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

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1Department of Medicine, St. Michael’s Hospital, University of Toronto, Ontario, Canada; 2Department of Medicine, St. Vincent’s Hospital, University of Melbourne, Victoria, Australia; 3Providence Medical Research Center, Sacred Heart Medical Center, Spokane, WA; 4Department of Medicine, University of Chicago, Chicago, IL; 5Department of Medicine, The University of Texas Southwestern Medical Center, Dallas, TX; 6Department of Medicine, Washington University School of Medicine, St. Louis, MO; 7Lilly Research Laboratories, Indianapolis, IN

Ruboxistaurin and TGF-β

Correspondence:
Richard E. Gilbert, M.D., Ph.D.
St. Michael’s Hospital
Room 6-138, 61 Queen Street, Toronto, Ontario
Canada, M5C 2T2
gilbert@medstv.unimelb.edu.au

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Excessive protein kinase C (PKC) β activity has been implicated in the pathogenesis of diabetic nephropathy (DN) (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 DN (7).

In contrast to albuminuria, thought to derive largely from plasma filtrate, urinary TGF-β mostly reflects its intra-renal production (8). In untreated patients with DN, urinary TGF-β is increased (8), parallels the magnitude of proteinuria (9), correlates with glycaemia (10), and falls 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 ruboxistaurin 32 mg/day in DN (11), in which the effect on urinary TGF-β was a pre-specified secondary objective. Patients were ≥30 years old, with type 2 diabetes and urinary albumin: creatinine ratio (ACR) 200-2000 mg/g despite stable blockade of the RAS with angiotensin converting enzyme inhibitors (ACEIs) and/or ARBs. Urine was collected before randomization (baseline) and at week 52 (endpoint). Samples were frozen, transported to a central laboratory (Covance, Indianapolis, IN), and stored at -70°C. Paired baseline and endpoint sample sets with sufficient urine (>2 ml) were available from 107/123 (87%) participants.

Prior to assay, 2.0 ml from each urine collection was thawed, placed in a filter unit (Centricon-10 filter, Amicon, Watford, UK), and concentrated 40-fold, by centrifugation for 60 min at 6500 rpm (12). Urinary TGF-β1 was assayed by solid-phase ELISA (Quantikine, R&D Systems, Abingdon, UK) (10). Intra- and inter-assay 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-endpoint changes in urinary TGF-β:creatinine ratio (TCR) were analyzed by ANOVA. Analyses of covariance enabled adjustments for baseline TCR and ACR.

**RESULTS**

Among placebo-treated patients, urinary TCR increased by 43% from baseline to endpoint (P<0.01). In comparison, ruboxistaurin-treated patients had a non-significant 19% increase in urinary TCR, less than half that observed for placebo (Figure 1). Analyses adjusted for baseline urinary TCR and ACR yielded similar results (placebo: +37%, P<0.01; ruboxistaurin: +24%, P=NS) (Figure 1).

**CONCLUSIONS**

Ethical and practical issues mostly preclude detailed tissue analyses in humans with DN. 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 indices of disease progression (13). Since 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 human diabetic context, 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 (eGFR) 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 eGFR nor a significant rise in urinary TCR over one 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 intra-renal production of this pro-fibrotic growth factor (8), the finding that TCR continued to rise in the placebo group of the present study, all of whom were receiving an ACEI 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 DN (6).

The present study has several limitations. The small study numbers permitted only within- rather than between-group analyses at endpoint. 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 DN, or those with type 1 diabetes. Notwithstanding these limitations, we speculate that by reflecting the intra-renal 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 DN already treated with agents that block the RAS.

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References


Figure 1. Percent increase from baseline in TCR after one year in placebo (white bars; N=56) and ruboxistaurin 32 mg/day- (black bars; N=51) treated patients with DN and type 2 diabetes. Both unadjusted analyses and analyses adjusted for baseline urinary TCR and baseline ACR are shown.