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Angiotensin converting enzyme inhibitor but not angiotensin receptor blockade or statin ameliorates murine adriamycin nephropathy

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Angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and statins have renoprotective effects. We studied the cellular mechanisms for this effect in adriamycin-treated mice receiving captopril, losartan, simvastatin, or their combinations. The mice developed albuminuria, renal insufficiency, and parenchymal inflammation/fibrosis accompanied by overexpression of intrarenal converting enzyme and angiotensin II. Only captopril consistently improved these abnormalities and reduced the cortical expression of several proinflammatory and profibrotic factors including transforming growth factor-β (TGF-β). These effects were independent of blood pressure, accompanied by increased urine *N*-acetylseryl-aspartyl-lysyl-proline (Ac-SDKP) levels, and the restoration of renal angiotensin-converting enzyme and angiotensin II to baseline levels. Losartan or simvastatin alone or together had no effect, and their addition to captopril did not enhance protection. *In vitro*, angiotensin II stimulated TGF-β in renal tubular cells via mitogen-activated protein kinase (MAPK) signaling. Captopril or Ac-SDKP suppressed angiotensin II-induced MAPK activation and TGF-β secretion. Angiotensin-converting enzyme inhibition confers renoprotection in adriamycin nephropathy by reducing intrarenal angiotensin II and augmenting Ac-SDKP expression that together attenuate MAPK signaling and its downstream proinflammatory and fibrogenic properties. The addition of receptor blocker and/or statin failed to potentiate such effects.

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Emerging evidence from animal and *in vitro* studies over the last decade indicates that tubulointerstitial lesions induced by proteinuria in chronic kidney disease (CKD) may be mediated through tubular epithelial cell activation.[1–7] One constant feature of proteinuric nephritis is the inevitable emergence of tubulointerstitial inflammation. The current therapeutic armamentaria that attempt to lower glomerular protein hyperfiltration in chronic glomerulonephritides of various primary and secondary etiologies are more often disappointing than successful. Thus, an unfulfilled need exists for reducing the progression of renal failure in patients with CKD.

Angiotensin II (AngII), the key peptide of the renin–angiotensin system , is now considered a growth factor that regulates cell proliferation, apoptosis, and fibrosis in the kidney.[8,9] Antagonizing these effects of AngII has become a key component of therapeutic strategies to halt progression. Data from models of renal injury suggest that blockade of AngII by angiotensin-converting enzyme inhibitor (ACEi), or AngII receptor subtype 1 (ATR1) blocker not only induces intraglomerular hemodynamic changes that favor reduction of glomerular protein ultrafiltration, but also attenuates inflammatory cell infiltration into the kidney.[10,11] These biological effects translate clinically into reduced rates of renal function deterioration by ACEi and angiotensin receptor blockade (ARB) therapy in diabetic and non-diabetic renal diseases.[12–14] In addition, the lipid-lowering 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors (statins) also possess profound anti-inflammatory properties[15] and confer renoprotective effects independent of their lipid-lowering capacity in specific experimental nephropathies.[16,17] At the cellular level, statins may downregulate monocyte chemoattractant protein-1 (MCP-1) in human glomerular mesangial cells[18] and suppress macrophage infiltration in rat mesangial proliferative nephritis.[19]

In this study, we explored the renoprotective and anti-inflammatory potential of combined angiotensin blockade and statin treatment in a murine model of adriamycin (ADR)-induced nephropathy, an experimental analogue of focal glomerulosclerosis in humans.[20] In this model, mice demonstrate features characteristic of chronic progressive renal disease in humans.[21] Overt proteinuria occurs and

persists from day 5.[22] Accumulation of macrophages begins to appear within the interstitium and glomeruli at week 2, followed by interstitial T-cell infiltration. By week 4, there is significant mesangial expansion, increased interstitial volume and monocytic infiltration, and enhanced cortical expression of MCP-1[23] and transforming growth factor-β (TGF-β).[24] Apart from MCP-1 and TGF-β, we also examined the participation of placental growth factor (PLGF), a member of the vascular endothelial growth factor family, which is associated with pathologic angiogenesis such as in renal cell carcinoma.[25] Its role in glomerulonephritis remains unclear. Our preliminary data suggest that PLGF promotes renal fibrosis via its proinflammatory rather than angiogenic effects. Indeed, emerging evidence suggests that PLGF plays a critical role in inflammation through monocyte activation and expression of cytochemokines.[26] These properties make PLGF a likely candidate in disorders characterized by intrarenal inflammation, including ADR nephropathy. Our specific objectives were to investigate the *in vivo* impact of applying combined ACE inhibition/ATR1 blockade and statin treatment on ADR nephropathy, and to dissect the cellular mechanisms of disease and therapy in cell culture systems.

RESULTS

Physical and biochemical parameters

All animals grew well and were alive prior to killing with steady body weight. Injection with ADR caused significant elevation of systolic blood pressure (Figure 1), and marked albuminuria, hypoalbuminemia, and renal failure after 4 weeks (Figure 2). The various pharmacologic interventions reduced blood pressure to a similar extent. However, only captopril treatment consistently alleviated albuminuria and renal failure. Losartan or simvastatin, alone, combined, or in addition to captopril, did not produce any synergistic effect.

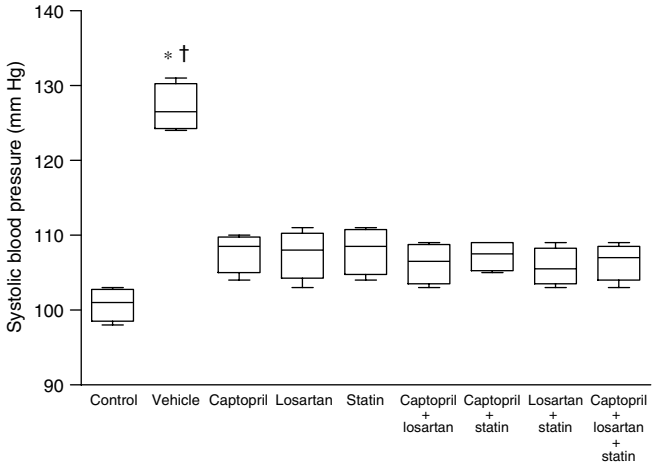

Figure 1 | Tailcuff systolic blood pressure at week 4 in control mice and mice with ADR nephrosis given different pharmacologic treatments; $N = 4$ in each group. *$P < 0.001$ vs control, †$P < 0.05$ vs all treatment groups. Box, line across, and whiskers indicate first and third quartiles, median, and range, respectively.

Use of simvastatin alone increased albuminuria numerically, albeit not significantly. Urinary levels of N-acetylseryl-aspartyl-lysyl-proline (Ac-SDKP) were markedly increased with captopril treatment (Figure 2d).

Morphological changes

Mice with ADR nephrosis exhibited severe lesions in both the glomerular and tubular compartments (Figure 3). There was also marked accumulation of interstitial α-smooth muscle actin (α-SMA) (Figure 4). These lesions were partially abrogated only by captopril. Losartan or statin alone or combined had no appreciable effects. Their addition to captopril did not reduce the extent of injury or fibrosis further.

Tubular cell proliferation and macrophage infiltration

Mice with ADR nephrosis exhibited marked increase in proliferating cell nuclear antigen (PCNA)-positive tubular cells ($P < 0.001$. vs control), which was abrogated only by protocols that contained captopril. Neither losartan nor simvastatin alone or combined enhanced the effect of captopril alone (Figure 5).

Macrophage infiltration into the interstitium was pronounced following ADR induction ($P < 0.001$ vs control). Treatment response was similar to that observed for tubular cell proliferation (Figure 6).

Expression of MCP-1, PLGF, TGF-β, and collagen I

Renal cortical western blots of MCP-1, PLGF, TGF-β, and collagen I were all markedly induced in ADR mice (Figure 7). Treatment with captopril alone significantly reduced the intensity of all four immunoblots. Losartan or simvastatin alone or combined did not reproduce such reduction. Their addition to captopril, either singly or together, did not further potentiate the suppressive effects observed with captopril alone.

Intrarenal ACE and AngII expression

Renal cortical ACE and AngII expression was elevated compared with controls (Figure 8). In response to captopril treatment, it was inhibited to levels comparable with baseline values. Losartan or simvastatin alone or combined did not inhibit ACE expression in the renal cortex.

Effect of AngII on the phenotype of PTECs

Acute exposure of proximal tubular epithelial cells (PTECs) to AngII with concentration above 10^{-9} M for 6 h upregulated the transcripts of TGF-β (Figure 9a). AngII had no effect on the mRNA expression of MCP-1 and PLGF in PTECs (data not shown). After 24 h of exposure, secretion of TGF-β protein, but not those of MCP-1 and PLGF, into culture supernatants was also stimulated with an induction threshold of 10^{-9} M (Figure 9b), which was progressively abrogated by concurrent treatment with captopril, but not losartan, in a dose-dependent manner (Figure 10). Similarly, simvastatin also had no effect on AngII-induced TGF-β release from PTECs (data not shown).