Inhibition of the renin-angiotensin-aldosterone system in diabetic nephropathy: focusing on renal fibrosis

Sandor Koszegi[1,4], Judit Hodrea[1,4], Lilla Lenart[1,4] , Adam Hosszu[1,4], Edgar Szkibinszkij[1], Laszlo Wagner[3] , Adam Vannay[2,4] , Tivadar Tulassay[2,4], Attila J Szabo[2,4], Andrea Fekete[1,4]

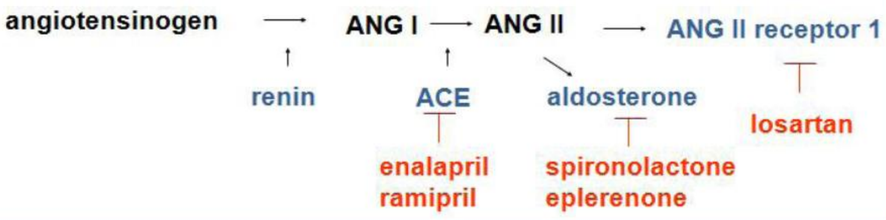
[1]MTA-SE „Lendület" Diabetes Research Group; [2]MTA-SE Pediatrics and Nephrology Research Group, [3]Department of Transplantation and Surgery; [4]1st Department of Pediatrics; Semmelweis University, Budapest

Introduction

- Nowadays 366 million people suffer from diabetes worldwide and this number will exceed 552 million by 2030. [1]
- In diabetes the **renin-angiotensin-aldosterone system (RAAS)** is highly activated. [2]
- The elevated renal **angiotensin (ANG) II** induces renal fibrosis via different growth factors. [3]
- One of the most characteristic markers of renal fibrosis is the **increased level of α-smooth muscle actin (αSMA)**. [4]
- **Platelet-derived growth factor (PDGF)** plays a key role in fibrosis. [5]
- During fibrosis, isoforms of PDGF B and D, and receptor PDGF ß are involved in the activation of fibroblasts. [6]

Aims

Our aim was to investigate the effect of various RAAS inhibitors on diabetes induced renal fibrosis.



Methods

- After 5 weeks of streptozotocin (65 mg / bwkg *ip*.) induced diabetes male Wistar rats were treated for 2 weeks with ACE inhibitor ***enalapril*** (40 mg x bwkg / day) or ***ramipril*** (10 µg / bwkg / day), ARB ***losartan*** (20 mg / bwkg / day) or aldosterone antagonists ***spironolactone*** or ***eplerenone*** (50-50 mg / bwkg / day). Untreated diabetic (D) and healthy animals (C) served as controls (n=6/group).
- Human kidney 2 (HK-2) proximal tubular cells were cultured in *normal* (5,5 mM) *or high glucose* solution (35 mM) or in *mannitol* (35 mM) as osmotic control.
- Normal rat kidney (NRK-49F) fibroblasts were treated with PDGF (10 ng/mL) and αSMA protein level was detected.
- Mesangial matrix expansion and tubulo-interstitial fibrosis were analyzed on PAS or Masson's trichrome stained kidney sections.
- Protein levels of αSMA in HK-2, NRK-49F and kidney tissue were measured by Western blot and the localization of PDGFRß and αSMA was detected by immunofluorescent staining

Summary - Conclusions

- **High glucose increases the PDGF production of proximal tubular cells, that in turn induces the αSMA production in renal fibroblast.**
- **This changes in the proximal cells are likely to be due to the presence of hyperglycemia than to glucose induced hyperosmolarity.**
- **This mechanism also contributes to the development of renal fibrosis seen in DNP.**
- **RAAS blockers ameliorate this process by directly acting on renal fibroblasts which could serve as a new therapeutic potential in the treatment of renal fibrosis.**

Results

1. RAAS inhibitors ameliorated diabetes induced renal damage

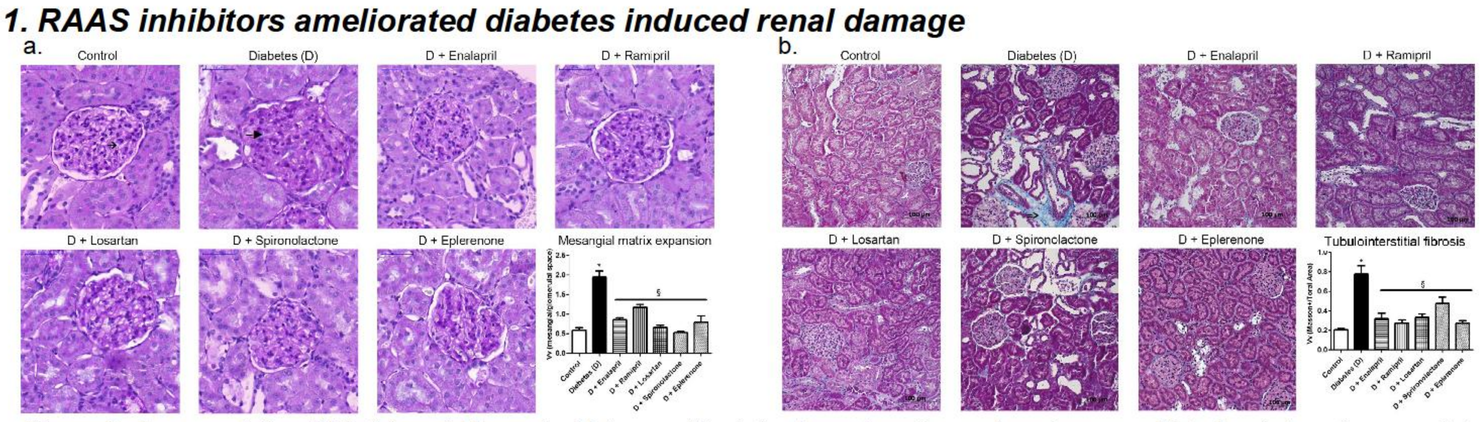


*Figure 1. Representative PAS (a) and Masson's trichrome (b) stained renal sections of each group. Diabetes induced mesangial matrix expansion and tubulo-interstitial fibrosis were ameliorated by each RAAS blocker. (a: 400x magnification, scale bar 50 µm, → : arteriola, –►: mesangial matrix; b: 200x magnification, scalebar 100 µm , => interstitial fibrosis). (*p<0.05 vs.Control; §p<0.05 vs. Diabetes (n=6/group).*

2. Glucose, but not mannitol increased αSMA level in HK-2 cells

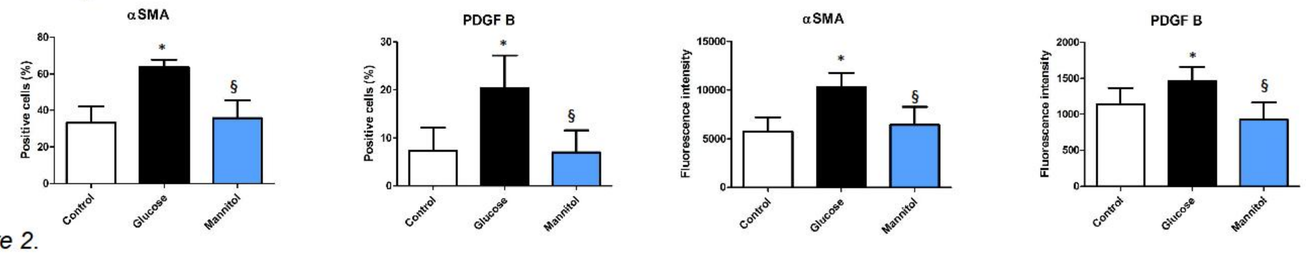


Figure 2.

*High glucose induced elevation of αSMA and PDGF B protein levels; while no change was observed in the case of osmotic control mannitol. (**p<0.05 *vs.* Control; §p<0.05 *vs.* Glucose (n=6/group))*

3. RAAS inhibitors decreased protein level of αSMA in vivo and in vitro

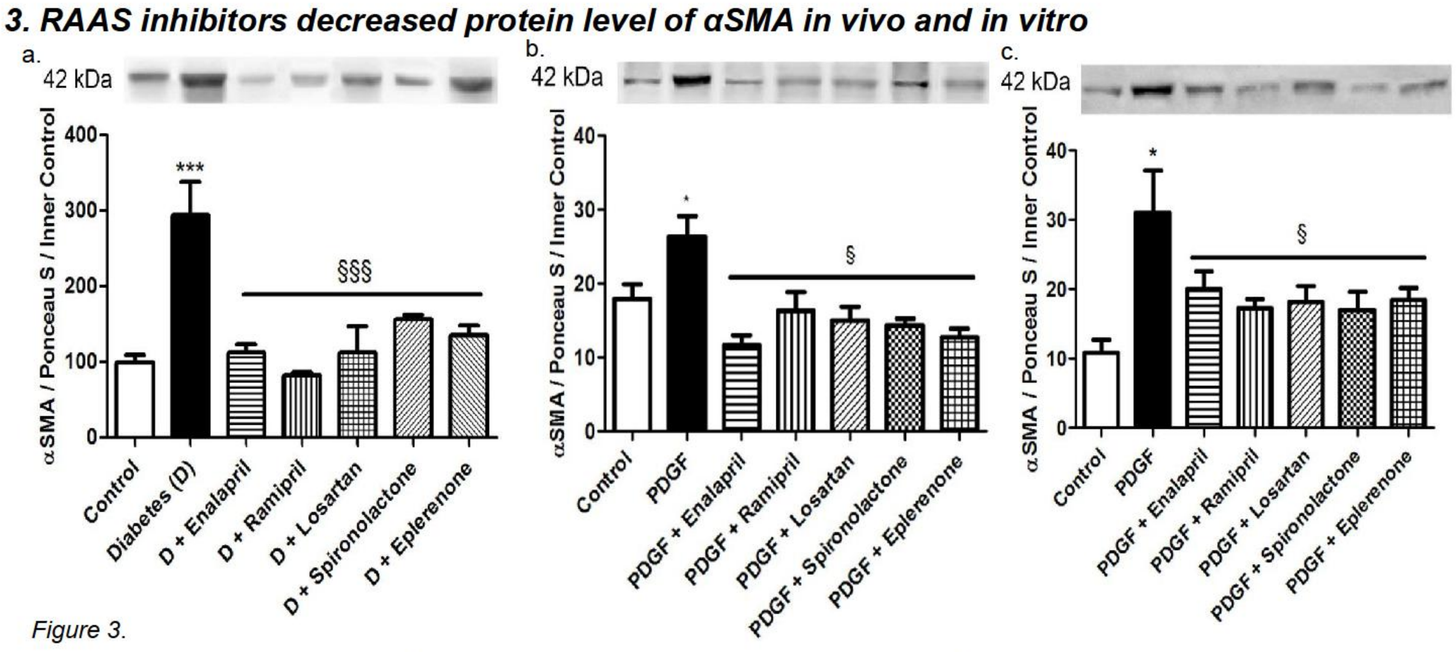


Figure 3.

*Diabetes induced elevation of αSMA protein level in the kidney (a) was diminished by various RAAS inhibitors (***p<0.001 vs. Control; §§§p<0.001 vs. Diabetes (n=6/group)). 24h (b) and 48h (c) PDGF- treated normal rat kidney fibroblasts (NRK-49F) produced more αSMA protein which decreased by RAAS blockers. (*p<0.05 vs. Control; §p<0.05 vs. PDGF (n=6/group)).*

4. Localisation of αSMA and PDGFRß in diabetic kidney

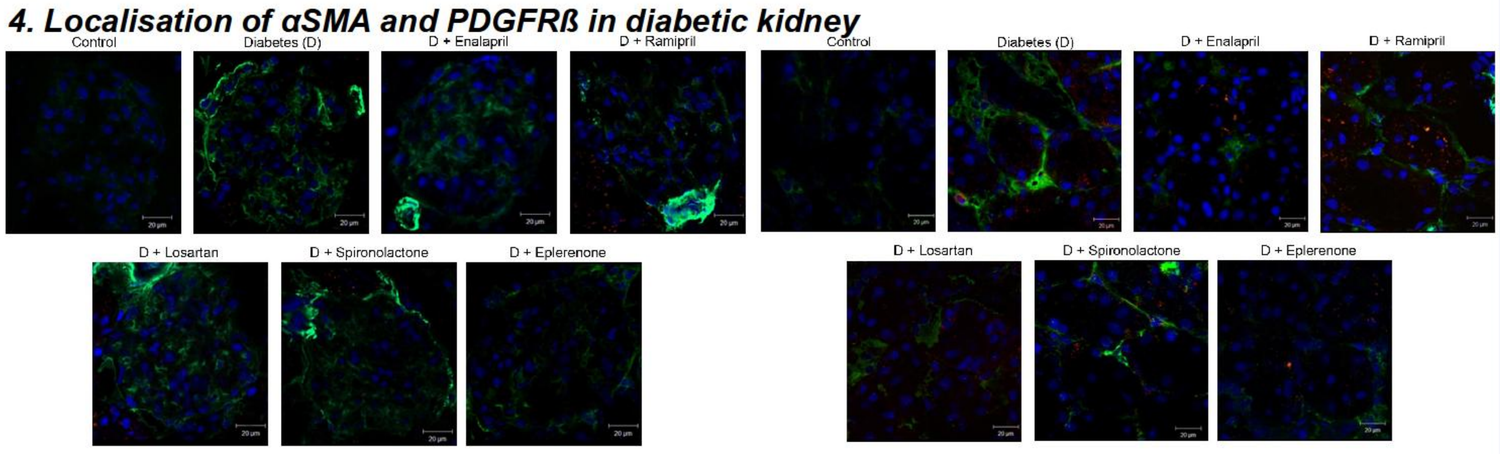


Figure 4.

Glomeruli showed no αSMA staining in controls, while slight increase was observed in diabetic rats. Each RAAS blocker treatment decreased αSMA within glomeruli. The staining of PDGF receptor was more intensive in tubuli. Tubular cells showed no αSMA staining in controls, while a definitive intracellular staining was visible in diabetic kidneys. Aldosterone antagonists decreased αSMA within tubuli and translocated the protein from the cytoplasm towards the basolateral membrane. The staining of the tubulo-interstitialy localized PDGF receptor was more intensive in diabetic rats than in controls. (630x magnification, scale bar 20 µm, green –αSMA, red – PDGFRß, blue – nuclei, Hoechst)

Acknowledgment:
This work was supported by *Lendület* Research Grant (LP008/2014) and grants of OTKA NK84087/2010, -K108688, KMR12-1-2012-0074.

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I) Diabetes. I1) Basic research in diabetes.
Sandor Koszegi
DOI: 10.3252/pso.eu.51era.2014
Poster presented at: 51 ERA-EDTA CONGRESS AMSTERDAM THE NETHERLANDS MAY31 JUNE2 2014
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