ANTIOXIDANTS & REDOX SIGNALING
Volume 7, Numbers 1 & 2, 2005

Forum Review

Isoprostanes and the Kidney

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ABSTRACT

Isoprostanes are not mere bystanders of oxidative injury, but possess potent biological activity and may thus contribute to the pathophysiology of various disorders associated with an increase in free radical formation. 15-F_{2t}-IsoP (8-iso-prostaglandin $F_{2\alpha}$) and 15-E_{2t}-IsoP (8-iso-prostaglandin E_2), two of the most abundant isoprostanes, are potent vasoconstrictors in various vascular beds, including the kidney. Since their discovery, numerous studies have aimed to define the receptors through which isoprostanes exert their effects. Whether the thromboxane receptor and/or other prostaglandin receptors mediate the actions of isoprostanes, or whether these compounds interact with their own unique receptors, remains to be clarified. Regardless of their exact mode of action, isoprostanes are being implicated in the pathophysiology of a variety of diseases, and their discovery might give rise to novel therapies for these diseases. Here we describe early studies that defined the vasoactive properties of isoprostanes in the kidney, and subsequent discoveries relating to their renal actions and pathophysiologic significance. *Antioxid. Redox Signal.* 7, 236–243.

EARLY STUDIES: RENAL VASOCONSTRICTOR ACTIONS OF 15-F_{2T}-ISOP

THE ISOPROSTANE 15-F_{2t}-IsoP is one of the most studied isoprostanes because of its natural abundance and early availability to investigators (6). It was discovered to act as a renal vasoconstrictor that selectively increases preglomerular vascular resistance, resulting in reduced transcapillary hydraulic pressure difference and leading to a dose-dependent reduction in single nephron plasma flow, glomerular filtration rate (GFR), and renal blood flow (RBF) (22). 15-F_{2t}-IsoP is a potent vasoconstrictor in low nanomolar concentrations, *i.e.*, one order of magnitude more potent than leukotriene D_4, the most potent renal vasoconstricting eicosanoid known (3). When infused in peripheral veins of rats at a dose of 5 μg/kg/min, 15-F_{2t}-IsoP resulted in 40.5 ± 0.9% reduction in RBF and 45.3 ± 0.9% reduction in GFR. These effects were not accompanied by an increase in systemic blood pressure, indicating a selective effect on renal vasculature at this dose (34 n*M*). Intrarenal arterial infusion at 0.5, 1, and 2 μg/kg/min also caused a parallel reduction in RBF and GFR in a dose-dependent manner. At the highest dose, 2 μg/kg/min, GFR and RBR fell to zero within 2 min of initiation of infusion (Fig. 1). Studies on 15-E_{2t}-IsoP have shown similar results to 15-F_{2t}-IsoP (25), although their related cyclooxygenase-derived prostaglandins (*i.e.*, PGE_2 and $PGF_{2\alpha}$) exert opposing (vasoconstrictor and vasodilator, respectively) effects on vascular smooth muscles.

DISCOVERY OF INTERACTIONS WITH THE THROMBOXANE A2 (TXA2) RECEPTOR *IN VIVO*

Experiments in the 1990s have shown that the vasoconstrictor effects of 15-F_{2t}-IsoP and 15-E_{2t}-IsoP can be abolished in rats by SQ29548, a TXA_2 receptor (TP receptor) antagonist (43). The TP receptor, a G protein-coupled transmembrane eicosanoid receptor, is the product of a single gene (1) but two alternative splice variants, and is named the platelet/placental (TP-P or TP-a) and endothelial (TP-E or TP-b) type receptors (31). Alternative splicing occurs selectively at the carboxyl terminus and confers association with different

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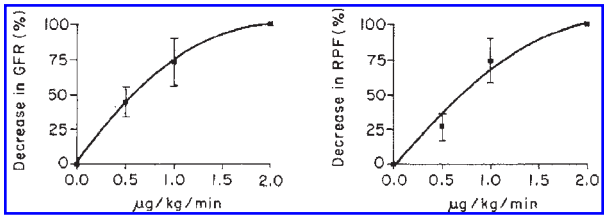

FIG. 1. Percent reduction in GFR and RBF during intrarenal infusion of 15-F_{2t}-IsoP in rats. Data are expressed as the means (n = 2) for the 0 and 2 μg/kg/min doses and the means ± SD (n = 4) for the 0.5 and 1.0 μg/kg/min doses.

G proteins, supporting the experimental finding that these receptors couple to both common and unique signaling pathways (12). TP-related signal transduction is consistently associated with calcium mobilization, inositol phospholipid turnover, and activation of protein kinase C (PKC) (28).

When the effect of isoprostanes on rat and human platelets was examined however, it was found that 15-F_{2t}-IsoP acts primarily as an antagonist at the TXA_2 receptor on platelets (23). Although it induces platelet shape change in humans and rats, it fails to produce irreversible aggregation and, at high doses, results only in weak reversible aggregation. These effects are inhibited by TXA_2 antagonists. Most importantly, 15-F_{2t}-IsoP prevented the irreversible platelet aggregation induced by TXA_2 receptor agonists and by arachidonic acid (23). The differential agonist/antagonist activity of 15-F_{2t}-IsoP on platelets and vascular smooth muscle cells could be explained by the presence of different TP receptor isoforms on these tissues. An alternative explanation is the presence of a unique isoprostane receptor on vascular smooth muscle cells, but not on platelets. Such a receptor likely bears structural similarities to the TP receptor, allowing TP receptor antagonists to bind and reverse the biological actions of isoprostanes.

Experiments on cultured rat aortic smooth muscle cells and human smooth muscle cells also suggested that 15-F_{2t}-IsoP has its own distinct receptor from TXA_2 (7). In rat aortic smooth muscle cells, 15-F_{2t}-IsoP was able to displace with significantly less potency TXA_2 agonists and antagonists from their specific binding sites. This differential affinity was especially marked in human cells successfully transfected with TXA_2 receptor cDNA; the COS-7 cells that had only the TXA_2 receptor were totally insensitive to the isoprostanes (Fig. 2). In addition, 15-F_{2t}-IsoP stimulated inositol 1,4,5-trisphosphate and DNA synthesis in cultured rat aortic smooth muscle cells with significantly greater potency than any TXA_2 agonist, and these effects were only partially inhibited by TXA_2 receptor antagonists (Fig. 3).

Further studies on platelets showed that the 15-F_{2t}-IsoP-induced platelet shape change is mediated via increases in inositol phosphate and intracellular calcium (33). In addition, and via secondary production of thromboxane, isoprostanes facilitate dose-dependent irreversible platelet aggregation in the presence of subthreshold concentrations of ADP (49), collagen, arachidonic acid, and analogues of TXA_2/PGH_2 (33). These findings are in contradistinction to the inhibitory effect of isoprostane on platelet aggregation described by Morrow *et al.* whereby isoprostanes appear to act as receptor antagonists to TXA_2 analogues (23). Binding studies on platelets supported the results obtained in similar studies on vascular smooth muscle cells (7) and favored the notion of a distinct isoprostane receptor on platelets as well. Of significance, 15-F_{2t}-IsoP is apparently, in part, enzymatically generated via cyclooxygenase in platelets (32), providing further evidence for its biological relevance.

An additional argument supporting the theory of an isoprostane receptor with a distinct biological profile from the thromboxane receptor with regard to specificity, cellular distribution, and binding characteristics came from further radioligand binding kinetics and functional studies, as well as molecular transfection studies (8). It was found that mesangial cells in rat glomeruli express the TXA_2 receptor, but not the isoprostane receptor, because they respond to TXA_2 agonists (and not isoprostanes) by contraction and increased inositol 1,4,5-trisphosphate production. These effects are abolished by a TXA_2 antagonist. In contrast, both isoprostane and TP receptors may be found on rat aortic smooth muscle cells, as these cells have both high- and low-affinity binding sites for isoprostanes (Fig. 4). At doses lower than those needed to activate the TP receptor, isoprostanes mediate activation of

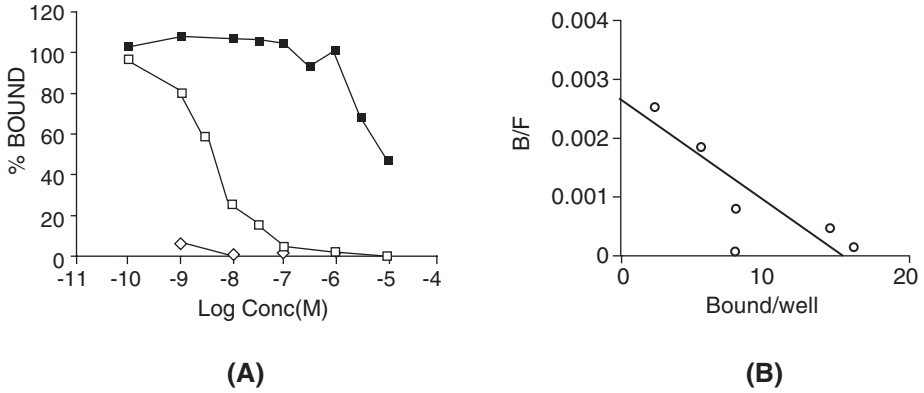

FIG. 2. Competitive binding assay of 5 n*M* [^{3}H]SQ29548 as a ligand in TXA_2 receptor cDNA-transfected COS-7 cells. (**A**) Displacement curve. (**B**) Scatchard analysis. Each point is expressed as the mean of three or four determinations. □, SQ29548; ■, 15-F_{2t}-IsoP in TXA_2 receptor cDNA-transfected cells; ◇, SQ29548 in vector-transfected cells.

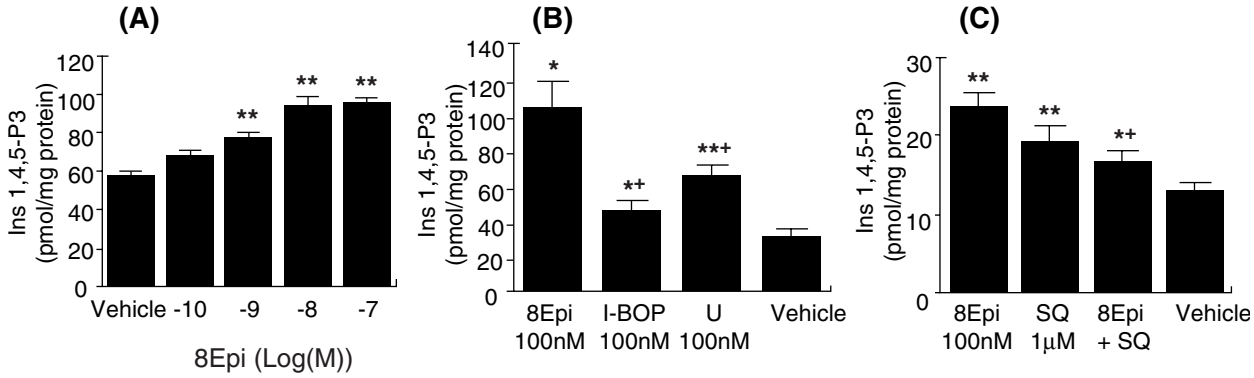

FIG. 3. Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] production at 10 s by 15-F_{2t}-IsoP (8-epi-$PGF_{2\alpha}$) in aortic smooth muscle cells. (**A**) Dose dependency. (**B**) Comparison of potencies of 15-F_{2t}-IsoP (8Epi), I-BOP, and U46619 (U) at 10^{-7} *M*. (**C**) Effect of 1 μ*M* SQ29548 (SQ) on Ins(1,4,5)P_3 by 10^{-7} *M* 15-F_{2t}-IsoP. Each value is expressed as the mean ± SE of three or four determinations. $^{**}p < 0.01$ vs. vehicle; $^{*}p < 0.05$ vs. vehicle; $^{+}p < 0.05$ vs. 15-F_{2t}-IsoP.

mitogen-activated protein kinase in a dose- and time-dependent manner, probably via the putative isoprostane receptor. Again, cells transfected with TP receptor cDNA failed to respond to physiologic doses of isoprostanes. At much higher concentrations, partial responses to F_2-isoprostanes are observed, likely through cross-activation of TXA_2 receptors (8).

The hypothesis of specific isoprostane receptors was challenged as studies on transgenic mice confirmed that cardiovascular responses to 15-F_{2t}-IsoP and 15-E_{2t}-IsoP are mediated via the TP receptor *in vivo*. Transgenic mice that overexpressed TP-b in the vasculature, but not in platelets, exhibited an exaggerated pressor response to infused 15-F_{2t}-IsoP compared with wild-type mice. This was blocked by TP antagonism. By contrast, both the pressor response to infused 15-F_{2t}-IsoP and its effects on platelet function were abolished in mice lacking the TP gene (2). The same holds true for 15-E_{2t}-IsoP except that 15-E_{2t}-IsoP is a more potent TP agonist, causing both irreversible aggregation in platelets and a stronger pressor response (2). In an effort to explain the differential binding affinities of isoprostanes and thromboxane agonists to the TP receptor previously described in many studies, it was speculated that isoprostanes and prostaglandins may activate both overlapping and distinct downstream signaling pathways linked to the TXA_2 receptor, possibly via different receptor–G protein interactions. Posttranscriptional modifications of the TXA_2 receptor for its intracellular target-

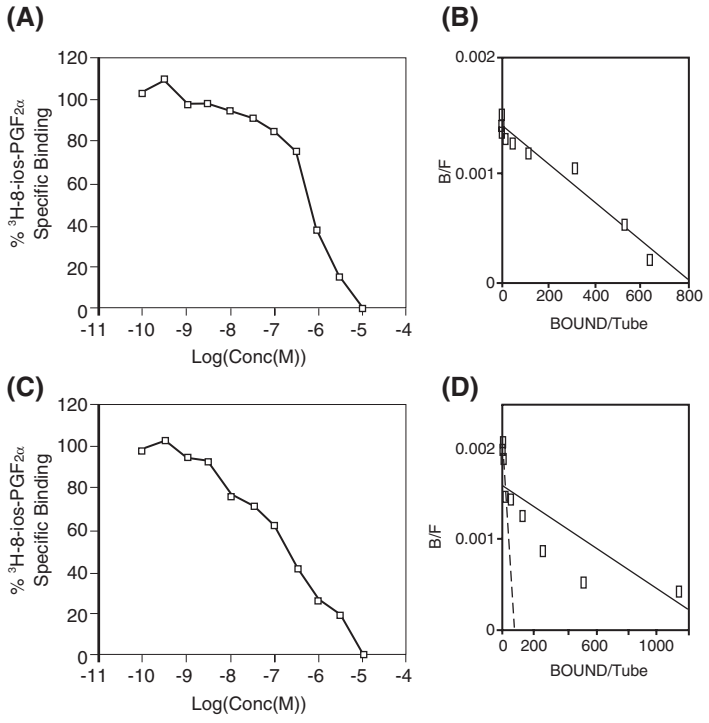

FIG. 4. Competitive binding assay using [^{3}H]8-iso-$PGF_{2\alpha}$ (15-F_{2t}-IsoP) (2.5 n*M*) as hot ligand in membrane fractions of rat mesangial cells and aortic smooth muscle cells. (**A**) Competitive binding curve in mesangial cell membrane fractions. (**B**) Scatchard analysis of A. (**C**) Competitive binding curve in aortic smooth muscle cell membrane fractions. (**D**) Scatchard analysis of C. ($n = 4$.)

ing may also explain the differences observed in binding and physiologic activities of isoprostanes and TXA_2, both of which would be abolished in TXA_2 receptor knockout mice.

A recent study using the transcription factor activator protein-1 (AP-1) luciferase activity as a reporter for TXA_2 receptor activation (48) has demonstrated that 15-E_{2t}-IsoP is an agonist at both TP-P and TP-E isoforms, when expressed in transfected CHO cells (47). The dose-response curve for the regulation of AP-1 by 15-E_{2t}-IsoP was comparable for both TP isoforms. In contrast, 15-F_{2t}-IsoP is not an agonist on these cells, findings consistent with the previous evidence from similar studies in transfected COS cells (7). Interestingly, treatment of naive CHO cells with 15-E_{2t}-IsoP (but not 15-F_{2t}-IsoP or TP agonists) was associated with an increase in AP-1 activity, which was not inhibited by TP antagonists. This was observed at concentrations of 15-E_{2t}-IsoP in the low nanomolar range, much lower than that required to activate AP-1 via TP, again suggesting that isoprostanes bind with high affinity to another receptor that leads to activation of AP-1 (47).

Many prostanoid receptors have since been implicated as candidate isoprostane receptors, including inositol phospholipid-coupled TP/EP_3 receptors (16), as well as the cyclic AMP-coupled EP_2 receptors (45). In addition, in some systems, isoprostanes activate pathways that release secondary mediators. For instance, 15-F_{2t}-IsoP was found to induce *de novo* synthesis of TXA_2 (13). Furthermore, 15-F_{2t}-IsoP itself is metabolized to biologically active products that up-regulate *de novo* TXA_2 synthesis via the same receptor as the parent compound (14). Therefore, one possible explanation for the differential actions of isoprostanes on cells is that each cell interacts with each isoprostane in a unique way that results from the combined effects of a variety of receptors and second messengers. The debate over the existence of a discrete isoprostane receptor may not be settled unless a unique isoprostane receptor can be positively identified and cloned. It also important to keep in mind that isoprostanes are initially formed esterified to membrane phospholipids, and their effect on cellular function is partly mediated by the alteration of membrane properties.

ISOPROSTANES IN RENAL INJURY MODELS AND HYPERTENSION

Diabetic nephropathy

The finding that oxidative stress plays an important role in tissue damage associated with diabetes (9, 50) has aroused interest in the role of isoprostanes in the development of diabetic nephropathy, the leading cause of renal failure in the industrialized world. As expected, diabetic rats had higher plasma levels and urinary excretion rates of F_2-isoprostanes. Dietary supplementation of vitamin E normalized plasma levels, decreased urine levels of F_2-isoprostanes, and concurrently decreased proteinuria and blood urea nitrogen levels (Figs. 5 and 6). Studies on cell culture in ambient high glucose revealed that isoprostanes are synthesized by glomerular endothelial and mesangial cells, independently of cyclooxygenase activity (20). It had also been shown earlier that high glucose induces the production of isoprostanes from smooth muscle cells (27). When added to mesangial cells in culture, isoprostanes up-regulated the synthesis of transforming growth factor-β (TGF-β), the transcription factor implicated in mediating the hypertrophic and prosclerotic effects of high glucose on glomerular mesangial cells in an autocrine fashion (41). The mechanism by which isoprostanes increase TGF-β in mesangial cells may be partly mediated via PKC activation, as PKC is known to be up-regulated by isoprostanes (46) and to increase TGF-β expression (17).

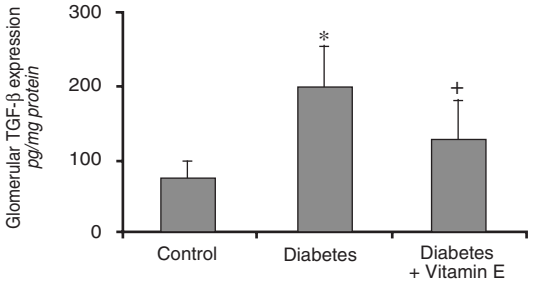

FIG. 5. Expression of TGF-β1 in total protein extracted from glomeruli isolated from control rats, diabetic rats, and diabetic rats treated after diet supplementation with vitamin E (1,000 U/kg diet) 4 weeks after the onset of diabetes. *$p < 0.05$ vs. control; $^+p < 0.05$ vs. diabetes. ($n = 5$.)

Isoprostanes may also participate in the pathogenesis of diabetic nephropathy via endothelin-1 up-regulation. The latter has been implicated in the development of diabetic nephropathy by virtue of the facts that endothelin-1 increases in diabetes (21) and renal function parameters improve upon administration of endothelin receptor antagonists (26).

Hypertension

15-F_{2t}-IsoP has also been found to increase in various forms of hypertension associated with increase in oxidative stress, including renovascular hypertension (18), pregnancy-induced hypertension (10), and obesity-associated hypertension (5). Endothelin has also been reported to increase in all

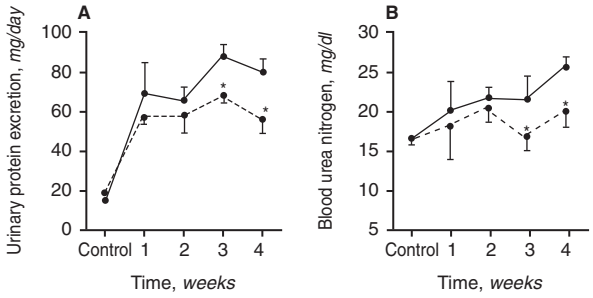

FIG. 6. Daily urinary protein excretion (A) and blood urea nitrogen levels (B) in diabetic rats (solid curve) and diabetic rats treated with vitamin E (dashed curve) 4 weeks after the onset of diabetes. *$p < 0.05$ vs. diabetes. Time zero values are from control nondiabetic rats.

the aforementioned hypertensive conditions (40). Endothelin-1 is a potent vasoconstrictor of vascular smooth muscle cells *in vivo* and *in vitro*; in the kidney, it decreases both renal plasma flow and GFR via vasoconstriction of both the afferent and efferent glomerular arterioles (4). 15-F_{2t}-IsoP has been shown to induce endothelin-1 release from bovine aortic endothelial cells, effects partly abolished by TXA_2 antagonists (51). A recent study by Sedeek *et al.* has demonstrated that chronic endothelin-1-induced hypertension in rats is associated with decreases in renal plasma flow and increases in renal vascular resistance, as well as increases in urinary 15-F_{2t}-IsoP excretion. All these responses are abolished by the addition of the antioxidant tempol, a superoxide dismutase mimetic, indicating that formation of reactive oxygen species may play an important role in mediating the changes in renal function and the hypertension induced by chronic elevation in endothelin (40).

Plasma levels of isoprostanes are also increased in spontaneously hypertensive rats and during angiotensin II-induced hypertension. Similarly, tempol administration decreases renal isoprostane excretion and lowers blood pressure in the spontaneously hypertensive rat (39). Isoprostanes have also been demonstrated to potentiate the vascular vasoconstrictor responses to angiotensin II and norepinephrine (38).

Chronic infusion of subpressor doses of angiotensin II has been shown to result in hypertension. The increase in blood pressure has been associated with an increase in oxidative stress (isoprostanes) and endothelin, and both effects were prevented or blunted by the administration of antioxidants (35). It was postulated that the angiotensin II-induced increase in endothelin is mediated by isoprostanes (29).

End-stage renal disease

Isoprostanes were also found to be elevated in end-stage renal disease, another condition associated with increased oxidative stress. Both uremia and dialysis favor the formation of reactive oxygen species (11) and may explain the high incidence of cardiovascular events in this population. In particular, patients on hemodialysis had significantly greater elevations in plasma levels of isoprostanes as compared with patients on continuous ambulatory peritoneal dialysis. As the majority of isoprostanes in blood are esterified and metabolized by the liver, the increase in isoprostanes could not be accounted for by a decrease in clearance (19).

Hepatorenal syndrome

The hepatorenal syndrome, a common complication in patients with advanced liver disease, is renal failure resulting from hypoperfusion secondary to both splanchnic vasodilation and intense constriction of the renal cortical vasculature. Given their powerful renal effect, isoprostanes are suspected of mediating the vasoconstriction seen in hepatorenal syndrome (24).

Acute tubular necrosis

In ischemic acute tubular necrosis, especially during the reperfusion period, urinary excretion rate of these eicosanoids is known to increase by >300% (30). Lazaroid, a 21-aminosteroid lipid-peroxidation inhibitor, was found to inhibit the F_2-isoprostane increase that is observed when hydrogen peroxide is added to cultures of renal proximal tubular cells, as well as to prevent the hydrogen peroxide-mediated cytolysis (36).

Kidney transplant/storage

Cold storage of rat kidney or renal tubular cells causes a time-dependent increase in the formation of isoprostanes, which may explain the immediate posttransplant renal vasoconstriction and dysfunction that occurs when the kidney has been subjected to an extended cold storage. Addition of desferrioxamine or the lazaroid compound 2-methylaminochroman to the preserving solution nearly prevents the increase of isoprostanes at 48 h (37). Rats transplanted with cold-stored kidneys treated with desferrioxamine, compared with untreated kidneys, had significantly greater renal function and less apoptotic and necrotic tubular injuries. Similarly, recipient rats of these kidneys had significantly greater GFR and RBF and less isoprostane excretion (15).

Aging

Isoprostanes also play a role in mediating renal damage associated with aging. The increase in oxidative stress associated with aging has been demonstrated by a threefold increase in renal F_2-isoprostanes along with an increase in oxidant-sensitive heme oxygenase and advanced glycosylation end products and their receptor. The decline in renal function that resulted from aging alone in rats (60% decrease of GFR) was decreased by up to 50% by the administration of a high-vitamin E diet. This improvement was associated with suppressed isoprostane levels and attenuated expression of heme oxygenase (34).

Glomerulonephritis

Enzymatic and nonenzymatic products of arachidonate metabolism play a crucial role in the pathogenesis and functional sequelae of glomerulonephritis. This was elegantly illustrated by Takahashi *et al.* when essential fatty acid deficiency was induced in weanling rats that were then subjected to antiglomerular basement membrane antibody-induced injury in adulthood (44). Glomerular dynamics and structure were essentially preserved as opposed to standard diet-fed controls despite the occurrence of proteinuria. The initial proteinuria was most likely secondary to neutrophil infiltration, a process largely complement-dependent; it later resolved as the glomeruli failed to develop an influx of macrophages and the subsequent histopathology. The mechanism by which essential fatty acid deficiency interferes with macrophage elicitation remains to be clarified, but it is speculated to involve the impairment of macrophage chemotactic responsiveness or the inhibition of the local generation of an arachidonate-related macrophage chemoattractant (44).

Of interest was the finding that this model exhibited normal glomerular hemodynamics, whereas cyclooxygenase inhibition alone during the autologous phase of glomerulonephritis caused a dramatic rise in the afferent arteriolar resistance. This suggests the presence of an arachidonate-

derived product in a cyclooxygenase-independent manner that increases selectively the preglomerular resistance and whose effect is normally masked by other cyclooxygenase-derived vasodilators like PGE_2 (42). As discussed above, 15-F_{2t}-isoP exerts a powerful constriction of preglomerular afferent arterioles and is therefore a likely candidate. Further studies are required to evaluate the role of isoprostanes in glomerulonephritis.

SUMMARY

A significant body of evidence supports the contention that isoprostanes play a role in certain forms of renal pathology. The initial discovery that 15-F_{2t}-IsoP is a potent vasoconstrictor in the renal vasculature led to the elucidation that this compound and 15-E_{2t}-IsoP exert their biological effects, at least in part, via interaction with the TXA_2 receptor. Whether distinct isoprostane receptors exist remains a matter of debate, although studies outlined herein have not completely ruled out this possibility. In addition to their biological effects, the quantitation of isoprostanes in certain renal disorders has provided significant support to the contention that a number of kidney diseases are associated with enhanced oxidant stress.

ABBREVIATIONS

AP-1, activator protein-1; GFR, glomerular filtration rate; IsoP, isoprostane; PG, prostaglandin; PKC, protein kinase C; RBF, renal blood flow; TGF-β, transforming growth factor-β; TP, thromboxane A_2-selective prostanoid receptor; TXA_2, thromboxane A_2.

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Received for publication April 30, 2004; accepted August 23, 2004.

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