concentration in all the AV samples, and the ratios were O1.6, O1.9, and O19 in the basal, low-dose, and standard-dose AVS respectively.

Laterality of aldosterone secretion

The side-to-side ratio of A/C is summarized in Table 3. Seven cases had the ratio in the basal AVS higher than the conservative cut-off (O4). In six of them, the ratio remained O4 in both the low- and standard-dose AVS. In the other case with the ratio of 6.62 (case 1), however, the ratio decreased to 2.46 (lower than the conservative cut-off, but higher than the aggressive cut-off) in the low-dose AVS. It decreased further to 0.63 and the laterality was changed in the standard-dose AVS, but its reciprocal ratio was lower than the aggressive cut-off (! 2).

Three cases had the ratio in the basal AVS lower than the conservative cut-off (! 4). In one case with the ratio higher than the aggressive cut-off (2.13, case 2), the laterality was changed in the low-dose AVS and the reciprocal ratio was 2.47 (lower than the conservative

Table 1 Baseline characteristics of patients.

Number (% female)	10 (30)
Age (years)	55G13 (34–71)
sCr (mmol/l)	73.4G15.6 (35.4–106.1)
sK^C (mmol/l)	3.4G0.6 (2.3–4.0)
ACTH (pg/ml)	16.1G7.2 (6.2–32.7)
PRA (ng/ml per hour)	0.2G0.1 (0.1–0.4)
PAC (ng/dl)	33.0G21.7 (13.5–66.8)
PAC/PRA	258G220 (55.5–668)

Values are expressed as meanGs.d. with their distribution given in parentheses, where appropriate. sCr, serum creatinine concentration; sK^C; serum potassium concentration; PRA, plasma renin activity; PAC, plasma aldosterone concentration.

Table 2 Results of adrenal vein sampling (AVS).

Case		Basal		Low dose		Standard dose	
		PAC	Cortisol	PAC	Cortisol	PAC	Cortisol
1	R/L	1558/177	22.1/16.6	1939/869	128/141	3643/3559	925/565
	IVC	9.7	3.7	16.6	7.1	25.9	22.0
2	R/L	401/136	18.4/13.2	317/508	171/111	648/7680	520/880
	IVC	11.8	2.8	11.9	5.5	21.6	13.9
3	R/L	61.7/20.3	84.0/54.2	594/221	341/288	2518/896	1159/785
	IVC	6.2	15.4	4.2	15.4	10.6	23.6
4	R/L	34.5/53.8	16.8/13.9	223/244	115/53	1310/2623	621/531
	IVC	9.3	6.9	11.2	8.2	16.4	18.9
5	R/L	498/17.5	18.2/17.1	2026/216	244/174	4432/592	443/492
	IVC	10.6	7.2	17.9	15.3	27	23.7
6	R/L	318/8.7	26/27	386/8.5	28.4/26	7667/447	1051/735
	IVC	7.5	15.7	8.1	13.5	20	25.5
7	R/L	2467/52.7	19.2/15.6	2269/125	20.9/304	10853/458	688/801
	IVC	61.3	5.5	72	10.1	80.0	18.2
8	R/L	563/17.7	17.9/17.1	1543/74.8	359/109	20006/373	521/438
	IVC	13.7	10.5	23.0	14.7	46.0	23.0
9	R/L	942/44.4	33.3/40.4	1964/177	404/424	3531/352	555/823
	IVC	42.9	8.9	45.5	15.0	60.5	26.2
10	R/L	34.9/2904	20.7/17.7	130/4001	275/362	475/8451	1354/1071
	IVC	39.6	2.6	51.3	7.6	79.5	25.5

PAC, plasma aldosterone concentration (ng/dl); cortisol, plasma cortisol concentration (mg/dl); R, right adrenal vein; L, left adrenal vein; IVC, inferior vena cava.

cut-off, but higher than the aggressive cut-off). The reciprocal ratio increased further to 7.01, higher than the conservative cut-off, in the standard-dose AVS. The other two cases (cases 3 and 4) had the ratio in the basal AVS lower than the aggressive cut-off (! 2). In these cases, the laterality was not changed, but the ratio increased to O2 in the low-dose AVS; the ratios in the basal/low-dose/standard-dose AVS were 1.88/2.38/2.40 and 1.96/2.27/1.90 respectively.

Suppression of aldosterone secretion

The suppression of aldosterone secretion, which was indicated by the AV/IVC of A/C ! 1, was also changed by the pre-stimulation in three cases (Table 4). Two cases (cases 3 and 5), which had the ratio of one side ! 1 in the basal AVS, had the ratio of the both sides O1 in both the low- and standard-dose AVS. Conversely, one case (case 2), which had the ratio of the both sides O1 in the basal AVS, had the ratio of one side ! 1 in both the low- and standard-dose AVS.

Table 3 The side-to-side ratio of aldosterone-to-cortisol ratio.

Case		Basal	Low dose	Standard dose
1	R/L (L/R)	6.62 (0.15)	2.46 (0.41)	0.63 (1.60)
2	R/L (L/R)	2.13 (0.47)	0.40 (2.47)	0.14 (7.01)
3	R/L (L/R)	1.96 (0.51)	2.27 (0.44)	1.90 (0.53)
4	R/L (L/R)	0.55 (1.88)	0.42 (2.38)	0.42 (2.40)
5	R/L (L/R)	26.8 (0.04)	6.68 (0.15)	8.32 (0.12)
6	R/L (L/R)	37.9 (0.03)	41.5 (0.02)	12.0 (0.08)
7	R/L (L/R)	38.1 (0.03)	264 (0.00)	27.6 (0.04)
8	R/L (L/R)	30.3 (0.03)	6.25 (0.16)	45.1 (0.02)
9	R/L (L/R)	25.7 (0.04)	11.7 (0.09)	14.9 (0.07)
10	R/L (L/R)	0.01 (97.7)	0.04 (23.4)	0.04 (22.5)

A/C, plasma aldosterone concentration (ng/dl)/plasma cortisol concentration (mg/dl); R, right adrenal vein; L, left adrenal vein.

Discussion

In this study, we showed that the diagnosis in the basal AVS could be affected by the physiologic fluctuation of ACTH at the time of AVS. The fluctuation could affect either the laterality or the suppression of aldosterone secretion at relatively high prevalence. In addition, we also showed that confirmation of successful sampling is difficult without sufficient pre-stimulation.

In the basal AVS, six cases had PAC of IVC ! 12 ng/dl, although we selected cases of the baseline PAC O12 ng/dl. The usage of calcium channel blockers, which can suppress aldosterone secretion, could have decreased the PAC of IVC in the basal AVS, because we used them to control blood pressure during AVS (17, 18). It is also possible that the difference between the PAC of IVC in the basal AVS and the baseline PAC reflected the physiologic fluctuation of PAC, because we performed AVS in the early afternoon (5). Performance of AVS in the early afternoon is the main limitation of this study. The difference between the basal and low-dose AVS might have been decreased if AVS were performed in the morning when ACTH is generally higher than in the afternoon.

For the pre-stimulation, we used cosyntropin, a synthetic corticotropin, which has corticotropic potency as the natural corticotropin (19). A bolus injection of 0.1 mg cosyntropin is expected to elevate its plasma concentration to w200 pg/ml (12). The insulin

Table 4 The adrenal vein/inferior vena cava of aldosterone-to-cortisol ratio.

Case		Basal	Low dose	Standard dose
1	R/L	27.0/4.07	6.54/2.66	3.39/5.42
2	R/L	5.26/2.47	0.85/2.10	0.85/5.63
3	R/L	1.83/0.93	6.28/2.81	4.84/2.54
4	R/L	1.52/2.87	1.42/3.37	2.44/5.70
5	R/L	18.5/0.69	7.08/1.06	8.79/1.06
6	R/L	25.6/0.68	22.7/0.55	9.29/0.77
7	R/L	11.5/0.30	15.2/0.06	3.60/0.13
8	R/L	24.1/0.79	2.75/0.44	19.2/0.43
9	R/L	5.87/0.23	1.60/0.14	2.79/0.19
10	R/L	0.11/10.8	0.07/1.63	0.11/2.53

AV, adrenal vein; IVC, inferior vena cava; A/C plasma aldosterone concentration (ng/dl)/plasma cortisol concentration (mg/dl); R, right adrenal vein; L, left adrenal vein.

hypoglycemic test, an acknowledged test to provoke maximum physiologic response of the pituitary–adrenal axis, can elevate plasma ACTH concentration to 100–300 pg/ml (20). Therefore, the low-dose pre-stimulation is thought to induce the maximum-physiologic but not supra-physiologic corticotropic response. Supporting this notion, the plasma cortisol concentration in the low-dose AVS was lower than its matching concentration in the standard-dose AVS.

After the low-dose AVS, we conducted the standard-dose AVS consecutively. The preceding stimulation could have modified the adrenal response to the following stimulation. However, the preceding low-dose AVS is not thought to have significantly modified the following standard-dose AVS in the present cases, because the adrenal response in the standard-dose AVS was several times higher than that in the low-dose AVS. The preserved adrenal response after the preceding 0.5 mg cosyntropin stimulation is also reported; the response to 250 mg stimulation after the preceding 0.5 mg stimulation is more than half of its maximum response (13).

The AV/IVC ratio of plasma cortisol concentration is widely used as an index for successful sampling. Its values from O1.1 to O5 are used for the judgment with lower values for the basal AVS than the pre-stimulated AVS (7, 11, 21, 22); the ratio O3 and O5 can be considered as conservative cut-offs for the former and the latter respectively. Using these conservative cut-offs, 9 out of 20 of the basal AVS samples and 3 out of 20 of the low-dose AVS samples are taken as unsuccessful, whereas all the standard-dose AVS samples are taken as successful. Consequently, only in half of the present cases, all three sets of AVS were taken successful by the conservative cut-offs. However, we verified the position of the catheter tip in each sampling by gentle injection of a small amount of contrast medium, and confirmed accomplishment of three sets of AVS at the same position in each case. Therefore, the result of basal and low-dose AVS samples being taken as unsuccessful by the conservative cut-offs indicates difficulty in confirming successful sampling without sufficient pre-stimulation. Difficulty in confirming successful sampling without pre-stimulation is also reported; nearly half of the basal AVS samples that the radiologist considered successful do not have the ratio O3 (21).

Unilateral form of aldosterone secretion is generally indicated in two ways: (i) laterality of secretion, which is judged by the side-to-side ratio of A/C either O4–5 (3, 7–9) or O2 (16) and (ii) suppression of secretion from the other side, which is judged by the AV/IVC of A/C ! 1 (7, 8, 10). The low-dose pre-stimulation affected either the laterality or the suppression in half of the present cases. In a subset of the cases in whom all three sets of AVS were taken as successful by the conservative cut-offs, the low-dose pre-stimulation also affected either the laterality or the suppression in two out of five cases. These results indicate that the diagnosis in the basal AVS is unreliable; the physiologic fluctuation of ACTH could change the diagnosis in the basal AVS (4). Supporting this notion, we experienced wide case-dependent difference of the basal ACTH stimulation (6).

Slight difference of each ratio is practically indistinguishable due to the variation of the intra-assay coefficients for PAC and plasma cortisol concentration, and the diagnosis in cases 3, 4, and 5 could be taken the same in all three types of AVS. However, the diagnosis in cases 1 and 2 could not be taken the same. The pre-stimulation amply changed the side-to-side ratio of A/C across the cut-offs, although both cases could be taken as bilateral lack of suppression independent of the pre-stimulation. These controversial cases did not undergo adrenalectomy, because they could be bilateral forms. Without histological confirmation, we could not determine which set of AVS was accurate, but we think that the diagnostic AVS should be performed under constant conditions such as after sufficient ACTH stimulation. Difficulty in confirmation of successful sampling without sufficient pre-stimulation also supports the necessity of ACTH stimulation in the diagnostic AVS.

In summary, the results of this study indicate that the physiologic fluctuation of ACTH can affect the diagnosis in the basal AVS. Although the enrollment was small, the high prevalence with diagnostic change by the physiologically attainable corticotropin indicates that the diagnosis in the basal AVS is unreliable in not a few cases. Difficulty in confirmation of successful sampling is another disadvantage of the basal AVS. Therefore, AVS with sufficient pre-stimulation, such as the standard-dose AVS, is preferable, especially when the basal AVS is difficult to be taken successful.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Hemodynamic Index of Atheromatous Renal Artery Stenosis for Angioplasty

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Background and objectives: Trans-stenotic pressure gradient across the constriction (PG), a hemodynamic variable of atheromatous renal artery stenosis (ARAS), is a widely used indicator for angioplasty, but its association with the outcome of angioplasty has not been fully investigated.

Design, setting, participants & measurements: In 34 hypertensive cases with unilateral ARAS, we evaluated hemodymanic variables of ARAS with reference to the systemic BP reduction after angioplasty as the outcome.

Results: In each phase, PG divided by its corresponding prestenotic arterial BP (PG/preBP) had better association with the outcome than PG. The mean phase PG/preBP had the largest area under the curve in the receiver operating characteristic analysis (0.794) with the sensitivity/specificity of 0.957/0.545 for its cut-off >0.15. Although the plasma renin activity, which reflects the perfusion to renal parenchyma, was higher in the angioplasty-efficacious cases than in the angioplasty-inefficacious cases before angioplasty (7.8 • 6.6 versus 3.4 • 3.8 ng/ml/h, P • 0.049), it was not generally reduced by angioplasty independent of the outcome.

Conclusions: As the index to select ARAS for angioplasty, PG/preBP was better than PG and the mean phase PG/preBP could be the best. However, other factors such as the microvascular kidney disease, which affect the perfusion to renal parenchyma, would influence the outcome.

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Impairment of renal perfusion by arterial constriction can induce systemic hypertension, known as renovascular hypertension, and atheromatous renal artery stenosis (ARAS) accounts for 70 to 90% of the cases (1,2). However, not all of the ARAS lesions impair renal perfusion, and the lesions that do not impair renal perfusion can be seen in hypertensive patients, especially in the elderly (3–5). Although angioplasty could improve renal perfusion, the procedure during angioplasty might deteriorate renal function. Therefore, angioplasty should be performed only for the lesions that could benefit by it.

At the perfusion-impairing lesion, hydrodynamic pressure drops. The drop is generally indicated by the trans-stenotic pressure gradients (PG), the difference of the arterial BP (BP) across the constriction, and PG of either the systolic or the mean phase is widely used to indicate the "hemodynamic significance" of ARAS (2,6). However, PG has not been sufficiently evaluated in accordance with the therapeutic outcome of angioplasty, and the hemodynamic variables to select the lesions that benefit by angioplasty remain to be elucidated. In this study, we evaluated hemodynamic variables of ARAS with reference to the systemic BP reduction after angioplasty as the outcome.

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Materials and Methods

Patients Selection

All of the patients who were referred to our department for suspicion of renovascular hypertension between January 1999 and December 2007 (n • 808) were enrolled in this study. Patients with at least one risk factor of atheroma (smoking, dyslipidemia, or DM) in addition to hypertension were evaluated by computed tomographic arteriography (CTA) or magnetic resonance arteriography (MRA), if they had relatively preserved renal function (serum creatinine concentration (sCr) • 176.8 mmol/L). Patients suspected of having ARAS based on the results of CTA and MRA were further evaluated by selective renal arteriography, and 34 patients with unilateral ARAS on one of the main trunks were included in the study. In all 34 patients, ARAS was treated by percutaneous transluminal renal angioplasty with the insertion of a stainless steel endoprosthesis (Palmaz stent with a diameter of 5 to 7 mm). Informed consent was obtained from all of the patients.

Data Collection

Baseline and postangioplastic information were recorded before the angioplasty and at 1 to 3 mo after the angioplasty, respectively. All blood samples were collected under fasting conditions in the morning. The sCr and the plasma renin activity (PRA) were enzymatically measured. The glomerular filtration ratio (GFR) was estimated by using the Modification of Diet in Renal Disease (MDRD) study equation modified for Japanese (7).

Therapeutic Outcome of Angioplasty

As the therapeutic outcome of angioplasty, we used the systemic BP reduction after angioplasty, which was defined as reduction in the total score of the administered antihypertensive agents with no increase in the office BP after angioplasty. For each patient, the total score was

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calculated before and after angioplasty as follows. Each antihypertensive agent was standardized by dividing the administered dose by its corresponding maximum approved daily dose. The sum of all of the standardized doses was used as the total score. The office BP reading was performed before and after angioplasty according to the guideline (6). The self-monitoring of the pre- and postangioplastic BP was also performed in all of the patients (8,9), and no increase in self-monitored BP was confirmed in the patients with no increase in the office BP.

Evaluation of the Arteriography

We evaluated the angiography of ARAS as described previously (10). In brief, the width of the renal artery was measured by a computer-based method on the arteriography with the narrowest image of ARAS. The width of the narrowest part was taken as the minimal lumen diameter, and the mean width of the first normal segments proximal and distal to ARAS was taken as the reference width. The % diameter reduction was calculated as "100 (1 • width of narrowest part/the reference width)."

Measurement of PG

We measured PG in each ARAS lesion using an end-hole 3-French catheter. The catheter was placed distal to the lesion, and the pressure measured there was recorded as the poststenotic arterial BP. Then the catheter was pulled back over the lesion and placed proximal to the lesion. The pressure measured there was recorded as the prestenotic arterial BP. PG was calculated by subtracting the poststenotic arterial BP from the prestenotic arterial BP in each phase. In all of the ARAS lesions, the systolic phase PG was decreased to • 10 mmHg after angioplasty.

Statistical Analysis

Continuous variables were expressed as the mean • SD (SD) and compared by using t test. Discrete variables were expressed as counts and compared by using •^{2} test. Statistical analysis was performed by using the Dr. SPSS II software package (SPSS, Chicago, IL). Probability values of P • 0.05 were considered statistically significant.

Results

Baseline Characteristics of the Patients

The baseline characteristics of the 34 cases examined in this study are summarized in Table 1. Angioplasty decreased the total score of antihypertensive agents in 23 cases (angioplasty-efficacious cases), but it did not decrease the score in the other 11 cases (angioplasty-inefficacious cases). The baseline systemic BP was lower and the baseline PRA was higher in the angioplasty-efficacious cases than in the angioplasty-inefficacious cases, while both had nearly the same estimated GFR and used nearly the same doses of antihypertensive agents. The angioplasty-efficacious cases were younger than the angioplasty-inefficacious cases, whereas the difference was not statistically significant.

The total score of the antihypertensive agents was generally high in the cases with low baseline systemic BP and high baseline PRA. However, the correlation of the score with either the systemic BP in each phase or the PRA was NS; the correlations with the systolic BP, the diastolic BP, and the PRA were • 0.097 (P • 0.584), • 0.212 (P • 0.229), and 0.269 (P • 0.123), respectively.

Hemodynamic Variables and the Angioplastic Outcome

Hemodynamic variables of ARAS are summarized in Table 2. We introduced PG divided by its corresponding prestenotic arterial BP (PG/preBP) as the variables. In each phase of BP, preangioplastic PG was higher in the angioplasty-efficacious cases than in the angioplasty-inefficacious cases. Preangioplas-

Table 1. Baseline characteristics of patients[a]

	All (n • 34)	Angioplasty		P
		Efficacious (n • 23)	Inefficacious (n • 11)	
Age (years)	58 • 7	57 • 6	61 • 8	0.072
Gender (% female)	29	26	36	0.54
Systemic BP (mmHg)				
Systolic	132 • 18	127 • 18	142 • 15	0.024
Diastolic	75 • 11	72 • 10	81 • 9	0.017
sCr (• mol/L)	83.2 • 25.9	85.3 • 29.8	78.8 • 15.0	0.50
GFR (ml/min/1.73 m^2)	60.9 • 21.6	61.6 • 23.4	59.5 • 18.1	0.79
PRA (ng/ml/h)	6.4 • 6.1	7.8 • 6.6	3.4 • 3.8	0.049
Antihypertensive, n (%)				
Total score	2.0 • 1.1	2.0 • 1.0	2.0 • 1.3	0.95
CCB	33 (97)	22 (96)	11 (100)	0.48
ACEI	9 (26)	6 (26)	3 (27)	0.94
ARB	14 (41)	11 (48)	3 (27)	0.25
ACEI or ARB	19 (56)	14 (61)	5 (45)	0.40
•-blocker	6 (18)	4 (17)	2 (18)	0.95
•-blocker	8 (24)	4 (17)	4 (36)	0.22
diuretic	8 (24)	6 (26)	2 (18)	0.61

[a]Data are presented as the mean • SD when appropriate. BP, blood pressure; sCr, serum creatinine; GFR, estimated glomerular filtration ratio; PRA, plasma renin activity; CCB, calcium channel blocker; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Table 2. Hemodynamic variables of ARAS[a]

	All (n • 34)	Angioplasty		P
		Efficacious (n • 23)	Inefficacious (n • 11)	
Pre-angioplasty				
PG (mmHg)				
Systolic	70 • 45	81 • 40	48 • 49	0.043
Mean	41 • 30	49 • 27	25 • 30	0.025
Diastolic	24 • 21	29 • 20	12 • 18	0.026
PG/preBP				
Systolic	0.45 • 0.27	0.53 • 0.24	0.28 • 0.24	0.009
Mean	0.38 • 0.26	0.46 • 0.24	0.22 • 0.24	0.016
Diastolic	0.31 • 0.26	0.38 • 0.25	0.15 • 0.22	0.009
Post-angioplasty				
PG (mmHg)				
Systolic	3.4 • 2.9	3.6 • 3.1	2.8 • 2.2	0.46
Mean	2.3 • 2.2	2.5 • 2.5	1.9 • 1.5	0.48
Diastolic	1.6 • 1.7	1.7 • 1.9	1.4 • 1.4	0.66

[a]Data are presented as the mean • SD. PG, trans-stenotic pressure gradient; preBP, prestenotic arterial blood pressure.

tic PG/preBP was also higher in the former than in the latter, and its difference was more significant than that of PG in each phase. In all of the cases, angioplasty was successful; sufficient stent-deployment and no residual stenosis were confirmed by postangioplastic renal arteriography. Although postangioplastic PG was slightly lower in the angioplasty-inefficacious cases than the angioplasty-efficacious cases, the difference was NS in each phase.

In both the systolic and mean phases, PG/preBP had larger area under the curve (AUC) in the receiver operating characteristic (ROC) analysis for the systemic BP reduction after angioplasty than PG (Figure 1). The mean phase PG/preBP had the largest AUC of 0.794; the other variables, the mean phase PG, the systolic phase PG/preBP, and the systolic phase PG, had AUC of 0.775, 0.764, and 0.749, respectively. The mean phase PG/preBP had also higher combination of sensitivity and specificity than the other variables. At a cut-off • 0.15, the sensitivity/specificity for the mean phase PG/preBP was 0.957/0.545. In comparison, the systolic phase PG had the sensitivity/specificity of 0.957/0.273 at a cut-off • 20 mmHg, one of the widely used threshold values for it to perform angioplasty.

The total score of the antihypertensive agents was generally high in the cases with high preangioplastic PG. However, its correlation with each variable of preangioplastic PG was NS, and even the correlation with the mean phase PG/preBP, which had the largest AUC for the outcome, was 0.218 (P • 0.216).

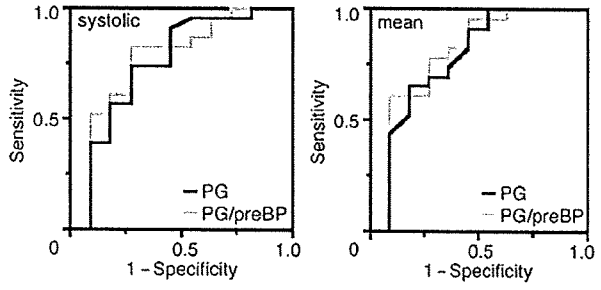

Figure 1. ROC for the angioplastic outcome. Variables in the systolic (left panel) and mean phases (right panel) are analyzed by ROC curve for the systemic BP reduction after angioplasty. Black and gray lines show analysis of PG and PG/preBP, respectively. PG, trans-stenotic pressure gradient; preBP, prestenotic arterial BP.

Angiographic Indices and PG/prePG

We further compared the mean phase PG/preBP with the angiographic indices (Figure 2). The cases with the mean phase PG/preBP • 0.15 had the minimal lumen diameter • 3 mm in 18/20 cases, and the % diameter reduction • 50% in 17/20

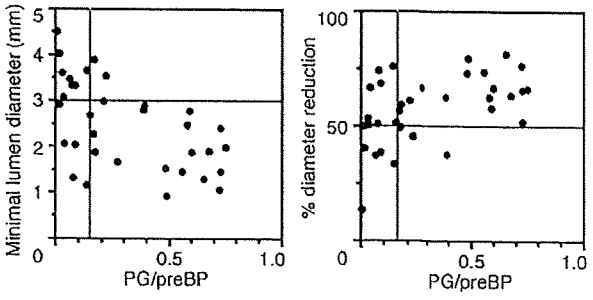

Figure 2. Correlation of hemodynamic index with angiographic indices Angiographic indices, minimal lumen diameter (left panel) and % diameter reduction (right panel), were plotted against the mean phase PG/preBP. Vertical lines indicate PG/preBP of 0.15. Horizontal lines in the left and right panels indicate the diameter of 3 mm and the reduction of 50%, respectively. PG/preBP, trans-stenotic pressure gradient/prestenotic arterial BP in the mean phase.

cases. Conversely, the cases with the minimal lumen diameter • 3 mm had the mean phase PG/preBP • 0.15 in 18/24 cases, and the cases with the % diameter reduction • 50% had the mean phase PG/preBP • 0.15 in 17/25 cases.

Association of PRA and the Angioplastic Outcome

As a variable that reflects the renal perfusion, we compared the baseline and postangioplastic PRA in the angioplasty-efficacious and the angioplasty-inefficacious cases (Table 3). PRA was generally not reduced after angioplasty, and it rather increased after angioplasty in many cases independent of the outcome. The postangioplastic PRA was still higher in the angioplasty-efficacious cases than in the angioplasty-inefficacious cases, and the mean ratio of the postangioplastic PRA to baseline PRA was same in both.

Discussion

The results of this study showed that the mean phase PG/preBP, a hemodynamic variable of ARAS introduced in this study, was the best index to select ARAS for angioplasty among hemodynamic variables examined. The angioplastic parameters such as residual stenosis could affect the angioplastic outcome. However, the difference of angioplastic parameters would not have affected the outcome in this study, because postangioplastic PG was not different independent of the outcome.

In this study, we used a micro-catheter as a device to measure PG, and the PG in "high-grade" stenosis could have been overestimated. However, "high-grade" stenosis would have high PG without overestimation, and the ARAS that has PG around the cut-offs for angioplasty would be "low-grade" stenosis. In these "low-grade" ARAS, a micro-catheter and PressureWire, one of the thinnest devices, is reported to give nearly same PG (11). Therefore, the obstructive effect by the device would not have affected the cut-offs of hemodynamic variables for angioplasty in this study.

The systolic phase PG is a generally used variable to indicate the "hemodynamic significance" of ARAS, although it has not been validated sufficiently with regard to the therapeutic outcome (6). The systolic phase PG • 15 or • 20 mmHg is widely considered to be "clinically significant" (2,4,6), but it was also reported that no cases with the systolic phase PG • 40 mmHg benefited by angioplasty (12). However, because the systemic BP before angioplasty was • 190/120 mmHg in the cases without benefit from angioplasty, the high prestenotic arterial BP is thought to have increased the PG in these cases.

Hydrodynamically, the prestenotic arterial BP affects PG; PG increases accordingly as the prestenotic arterial BP increases (13,14). Therefore, owing to the fluctuation of the prestenotic arterial BP, PG would be inconsistent (4). PG/preBP, which has linear relation to PG (15), is thought to cancel out the effect of the prestenotic BP on PG and to be a better index for the "hemodynamic significance" of ARAS than PG. Supporting this notion, it is reported that renin in the renal vein of the kidney with stenosis increases when poststenotic arterial BP/prestenotic arterial BP is • 0.90 (PG/preBP • 0.10) (15).

The mean phase PG/preBP had larger AUC in the ROC analysis than the systolic phase PG/preBP. The systolic and mean phases of BP correspond to the peak and mean of the ejected bolus volume of cardiac contraction, respectively (16), and the latter reflects more closely the total amount of blood supply to the organs. Therefore, the poststenotic arterial BP in the mean phase is thought to reflect the total blood supply to the kidney. The PG, the poststenotic arterial BP subtracted from the prestenotic arterial BP, in the mean phase is thought to reflect the reduction of the total blood supply to the kidney, and it would be suitable to indicate "hemodynamic significance" of ARAS.

Previously, we showed that the minimal lumen diameter • 3 mm could be a good angiographic index to select ARAS for angioplasty; it could select the angioplasty-efficacious ARAS more adequately than % diameter reduction • 50% and • 75%, widely used angiographic indices for ARAS (10). Supporting this notion, most of ARAS with the mean phase PG/preBP • 0.15 could be selected by the minimal lumen diameter • 3 mm. These results indicate that ARAS could be selected for PG-measurement by the angiographic index of the minimal lumen diameter • 3 mm, and then selected for angioplasty by the hemodynamic index of the mean phase PG/preBP • 0.15.

The AUC was only 0.794 even for the mean phase PG/preBP. This indicates that the systemic BP reduction after angioplasty cannot be predicted only by "hemodynamic significance" of the stenosis. Because ARAS is frequently accompanied with atheromatous lesions in the intrarenal arteries (2,17), the microvascular kidney disease is thought to have affected the outcome in ARAS. PRA is known to increase accordingly as perfusion to the viable renal parenchyma decreases (18,19), and PRA in the cases of ARAS would reflect the ischemia caused by not only ARAS lesions but also the microvascular kidney disease. The baseline PRA in this study, which was higher in the angioplasty-efficacious cases than in the angioplasty-inefficacious cases, indicated that more amount of viable renal parenchyma was ischemic in the former than in the latter. No reduction of

Table 3. PRA with reference to the angioplastic outcome[a]

	All (n • 34)	Angioplasty		P
		Efficacious (n • 23)	Inefficacious (n • 11)	
Pre-angioplastic PRA (ng/ml/h)	6.4 • 6.1	7.8 • 6.6	3.4 • 3.8	0.049
Post-angioplastic PRA (ng/ml/h)	6.0 • 9.2	8.0 • 10.7	1.8 • 1.6	0.066
Post/Pre ratio	1.3 • 1.5	1.3 • 1.5	1.3 • 1.6	0.992

[a]Data are presented as the mean • SD. PRA, plasma renin activity.

postangioplastic PRA not only in the latter but also in the former indicated persistence of the ischemia even after angioplasty in both. The ischemia would have been caused mostly by the microvascular kidney disease in many cases independent of the outcome. The usage of the variables that could reflect the microvascular kidney disease might improve prediction of the outcome in ARAS (1,2,17).

The angioplasty-efficacious cases had lower baseline systemic BP than the angioplasty-inefficacious cases. This would indicate that the angioplasty-efficacious cases had more medication-controllable hypertension, whereas angioplasty is generally recommended for the cases with medication-uncontrollable hypertension (3,20,21). However, the angioplasty-efficacious cases used the antihypertensive agents that can inhibit the renin-angiotensin cascade at higher prevalence than the angioplasty-inefficacious cases, although the baseline total antihypertensive score was the same in both. The usage of these agents might have effectively reduced the systemic BP in the angioplasty-efficacious cases. The effectiveness of these agents and younger age in these cases might have indicated that a larger amount of viable renal parenchyma was preserved in them.

Conclusion

The mean phase PG/preBP would be a better index to select ARAS for angioplasty than the systolic phase PG, a widely used indicator. However, the efficacy of angioplasty could be influenced by the factors that affect the intrarenal perfusion, such as the microvascular kidney disease. To predict the efficacy of angioplasty, appropriate evaluation of these factors would be necessary. The analysis in future studies with the variables that reflect these factors could afford more information to select treatment for ARAS.

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Disclosures

None.

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