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Endothelin-1 receptor blockade prevents renal injury in experimental hypercholesterolemia

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Background. The potent vasoconstrictor endothelin-1 is involved in regulation of renal function, and is up-regulated in hypercholesterolemia (HC), a risk factor for renal disease that increases oxidative stress and impairs renal hemodynamic responses. However, the involvement of endothelin (ET) in this disease process is yet unknown.

Methods. Regional renal hemodynamics and function in vivo were quantified in pigs at baseline and during infusion of acetylcholine using electron beam computed tomography after a 12-week normal diet ($N = 6$), HC diet ($N = 6$), and HC diet orally supplemented (4 mg/kg/day) with the selective ET receptor-A (ET-A) blocker ABT-627 (HC+ET-A, $N = 6$). Plasma levels of 8-epi-PGF2-α-isoprostanes, markers of oxidative stress, were measured using enzyme immunoassay, and renal tissue was studied ex vivo using Western blotting, electrophoretic mobility shift assay, and immunohistochemistry.

Results. Total and low-density lipoprotein (LDL) cholesterol were similarly increased, but isoprostanes were decreased in HC+ET-A compared to HC alone. Basal renal perfusion was similar among the groups, while glomerular filtration rate (GFR) increased in HC+ET-A compared to HC. Stimulated perfusion and GFR were blunted in HC, but normalized in HC+ET-A. Moreover, ET blockade increased expression of endothelial nitric oxide synthase, and decreased endothelial expression of the oxidized-LDL receptor LOX-1, as well as tubular immunoreactivity of inducible nitric oxide synthase, nitrotyrosine, nuclear factor-κB, transforming growth factor-β, and tubulointerstitial and perivascular trichrome staining.

Conclusion. ET-A blockade improves renal hemodynamic and function in HC, and decreases oxidative stress, and renal vascular and tubulointerstitial inflammation and fibrosis. These findings support a role for the endogenous ET system in renal injury in HC and atherosclerosis.

Key words: hypercholesterolemia, endothelin, regional blood flow, kidney.

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The predominant isoform of the endogenous endothelin system is endothelin (ET)-1, a potent and long-lasting renal vasoconstrictor involved in the regulation of vascular tone [1] with pronounced atherogenic and mitogenic properties [2], whose actions are mediated by the specific ET-A and ET-B receptors [3]. ET-1 production is increased in systemic cardiovascular disease like early atherosclerosis [2, 3] and hypercholesterolemia (HC) [4, 5], which can disrupt the delicate balance between endothelium-derived vasoactive factors. Indeed, coronary [4] and peripheral [6] endothelial function was restored in experimental HC during ET blockade, supporting the role of ET-1 as an early contributor to endothelial dysfunction in HC [5].

We have previously shown [7–10] that diet-induced HC in the swine was associated with impaired renal perfusion and tubular responses to challenge, accompanied by increased generation of reactive oxygen species (ROS), or increased oxidative stress. HC and ROS have been implicated in the pathogenesis of renal injury by direct cellular toxicity, partly through liberation of vasoconstrictor-bioactive lipids and inactivation of nitric oxide (NO) [11]. Furthermore, ROS increase synthesis of ET-1 [12] and act as second messengers of this peptide [13]. ET-1 may, in turn, increase superoxide anion (O^{2-}) and peroxynitrite production [14, 15], and promote uptake of oxidized low-density lipoprotein (ox-LDL) by its specific receptor LOX-1 [16], thereby contributing to development and progression of endothelial dysfunction and atherosclerosis. Indeed, ET could conceivably mediate some of the renal effects of HC. However, the possible role of ET-1 in renal functional impairment and the potential beneficial effects of endothelin receptor blockade on the kidney in early HC remain elusive.

Electron beam computed tomography (EBCT) provides accurate and noninvasive measurements of single-kidney regional hemodynamics and function in vivo,

which agree well with conventional measures [17]. Therefore, EBCT enables quantification of subtle alterations in the hemodynamics and function of the intact kidney [8, 10, 17, 18]. This scanner affords a unique opportunity to study the intrarenal effects resulting from endogenous endothelin blockade in HC. Therefore, the present study was designed to examine whether blockade of the endogenous endothelin system in HC pigs improved renal hemodynamics and functional responses, as well as renal tissue injury.

METHODS

Procedures were approved by the Institutional Animal Care and Use Committee. Eighteen domestic crossbred pigs of similar body weight (50 to 60 kg each) were studied after 12 weeks of normal ($N = 6$), 2% HC ($N = 6$) [4, 8, 10], or HC diet orally supplemented with the selective endothelin receptor-A blocker ABT-627 (HC+ET-A, $N = 6$) on a weight-adjusted scale to maintain a dose of 4 mg/kg per day [4]. ABT-627 is an orally active, nonpeptide selective ET-A receptor antagonist that has been fully characterized and has a binding Ki for the ET-A receptors approximately 2000-fold greater than for the ET-B receptors (0.035 nmol/L and 69.5 nmol/L, respectively) [19, 20]. The dosage of ABT-627 was based on drug level determinations in prior in vivo studies that demonstrated an attenuated blood pressure response to ET-1 infusion [4, 21, 22].

Following completion of 12 weeks of diet, in vivo EBCT studies were performed for assessment of renal regional perfusion, renal blood flow (RBF), glomerular filtration rate (GFR), and tubular function. On the day of the study, each animal was anesthetized with 0.5 g of intramuscular ketamine and xylazine, intubated, and mechanically ventilated with room air. Anesthesia was maintained with a mixture of ketamine (0.2 mg/kg/min) and xylazine (0.03 mg/kg/min) in normal saline, administered via an ear vein cannula (0.05 mL/kg/min) as previously described [8, 9]. Catheters were placed in the aorta (a few centimeters above the level of the renal arteries) and the superior vena cava. Forty consecutive EBCT scans (over 3 minutes) were subsequently performed at variable time intervals after a central venous bolus injection (0.5 mL/kg) of the nonionic, low-osmolar contrast medium iopamidol (Isovue-370®; Squibb Diagnostics, Princeton, NJ, USA). The studies were performed at baseline, and then repeated during intra-aortic infusion of the endothelium-dependent vasodilator acetylcholine (Ach, 4.5 μg/kg/min). The 8-epi-PGF2-α isoprostanes, considered the most ubiquitous and reliable oxidative stress markers [18, 23, 24], were measured in systemic venous plasma using an enzyme immunoassay (EIA) (Cayman, Ann Arbor, MI, USA) [18, 23, 24]. This assay has high accuracy (correlation coefficient of 0.99 on a standard curve), specificity of 98%, and interassay variability <10% [23, 25].

Pigs were then euthanized with intravenous sodium pentobarbital (100 mg/kg) and in vitro studies performed. Renal morphology was evaluated in 5 μm cross-sections stained with hematoxylin and eosin (H&E) and trichrome [8]. Protein expression of transforming growth factor (TGF)-β and the specific oxidized-LDL receptor LOX-1 was measured using Western blot and immunohistochemistry. Expression of the pro-inflammatory nuclear factor kappa B (NFκB) was evaluated using immunohistochemistry, and confirmed using electrophoretic mobility shift assay (EMSA). In addition, using either frozen or deparaffinized 5 μm-thick cross-sections, as detailed previously [8], to evaluate immunoreactivity for endothelial- (eNOS) and inducible- (iNOS) nitric oxide synthase, and nitrotyrosine (footprint for peroxynitrite formation).

Protein extraction and Western blotting

Frozen renal tissue was pulverized and homogenized at 4°C in chilled protein extraction buffer. The homogenate was incubated in buffer for 1 hour at 4°C, and the homogenized lysates then centrifuged for 15 minutes at 14,000 rpm. The supernatant was removed and the protein concentration determined by a Coomassie assay. The lysate was diluted 1:4 in 1× Page Sample Buffer, sonicated, and heated at 95°C to denature the proteins. The lysate was then loaded onto a gel, and subsequently run as per standard Western blotting protocols using specific antibodies against TGF-β and LOX-1 (1:200 each; Santa Cruz Biotechnology, CA, USA). The membrane was finally exposed to x-ray film (Kodak, Rochester, NY, USA), which was subsequently developed [26].

Electrophoretic mobility shift assay (EMSA)

EMSA was performed according to established protocols [27, 28]. Renal tissue was homogenized in a buffer [N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) 10 mmol/L (pH 7.8), KCl 15 mmol/L, $MgCl_2$ 2 mmol/L, ethylenediaminetetraacetic acid (EDTA) 0.1 mmol/L, dithioreitol (DTT) 1 mmol/L, and phenylmethylsulfonyl fluoride (PMSF) 1 mmol/L] and centrifuged (4000 *g* for 10 min). The pellet was resuspended in a homogenization buffer with KCl concentration of 0.39 mol/L, at 4°C for 1 hour. The probe was subsequently ultracentrifuged at 100,000 *g* for 30 minutes and the supernatant dialyzed overnight in a buffer [HEPES 50 mmol/L (p.H. 7.8), KCl 50 mmol/L, EDTA 0.1 mmol/L, DTT 1 mmol/L, PMSF 1 mmol/L, and 10% glycerol]. The dialyzed supernatant was analyzed for protein concentration by a Coomassie assay and stored at –80°C for subsequent gel shift assays. These were performed as two competition assays [unlabeled nonspecific (SP1) and specific (NFκB) consensus oligos] with a commercially

available kit (Promega, Madison, WI, USA) and nuclear extracts from HeLa cells (Promega) as positive sample controls. Probes of tissue samples and nuclear extracts were loaded to 5% TBE gels and run for 45 minutes at 250 V at 4°C. Radioactivity in each probe was adjusted to 250,000 cpm and protein content to 5 μg. Following electrophoresis, gels were placed on filter paper, dried on a gel dryer, and exposed to an x-ray film (Kodak) with an intensifying screen for 1 hour at –80°C. X-ray films were developed using an automated film-developing machine.

Immunostaining for eNOS, nitrotyrosine, and LOX-1 was performed in frozen 5 μm-thick cross-sections, as described previously [8]. Monoclonal antibodies for eNOS (1:500; Transduction-Laboratories, Lexington, KY, USA), nitrotyrosine residues (1:20; Cayman) [8], and for LOX-1 (1:280) served as primary antibodies. The Vectastain-Elite ABC Kit (Vector Laboratories, Burlingame, CA, USA) was used following vendor's instructions.

Immunostaining for iNOS, NFκB, and TGF-β was performed on deparaffinized renal 5 μm-thick cross-sections. Polyclonal iNOS (1:500; Affinity Bioreagents, Golden, CO, USA), and TGF-β (1:10; Santa Cruz Biotechnology, Inc.), and monoclonal NFκB (1:50; Santa Cruz Biotechnology Inc.) primary antibodies were utilized. The secondary antibody, immunoglobulin (Ig) G (Envision Plus; Dako, Carpinteria, CA, USA), was followed by staining with the Vector NovaRED substrate kit (Vector Laboratories), and slides were counterstained with hematoxylin [8].

Data analysis

Renal regional perfusion (mL/minute/g), intratubular fluid concentration (ITC), which reflects the concentration of contrast media and serves as an index of tubular fluid-reabsorption, single-kidney GFR, and RBF were calculated from EBCT time-density curves obtained from renal cortex and medulla using previously validated methods [8, 10, 17, 18]. Representative histologic mid-hilar cross sections of the kidney (1 per animal) were examined using a computer-aided image analysis program (Meta Imaging Series 4.6, MetaMorph, Universal Imaging Corporation, Downingtown, PA, USA). In each representative slide, immunostaining or trichrome staining was semi-automatically quantified in 15 to 20 fields by the computer program, and expressed as percentage of staining of total surface area, and the results from all fields averaged [8]. The H&E slides were analyzed visually and qualitatively for the presence of glomerulosclerosis. Renal arteriolar media-to-lumen ratio was assessed following standard techniques [8, 29].

Statistical analysis

Data are mean ± SEM, and in vivo data were compiled from both kidneys. Comparisons among the groups were performed using analysis of variance (ANOVA) with the Bonferroni correction and unpaired Student *t* test, and within groups using paired Student *t* test. Statistical significance was accepted for $P \leq 0.05$.

RESULTS

Serum cholesterol levels were similarly elevated in HC and HC+ET-A (Table 1). Total isoprostanes were higher in HC compared to normal, but not different from normal in HC+ET-A (Table 1). There were no significant differences among the groups in mean arterial pressure (Table 1).

Renal hemodynamics and function

Basal RBF, cortical and medullary perfusions were similar among the groups, while in HC+ET-A GFR was increased compared to HC (Table 1). Basal ITC was similar in normal and HC, whereas in HC+ET-A proximal tubular ITC was lower compared to HC, and tended to be lower compared to normal, as well (Table 1).

In normal animals, Ach increased RBF, GFR, and cortical and medullary perfusions (Fig. 1). In HC, GFR significantly increased, but the increase was attenuated compared to normal, and RBF and regional perfusion remained unchanged. On the other hand, in HC+ET-A pigs Ach increased RBF, GFR, cortical and medullary perfusion (Fig. 1 A to D). Renal tubular response was also different among the groups. In normal animals, Ach tended to decrease ITC in the distal and collecting tubules ($P = 0.07$, data not shown), while in the proximal tubule and the loop of Henle, ITC remained unchanged. In HC, Ach induced a significant decrease in ITC in the proximal and distal tubules ($P < 0.03$ for both), while in HC+ET-A, ITC significantly decreased in loop of Henle, distal tubules, and collecting duct ($P < 0.02$ for all).

Renal tissue

H&E slides did not reveal overt glomerular or vascular sclerosis in HC. However, the media-to-lumen ratio in HC was significantly greater than normal, and trichrome staining showed increased perivascular and tubulointerstitial fibrosis (Table 2, Fig. 2A), which were associated with significantly increased tubular and glomerular TGF-β expression. These findings were significantly attenuated in HC+ET-A (Table 2, Fig. 3A).

Immunoreactivity of eNOS in renal arteriolar endothelial cells was attenuated in HC compared to normal, but normalized in HC+ET-A (Fig. 2B), suggesting increased potential for NO production. Proximal and distal tubular immunoreactivity for iNOS, and global tubular and glomerular expression of nitrotyrosine (the marker for peroxynitrite formation in vivo), were higher in HC compared with normal, suggesting pro-inflammatory changes and increased generation of ROS, but were largely de-

Table 1. Systemic and renal characteristics in normal, hypercholesterolemic (HC), and HC+ET-A pigs

	Normal ($N = 6$)	HC ($N = 6$)	HC+ET-A ($N = 6$)
Total cholesterol *mmol/L*	1.67 ± 0.16	10.6 ± 0.9[a]	10.7 ± 1.1[a]
Low-density lipoprotein *mmol/L*	0.58 ± 0.12	7.6 ± 0.8[a]	7.8 ± 0.9[a]
Mean arterial pressure *mm Hg*	112 ± 5.7	118.2 ± 4.3	116 ± 5.5
Isoprostanes *pg/mL*	116.4 ± 15.5	238 ± 29.7[a]	154.4 ± 14.4[b]
Renal blood flow *mL/min*	542.8 ± 26.1	545.9 ± 48.6	518.2 ± 37.6
Glomerular filtration rate *mL/min*	75.4 ± 3.1	63.1 ± 4.7	84.5 ± 6.6[b]
Perfusion *mL/min/cc*			
Cortex	4.3 ± 0.3	4.4 ± 0.4	4.0 ± 0.3
Medulla	2.8 ± 0.3	3.1 ± 0.5	3.2 ± 0.4
Intratubular contrast concentration *arbitrary units*			
Proximal tubule	3.7 ± 0.2	4.5 ± 0.2	3.1 ± 0.3[b,c]
Henle's loop	8.7 ± 1.2	7.1 ± 0.4	9.1 ± 1.3
Distal tubule	7.0 ± 0.9	7.0 ± 0.4	7.1 ± 0.6
Collecting duct	11.9 ± 1.4	12.0 ± 1.8	9.1 ± 1.7

ET-A is endothelin receptor A. Data are mean ± SEM.
[a] $P < 0.05$ vs. normal
[b] $P < 0.05$ vs. HC
[c] $P < 0.1$ vs. normal

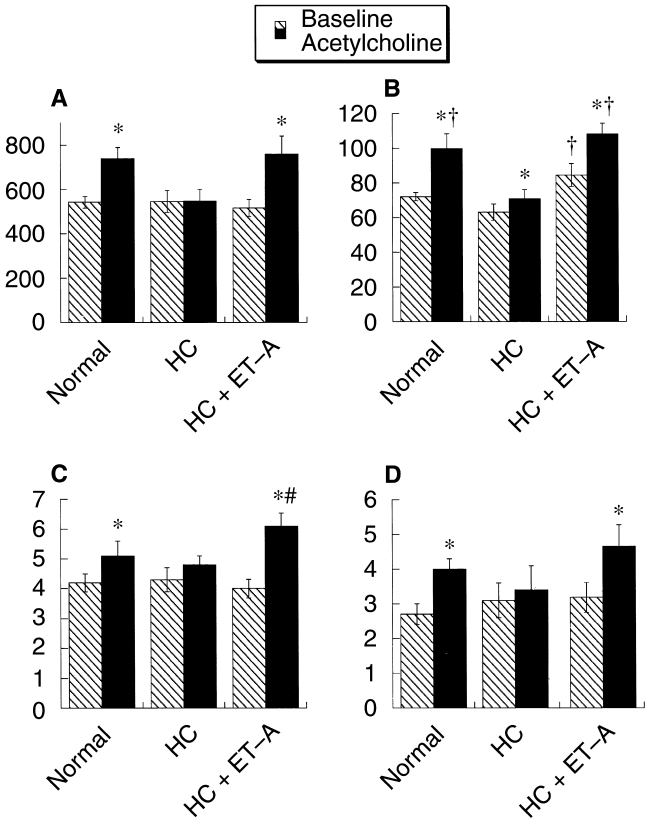

Fig. 1. (*A*) Renal blood flow, (*B*) glomerular filtration rate, (*C*) cortical, and (*D*) medullary perfusion under basal conditions and in response to acetylcholine in normal, hypercholesterolemic (HC), and HC+ET-A groups. *$P < 0.05$ vs. baseline; †$P < 0.05$ vs. HC; #$P < 0.05$ vs. normal.

creased in HC+ET-A (Fig. 2 C to D). A similar pattern was also observed in the expression of NFκB in proximal tubules (Table 2, Fig. 4), which was subsequently verified by EMSA (Fig. 4). Moreover, expression of the LOX-1 receptor was increased in HC renal endothelial cells, and

Table 2. Tissue staining (% of renal area), in normal, hypercholesterolemic (HC), and HC+ET-A kidneys

	Normal ($N = 6$)	HC ($N = 6$)	HC+ET-A ($N = 6$)
Trichrome	3.2 ± 0.06	5.8 ± 0.6[a]	3.8 ± 0.2[a,b]
Media-lumen ratio	0.2 ± 0.01	0.27 ± 0.02[a]	0.17 ± 0.01[a,b]
Transforming growth factor-β	1.0 ± 0.06	2.3 ± 0.2[a]	1.2 ± 0.07[a,b]
Endothelial NOS	7.6 ± 0.3	4.9 ± 0.4[a]	8.1 ± 0.3[b]
Inducible NOS	3.4 ± 0.4	8.9 ± 0.6[a]	5.1 ± 0.3[a,b]
Nitrotyrosine	7.4 ± 0.3	8.9 ± 0.1[a]	6.5 ± 0.3[a,b]
Nuclear factor-κB	0.3 ± 0.02	1.9 ± 0.3[a]	0.7 ± 0.04[a,b]
LOX-1	2.2 ± 0.2	3.2 ± 0.2[a]	2.7 ± 0.2[c]

Abbreviations are: ET-A, endothelin receptor A; NOS, nitric oxide synthase; LOX, lectin-like oxidized LDL receptor. Data are mean ± SEM.
[a] $P < 0.05$ vs. normal
[b] $P < 0.05$ vs. HC
[c] $P = 0.06$ vs. normal and HC

decreased in HC+ET-A (Table 2, Fig. 3B), suggesting that ET-A blockade decreased the potential for ox-LDL uptake in renal tissue.

DISCUSSION

This study demonstrates that endothelin A-receptor blockade in HC elevates GFR and improves renal hemodynamic and functional responses to an endothelium-dependent vasodilator, implying involvement of the endothelin system in the blunted renal responses observed in HC. These may be achieved by correction of the vasoconstrictor/vasodilator imbalance, which characterizes HC and atherosclerosis. Furthermore, these beneficial effects were accompanied by decreased oxidative stress, increased eNOS, and decreased endothelial LOX-1 expression, decreased tubular iNOS, nitrotyrosine, NFκB, and TGF-β immunoreactivity, and decreased perivascular and tubulointerstitial trichrome staining. Thus, blockade of the endogenous endothelin system in HC decreased vascu-

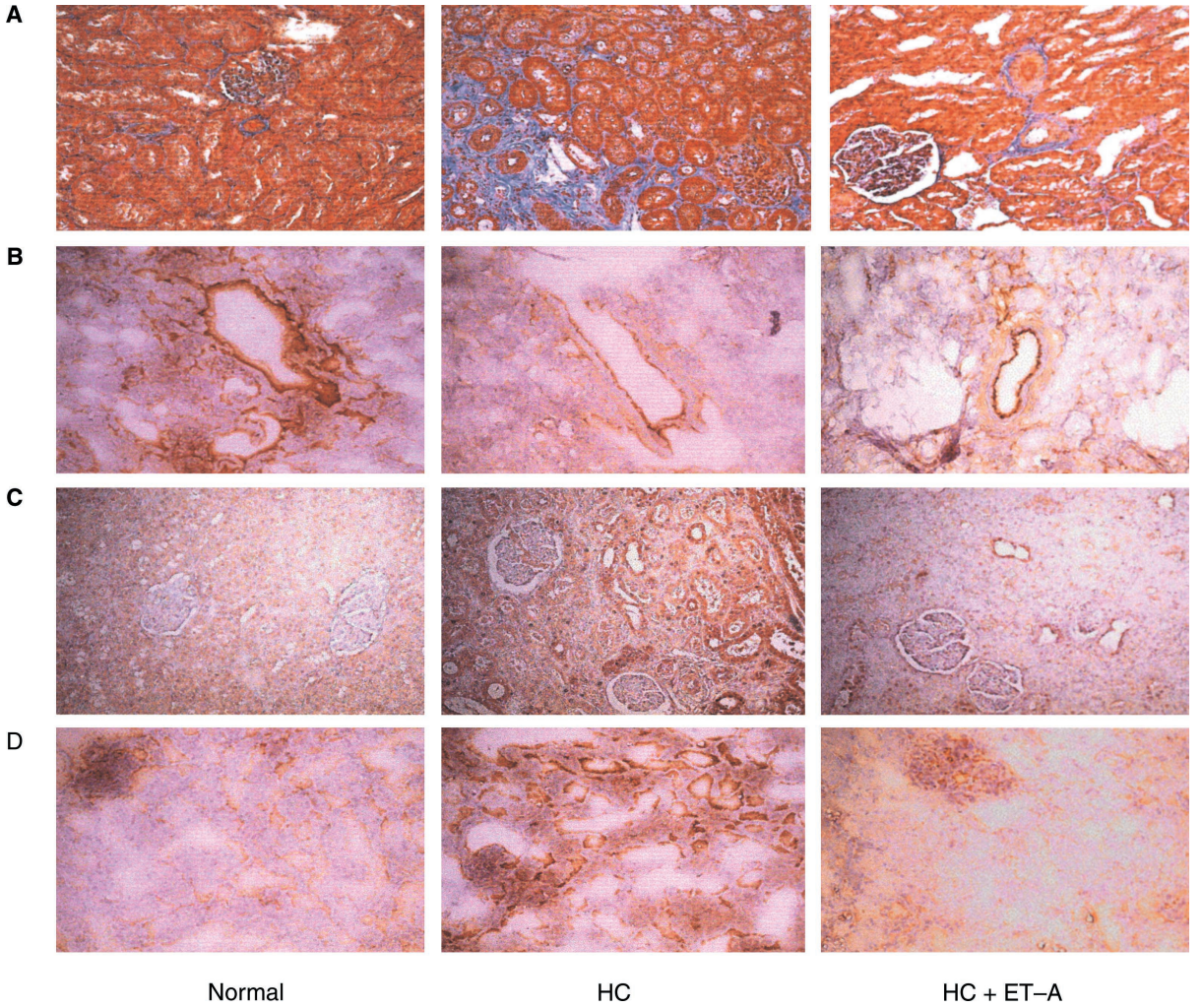

Fig. 2. Representative renal staining for (*A*) trichrome, (*B*) eNOS, (*C*) iNOS, and (*D*) nitrotyrosine in normal, hypercholesterolemic (HC), and HC + ET-A groups. These show decreased perivascular and tubulointerstitial fibrosis and suggest increased availability of NO and decreased inflammation in HC+ET-A. (Magnification, ×20).

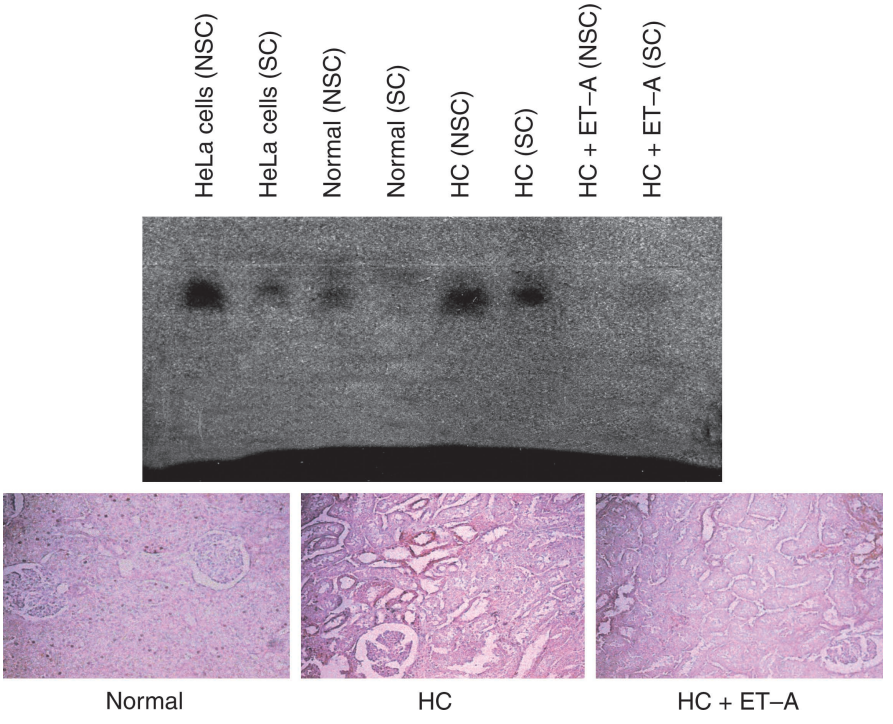

Fig. 4. Representative renal immunohistochemistry and electrophoretic mobility shift assay (EMSA) that shows increased activation of NFκB in hypercholesterolemic (HC) pigs, which was substantially attenuated in HC + ET-A. NSC, nonspecific competitor; SC, specific competitor. Magnification, ×20.

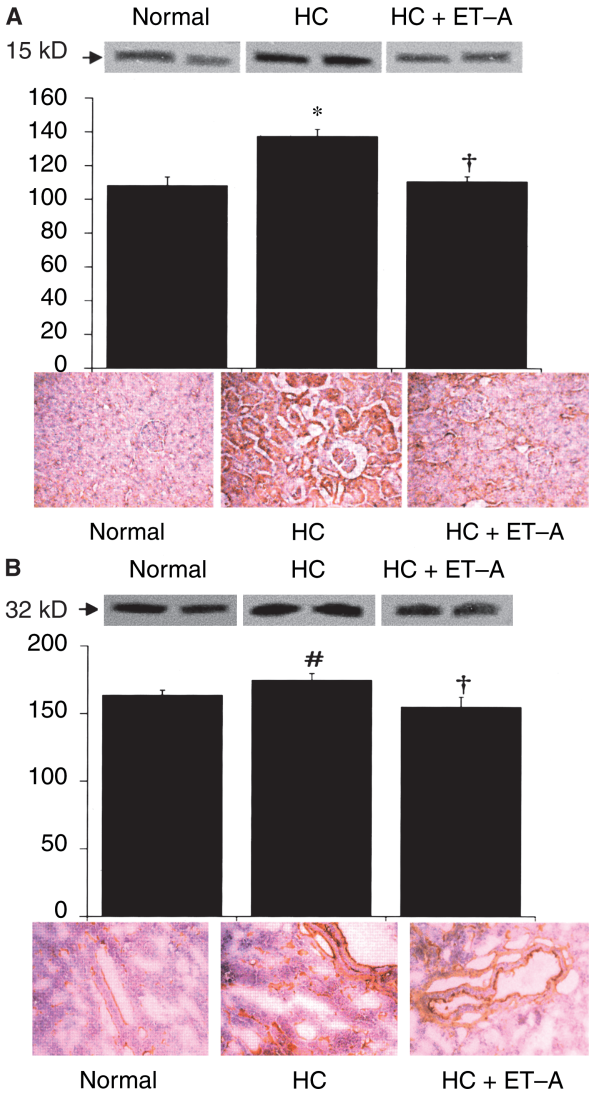

Fig. 3. Representative renal immunoblots and immunohistochemistry demonstrating protein expression of (*A*) the profibrotic transforming growth factor (TGF)-β and (*B*) the ox-LDL receptor LOX-1 in normal, hypercholesterolemic (HC), and HC+ET-A groups. *$P < 0.05$ vs. normal; †$P < 0.05$ vs. HC; #$P = 0.06$ vs. normal. Magnification, ×20.

lar, glomerular, and tubulointerstitial renal inflammation and fibrosis. This study supports a role for the endogenous endothelin system in the mechanism of renal injury in early atherosclerosis.

Experimental evidence suggests that lipid abnormalities are associated with a decline in renal function and participate in the progression of renal disease [30]. Accumulating evidence has shown increased activity of the endogenous ET system in both human and experimental HC and atherosclerosis [5, 31, 32]. ET-1 is a potent vasoconstrictor, which exerts its biological effect through activation of specific ET-A and -B receptors [3]. ET-A receptors mediate vasoconstriction and cell proliferation, whereas ET-B receptors are involved in ET-1 clearance, inhibition of endothelial apoptosis, release of NO and prostacyclin, inhibition of endothelin converting-enzymes expression [32, 33], and coronary vasoconstriction [34]. Recent studies showed favorable vascular effect of selective ET-A receptor antagonists, both in vivo and in vitro, in experimental models of HC and atherosclerosis [4, 22], suggesting that activation of the local ET system contributes to endothelial dysfunction and vascular remodeling, mainly through ET-A receptors. Notably, the renal microcirculation is particularly susceptible to ET-1, and its ET-A receptor induces NFκB expression and inflammation [35], likely mediates some of the vascular effects of angiotensin II, and regulates renal vascular resistance [1, 36, 37]. On the other hand, the renal ET-B receptor participates in regulation of fluid excretion in the distal nephron [38] and inhibits sodium reabsorption in a NO-dependent fashion [39]. Indeed, previous studies have shown that selective chronic ET-A blockade decreases renal injury and fibrosis [40], and in ischemic acute renal failure provides greater long-term functional and morphologic benefits than blockade of both ET-A and ET-B [39, 41]. However, the potential role of endothelin in instigation of renal injury in HC, and the potential benefits of ET-A blockade on renal hemodynamics and function, has not been tested.

Our study showed that blockade of the ET-A receptor led to a significant increase in basal GFR in HC+ET-A compared to HC. This effect may be due to reduction in afferent vasoconstriction, or reduced constriction of mesangial cells and a consequent increase in ultrafiltration coefficient [42]. Furthermore, Ach-stimulated RBF, GFR, and renal-regional perfusion were largely restored. This improvement in renal function may also result from increased bioavailability of endogenous NO [43], consequent to the augmented expression of eNOS and/or decrease in oxidative stress-mediated degradation of NO in HC+ET-A. Recent studies have shown that ET directly stimulates O_2^- production [15]. O_2^- may act as an intracellular signal linked to growth related responses, and also avidly interacts with NO [44], leading to vasoconstriction and formation of peroxynitrite, which may further contribute to cellular injury [45]. In support of this notion, plasma levels of isoprostanes (in vivo markers of oxidative stress) [24], as well as peroxynitrite formation (reflecting the presence of O_2^- were decreased in the HC+ET-A group, suggesting that ET-A blockade attenuates both systemic and tissue oxidative stress in HC. Furthermore, ET-A blockade may restore the vasoconstriction/vasodilation balance, which is disrupted in HC and impairs the regulation of vascular tone [46].

In addition, we have previously shown that HC was associated with decreased proximal reabsorption in response to Ach, possibly due to tubular inflammatory changes and iNOS-derived NO [8–10]. In the current study, the decrease in basal proximal fluid reabsorption observed in HC+ET-A may have been a result of blocking

the effects of AII and/or ET in the proximal tubule [47], similar to our observation using an angiotensin II type 1 (AT1) receptor blocker [7]. However, the proximal tubular response to Ach in HC+ET-A was similar to that observed in normal animals, conceivably due to attenuated proximal tubular expression of iNOS. On the other hand, similar to HC, the decrease in fluid reabsorption in the distal nephron in response to Ach remained enhanced in HC+ET-A, and might have resulted from increased activation of the ET-B receptor in the collecting duct [48] in the face of ET-A blockade.

In addition to its functional effects, ET-1 is also involved in cell proliferation, inflammation [35], and growth factor expression that may lead to renal fibrosis [49]. We observed in HC increased expression of iNOS (in the proximal and distal tubules) and NFκB (in the glomeruli and proximal tubule), which are involved in renal inflammation [8]. Furthermore, this was accompanied by increased expression in renal arterioles of LOX-1, the specific receptor that facilitates the uptake of the cytotoxic ox-LDL, which may also contribute to renal injury. Indeed, a previous study has shown that ET-1 increases the expression of LOX-1 in human endothelial cells in vitro [16]. Our study demonstrates, for the first time, that ET-A blockade in diet-induced HC attenuates not only renal iNOS and NFκB, but also LOX-1 expression in vivo, which might serve as a mechanism for renal protection. Moreover, this was associated with markedly decreased tubular and glomerular expression of TGF-β and attenuated perivascular and tubulointerstitial fibrosis, suggesting an overall decrease in renal injury in HC+ET-A.

CONCLUSION

The current study demonstrates, for the first time, that ET-A blockade augments GFR and preserves renal hemodynamic responses in the intact kidney of pigs with diet-induced HC associated with decreased oxidative stress, inflammation, and fibrosis in renal tissue. Further studies will be needed to evaluate the potential of ET-blockade to reverse pre-established renal injury in longer duration of HC and the effect of ET-A blockade on the normal kidney. Our findings suggest involvement of the endothelin system in the mechanism of renal injury in HC and atherosclerosis and support a role for these agents in renal protection.

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