Abstract – Diabetes UK, 2018

A role for ATP in renal fibrosis as a downstream mediator of TGF-β1-evoked changes in hemichannel activity

Price GW, Potter JA, Squires PE, Hills CE,
Joseph Banks Laboratories, University of Lincoln, LN6 7DL

Aims: Data from our group confirms that increased expression of Connexin-26 and Connexin-43 in biopsy material from patients with proven nephropathy is paralleled by TGF-β1-evoked changes in hemichannel-mediated ATP release. With recent studies linking increased ATP to fibrosis in multiple tissue types, we have previously confirmed that incubation of proximal tubule cells with ATPγS (1-100μM) evokes increased expression of ECM markers. In the current study, we provide evidence of a direct role for ATP and associated downstream purinergic signalling as a mediator of TGF-β1 induced altered hemichannel activity in ECM expression.

Methods: Human kidney (HK2) proximal tubule cells were treated for 48hrs with either TGF-β1(10ng/mL) ± nucleotidase; apyrase (100μM) or ATPγS (10μM) ± purinergic receptor antagonist suramin (100μM). Expression of Collagen I, Collagen IV, Fibronectin and Laminin were determined by immunoblotting.

Results: Immunoblotting confirmed that apyrase negated TGF-β1 upregulation of Collagen I, from 366.0±13.0% of control to 119.5% (n=3 P<0.001) and reversed loss of Collagen IV expression from 30.1±12.1% of control to 123.3±9.8% (n=3 P<0.01). The nucleotidase also negated an upregulation of Fibronectin, from 201.5±3.7% to 156.7±27.6% (n=3 P<0.01) and Laminin, from 339.5±43.1% to 173.4±42.8% (n=3 P<0.05). Suramin inhibited ATPγS-induced changes in expression of ECM, reducing expression of Collagen I from 452.9±20.6% of control to 192.1±16.0%; Collagen IV from 157.2±17.4% to 93.6±12.8%; Fibronectin from 222.6±9.5% to 103.7±5.2% and Laminin from 177.8±25.0% to 86.4±11.7% (n=3 P<0.05).

Conclusions: The current study confirms that TGF-β1 induced changes in hemichannel mediated ATP release may in part, contribute to tubular fibrosis in the diabetic kidney.

Acknowledgement: This work is supported by Diabetes UK (BDA:16/0005427 and BDA:16/0005509).