

Effect of Ruboxistaurin on Urinary Transforming Growth Factor- β in Patients With Diabetic Nephropathy and Type 2 Diabetes

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Excessive protein kinase C- β (PKC- β) activity has been implicated in the pathogenesis of diabetic nephropathy (1–5) such that its selective inhibition might be a useful strategy in treating patients with this complication.

Ruboxistaurin mesylate, a bisindolylmaleimide, is a specific and selective inhibitor of PKC- β isoforms that, in preclinical studies, attenuates overexpression of transforming growth factor- β (TGF- β) (6), a key mediator of the glomerulosclerosis and tubulointerstitial fibrosis characterizing diabetic nephropathy (7).

In contrast with albuminuria, thought to derive largely from plasma filtrate, urinary TGF- β mostly reflects its intrarenal production (8). In untreated patients with diabetic nephropathy, urinary TGF- β is increased (8), parallels the magnitude of proteinuria (9), correlates with glycemia (10), and decreases with

angiotensin receptor blocker (ARB) therapy (10). The effects of agents beyond those that block the renin-angiotensin system (RAS), such as PKC- β inhibition, on urinary TGF- β are unknown.

RESEARCH DESIGN AND METHODS

We obtained urine from participants in a prospective, double-blind, placebo-controlled study of the effects of ruboxistaurin 32 mg/day in patients with diabetic nephropathy (11), in which the effect on urinary TGF- β was a prespecified secondary objective. Patients were ≥ 30 years old with type 2 diabetes and urinary albumin-to-creatinine ratio (ACR) 200–2000 mg/g despite stable blockade of the RAS with ACE inhibitors and/or ARBs. Urine was collected before randomization (baseline) and at week 52 (end point). Samples were frozen, transported to a central laboratory (Covance, Indianapolis, IN), and stored at -70°C .

Paired baseline and end point sample sets with sufficient urine (>2.0 ml) were available from 107 of 123 (87%) participants.

Before assay, 2.0 ml from each urine collection were thawed, placed in a filter unit (Centricon-10 filter; Amicon, Watford, U.K.), and concentrated 40-fold by centrifugation for 60 min at 6,500 rpm (12). Urinary TGF- β 1 was assayed by solid-phase enzyme-linked immunosorbent assay (Quantikine; R&D Systems, Abingdon, U.K.) (10). Intra- and interassay coefficients of variation were 7.5 and 12.2%, respectively. Results were expressed relative to urinary creatinine concentration, measured by autoanalyzer.

Within-subject, baseline-to-end point changes in urinary TGF- β -to-creatinine ratio (TCR) were analyzed by ANOVA. ANCOVAs enabled adjustments for baseline TCR and ACR.

RESULTS— Among placebo-treated patients, urinary TCR increased by 43% from baseline to end point ($P < 0.01$). In comparison, ruboxistaurin-treated patients had a nonsignificant 19% increase in urinary TCR, less than half that observed for placebo (Fig. 1). Analyses adjusted for baseline urinary TCR and ACR yielded similar results (placebo: +37%, $P < 0.01$; ruboxistaurin: +24%, $P = \text{NS}$) (Fig. 1).

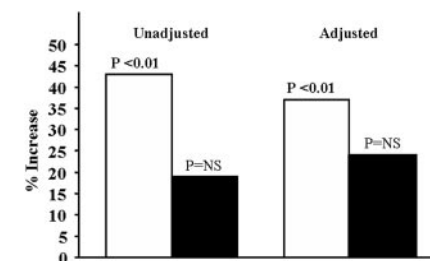


Figure 1—Percentages of increase from baseline in TCR after 1 year in patients with diabetic nephropathy treated with placebo (\square , $n = 56$) and ruboxistaurin 32 mg/day (\blacksquare , $n = 51$). Unadjusted analyses and analyses adjusted for urinary TCR and ACR at baseline are shown.

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Abbreviations: ACR, albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; PKC- β , protein kinase C- β ; RAS, renin-angiotensin system; TCR, TGF- β -to-creatinine ratio; TGF- β , transforming growth factor- β .

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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CONCLUSIONS— Ethical and practical issues mostly preclude detailed tissue analyses in humans with diabetic nephropathy. Accordingly, plasma creatinine and urinary protein excretion are used to predict prognosis and therapeutic response. Recently, several protein and cell markers reflecting disease pathogenesis have been suggested as indexes of disease progression (13). Because many renal diseases are characterized by fibrosis, urinary excretion of fibrogenic growth factors, such as TGF- β , has been of particular interest (9,10,14,15). Indeed, by stimulating fibrogenesis in epithelial cells, the tubular passage of TGF- β has also been implicated in the development of the tubulointerstitial fibrosis characterizing proteinuric renal diseases (14). This may be particularly important in the context of human diabetes, where the extent of tubulointerstitial disease is a close correlate of declining renal function (16,17) and therapeutic response (18).

Among placebo patients in the ruboxistaurin study (11), ACR remained stable with good blood pressure control and blockade of the RAS. However, despite these measures, estimated glomerular filtration rate still declined significantly (11), in association with a continued increase in TCR, as shown in the present report. In contrast, ruboxistaurin patients experienced neither a significant fall in estimated glomerular filtration rate nor a significant rise in urinary TCR over 1 year.

While RAS blockade is highly effective in reducing proteinuria, renal dysfunction continues to progress in the majority of patients (19). Since urinary TGF- β likely reflects intrarenal production of this profibrotic growth factor (8), the finding that TCR continued to rise in the placebo group of the present study, all of whom were receiving an ACE inhibitor or ARB with stable albuminuria, suggests that TCR may be a useful marker of continued renal fibrogenesis and consequent dysfunction. Indeed, while albuminuria is conventionally viewed as a marker of glomerular injury, the tubulointerstitium, given its large relative volume, appears to be the major source of TGF- β in the diabetic kidney (20). Accordingly, we speculate that the changes in TCR with ruboxistaurin in this study may reflect a relative reduction in tubulointerstitial TGF- β (and consequently fibrosis), as seen in preclinical studies of diabetic nephropathy (6).

The present study has several limitations. The small study numbers permitted

only within- rather than between-group analyses at end point. Furthermore, it is unclear whether the same effects on urinary TCR would be observed in a wider population of patients with type 2 diabetes and diabetic nephropathy, or in those with type 1 diabetes. Notwithstanding these limitations, we speculate that by reflecting the intrarenal production of this key fibrogenic growth factor, urinary excretion of TGF- β might serve as a useful biomarker of disease progression (and response to therapeutic intervention) in patients with diabetic nephropathy already treated with agents that block the RAS.

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