RESISTANT STARCH AMELIORATES ADVANCED GLYCATION ENDPRODUCT-INDUCED ALBUMINURIA IN A MOUSE MODEL OF TYPE 2 DIABETES

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Aim: To investigate whether excess consumption of dietary advanced glycation endproducts (AGEs) cause gut dysbiosis, exacerbating renal injury in a type 2 diabetes model.

Background: Long-term excess intake of dietary AGEs contributes to chronic renal injury. Recent research implicates gut dysbiosis in the progression of diabetic nephropathy. However, the role of dietary AGEs in gut dysbiosis and renal dysfunction in the context of diabetes has not yet been explored.

Methods: Six week old diabetic (db/db) and non-diabetic (db/h) mice were randomized (n = 12/group) to receive a low AGE (LAGE, unbaked rodent chow) or a high AGE diet (HAGE, baked at 160 °C for 1 hour), with or without resistant starch (RS) for 10 weeks. 24-hour urine was collected for the assessment of albuminuria. Intestinal permeability was assessed in-vivo by the clearance of FITC-labelled dextran (500 mg/kg body weight).

Results: The high AGE diet exacerbated albuminuria in db/db mice (874.4 ± 154.8 vs 536.2 ± 96.53 μg/24 h, $P < 0.05$, db/db HAGE vs db/db LAGE), and this AGE-induced increase in albuminuria was attenuated by RS (874.4 ± 154.8 vs 515.5 ± 71.88 μg/24 h, $P < 0.05$, db/db HAGE vs db/db HAGE + RS). Db/db mice had greater gut permeability compared to db/h mice (2.38 ± 0.32 vs 1.05 ± 0.11 μg/ml, $P < 0.01$, db/db LAGE vs db/h LAGE). Furthermore, the high AGE diet tended to increase gut permeability of db/db mice (3.43 ± 0.43 vs 2.38 ± 0.32 μg/ml, $P = 0.06$, db/db HAGE vs db/db LAGE), an effect not observed in RS-fed db/db mice.

Conclusions: A high AGE diet led to increased intestinal permeability, which was associated with worsening albuminuria in db/db mice. Resistant starch was protective against high AGE induced albuminuria in db/db mice. These preliminary studies support the notion that dietary AGEs contribute to renal disease via alterations in gut homeostasis.

PROTEASE ACTIVATED-RECEPTOR-2 INDUCED INFLAMMATION AND FIBROSIS IS MEDIATED IN PART THROUGH ACTIVATION OF TRANSFORMING GROWTH FACTOR-β SIGNALLING

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Aim: To explore the pro-inflammatory and pro-fibrotic responses of primary human kidney tubular epithelial cells (HTECs) following activation of protease-activated receptor-2 (PAR-2) and/or the transforming growth factor-β (TGF-β) receptor.

Background: Tubulointerstitial inflammation and fibrosis are observed in all progressive forms of chronic kidney disease (CKD) irrespective of the initiating insult. Recent evidence suggests that serine proteases, commonly produced following kidney injury, promote and exacerbate these responses by activating PAR-2 and TGF-β receptors.

Methods: Confluent HTECs were treated with the synthetic PAR-2 agonist 2f-LIGRLO-NH_2 (1 μM) and/or TGF-β1 (5 ng/mL) in the presence or absence of the pan-protein kinase C (PKC) inhibitor Gö6983, the TGF-β receptor kinase inhibitor SB-431542, and PAR-2 antagonist GB88. Inductions of pro-inflammatory and pro-fibrotic molecules [e.g. CSF2, TNF-α, PAI-1, MMP-1, MMP-9, and MMP-10] was measured at the mRNA and protein level by qPCR, western blot, and ELISA.

Results: Alone, 2f-LIGRLO-NH_2 significantly induced production range of molecules including CSF2, TNF-α, CTGF, PAI-1, MMP-1, MMP-9, and MMP-10 from HTEC. Smad2 was also activated by this treatment. TGF-β1 treatment induced production CSF2, CTGF, and PAI-1 although to a lesser extent than with PAR-2 activation. Concurrent activation of PAR-2 and TGF-β receptors gave a synergistic increase in the production of a number of these factors, particularly CSF2 and MMP-9. GB88, Gö6983 and SB-431542 significantly reduced production of molecules CTGF, CSF2, PAI-1, and MMP-9.

Conclusions: PAR-2 activation induces pro-inflammatory and pro-fibrotic responses in HTECs which involve PKC and TGF-β signalling pathways. PAR-2 antagonists may be useful for pharmacotherapy of CKD, and are currently being tested for efficacy in animal models.