LOXL2 as a potential treatment target in diabetic nephropathy

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Introduction

Fibrotic changes in diabetic nephropathy arise from disorganized and exaggerated deposition of extracellular matrix (ECM) and loss of normal renal parenchyma. Lysyl oxidase-like 2 (LOXL2) is a copper-dependent amine oxidase that belongs to the lysyl oxidase family. LOXL2 plays a key role in ECM stabilization and is upregulated in many diseases with fibrotic response, primarily by facilitating collagen crosslinking. In addition, it has been linked to fibroblast activation and epithelial to mesenchymal transition (EMT).

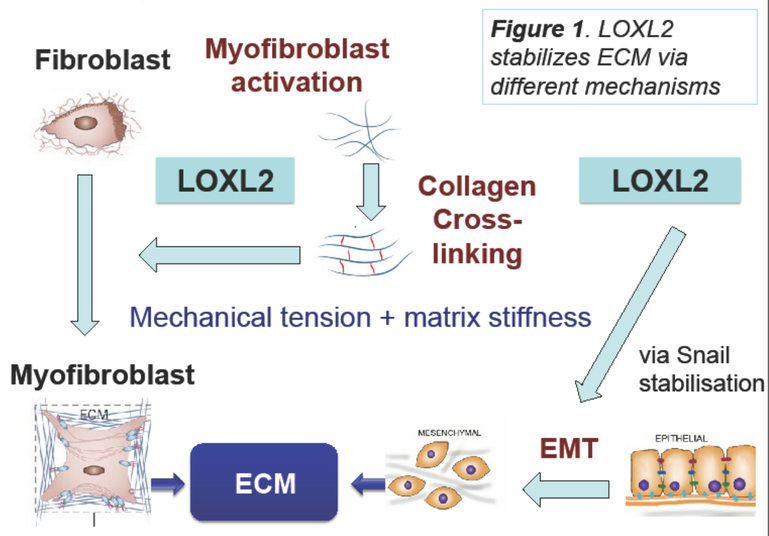


***Figure 1**. LOXL2 stabilizes ECM via different mechanisms*

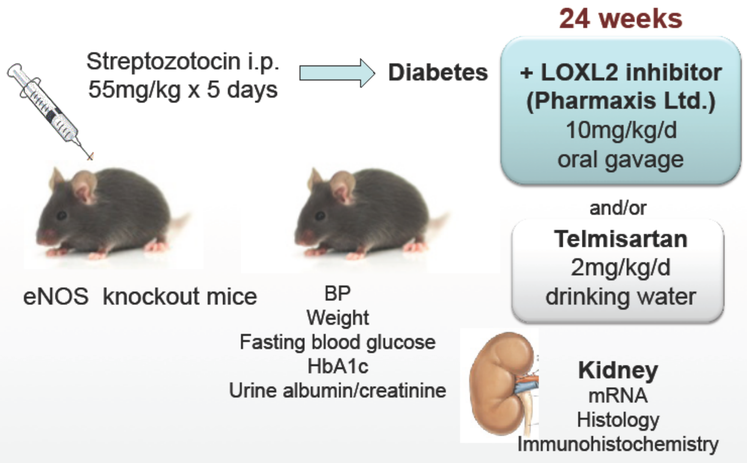
Increased LOXL2 levels have been found in kidney biopsies of patients with diabetic nephropathy[1].

Aim

To investigate the renoprotective effect of a LOXL2-inhibitor in a mouse model of diabetic nephropathy

Methods

Diabetic mouse model



Diabetes was induced in eNOS knockout mice (eNOS -/-) on C57 BL/6 background at 6-9 weeks of age by low dose streptozotocin. Diabetic mice received a highly selective small molecule LOXL2 inhibitor (courtesy of Pharmaxis Ltd) per daily oral gavage or Telmisartan as comparative limb of current best practice. 24 hour urine was collected in metabolic cages and mice were sacrificed after 24 weeks of treatment. Kidneys were examined for histology and cortical expression of fibrosis, EMT and inflammatory markers.

Results

Metabolic Parameter

	Ctrl	DM	DM +LOXL2i	DM +Telmi	DM +LOXL2i +Telmi
Body weight (g)	24.7±0.4	21.5±0.9**	22.2±0.4*	23.1±0.62	22.7±0.6
Average Fasting BSL	9.7±0.4	20.6+0.4**	23.9±0.7##	26.2±0.5##	24.7±0.7##
HbA1c	4.3±0.1	8.1±0.2**	8.4±0.1**	8.7±0.2**	8.9±0.5**

***Table 1**. Metabolic parameter at 24 weeks of treatment, mean±SEM, * P<0.05 vs. Control (Ctrl),**P<0.01 vs. Ctrl, ## P<0.01 vs. diabetes (DM)*

1. Albuminuria

Diabetic mice treated with the LOXL2 inhibitor had significant reduction in albuminuria

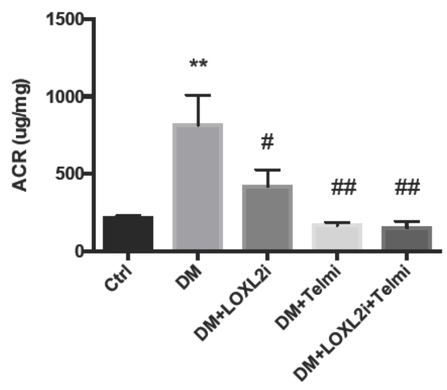
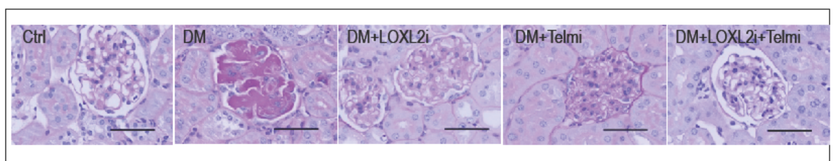


***Figure 2.** Albumin/creatinine ratio from 24 hour-urine after 24 weeks of treatment. Mean±SEM, **P<0.01 vs. Ctrl, #P< 0.05 vs. DM, ##P<0.01 vs. DM, n=5-8*

2. Histology



LOXL2 inhibition ameliorated glomerulosclerosis.

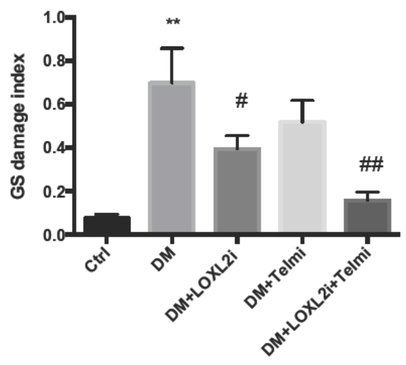
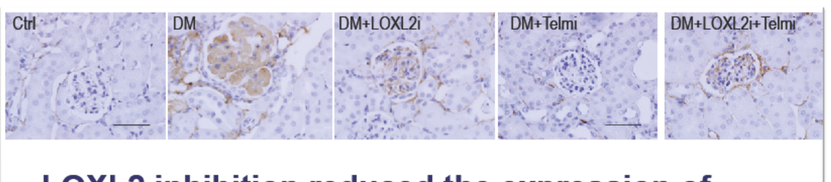


***Figure 3.** PAS-stained paraffin sections indicating the extent of glomerulosclerosis. 20 glomeruli were captured and graded on a scale from 0-4 in a blinded manner. Mean±SEM, **P<0.01 vs. Ctrl, #P<0.05,##P<0.01 vs. DM, n=5-8, scale bar 50µm.*

3. Fibrosis markers



LOXL2 inhibition reduced the expression of fibronectin in diabetic mice.

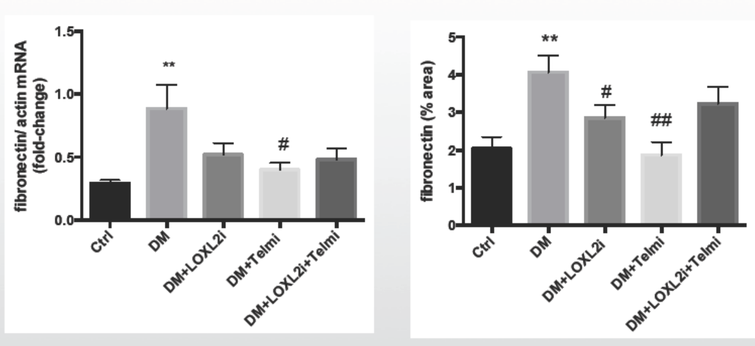


***Figure 4**. mRNA expression and immunohistochemistry of fibronectin, mean±SEM, **P<0.01 vs. Ctrl, #P<0.05 vs. DM ##P<0.01 vs. DM, n=5-8*

Results

LOXL2 inhibition reduced the expression of collagen I in diabetic mice.

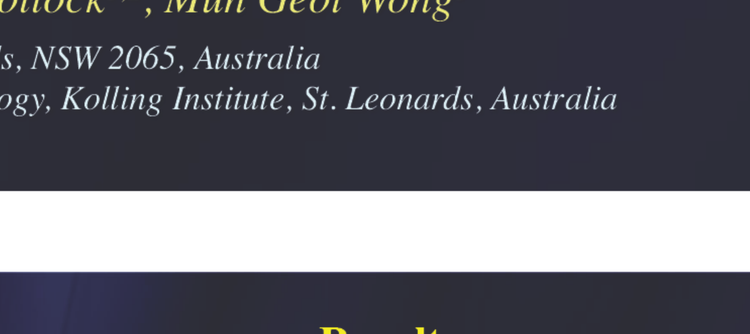


***Figure 5**. mRNA expression and immunohistochemistry of collagen I, mean±SEM, *P<0.05 vs. Ctrl,**P<0.01 vs. Ctrl, #P<0.05 vs. DM, n=5-8*

4. Myofibroblast activation

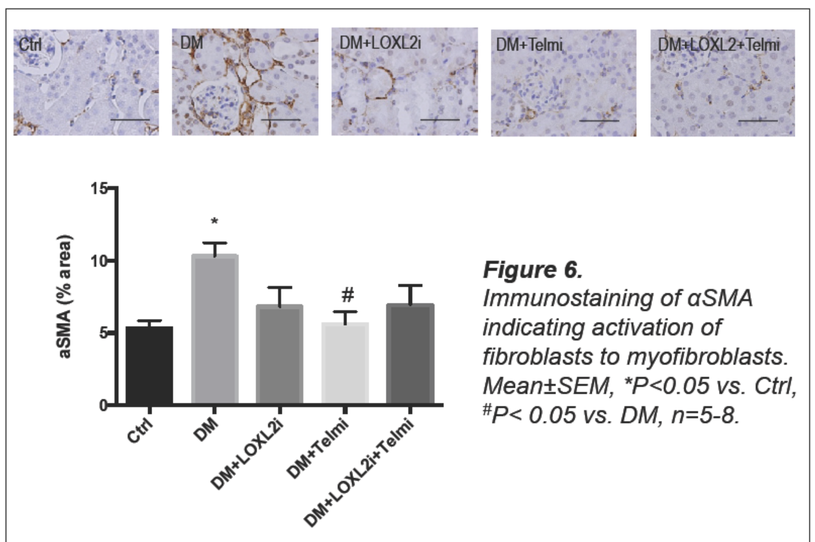


***Figure 6.** Immunostaining of αSMA indicating activation of fibroblasts to myofibroblasts. Mean±SEM, *P<0.05 vs. Ctrl, #P< 0.05 vs. DM, n=5-8.*

- There was a trend towards reduction in αSMA after LOXL2 inhibition.
- There was no difference in E-Cadherin and Snail mRNA expression as well as E-Cadherin immunostaining between treated and untreated diabetic mice.
- LOXL2 inhibition had no impact on markers of inflammation (MCP-1mRNA, F4/80 IHC).

Conclusion

Selective LOXL2 inhibition reduced markers of fibrogenesis in a mouse model of diabetic nephropathy. There was a significant reduction in albuminuria and amelioration of glomerulo-sclerosis. Thus, it had a beneficial effect on preserving glomerular structure and function and might prove to be a potential therapeutic strategy in diabetic nephropathy and renal fibrosis of other aetiologies.

Acknowledgement

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References:
1. Higgins DF et al. J Clin Invest 117: 3810-3820, 2007

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