Functional Significance of Renal Prostacyclin and Thromboxane A_2 Production in Patients with Systemic Lupus Erythematosus

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Abstract

We have examined the urinary excretion of stable immunoreactive eicosanoids in 23 female patients with systemic lupus erythematosus (SLE), 16 patients with chronic glomerular disease (CGD), and 20 healthy women. SLE patients had significantly higher urinary thromboxane B_2 (TXB_2) and prostaglandin (PG) E_2 excretion and significantly lower 6-keto-$PGF_{1\alpha}$ than did healthy women. In contrast, CGD patients only differed from controls for having reduced 6-keto-$PGF_{1\alpha}$ excretion. The group of SLE patients with active renal lesions differed significantly from the group with inactive lesions for having a lower creatinine clearance and urinary 6-keto-$PGF_{1\alpha}$ and higher urinary TXB_2. Higher urinary TXB_2 excretion was associated with comparable platelet TXB_2 production in whole blood, undetectable TXB_2 in peripheral venous blood, and unchanged urinary excretion of 2,3-dinor-TXB_2. A significant inverse correlation was found between urinary TXB_2 and creatinine clearance rate (C_{Cr}). In contrast, the urinary excretion of 6-keto-$PGF_{1\alpha}$ showed a significant linear correlation with both C_{Cr} and para-aminohippurate clearance rate (C_{PAH}). In four SLE and seven CGD patients, inhibition of renal cyclooxygenase activity by ibuprofen was associated with a significant reduction in urinary 6-keto-$PGF_{1\alpha}$ and TXB_2 and in both C_{Cr} and C_{PAH}. However, the average decrease in both clearances was 50% lower in SLE patients than in CGD patients, when fractionated by the reduction in urinary 6-keto-$PGF_{1\alpha}$ or PGE_2 excretion.

We conclude that the intrarenal synthesis of PGI_2 and TXA_2 is specifically altered in SLE. Such biochemical alterations are associated with changes in glomerular hemodynamics and may play a role in the progression of SLE nephropathy.

Introduction

Prostaglandin (PG)[1] I_2 or prostacyclin and thromboxane (TX) A_2 are labile oxygenated metabolites of arachidonate with contrasting effects on platelet function, vascular tone, and glomerular function (1, 2). In humans, the corresponding stable hydration products, 6-keto-$PGF_{1\alpha}$ and TXB_2, respectively, are synthesized by renal cortical microsomes (3), represent the most abundant cyclooxygenase products of arachidonate in isolated glomeruli (4), and can be measured in urine (5, 6).

The glomerular synthesis of vasodilator PGI_2 and vasoconstrictor TXA_2 might theoretically mediate and/or be regulated by changes in glomerular hemodynamics associated with progressive glomerular sclerosis (7), by virtue of their effects on glomerular capillary plasma flow rate (Q_A) and on glomerular ultrafiltration coefficient (Kf) (2, 8). To the extent that the urinary excretion of 6-keto-$PGF_{1\alpha}$ and TXB_2 is primarily a reflection of the renal synthesis of the unstable parent compounds (9), we have recently suggested that patients with systemic lupus erythematosus (SLE) and active renal disease can be characterized as having enhanced renal synthesis of TXA_2 (10), while sharing with other forms of chronic glomerular disease a reduced glomerular synthesis of PGI_2 and PGI_2-dependent renal function (11).

The aims of the present studies were: (*a*) to define the status of cyclooxygenated products of renal arachidonate metabolism in SLE patients as compared with healthy controls and patients with chronic glomerulonephritis; (*b*) to characterize the nature of enhanced urinary TXB_2-like immunoreactivity detected in SLE patients; (*c*) to assess the renal vs. extrarenal sources of urinary TXB_2 in SLE; (*d*) to correlate changes in eicosanoid excretion with SLE renal lesions; and (*e*) to explore the functional significance of renal PGI_2 and TXA_2 synthesis by simultaneous

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1. *Abbreviations used in this paper:* C_{Cr}, creatinine clearance rate; C_{PAH}, para-aminohippurate clearance rate; CGD, chronic glomerular disease; GC/MS, gas chromatography/mass spectrometry; PAH, para-aminohippurate; PG, prostaglandin; SLE, systemic lupus erythematosus; TLC, thin-layer chromatography; TX, thromboxane.

measurements of eicosanoid excretion and renal function, performed under basal conditions and following administration of a cyclooxygenase inhibitor.

Methods

A total of 59 female subjects were studied on several occasions. 23 SLE patients, aged 14–62 yr (mean±SD, 34±14), were studied at three different institutions during a 3-yr period. 20 healthy women, aged 23–40 yr (mean±SD, 29±5), without personal or family history suggestive of an autoimmune state, and 16 patients aged 19–67 yr (mean±SD, 41±17) with clinically and bioptically ascertained chronic glomerulonephritis, were studied as controls. The nature and purpose of the study was explained to each subject, and informed consent was obtained from all subjects who entered this study. SLE patients included in the present study met the following criteria: (*a*) diagnosis of SLE as defined by the American Rheumatism Association (12); and (*b*) histologic verification of lupus nephritis provided by percutaneous renal biopsy. All biopsies were examined by light and electron microscopy, and by immunofluorescence. The characteristics of the examined tissue were defined according to the World Health Organization classification (13), as no glomerular changes (class I), mesangial (class II), focal proliferative (class III), diffuse proliferative (class IV), and membranous (class V) glomerulonephritis. Thirteen patients satisfied criteria for clinical and renal activity of SLE, i.e., active renal lesions and one or more of the following: arthralgia, pleuritis, pericarditis, vasculitis, myalgia, and cerebral involvement. Active renal disease was defined on the basis of histologic evidence of active nephritis (glomerular hypercellularity; foci of necrosis of glomerular tufts; tubular, interstitial, and vascular changes) and either low serum C_3 levels or the presence of serum antibodies to native DNA. 12 out of these 13 patients had diffuse proliferative and necrotizing glomerulonephritis (class IV B), while one had focal segmental and necrotizing glomerulonephritis (class III A). Class IV B lesions were also characterized by infiltration of mononuclear cells, and/or immune deposits in the tubular basement membrane, interstitial tissue, and peritubular capillaries. Glomerulosclerosis, intimal sclerosis of vessels, and interstitial fibrosis were considered inactive lesions. The histologic findings in the 10 patients with inactive renal lesions included minor glomerular abnormalities (1, class I B), mesangial proliferative (1, class II B), focal segmental (2, class III C), diffuse proliferative (3, class IV A), and diffuse membranous (3, class V A) glomerulonephritis. 7 of the 13 SLE patients with active renal lesions and 4 of the 10 patients with inactive lesions had mild to severe hypertension (diastolic blood pressure, 90–115 mmHg). The histologic diagnosis in the 16 patients with other forms of chronic glomerular disease included membranoproliferative glomerulonephritis, membranous glomerulonephritis, IgA nephropathy, mesangial IgM glomerulonephritis, Alport's disease, and renal amyloidosis. Four of these patients had mild to moderate hypertension. Three of the SLE patients with active renal lesions and two of the patients with chronic glomerular disease had the nephrotic syndrome. All patients were studied while hospitalized and kept on a controlled diet (sodium, 100 mmol/d; potassium, 80 mmol/d). None of the subjects was on cytotoxic drugs, nonsteroidal antiinflammatory drugs, or other drugs known to interfere with renal or platelet arachidonate metabolism, at the time of study.

Basal study. Each patient was studied at least on three consecutive occasions with biochemical and functional measurements. The renal synthesis of PGE_2, $PGF_{2\alpha}$, PGI_2, and TXA_2 was investigated by measuring the urinary excretion of PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and TXB_2, respectively, in 8–24-h urine collections, as previously described (6, 14, 15). Simultaneous measurements of creatinine clearance were obtained on three consecutive days. Extrarenal TXB_2 production was assessed by measurement of the urinary excretion of 2,3-dinor-TXB_2 (16) and of plasma and serum concentrations of TXB_2. Venous blood samples (5 ml) were drawn at 10 a.m., 2 h after a light breakfast, on the same days of urine collection. 2 ml of blood were promptly distributed into iced tubes containing EDTA (0.02 ml of a 5% solution per milliliter of blood) and fenoprofen sodium salt (10 μg per milliliter of blood; Eli Lilly & Co., Indianapolis, IN), to prevent platelet TXA_2 synthesis and release after sampling. All tubes were promptly centrifuged in a refrigerated centrifuge, and supernates were frozen for subsequent analysis. Platelet TXA_2 production in response to endogenous thrombin was studied by letting triplicate 1-ml aliquots of whole blood clot at 37°C for 60 min, as previously described (17, 18).

Ibuprofen study. Five healthy women, four SLE patients (three with inactive renal lesions and one with active renal lesions), and seven patients with chronic glomerular disease gave informed consent to participate in a study of the functional and biochemical effects of a nonselective cyclooxygenase inhibitor, i.e., ibuprofen (1,200 mg/d) given orally for 7 d (11). 24-h urine specimens and blood samples were obtained on the fourth, fifth, and sixth days of drug therapy for eicosanoid measurements and creatinine clearance determinations, and paraaminohippurate (PAH) clearance was measured on the seventh day, as described (11). PAH clearance was not determined in healthy women.

Analyses. Levels of serum and urinary creatinine were measured by the Jaffe chromogen reaction. The method of Bratton and Marshall was used to determine the levels of serum and urinary PAH. All clearance values were corrected for body-surface area. Urinary PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and TXB_2 were measured by previously described radioimmunoassay (RIA) techniques (6, 14, 15), after extraction and silicic acid column chromatography. Because of the instability of PGE_2 in human urine, urine specimens were frozen immediately after collection and were kept at −20°C until extracted. Validation of urinary RIA measurements was obtained by several independent criteria, i.e., dilution and recovery studies; comparison among multiple antisera; characterization of the chromatographic pattern of distribution of the extracted PG-like and TX-like immunoreactivity on thin-layer chromatography (TLC); comparison with gas chromatography/mass spectrometry (GC/MS) determinations. These techniques are described in detail elsewhere (6, 14, 15). For comparison of urinary TXB_2-like immunoreactivity with GC/MS determination, a 200-ml sample pooled from three consecutive urine collections obtained from a patient with active SLE was subjected to extraction and silicic acid column chromatography. The column eluate corresponding to TXB_2 was then subjected to TLC. Silica gel corresponding to the TXB_2 area, as determined by comparison with cochromatographed authentic TXB_2, was scraped off and eluted with methanol. This was divided into two identical aliquots and evaporated to dryness. TXB_2 concentrations were determined by RIA in our laboratory, and by GC/MS in the Burroughs Wellcome and Co. Research Laboratories (Beckenham, Kent, England) through the courtesy of Dr. John Salmon. The eluates obtained from a side lane of the same plate were subjected to the same procedure and used as a blank. For GC/MS analysis, the urine extract, the standard, and the blank were derivatized by reaction with freshly prepared diazomethane, methoxylamine hydrochloride, and finally bis-(trimethyl silyl)-trifluoroacetamide containing 1% trimethylchlorosilane. The derivatives were injected into a gas chromatograph (model 5730; Hewlett-Packard Co., Palo Alto, CA) combined with a mass spectrometer (VG Micromass 16 F; Hewlett-Packard Co). The gas chromatograph was fitted with a 1% OV1 column operated at 240°C. Selected ion monitoring of mass to charge ratio (m/z) 301 was performed at 28 eV; the source current and temperature were 200 μA and 240°C, respectively. The urine extract, as well as the cochromatographed authentic TXB_2, produced the largest ion at m/z 301 at the same retention time as the derivatized standard. No such ion was detected in the blank.

The above RIA systems use 5,000 dpm of [^{3}H]TXB_2 (140 Ci/mmol; New England Nuclear, Boston, MA), [^{3}H]PGE_2 (160 Ci/mmol; Amersham Radiochemical Centre, Amersham, England), [^{3}H]6-keto-$PGF_{1\alpha}$ (151 Ci/mmol; Amersham Radiochemical Centre), and [^{3}H]$PGF_{2\alpha}$ (180 Ci/mmol; Amersham Radiochemical Centre), respectively.

A novel RIA technique was developed for measuring urinary 2,3-dinor-TXB_2, the major urinary metabolite of infused TXB_2 in man (16). This uses 5,000 dpm of L-[4,5-^{3}H]leucine (131 Ci/mmol; Amersham Radiochemical Centre) coupled to 2,3-dinor-TXB_2 and an anti-2,3-dinor-TXB_2 serum diluted 1:40,000 in a final volume of 2.0 ml. To prepare

the tritium-labeled 2,3-dinor-TXB_2, 0.2 ml of thionylchloride is added to 2 ml of methanol chilled on dry ice. L-[4,5-^{3}H]leucine-methylester is prepared by incubating 1.0 μCi of L-[4,5-^{3}H]leucine in 0.5 ml of this mixture overnight at room temperature. Methylation is verified on a silica-gel TLC plate (60 F 254; E. Merck, Darmstadt, West Germany) developed using the ascending technique in chloroform/methanol (9:1, vol/vol). Thereafter, the incubation mixture is evaporated to dryness, recovered with 50 μl of ethanol/water (5:1, vol/vol), and added to 100 μg of authentic 2,3-dinor-TXB_2. *N,N'*-dicyclo-hexyl-carbodiimide is added and the mixture is incubated overnight at room temperature. The conjugate is separated from free L-[4,5-^{3}H]leucine by running the mixture on a sep-pak C18 cartridge (Waters Associates, Milford, MA). The column is eluted with 10 ml of water, 10 ml of *n*-hexane, and 10 ml of ethyl-acetate. The conjugate and free 2,3-dinor-TXB_2 are recovered in the ethyl-acetate fraction. A further purification is performed by Sephadex G 10 (Pharmacia Fine Chemicals, Uppsala, Sweden) column chromatography. The mixture is evaporated to dryness and recovered with 1 ml 0.01 M phosphate buffer, pH 7.5, and applied to a column 1 × 40 cm. 1-ml aliquots are collected separately from the column effluent by a fraction collector, and a 20-μl aliquot of each sample is counted in a liquid scintillation counter. The immunoreactivity of each fraction eluted from the column is tested in the RIA system by incubating a properly diluted aliquot with the anti-2,3-dinor-TXB_2 serum diluted 1:25,000 in a final volume of 1.5 ml for 24 h at 4°C. The fractions eluted from the column showing the best binding with antiserum (generally 3–4 fractions) are pooled and properly diluted for routine assays. ~40% binding of the tracer is obtained after 16–24 h incubation at 4°C. Separation of free from antibody-bound [^{3}H]2,3-dinor-TXB_2 is obtained by rapid addition of 10 mg of uncoated charcoal (Norit A, Fisher Scientific Co., Fair Lawn, NJ) and subsequent centrifugation at the same temperature. The binding of [^{3}H]2,3-dinor-TXB_2 is inhibited by unlabeled 2,3-dinor-TXB_2 in a linear fashion, over the range of 10–500 pg/ml, with an IC_{50} (concentration required to displace 50% of bound [^{3}H]2,3-dinor-TXB_2) of 180 pg/ml. The least detectable concentration that can be measured with 95% confidence (i.e., 2 SD at zero) is 20 pg/ml. The cross-reactivity of other eicosanoids is as follows: TXB_2, 51%; PGD_2, 2.1%; 2,3-dinor-6-keto-$PGF_{1\alpha}$, 0.13%; 6-keto-$PGF_{1\alpha}$, <0.07%; PGE_2 and $PGF_{2\alpha}$, <0.04%; 11,15-bis-dehydro-TXB_2, <0.02%. Urinary 2,3-dinor-TXB_2 was extracted from 20-ml urine samples run on sep-pak C18 cartridges (Water Associates) and eluted with ethyl-acetate. Eluted 2,3-dinor-TXB_2 was separated from TXB_2 on silica gel TLC plates (60 F_{254}; E. Merck, Darmstadt, West Germany). The plates were developed using the ascending technique in chloroform/methanol/acetic acid/water (90:8:1:0.8). Silica gel corresponding to the 2,3-dinor-TXB_2 area, as determined by comparison with cochromatographed authentic 2,3-dinor-TXB_2, was scraped off and eluted with 0.02 M PO_4 buffer. The overall recovery as assessed by adding a known amount of authentic 2,3-dinor-TXB_2 or [^{3}H]TXB_2 to urine samples averaged 45±15% (mean±SD, n = 34). This technique has been validated by comparison with GC/MS in the selected ion monitoring mode (19). In 61 samples, including urine obtained from aspirin-treated and TXB_2-infused healthy subjects ranging in 2,3-dinor-TXB_2 excretory rates between 46 and 11,782 (GC/MS) vs. 54 and 14,338 (RIA) pg/mg creatinine, a statistically significant correlation was found between the two methods: $r = 0.933$, $P < 0.001$ (G. Ciabattoni, C. Patrono, I. Blair, and G. A. FitzGerald, manuscript submitted for publication). Serum and plasma concentrations of TXB_2 were measured by RIA (17, 18). Unextracted samples were diluted in the standard diluent of the assay (0.02 M PO_4 buffer, pH 7.4) and assayed in a volume of 1.5 ml at a final dilution of 1:15 (plasma) or 1:500–1:15,000 (serum). The least detectable concentration that could be measured with 95% confidence was 1 pg of TXB_2 per ml of incubation mixture. Therefore, the detection limit of the assay was 15 or 500 pg/ml of plasma or serum, respectively.

Results were analyzed using one-way analysis of variance, for multiple comparisons, and t test for single comparisons. Moreover, eicosanoid measurements were correlated with functional measurements by stepwise regression analysis and multiple linear regression. All values are reported as means±1 SD. Statistical significance was defined as $P < 0.05$.

Results

The basal measurements of platelet and renal arachidonate metabolites obtained in the four groups of subjects are summarized in Table I. The intra-subject variability of urinary excretory rates of the four eicosanoids, measured on one to six occasions, ranged between 16 and 21%, and did not differ among the four groups of subjects. When compared with healthy women, both groups of SLE patients had significantly higher urinary TXB_2 and PGE_2 excretion and significantly lower 6-keto-$PGF_{1\alpha}$. However, while all but one patient in each group had urinary TXB_2 > 2 SD of the normal mean, only six patients with active-renal lesions and two patients with inactive lesions had urinary PGE_2 > 2 SD of the normal mean. No statistically significant correlation was found between excretory rates of these eicosanoids in either group of SLE patients. In contrast, patients with chronic glomerular disease only differed from healthy women for having significantly reduced 6-keto-$PGF_{1\alpha}$ excretion. The group of SLE patients with active renal lesions differed significantly from the group with inactive lesions for having a lower creatinine clearance (69±29 vs. 92±18 ml/min per 1.73 m^2), a lower urinary 6-keto-$PGF_{1\alpha}$ (2.0±1.0 vs. 3.4±0.4 ng/h) and higher urinary TXB_2 (9.4±4.5 vs. 4.6±1.6). Such higher excretion of TXB_2 was associated with comparable platelet TXB_2 production in whole blood (179±85

Table I. Summary of Basal Characteristics and Eicosanoid Measurements (Mean±SD) in Healthy Women (Group 1), Patients with Chronic Glomerular Disease (Group 2), SLE Patients with Active (Group 3), or Inactive (Group 4) Renal Lesions

	Age	Creatinine clearance	Serum TXB_2	Urinary TXB_2	Urinary 6-keto-$PGF_{1\alpha}$	Urinary PGE_2	Urinary $PGF_{2\alpha}$
	yr	*ml/min/1.73 m^2*	*ng/ml*	*ng/h*	*ng/h*	*ng/h*	*ng/h*
Group 1 n = 20	29±5	110±5	330±136	2.0±0.6	4.1±0.9	7.6±2.7	18.1±6.8
Group 2 n = 16	41±17	91±19	282±132	1.9±0.4	2.3±1.0	7.4±2.4	15.7±7.1
Group 3 n = 13	34±16	69±29	179±85	9.4±4.5	2.0±1.0	15.0±10.2	16.4±6.2
Group 4 n = 10	34±11	92±18	217±91	4.6±1.6	3.4±0.4	10.9±4.7	15.9±4.2
P 1 vs. 2	<0.01	<0.01	NS	NS	<0.0005	NS	NS
P 1 vs. 3	NS	<0.002	<0.005	<0.0005	<0.0005	<0.05	NS
P 1 vs. 4	NS	<0.01	<0.01	<0.0005	<0.005	<0.05	NS
P 2 vs. 3	NS	<0.05	<0.05	<0.0005	NS	<0.05	NS
P 2 vs. 4	NS	NS	NS	<0.0005	<0.0005	<0.05	NS
P 3 vs. 4	NS	<0.05	NS	<0.0005	<0.0005	NS	NS

vs. 217±91 ng/ml) and undetectable (i.e., <15 pg/ml) TXB_2 in peripheral venous blood (not shown). Individual urinary eicosanoid measurements performed in SLE patients are detailed in Table II.

To characterize the nature of this enhanced urinary TXB_2-like immunoreactivity, 18 urinary samples from 12 SLE patients were analyzed for TXB_2 by three different anti-TXB_2 sera. Two of these, which showed a limited cross-reactivity (1.3 and 2.0%, respectively) with 2,3-dinor-TXB_2 (18), gave almost identical results ($r = 0.97$, $P < 0.001$). A third anti-TXB_2 serum, which had ~50% cross-reactivity with 2,3-dinor-TXB_2, gave twofold higher estimates of TXB_2-like immunoreactivity, though these correlated with the former in a statistically significant fashion ($r = 0.83$ and 0.89, respectively; $P < 0.01$). When a pooled urinary extract from SLE patients was subjected to TLC, anti-TXB_2 serum 3 revealed the presence of two distinct peaks of immunoreactivity, co-migrating with authentic TXB_2 and 2,3-dinor-TXB_2, respectively (Fig. 1). In contrast, anti-TXB_2 sera 1 and 2 detected a single peak of immunoreactivity co-migrating with authentic TXB_2. The qualitative as well as the quantitative pattern of distribution of TXB_2-like immunoreactivity in the region of authentic TXB_2 was substantially identical with the three antisera (Fig. 1). This finding strongly supports the identification of the urinary immunoreactive material as TXB_2. Further evidence for this conclusion was obtained from GC/MS analysis. Thus, RIA detected 51 ng of TXB_2 in the same region of the TLC plate where GC/MS measured 45 ng.

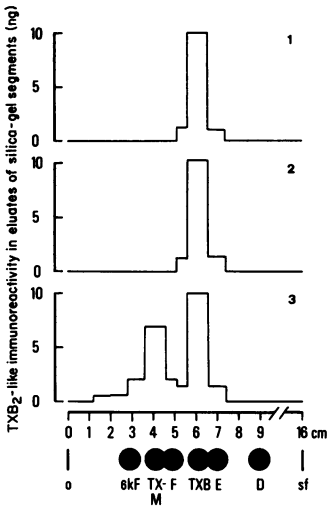

Figure 1. Characterization of urinary TXB_2-like immunoreactivity of SLE patients. Pooled purified extracts, prepared from urine obtained under basal conditions, were subjected to thin-layer chromatography. The whole lane of the plate was divided into 1-cm segments, and the silica gel was scraped off and eluted with methanol. All the eluates were assayed for TXB_2 with three anti-TXB_2 sera of different specificities (see ref. 18 for details). The vertical marks on the abscissa indicate the origin (o) and the solvent front (sf) of the plate. The dots indicate the location of cochromatographed authentic PGs and TXs: 6kF, 6-keto-$PGF_{1\alpha}$; TX-M, 2,3-dinor-TXB_2; F, $PGF_{2\alpha}$; TXB, TXB_2; E, PGE_2; D, PGD_2.

Table II. Renal Eicosanoid Excretion and Creatinine Clearance (C_{Cr}) in 23 SLE Patients

Patient	W.H.O.* class	TXB_2	6-keto-$PGF_{1\alpha}$	PGE_2	$PGF_{2\alpha}$	C_{Cr}
		ng/h	*ng/h*	*ng/h*	*ng/h*	*ml/min/1.73 m²*
1	IVB	5.6	2.0	15.0	19.3	89
2	IVB	8.4	3.0	32.7	25.7	120
3	IVB	10.5	2.3	10.8	16.9	95
4	IVB	10.7	1.2	11.0	11.0	73
5	IVB	10.4	1.1	14.5	10.9	40
6	IVB	6.5	1.3	10.0	17.8	78
7	IIIA	6.6	2.5	5.7	9.9	100
8	IVB	16.2	4.3	11.7	19.9	45
9	IVB	2.7	0.4	1.2	2.6	38
10	IVB	10.9	3.1	37.6	20.5	69
11	IVB	10.3	2.2	13.7	18.6	52
12	IVB	18.9	1.6	22.0	21.9	19
13	IVB	5.0	1.2	9.3	18.6	74
14	IVA	5.3	3.2	9.8	10.5	105
15	VA	3.3	3.8	21.8	20.5	99
16	VA	4.5	3.3	15.1	12.9	112
17	VA	5.7	3.0	10.2	17.5	114
18	IIB	0.7	2.9	6.2	11.2	85
19	IIIC	5.7	3.7	9.0	18.5	83
20	IVA	4.6	3.8	6.5	12.2	92
21	IIIC	4.5	3.7	12.9	23.0	99
22	IB	6.1	2.6	8.4	14.9	53
23	IVA	5.6	3.7	9.3	17.6	82

* W.H.O., World Health Organization.

To provide further evidence for the intrarenal vs. extrarenal source of enhanced urinary TXB_2 detected in SLE patients, we have measured the urinary excretion of 2,3-dinor-TXB_2, the major urinary metabolite of infused TXB_2 in man (16), primarily reflecting systemic, i.e., extrarenal TXB_2 production (9). As shown in Table III, both groups of SLE patients excreted amounts of 2,3-dinor-TXB_2 comparable to that found in a group of age-matched healthy women, despite markedly different excretory rates of TXB_2. No statistically significant correlation was found between the two sets of measurements in any of the three groups considered.

To assess the possible association of changes in vasoactive eicosanoid production with the basal status of renal function in SLE patients, urinary excretory rates of the four major cyclooxygenase products were correlated with the creatinine clearance rate (C_{Cr}) and PAH clearance rate (C_{PAH}). A significant inverse correlation was found between urinary TXB_2 excretion and C_{Cr} ($y = 112.1–4.18x$; $r = -0.64$; $n = 28$; $P < 0.005$). A similar trend was noted for C_{PAH}, though the small number of patients examined ($n = 8$) precluded statistical significance ($r = -0.478$). Urinary TXB_2 did not correlate with the degree of proteinuria. In contrast, the urinary excretion of 6-keto-$PGF_{1\alpha}$ showed a significant positive correlation with both C_{Cr} ($y = 45.1 + 14.2x$; $r = 0.56$; $n = 28$; $P < 0.005$) and C_{PAH} ($y = 75.5 + 238x$; $r = 0.70$; $n = 8$; $P < 0.05$). When the ratio of urinary TXB_2 to

Table III. Urinary Excretion (Mean±SD) of TXB_2 and 2,3-dinor-TXB_2 in Healthy Women and in SLE Patients with Active or Inactive Renal Lesions

Subjects		Urinary TXB_2	Urinary 2,3-dinor-TXB_2
n		*ng/h*	*ng/h*
HW	8	2.2±0.3	13.4±4.5
SLE-A	8	9.6±5.6	14.7±4.7
SLE-I	7	4.7±1.9	13.1±4.4

HW, healthy women; SLE-A, SLE patients with active renal lesions; SLE-I, SLE patients with inactive renal lesions.

6-keto-$PGF_{1\alpha}$ was plotted as a function of C_{Cr}, a statistically significant inverse relationship was found in SLE patients as depicted in Fig. 2. Urinary PGE_2 and $PGF_{2\alpha}$ excretion did not correlate with either C_{Cr} ($r = -0.067$ and 0.218, respectively) or C_{PAH} to any statistically significant extent.

Evidence for a functional prevalence of vasodilator PGI_2 over vasoconstrictor TXA_2 is provided by the short-term effects of ibuprofen. Fig. 3 compares changes in urinary 6-keto-$PGF_{1\alpha}$ and TXB_2 and in C_{Cr} and C_{PAH}, associated with 1-wk dosing with this nonselective cyclooxygenase inhibitor in four SLE patients and five healthy women. In SLE patients, pharmacologic inhibition of renal cyclooxygenase activity was associated with a significant ($P < 0.01$) reduction in urinary 6-keto-$PGF_{1\alpha}$ by 77% and in TXB_2 by 67%, and in both C_{Cr} and C_{PAH} by 26% and 34%, respectively. In healthy women, a similar reduction of urinary 6-keto-$PGF_{1\alpha}$ and TXB_2 by 73% and 66%, respectively, was not associated with any detectable change in C_{Cr}.

Moreover, we have compared the ibuprofen-induced biochemical and functional changes in these SLE patients with those measured in seven patients with chronic glomerular disease (Table IV) displaying a similar base-line C_{Cr} (92±19 vs. 85±31 ml/min per 1.73 m^2) and a significantly lower urinary TXB_2 excretion (2.1±0.4 vs. 6.1±5.0). Although the two groups of patients showed a similar percentage reduction of both C_{Cr} (25% vs. 26%) and C_{PAH} (35% vs. 34%), associated with a comparable suppression of urinary 6-keto-$PGF_{1\alpha}$ excretion (67% vs. 77%), a significant difference became apparent when calculating individual changes in C_{Cr} and C_{PAH} per unit change in urinary 6-keto-$PGF_{1\alpha}$ excretion (Table V). Thus, the average decrease in both clearances was ~50% lower in SLE patients than in patients with chronic glomerular disease, when fractioned by the reduction in urinary 6-keto-$PGF_{1\alpha}$ excretion. Similar results were obtained when individual changes in C_{Cr} and C_{PAH} were fractioned by the reduction in urinary PGE_2 excretion (Table VI). Thus, simultaneous suppression by ibuprofen of enhanced renal TXA_2 production might possibly account for such pattern, by attenuating the consequences of reduced vasodilatory PGs. Such interpretation is supported by the finding that the actual reduction in C_{Cr} measured in the 11 patients treated with ibuprofen (−22.4±7.4 ml/min per 1.73 m^2) did not differ significantly from the expected value (−24.1±15.6 ml/min per 1.73 m^2) calculated on the basis of theoretical changes in C_{Cr} associated with the observed changes in U-6-keto-$PGF_{1\alpha}$ and U-TXB_2 excretion.

Discussion

Several experimental models of nonimmune as well as immune-mediated renal damage are associated with quantitative and qualitative alterations of renal arachidonate metabolism (20). Thus, a ureteral obstruction model of hydronephrosis (21), renal venous occlusion (22), and glycerol-induced acute renal failure in rabbits (23) all share a marked enhancement of renal PG-synthesis and the induction of TXA_2 production. Indirect pharmacologic evidence, largely derived from the use of selective TX-synthase inhibitors, suggests a pathophysiologic role for such vasoactive eicosanoids in mediating changes in renal vascular resistance and regional blood flow (20). Similarly, in antiglomerular basement membrane antibody-induced glomerulonephritis in the rat, there is enhanced synthesis of TXA_2 and PGs in the glomerulus, that mediate changes in renal hemodynamics (24). Because both vasodilator and vasoconstrictor arachidonate derivatives are synthesized by human glomeruli (3, 4), and their stable hydration products can be measured in urine (5, 6), we initiated a search for alterations in their relative production in human models of glomerular disease.

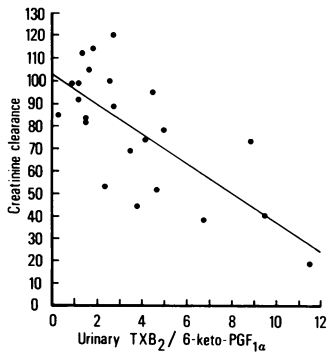

Figure 2. Correlation between creatinine clearance (ml/min per 1.73 m^2) and the ratio of urinary TXB_2/6-keto-$PGF_{1\alpha}$ excretion in SLE patients. Individual data for groups 3 and 4 are given in Table II. $n = 23$; $r = -0.72$; $P < 0.01$.

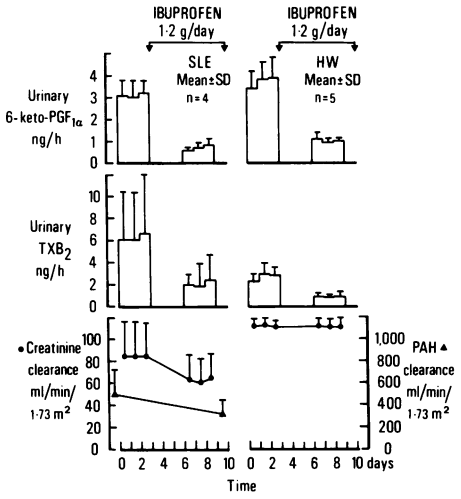

Figure 3. Biochemical and functional changes related to renal arachidonate metabolism, in four SLE patients and five healthy women (HW), associated with administration of a cyclooxygenase inhibitor. Urinary 6-keto-$PGF_{1\alpha}$ (*top*), urinary TXB_2 (*middle*), creatinine, and PAH clearances (*bottom*) were measured on three consecutive days before and during 1-wk treatment with ibuprofen (1.2 g/d). Each measured function is expressed as mean±1 SD. The arrows indicate the duration of drug treatment. With the exception of creatinine clearance in healthy women, all changes associated with ibuprofen administration were statistically significant ($P < 0.01$) with respect to predose control values, by analysis of variance.

Table IV. Changes (Mean±SD) in C_{Cr}, C_{PAH}, and Urinary 6-keto-$PGF_{1\alpha}$ Excretion After Ibuprofen in Patients with CGD and SLE Patients

Patients	C_{Cr}	C_{PAH}	Urinary 6-keto-$PGF_{1\alpha}$
	ml/min/1.73 m^2	*ml/min/1.73 m^2*	*ng/h*
SLE before (group 5)	85±31	499±225	3.1±0.6
SLE after (group 6)	63±22	331±116	0.7±0.2
CGD before (group 7)	92±19	525±125	2.1±0.8
CGD after (group 8)	69±13	342±93	0.7±0.3

$P < 0.01$ for all values after ibuprofen vs. control measurements. Ibuprofen dose, 1,200 mg/d × 7 d. CGD, $n = 7$; SLE, $n = 4$.

Table V. Change (Mean±SD) in C_{Cr} and C_{PAH} per ng/h Change in Urinary 6-keto-$PGF_{1\alpha}$ After Ibuprofen Treatment in Patients with CGD and SLE Patients

Patients	$\Delta C_{Cr}/\Delta$6-K-$PGF_{1\alpha}$	$\Delta C_{PAH}/\Delta$6-K-$PGF_{1\alpha}$
n	$ml \cdot min^{-1} \cdot ng \cdot h^{-1}$	$ml \cdot min^{-1} \cdot ng \cdot h^{-1}$
CGD 7	18.1±8.4	147±61
SLE 4	9.3±2.8	73±50

$P < 0.05$ SLE vs. CGD for both clearances. Ibuprofen dose, 1,200 mg/d × 7 d.

We report increased urinary excretion of TXB_2 and decreased urinary excretion of 6-keto-$PGF_{1\alpha}$ in female patients with SLE as the most prominent and specific alteration of renal arachidonate metabolism and provide evidence for its functional significance. The identification of the immunoreactive material present in the urine of SLE patients as TXB_2 was obtained by several independent criteria: (*a*) its identical immunochemical behavior with authentic TXB_2 upon dilution; (*b*) its identical chromatographic behavior with authentic TXB_2 upon TLC separation; (*c*) its detection by three different anti-TXB_2 sera; and (*d*) its detection by GC/MS.

It is generally accepted that the urinary excretion of unmetabolized PGE_2 and $PGF_{2\alpha}$ largely reflects the renal synthesis of these compounds in healthy women (25), as primary PGs are not detectable in the systemic circulation and are currently viewed as locally active modulators. Despite early claims to the contrary, this seems to apply also to PGI_2 and TXA_2 (9). However, inasmuch as a small fraction of exogenously infused TXB_2 can be recovered unchanged in urine (16), the possible contribution of extrarenal sources of TXA_2 production should be considered when claiming an intrarenal origin of urinary TXB_2 in disease states. Platelets represent a major source of TXB_2 in the human body, inasmuch as the urinary excretion of its major metabolite 2,3-dinor-TXB_2 is largely suppressed by daily low-dose aspirin administration (19), which does not affect renal cyclooxygenase activity (18). The finding of (*a*) slightly reduced TXB_2 production in whole blood, (*b*) undetectable TXB_2 in peripheral venous plasma, and (*c*) urinary 2,3-dinor-TXB_2 excretion in the normal range, makes it unlikely that extrarenal platelet activation can account for enhanced urinary TXB_2 excretion in SLE patients. Intrarenal platelet activation (26) can also be excluded on the basis of our earlier observation (15) that neither low-dose aspirin nor sulindac, i.e., two inhibitors sparing renal cyclooxygenase activity by different mechanisms (11, 18), reduced significantly urinary TXB_2 in SLE patients despite profound suppression of platelet cyclooxygenase activity. Thus, a nonplatelet intrarenal source of enhanced TXA_2 production seems most likely, though we cannot define the precise cellular origin within the kidney. As for the mechanism(s) underlying enhanced renal TXA_2 production in SLE, several possibilities should be considered. An inflammatory response involving fibroblast-like cells and mononuclear cells (27) seems unlikely, as this would be associated with markedly enhanced PGE_2 synthesis and might also occur in other forms of chronic glomerular disease. Chronic joint inflammation in rheumatoid arthritis is associated with consistently elevated levels of both TXB_2 and PGE_2 in synovial fluid (28). In contrast, enhanced urinary PGE_2 excretion was found in only 35% of SLE patients. Moreover, the absence of any statistically significant correlation between urinary PGE_2 and TXB_2 suggests a different cellular origin of these eicosanoids within the kidney of SLE patients.

Table VI. Change (Mean±SD) in C_{Cr} and C_{PAH} per ng/h Change in Urinary PGE_2 After Ibuprofen Treatment in Patients with CGD and SLE patients

Patients	$\Delta C_{Cr}/\Delta PGE_2$	$\Delta C_{PAH}/\Delta PGE_2$
n	$ml \cdot min^{-1} \cdot ng \cdot h^{-1}$	$ml \cdot min^{-1} \cdot ng \cdot h^{-1}$
CGD 7	5.3±2.2	42.4±17.4
SLE 4	2.2±1.1	16.8±11.0

$P < 0.05$ SLE vs. CGD for both clearances. Ibuprofen dose, 1,200 mg/d × 7 d.

The possibility that filtered protein traps TXB_2 in the kidney and hence enhances urinary TXB_2 excretion is unlikely in view of the absence of any statistically significant correlation between protein and TXB_2 excretion in SLE patients, and of the normal TXB_2 excretion in two patients with chronic glomerulonephritis (type I membranoproliferative and mesangial IgM glomerulonephritis, respectively) who had the nephrotic syndrome.

Finally, one might consider the occurrence of an IgM autoantibody directed against lipomodulin (a phospholipase A_2 inhibitory protein), described by Hirata et al. (29) in sera of SLE patients and New Zealand Black × New Zealand White F_1 mice. The antilipomodulin antibody potentiates the stimulatory activity of bradykinin on the release of arachidonate from human fibroblasts (29), and might similarly affect intrarenal TXA_2-producing cells. Interestingly, in New Zealand Black × New Zealand White F_1 mice there is also increased renal production of TXB_2 but not of PGE_2 (V. E. Kelley, personal communication).

The finding that both groups of SLE patients had significantly lower 6-keto-$PGF_{1\alpha}$ excretion than did healthy women is consistent with our recent observation that the vast majority of patients with chronic glomerular disease display such biochemical abnormality, regardless of the nature of the glomerular involvement (11). At least two distinct mechanisms may account for a reduction in the glomerular synthesis of PGI_2, i.e., (*a*) inhibition of PGI_2-synthase by hydroperoxy-derivatives of arachidonate (30), or (*b*) feedback regulation of PGI_2 synthesis by the hemodynamic changes associated with progressive glomerular sclerosis (7). Although no specific recognition pattern can be ascribed to either enhanced urinary TXB_2 or reduced urinary 6-keto-$PGF_{1\alpha}$ excretion alone, the simultaneous occurrence of the two in SLE patients had a high discriminating power in our limited series.

The functional significance of biochemical changes detected in SLE patients is borne out by significant correlations with C_{Cr} and C_{PAH} in the basal state and by changes of these parameters associated with cyclooxygenase inhibition. As for the former, these are consistent with the known influences of TXA_2 and PGI_2 on glomerular hemodynamics and on the contractility of glomerular mesangial cells (2, 8). Within the limits of the assumption that changes in the urinary excretion of TXB_2 and 6-keto-$PGF_{1\alpha}$ reflect changes in renal TXA_2 and PGI_2 synthetic rates (31), respectively, the vasodilatory influence of the latter seems to outweigh the vasoconstrictor and contractile actions of the former. Evidence supporting this conclusion derives from

the following observations in SLE patients: (*a*) the slope of the regression line of urinary 6-keto-$PGF_{1\alpha}$ over C_{Cr} was approximately fourfold greater than the corresponding value for the regression of urinary TXB_2; (*b*) administration of a nonselective cyclooxygenase inhibitor, suppressing renal TXA_2 and PGI_2 synthesis to a similar extent, significantly reduced C_{Cr} and C_{PAH}. In the present study, there is a linear correlation between urinary 6-keto-$PGF_{1\alpha}$ excretion and C_{Cr} when data from all the groups are considered (Fig. 4). Our observation is consistent with recent findings of Stork and Dunn (32) that, in the autologous phase of nephrotoxic serum nephritis in the rat, acute infusion of cyclooxygenase inhibitors was associated with significant reductions in glomerular filtration rate and renal plasma flow, despite concomitant suppression of abnormally elevated glomerular TXB_2 production. However, that enhanced urinary TXB_2 excretion is not merely a marker of progressively deteriorating renal function in SLE is indirectly suggested by ibuprofen-induced fractional changes of C_{Cr} and C_{PAH} in SLE patients vs. patients with chronic glomerular disease (Tables V and VI). Thus, the reduction in both clearances associated with a given change in urinary 6-keto-$PGF_{1\alpha}$ or PGE_2 was significantly attenuated in SLE patients when compared with the latter group. Such a different pattern of response is consistent with the concomitant suppression by the drug of vasoconstrictor and contractile influences exerted by enhanced intrarenal TXA_2. A similar conclusion can be reached by theoretical calculations of the expected change in C_{Cr} and C_{PAH} on the basis of the observed reduction in urinary 6-keto-$PGF_{1\alpha}$ and TXB_2, after ibuprofen administration.

On the other hand, our results do not provide evidence for enhanced renal synthesis of vasodilator PGE_2 as a consistent hallmark of the cyclooxygenase-dependence of renal function in SLE, as suggested by the early observations of Kimberly et al. (33). However, it should be pointed out that enhanced glomerular PGE_2 production may not be reflected by urinary PGE_2 excretion, which largely reflects a medullary origin. Thus, decrements in renal function after ibuprofen may well depend upon reductions in the cortical synthesis of both PGI_2 and PGE_2.

More direct evidence for the functional significance of renal TXA_2 synthesis in SLE would require the use of a selective TX-synthase inhibitor or a TXA_2-receptor antagonist. However, three currently available imidazole-analogue TX-synthase inhibitors, i.e., dazoxiben (34), OKY-046 (35), and UK-38,485 (36) only marginally (<40%) reduced urinary TXB_2 excretion when given to healthy subjects at doses fully suppressing platelet TXB_2 production. Similarly, differential inhibition of platelet vs. glomerular TX-synthase detected in vitro (34) was also observed ex vivo in rats with nephrotoxic serum nephritis (24). Whether a tissue-selective TX-synthase inhibitor or a TXA_2-receptor antagonist may indeed improve renal function in SLE patients remains to be determined.

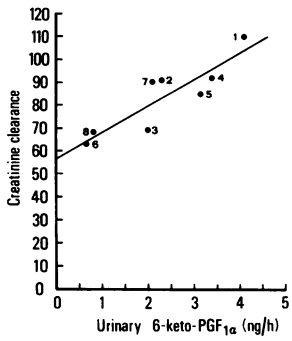

Figure 4. Correlation of creatinine clearance and urinary 6-keto-$PGF_{1\alpha}$ excretion. Data given in Tables I and IV are correlated to show the relationship between glomerular filtration rate and renal PGI_2 production of subjects from each of the experimental groups ($r = 0.870$; $P < 0.01$). The numbers 1 through 8 designate different groups, as detailed in Tables I and IV.

We conclude that, in SLE patients (*a*) a complex alteration in renal arachidonate metabolism exists, the most consistent and specific features of which are reflected by enhanced urinary excretion of TXB_2 and decreased urinary excretion of 6-keto-$PGF_{1\alpha}$; (*b*) the severity of such biochemical alterations correlates with the activity of renal lesions and with deteriorating renal function; (*c*) PGI_2 is a determinant of glomerular hemodynamics and its suppression by cyclooxygenase inhibitors accounts for the reduction in renal function associated with these drugs; (*d*) the simultaneous suppression of intrarenal TXA_2 synthesis partially attenuates the functional consequences of cyclooxygenase inhibition, as compared with other forms of chronic glomerular disease with normal TXB_2 excretion; and (*e*) the long-term consequences of selective vs. nonselective pharmacologic modulation of renal arachidonate metabolism remain to be determined.

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Note added in proof. These results were further confirmed by quantitative analysis of the samples for thromboxane B_2 employing a stable isotope dilution assay. Thromboxane B_2 was derivatized as the methoxime, trimethylsilyl ether, pentafluorobenzyl ester, and was analyzed by negative spectrometry in the selected ion monitoring mode (Lawson, J., A. Brash, J. Doran, and G. A. Fitzgerald. 1985. *Analyt. Biochem.* In press.). Thromboxane B_2 excretion was not significantly different in the lupus patients when measured by GC/MS (7.3 + 3.9 ng/h) or by RIA (10.0 + 4.4 ng/h).

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