Eicosanoid Excretion in Hepatic Cirrhosis

Predominance of 20-HETE

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Abstract

The cytochrome P450 system transforms AA to hydroxyeicosatetraenoic acid (HETE) metabolites that are vasoactive and affect transport in several nephron segments. A principal product of this system, 20-HETE, participates in key mechanisms that regulate the renal circulation and extracellular fluid volume. We hypothesized that excess production of 20-HETE, which constricts the renal vasculature, contributes to the renal functional disturbances in patients with hepatic cirrhosis, particularly the depression of renal hemodynamics. The development of a precise and sensitive gas chromatographic/mass spectrometric method makes it possible to measure 20-HETE and the subterminal HETEs (16-,17-,18-, and 19-HETEs) in biological fluids. As 20-HETE was excreted as the glucuronide conjugate, measurement of 20-HETE required treatment of urine with glucuronidase. We measured HETEs in the urine of patients with cirrhosis, and compared these values to those of normal subjects. Urinary excretion rate of 20-HETE was highest in patients with ascites; 12.5±3.2 ng/min vs. 5.0±1.5 and 1.6±0.2 ng/min in cirrhotic patients without ascites and in normal subjects, respectively. Excretion of 16-, 17-, and 18-HETEs was not increased. In patients with cirrhosis, the excretory rate of 20-HETE was several-fold higher than those of prostaglandins and thromboxane, whereas in normal subjects 20-HETE and prostaglandins were excreted at similar rates. Of the eicosanoids, only increased excretion of 20-HETE in subjects with cirrhosis was correlated ($r = -0.61$; $P < 0.01$) with reduction of renal plasma flow (RPF). (*J. Clin. Invest.* 1997. 100:1264–1270.) Key words: hepatorenal syndrome • 16-,17-,18-HETEs • arachidonate metabolites • cytochrome P450 • renal eicosanoids • thromboxane

Introduction

During the course of cirrhosis, renal function progressively deteriorates without evidence of organic nephropathy (1). Functional renal impairment in compensated cirrhosis is subclinical, the alterations being a reduction of renal blood flow (RBF),[1] (2, 3, 4), in particular the cortical component (5, 6), usually without depression of GFR (3, 4), a blunted response to a sodium load (7), and increased proximal tubular sodium reabsorption (8). When ascites develops, sodium and water excretion are demonstrably impaired, and RBF is decreased further (3, 4), effects that may be associated with reduced GFR. Subjects with cirrhosis and ascites show activation of the principal antidiuretic-antinatriuretic neurohumoral systems, the renin-angiotensin-adrenergic-aldosterone ADH axis (3, 9, 10). Renal vasodilator-diuretic systems, such as prostaglandins (3, 11–13), kallikrein-kinin (14, 15), and nitric oxide (16), are also activated in cirrhotic subjects with ascites. A vascular mechanism involving prostaglandins E_2 (PGE_2) and I_2 (PGI_2) (3, 12, 13) contributes to maintenance of RBF and GFR in patients with far advanced cirrhosis and ascites. The importance of a prostaglandin-dependent mechanism in cirrhosis with ascites is evident in the rapid decline in renal function produced by inhibition of cyclooxygenase with aspirin-like drugs (17). Thromboxane A2 (TxA_2), on the other hand, may contribute to deterioration in renal function (12, 18).

Metabolites of AA that are generated by the cytochrome P450 (CYP) system have also been identified as major products of AA metabolism in the kidney (19). One of these products, 20-hydroxyeicosatetraenoic acid (20-HETE), that is produced in large quantities by the kidney, is vasoactive (20–23), and affects renal transport mechanisms (24, 25). 20-HETE is a principal product of AA metabolism in the proximal tubules (26), medullary thick ascending limb (mTAL) (27), and preglomerular microvessels (28). 20-HETE is a major component in several key mechanisms that regulate the renal circulation and salt and water excretion, and that are affected in cirrhotic subjects. 20-HETE has been shown to: (*a*) modulate the Na^+-K^+-Cl^- cotransporter in the mTAL (24); (*b*) participate in tubuloglomerular feedback (29), and, (*c*) contribute to blood pressure elevation when produced in excess as in the spontaneously hypertensive rat (30). In terms of the genesis of renal dysfunction in association with hepatic cirrhosis, 20-HETE is produced in large amounts by preglomerular arterioles at which site it acts in the autoregulatory mechanism to constrict renal arterioles in response to increased perfusion pressure (28). 20-HETE has been identified in the human kidney, and is excreted conjugated to glucuronide (31); treatment with glucuronidase is required for measurement of the free form of urinary 20-HETE.

We have recently demonstrated that 20-HETE as well as 19-HETE and the subterminal HETEs, 16-, 17-, and 18-, are released selectively from the rabbit kidney in response to angiotensin II (AII); AVP and bradykinin were without effect

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1. *Abbreviations used in this paper:* 20-HETE, 20-hydroxyeicosatetraenoic acid; CYP, cytochrome P450; GC/MS, gas chromatographic/mass spectrometric; mTAL, medullary thick ascending limb; PAH, *p*-aminohippurate; PRA, plasma renin activity; RBF, renal blood flow; RPF, renal plasma flow.

(32). HETEs effluxed primarily into the urine, venous efflux being a small component of the total (33). The subterminal HETEs were shown to be vasoactive, and to affect renal ion transport (32). This was the first report that an intact organ responded to hormonal stimulation by releasing HETEs.

The simultaneous measurement of 20-HETE and the subterminal HETEs (16-, 17-, 18-, and 19-) in biological fluids is now possible because of the development of a precise and sensitive gas chromatographic/mass spectrometric (GC/MS) method that combines the specificity of capillary column gas chromatography and the sensitivity of electron capture ionization mass spectrometry (32). We have applied this method to measuring HETEs in the urine of normal subjects and those with cirrhosis of varying severity. We designed this study to define a possible role of 20-HETE and related CYP-derived HETEs in the evolution of renal functional deterioration in patients with cirrhosis and ascites. Cirrhotic subjects with overt renal failure, as defined by serum creatinine levels > 2 mg/dl, were excluded because the focus of the study was renal dysfunction secondary to hepatic cirrhosis. Cirrhotic subjects similar to those in the present study have been reported to show either normal or slightly reduced GFRs associated with modest reductions in RBF (3, 4). In view of the considerable capacity to produce 20-HETE by that section of the renal vasculature (the preglomerular microvessels) primarily responsible for effecting changes in renal vascular resistance (28) as well as the vasoconstrictor potency of 20-HETE (23), we hypothesize that renal 20-HETE levels are increased in subjects with cirrhosis. This increase measured as urinary excretion of 20-HETE was correlated with the progression of liver failure and associated renal dysfunction. Of the HETEs, urinary excretion of 20-HETE was increased selectively in cirrhotic subjects; 20-HETE exceeded by threefold the excretion of thromboxane, the predominant urinary cyclooxygenase product in cirrhosis with ascites.

Methods

Study population. 16 cirrhotic patients (8 ascites, 8 nonascites; 4 females, and 12 males; mean age 51±11 yr) were included in this study. The diagnosis of cirrhosis was histological in all cases; the etiology was alcoholic in 10 and posthepatitic in 6. Among cirrhotic patients, eight were without ascites and eight had ascites with clinical and sonographic evidence of ascites. According to Child-Pugh criteria (34), five patients were grade A, five patients were grade B, and six were grade C. All patients had endoscopically demonstrated esophageal varices. Cirrhotic subjects were excluded if they had hyperglycemia (serum glucose > 120 mg/dl), renal failure (serum creatinine > 2 mg/dl), hyponatremia (serum sodium < 125 mEq/liter), hypokalemia (serum potassium < 3.0 mEq/liter), hyperkalemia (serum potassium > 5.0 mEq/liter), hypertension, or heart failure. At the time of the study, patients were stable; those with alcoholic liver disease had been abstinent for at least 2 mo before the study, and gastrointestinal hemorrhage had not occurred in the month before the study. Diuretics were discontinued 10 d before the study. The control population included five normal male subjects and three female (mean age 45±8 yr). History, physical examination, electrocardiogram, and routine chemical analyses excluded cardiovascular, renal, and hepatic dysfunction. None of the control subjects were on medication. The study was designed and performed according to the principles of the Declaration of Helsinki. The nature of the study was fully explained to the patients, and verbal informed consent was obtained in all patients.

Study design. Patients and controls were maintained for at least 5 d before the study on a normocaloric diet with a sodium intake of 80 mmol/d; 24-h urinary sodium excretion was used to control the diet. Water intake was restricted to 1 liter per day. For the 24 h before and throughout the study, beverages containing methylxanthines were excluded. At 9:00 pm of the day before the study, 600 mg of lithium carbonate was administered per osmole. On the following morning after an overnight fast, a urine collection period was started at 6:00 am and an intravenous priming dose of *p*-aminohippurate (PAH) (8 mg/kg) was given, followed by constant infusion of 5% dextrose/water at 1.5 ml/min for at least 5 h. The amount of PAH infused was calculated to maintain a serum PAH concentration of ~ 1.5 mg/dl. Blood samples were taken at the beginning, at midpoint, and at the end of the urine collection period (which lasted at least 7 h). Urine was collected by spontaneous voiding. To maintain an adequate diuresis, tap water 100–200 ml/hr was given. Patients remained supine throughout the study. Blood samples at the beginning and at the end of the urine collection period were analyzed for sodium (Na), lithium (Li), creatinine (Cr), and PAH concentrations. Blood samples at the midpoint of the clearance period were analyzed for plasma renin activity (PRA). Urine samples were analyzed for Na, Li, Cr, and PAH concentrations. Samples were also immediately frozen and kept at −70°C for assay of PGE_2, 6ketoPGF$_{1\alpha}$, TxB_2, and CYP-dependent HETE.

Analytic procedures. Na and Li were measured by flame photometry (Instrumentation Laboratory, Inc., Lexington, MA). Cr and PAH were determined colorimetrically. PRA was determined by RIA of angiotensin I (Renin Maia Kit; Bio/Data Corp., Hatboro, PA).

CYP-dependent HETEs. 25 ng of 19-HETE-d_3 was added as an internal standard to 5-ml urine samples. Samples were allowed to equilibrate for 20 min at room temperature, and then incubated for 2 h at 37°C with 0.5 mg of β-glucuronidase (in 0.075 M potassium phosphate buffer, pH 6.8). Incubation with β-glucuronidase has been shown to increase urinary levels of 20-HETE $\sim$ 20-fold (31), a result confirmed by preliminary experiments performed in our laboratory (see Fig. 1 *B*) After adjusting the pH to 3.0, each urine sample was applied to a solid-phase extraction column (Tox Elut; Varian Inc., Harbor City, California), allowed to sit for 15 min, and then eluted with 15 ml of dichloromethane. After evaporation to dryness, the residue was dissolved in methanol (20 μl), and purified by reverse-phase HPLC on a Ultrasphere ODS (250 × 4.6 mm, 5 nm; Beckman Instruments Inc., Fullerton, CA) column, with a linear gradient running from acetonitrile:water:acetic acid 62.5:37.5:0.5 to acetonitrile 100% at a flow rate of 1 ml/min for 20 min. Fractions corresponding to elution of 16-, 17-,18-,19-, and 20-HETE standards and 19-HETE-d3 (8 and 9 min), were collected, dried, and dissolved in acetonitrile (100 μl). For GC/MS analysis, samples were converted to pentafluorobenzyl ester trimethylsylyl ethers by the addition of 30 μl of α-bromo-2,3,4,5,6-pentafluoro-toluene and 30 μl of *N*,*N*-diisopropylethylenamine for 30 min. After evaporation to dryness under nitrogen, trimethylsylyl ethers of free hydroxyl groups were prepared by reaction with 80 μl of bis(trimethylsylyl)-trifluoracetamide and 20 μl of pyridine for 30 min. Samples were dissolved in isooctane, and 1-μl aliquots were analyzed by GC/MS. Gas chromatography (HP5890) was performed on a DB-1 column (15 m; 0.25-mm inner diameter, 0.25-μm film thickness; J & W Sci., Folsom, CA) using a temperature program ranging from 150–300°C at a rate of 15°C/min. The mass spectrometer (5989A; Hewlett-Packard Co., Palo Alto, CA) was operated in the negative ion chemical ionization mode (electron capture) with methane as the reagent gas at a service pressure of 1.6 Torr. The CYP-HETEs were identified by comparison of gas chromatographic retention times with authentic standards of 16- to 20-HETEs and quantitated by calculating the ratio of relative abundance with 19-HETE-d_3.

Prostaglandins. PGE_2, 6ketoPGF$_{1\alpha}$, and TxB_2 were measured in unextracted urine with the use of second-antibody, solid-phase, ELISA (35, 36). Purification by solid-phase column extraction, performed as previously described, did not improve the quality of the assay. Microtiter plates (immunoplate II; Nunc Inc., Naperville, IL) were coated with goat anti–rabbit IgG (200 μl per well, Boehringer Mannheim Biochemicals, Indianapolis, IN) at a concentration of 10

μg/ml, diluted with 50 mmol/liter potassium phosphate buffer, pH 7.4. After an overnight incubation at 4°C, plates were washed three times with successive 300-μl vol of wash buffer composed of 0.05% Tween 20 in 10 mmol/liter potassium phosphate buffer, pH 7.4. Plates were then filled with enzyme immunoassay buffer of the following composition: 0.1 mol/liter potassium buffer, 0.4 mmol/liter NaCl, 85 mmol/liter Na_4EDTA, 0.01% wt/vol NaN_3, and 0.1% wt/vol BSA. Plates were then covered with microtest plastic film (Fisher Scientific Co., Santa Clara, CA) and stored at 4°C for a minimum of 18 h before their use in the competitive binding step. Coated plates could be stored in this manner for several weeks. After buffer removal, 50-μl aliquots of enzymatic tracer, antibody, and either prostaglandin standard (4–2,000 pg/ml) or samples, diluted as appropriate with buffer, were placed in duplicate in the coated wells. Nonspecific binding was determined by replacement of antibody with buffer and maximal binding by equilibration in the absence of prostanoids. After overnight equilibration at 4°C, plates were washed three times with 300 μl of enzyme immunoassay buffer. Wells were filled with 200 μl of Ellman's reagent. The concentrated stock solution was diluted 1:100 as required. After 1–2 h of shaking, absorbance was read at 405 nm with automated subtraction of readings at 630 nm to correct for nonspecific absorbance (Microplate EL309; Bio-Tek Instruments Inc., Winooski, VT). Microtitration and washing steps were carried out with an automatic dispenser (Pro/Pette; Perkin Elmer Corp., Norwalk, CT). Curve fitting was done with computerized mass action smoothed spline interpolation (Packard Instruments, Meriden, CT), and sample prostaglandin concentrations were automatically calculated by reference to the standard curve.

Calculations. Mean arterial pressure was calculated as diastolic pressure plus one-third of pulse pressure. Clearances of Cr, Na, Li, and PAH were calculated by the conventional formula $CA = (UA \times V)/PA$ where CA is clearance of A. UA and PA are urine and plasma concentrations of A, respectively, and V is the urinary output. Clearance of PAH and Cr were used as measures of renal plasma flow (RPF) and GFR, respectively.

Statistical analysis. Results were expressed as mean±SE. Comparison between cirrhotic and control subjects was carried out by ANOVA and then the Bonferroni test. The 5% probability level was regarded as significant.

Results

The hemodynamic and renal functional indices in patients with cirrhosis with and without ascites and in control subjects are shown in Table I. Blood pressure did not differ among the three groups as it was recorded in the supine position. In cirrhotic subjects with ascites, RPF was reduced and GFR was depressed (the latter not significantly) when associated with decreased lithium clearance, an index of proximal tubular reabsorption (37), and reduced urinary sodium excretion and clearance; PRA was marginally elevated.

Table I. Renal Hemodynamic and Functional Parameters In Control and Cirrhotic Subjects

		Cirrhotic patients	
	Control subjects	Without ascites	With ascites
MAP (mmHg)	98±3	96±2	95±3
Plasma sodium (mEq/liter)	141±2	140±3	138±3
Sodium excretion (μEq/min)	156±13	161±33	114±13*
GFR (ml/min)	104±17	118±11	91±4
RPF (ml/min)	614±50	513±39	419±37*
Sodium clearance (ml/min)	1.25±0.12	1.55±0.34	0.83±0.12*
Lithium clearance (ml/min)	32.2±3.5	27.4±3.7	22.5±3.2*
PRA (ng/ml/h)	1.7±0.3	1.0±0.3	2.6±0.8

MAP, mean arterial pressure; RPF, renal plasma flow; PRA, plasma renin activity. *$P < 0.05$ vs controls.

Separation of CYP-HETE standards is shown in Fig. 1 *A*. CYP-dependent HETEs were virtually undetectable in urine before incubation with β-glucuronidase (Fig. 1 *B*). When urine from control and cirrhotic subjects was preincubated with β-glucuronidase, four CYP-derived HETEs (16-, 17-, 18-, and 20-HETE) were readily demonstrated (Fig. 1 *B*). Two unidentified peaks migrating between 19- and 20-HETEs did not correspond to any known HETE standard. Lipoxygenase-derived HETEs such as 12- and 15- HETEs were separated from CYP-related HETEs during the extensive purification with HPLC. Any unseparated lipoxygenase-derived HETEs would have eluted at 7.4 min (Fig. 1). The two compounds, which were not characterized structurally, have to be considered as impurities,

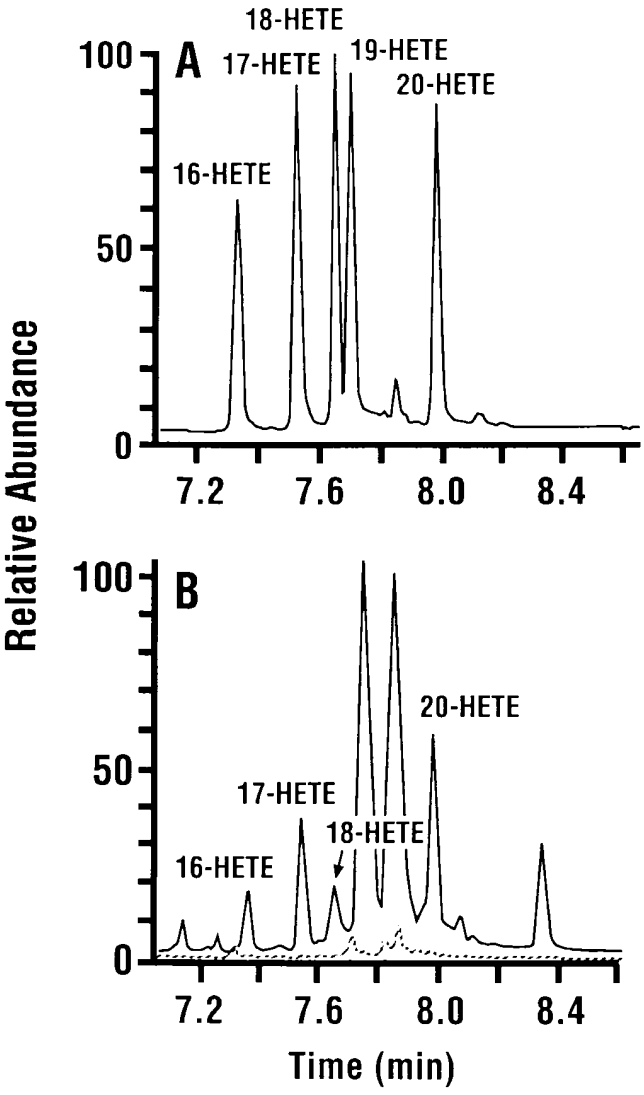

Figure 1. Comparison of chromatograms obtained during gas chromatographic/mass spectrometric analyses of a mixture of authentic standards of HETEs (16-,17-, 18-, 19-, 20-HETE) (*A*) and HETEs resulting from 5-ml urine samples preincubated (-) or not preincubated (---) with β-glucuronidase (*B*) as described in Methods.

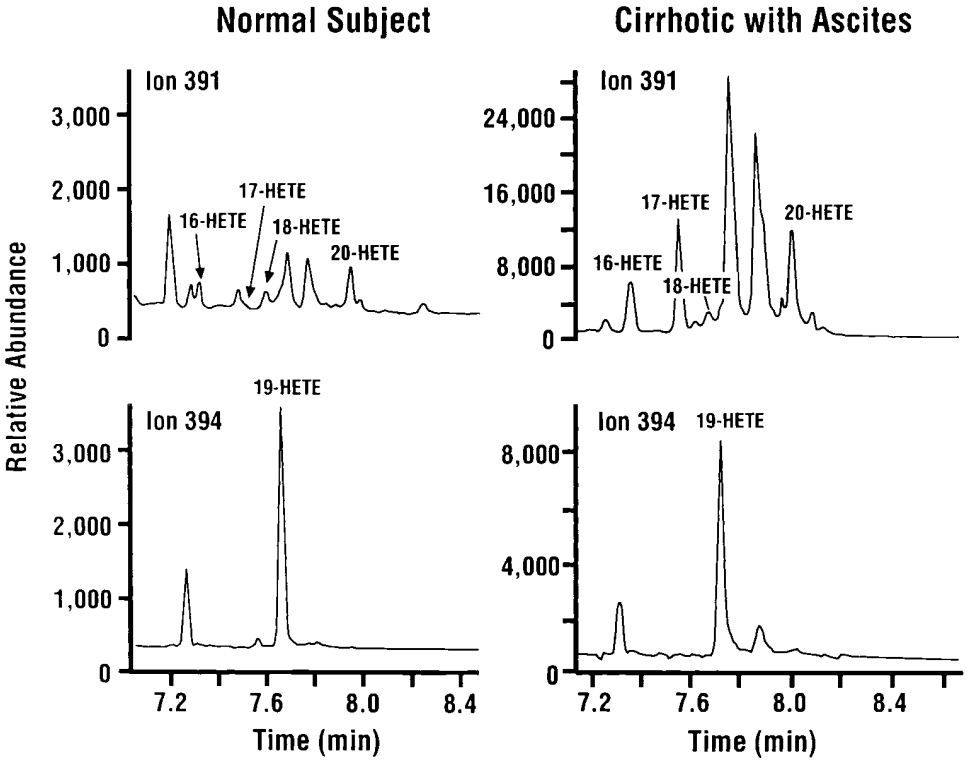

Figure 2. Gas chromatographic/mass spectrometric analysis of 5-ml urine samples from a normal subject (*left*) and from a cirrhotic patient with ascites (*right*), preincubated with β-glucuronidase. Ions m/z 391 (*top chromatograms*) and m/z 394 (*bottom chromatograms*), correspond to endogenous HETEs and internal standard 19-HETE-d_3 (5 ng/ml), respectively.

and were most evident in urine from cirrhotic subjects. 19-HETE could not be demonstrated in the urine of either control or cirrhotic subjects. Fig. 2 shows the difference in gas chromatographic analysis of urine from a control and a cirrhotic subject.

Urinary excretion of 20-HETE was significantly increased in cirrhotic patients, the largest increase occurring in those with ascites, whereas excretion rates of 16-, 17- and 18-HETE were not increased significantly in cirrhotic patients when compared to control subjects (Fig. 3). Renal plasma flow (RPF), which was significantly decreased only in cirrhotic subjects with ascites (Table I), correlated negatively with 20-HETE excretion ($r = -0.61$; $P < 0.01$) (Fig. 4), but did not correlate with excretion of 16-, 17- and 18-HETEs. 3 of the 16 subjects with cirrhosis, and 2 of the 8 control subjects were not included in Fig. 4 because renal clearances could not be ob-

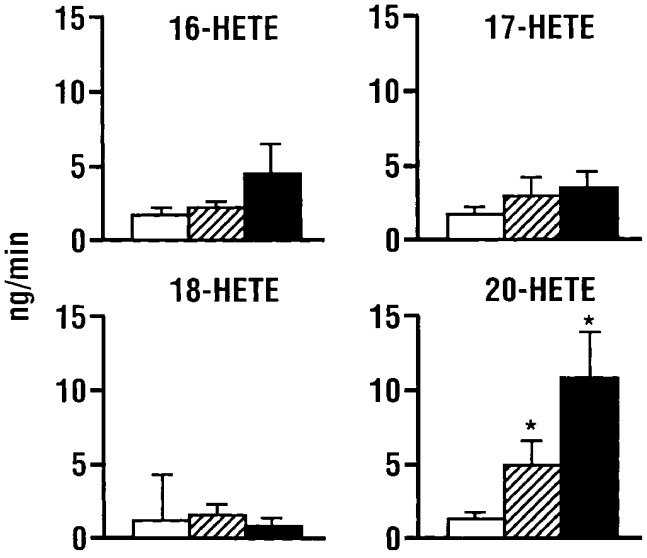

Figure 3. Urinary excretion of 16-, 17-, 18-, 20-HETEs in normal subjects and in cirrhotic patients with and without ascites. *$P < 0.05$ compared to controls. *White bars*, controls; *striped bars*, nonascitic patients; *black bars*, ascitic patients.

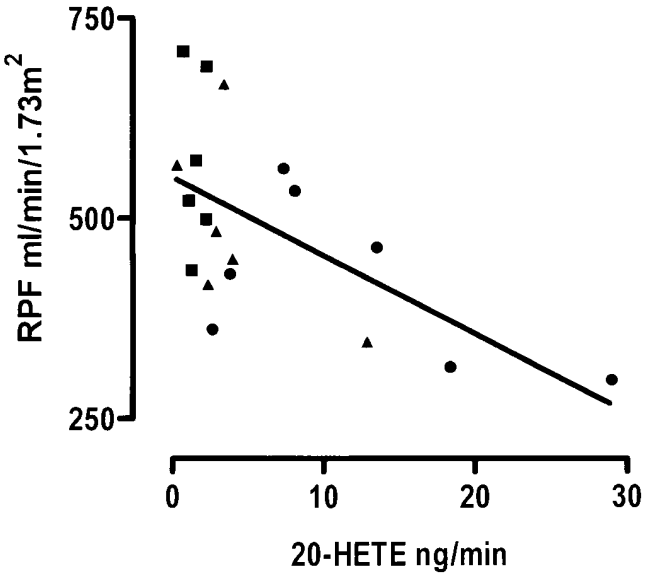

Figure 4. Correlation of RPF (ml/min) and 20-HETE urinary excretion (ng/min) for normal subjects (■), and subjects having cirrhosis with (●) and without (▲) ascites. $r = -0.61$; $P < 0.01$.

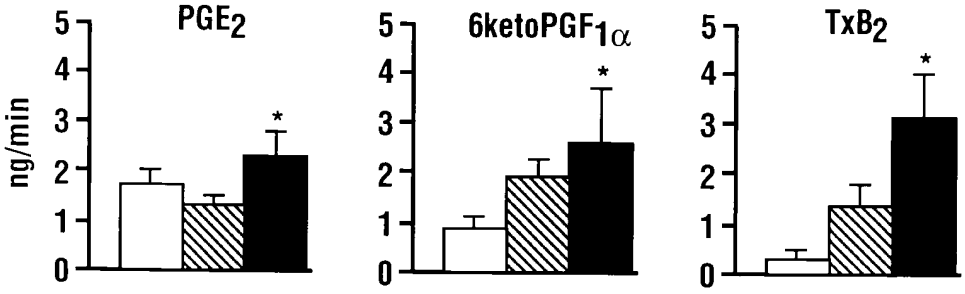

Figure 5. Urinary excretion of PGE_2, and the hydrolysis products of PGI_2 and TxA_2, 6-keto-prostaglandin $F_1\alpha$ (6-keto-$PGF_1\alpha$,) and thromboxane B2 (TxB_2) respectively, in normal subjects and in cirrhotic patients with and without ascites. $*P < 0.05$ compared to controls. *White bars*, controls; *striped bars*, nonascitic patients; *black bars*, ascitic patients.

tained for technical reasons. Nonetheless, the 20-HETE excretion rates for these subjects clearly separated normal from cirrhotic subjects. 20-HETE values for the three subjects with cirrhosis for which RPFs were not available were 4.42, 9.91, and 6.64 ng/min vs. 1.17 and 1.45 ng/min for the two normal subjects. Neither female subjects with cirrhosis (4 of 16) nor female control subjects (3 of 8) differed from corresponding male subjects in terms of HETE excretion. For example, the range of 20-HETE excretory rates for female vs. male patients with cirrhosis was 2.3–18.4 ng/min and 0.3–29.0 ng/min, respectively; and for female vs. male control subjects 1.1–2.2 ng/min and 0.7–2.2 ng/min, respectively.

Urinary excretion of PGE_2, PGI_2, and TxA2 (measured as their hydrolysis products, 6-keto-$PGF_{1\alpha}$, and TxB_2, respectively), was significantly increased only in cirrhotic patients with ascites (Fig. 5), the largest increase over control, (eightfold) occurring for TxB_2. In contrast to 20-HETE, however, changes in RPF for cirrhotic subjects were not significantly correlated with excretion of TxB_2 ($r = -0.40$; $P > 0.05$). Urinary excretion of prostaglandins was not different in male and female subjects. The range of PGE_2 excretory rates for female vs. male subjects with cirrhosis was 1.3–3.2 ng/min and 0.2–4.0 ng/min, respectively; for female vs. male control subjects, 2.1–2.3 ng/min and 0.6–2.1 ng/min, respectively.

Discussion

The key finding in this study was the selective increase in urinary excretion of 20-HETE in subjects with cirrhosis when compared to 16-, 17-, and 18-HETEs, and a further increase in the excretion of 20-HETE with the development of ascites. Urinary excretion of the subterminal HETEs: 16-,17-, and 18-; was not increased. Falck et al. first described oxidation of AA by a hepatic CYP-dependent enzyme to 16-, 17-, and 18-HETEs (38). Our study is the first report of the excretion of 16-, 17- and 18-HETEs by humans, and confirms the important observation of Prakash et al. (31) that 20-HETE is excreted primarily as the glucuronide conjugate, as were the 16-, 17-, 18-, and 19-HETEs. Thus, measurement of CYP-derived HETEs in the urine requires incubation of samples with β glucuronidase, and yielded values in normal subjects comparable to those reported by Prakash et al. (31). The quantity of 20-HETE excreted per unit time in cirrhotic subjects with ascites exceeded that of prostanoids by a factor of three- to five-fold. The excretory rate of TxB_2, the stable hydrolysis product of TxA_2 that demonstrated the largest increase of the cyclooxygenase products in subjects with cirrhosis and ascites, was less than one-third that of 20-HETE.

Urinary 20-HETE levels probably reflect renal synthesis. Carroll et al. measured efflux of 20-HETE as well as 16-, 17-, 18- and 19-HETEs from the rabbit isolated kidney (32). In the rabbit, AII increased renal efflux of CYP-related HETEs, 20-HETE exhibiting a seven-fold increase. The finding that AII selectively increased renal efflux of HETEs in the rabbit provides a clue as to a possible determinant of enhanced excretion of 20-HETE in cirrhosis, namely, activation of the renin-angiotensin-aldosterone axis, a feature of cirrhosis with ascites (3). PRA, however, was only marginally elevated in cirrhotic subjects with ascites, although conditions that would promote activation of PRA and accentuate differences between cirrhotic subjects and normal subjects were minimized. Thus, PRA was measured on blood samples drawn in the supine position, which is associated with a 50% or greater reduction in PRA (39). An additional consideration, regarding hormonal stimulation of 20-HETE production, is based on the findings of Moore et al. that endothelin plasma levels were greatly elevated in liver disease, particularly in patients with the hepatorenal syndrome (40). We have recently reported that a cytochrome P450 product, probably 20-HETE, participates in the renal vasoconstrictor action of ET-1 (41). ET-1 evoked a three to fourfold increase in efflux of 20-HETE from the rat kidney, and inhibition of CYP-dependent AA metabolism greatly reduced the renal vascular response to ET-1. Thus, 20-HETE may contribute to the renal vasoconstrictor responses to both ET-1 and AII. 20-HETE, in concentrations of 10 nM, constricted renal afferent arterioles (42). The vasoconstrictor properties of 20-HETE (43), its major site of synthesis in the preglomerular microvasculature (28), and its release by AII and ET-1 (32, 41), argue for its participation in the renal functional abnormalities associated with cirrhosis, particularly the initial reduction in RPF and the later depression of GFR.

Conjugation of endogenous compounds with glucuronic acid, catalyzed by microsomal glucuronyl transferase, converts lipid-soluble hormones such as steroids into glucuronides, which can be more easily excreted (44). The glucuronide conjugate is usually biologically inactive (44), which appears to be the case for the 20-HETE glucuronide conjugate, as it was shown to be devoid of renal vasoactivity (M.A. Carroll, Department of Pharmacology, New York Medical College; personal communication). Moreover, the renal proximal tubules as well as the liver demonstrate high specific activity of glucuronyl transferase (45).

Infusion of 20-HETE has been reported to promote diuresis and natriuresis (22), effects at odds with the pathophysiologic abnormalities involving retention of salt and water in cirrhosis (1). There are however, as many as 12 isoforms of the CYP 4A enzyme family that catalyze ω-hydroxylation of fatty acids including AA, the latter resulting in formation of 20-

HETE (46). These isoforms are localized in several renal structures, and differ in their inducibility by agents, and therefore vary in their response to regulatory factors. Indeed, the CYP 4A2 isoform is expressed primarily in the preglomerular microcirculation of the rat (42), and was shown to generate 20-HETE in response to AII (47). In contrast, the CYP 4A1 and three isoforms of ω hydroxylase that are highly expressed in proximal tubules (48), did not respond to AII challenge by increasing production of 20-HETE, whereas parathyroid hormone and epidermal growth factor did stimulate release of 20-HETE from proximal tubules (26). AII, however, when given to the intact kidney, produced a sevenfold increase in renal efflux of 20-HETE (32), suggesting that sites other than the nephron were responsible for the large efflux of 20-HETE in response to AII. More to the point, the capacity of AII to release large quantities of 20-HETE from preglomerular arterioles was reported recently (47). In view of the large capacity of renal arterioles and arteries to produce 20 HETE (42), it is reasonable to consider the renal preglomerular vasculature as important sources of urinary 20-HETE. The progressive decline in RPF associated with evolving hepatic cirrhosis (Table I) correlated negatively with urinary excretion of 20-HETE (Fig. 4), suggesting a close relationship between 20-HETE production by preglomerular arterioles and reduced RPF. The preglomerular vascular effects of 20-HETE alone can account for salt and water retention seen in subjects with cirrhosis as constriction of the preglomerular microcirculation, of itself, may promote salt and water reabsorption by decreasing renal interstitial hydrostatic pressure, a major determinant of reabsorption in deep nephrons (49).

In conclusion, urinary excretion of 20-HETE, a potent renal vasoconstrictor eicosanoid, is selectively elevated amongst the CYP-related HETEs in subjects with cirrhosis, achieving its highest levels in the presence of ascites. The urinary levels of 20-HETE exceeded by threefold those of TxB_2, the major urinary prostanoid product in cirrhotic subjects. In view of the multiplicity of hormonal and neurohumoral derangements in cirrhosis, it seems likely that, as indicated by Epstein, the renal functional abnormalities require the participation of several hormonal and/or neural effectors, acting in concert (1). The present study has identified 20-HETE as a possible effector of the renal functional abnormalities associated with progression of hepatic cirrhosis.

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