URAT-1 inhibition protects humans proximal tubular cells from apoptotic damage induced by uric acid

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Introduction: Uric acid (UA) has been implicated in chronic kidney disease progression and development of hypertension, but the underlyne mechanisms are not completely understood. Increased UA levels has been associated with albuminuria, increased renal resistite index [1] and tubular atrophy[2]. The suggested pathways include RAAS stimulation[3], NO inactivation and oxidative stress. UA enters proximal tubular cells via specific transporter; the most studied is URAT-1, a target molecule of uricosuric drugs, including Probenecid and Losartan[4].

Aims:

- To investigate the role of UA to promotes apoptosis in a line of human proximal tubular cells (HK-2);
- To analyze the specific pathways involved in the apoptosis induced by uric acid.

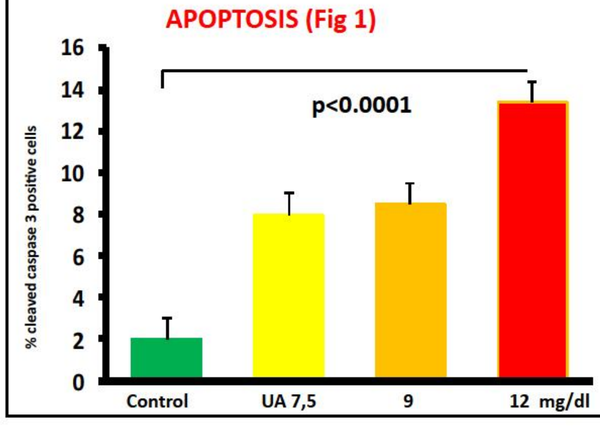
Results: Uric acid promotes apoptosis already at 7,5 mg/dl and significantlly more at 12 mg/dl (14%±0.35 vs 2%±0.87, p=0,0001;fig.1). Increasing UA concentrations reduce cells viability (Control VS UA 12mg/dl-30%, p=0.015 fig.2). Inhibition of Caspase 9, but not Caspase 8, protects against uric acid induced apoptosis (CASP9 In VS UA P< 0,0001; CASP8 In VS UA P=NS, Fig 3). High concentrations of UA upregulate BAX (+60%, P<0.05%) and downregulate XIAP (-30%, P<0,05) (Fig 4). Mitochondria exposed to UA was significantly more damaged than control (70% vs 95 %; p<0.05)(Fig 5). Cells exposed to elevated levels of UA show an increased production of ROS (10.90% ± 1.550 of UA trated cells was DCFH-DA positive VS 2.200% ± 0.4789 of control cells; P=0.003) (Fig 6). Inhibiting ROS production with DPI, a NADPH inhibitor, significantly reduce apoptosis in UA exposed cells (-70%; P=0.0001) (Fig. 7). Blockade of UA transporter with Probenecid or Losartan significantly reduce apoptosis (Probenecid 20 µM VS control P<0.01; Losartan 10 µM VS control P<0.001) (Fig 8).

Materials and Methods

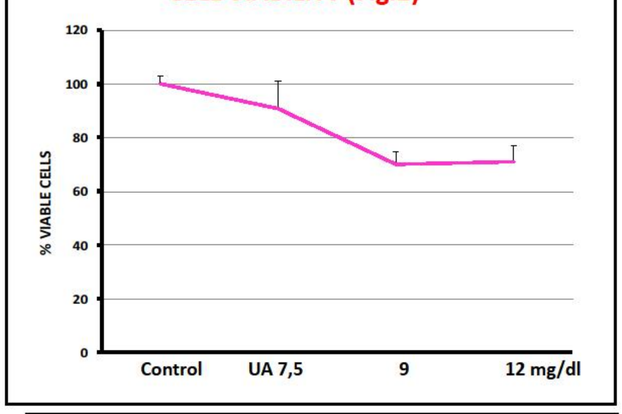
Cell culture and treatments: HK-2 treated for 48 hours with 7,5-12 mg/dl uric acid. Caspase inhibitors (-8,-9; 50 mmol), diphenylene iodonium (10 µmol/L added to cultures to identify effects and apoptotic pathways that could modulate cell survival).
Cell Viability: MTT test. ***Apoptosis assay:*** Cleaved Caspase-3 fragment immunostaining. ***Evaluation pro-/anti-apoptotic proteins:*** Western Blot. ***Mitochondrial transmembrane potential evaluation:*** MitoCaptureTM detection kit. ***Statistical analysis:*** The ANOVA and the Tukey-Kramer multiple comparison test were used. Results are expressed as mean±SEM. Differences were considered statistically significant if $p < 0.05$.

Results

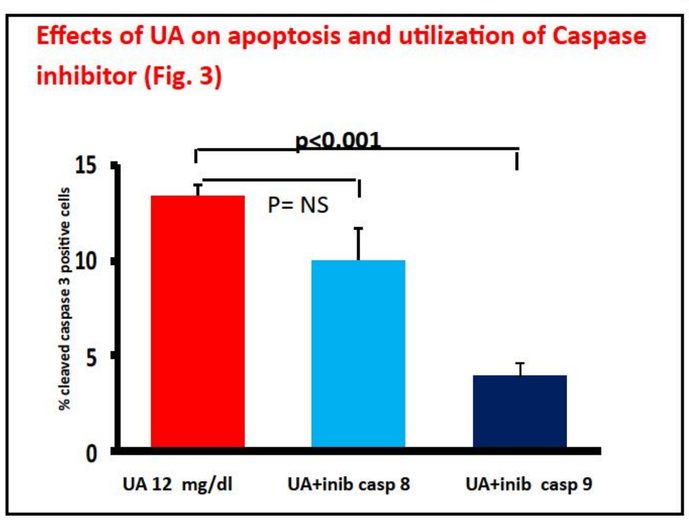
APOPTOSIS (Fig 1)



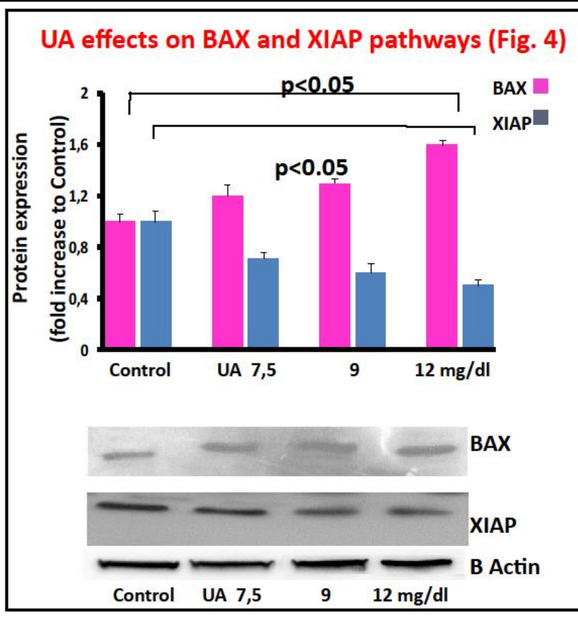
CELL VIABILITY (Fig.2)



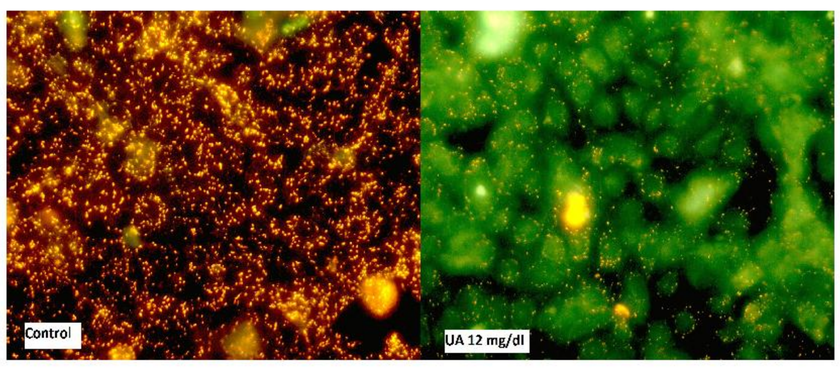
Effects of UA on apoptosis and utilization of Caspase inhibitor (Fig. 3)



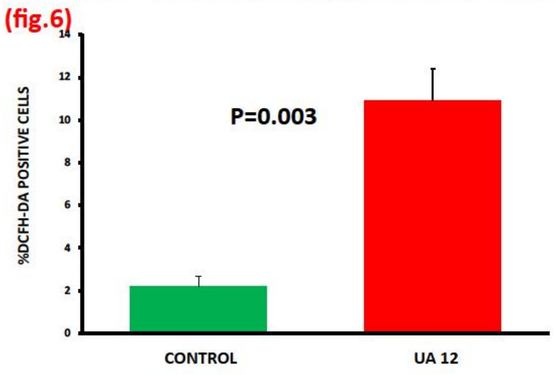
UA effects on BAX and XIAP pathways (Fig. 4)



DYSRUPTION OF TRANSMENBRANE POTENTIAL AFTER 48 HRS UA 12 mg/dl (FIG. 5)

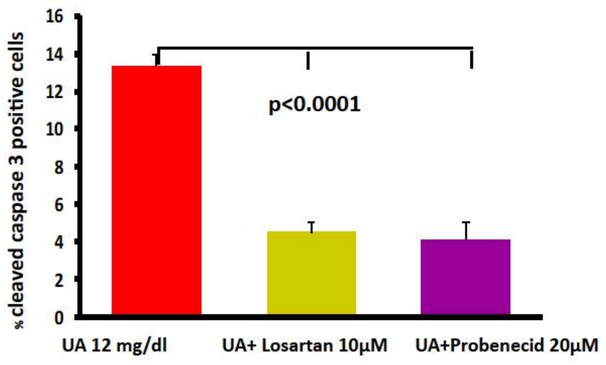


EFFECTS OF URIC ACID 12 mg/dl ON ROS PRODUCTION (fig.6)



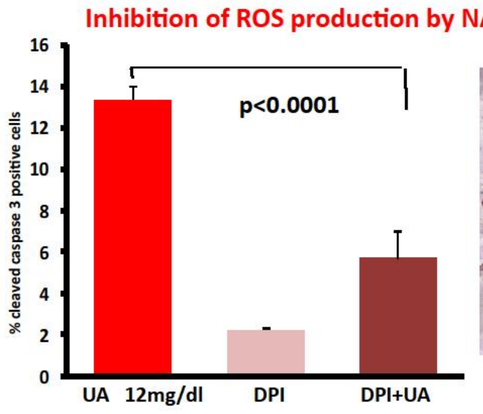
AU 12 mg/dl induced intracellular ROS production as detected by the oxidation-sensitive probe DCFH-DA .

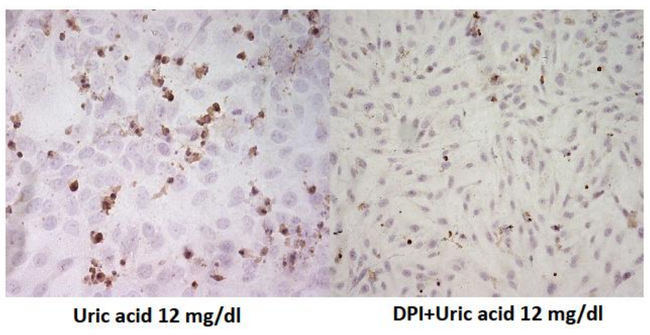
Effects of UA transporter blockade (Fig. 8)



UA (7,5-12 mg/dl) induced apoptosis at 48 hours. It was blunted by losartan and probenecid.

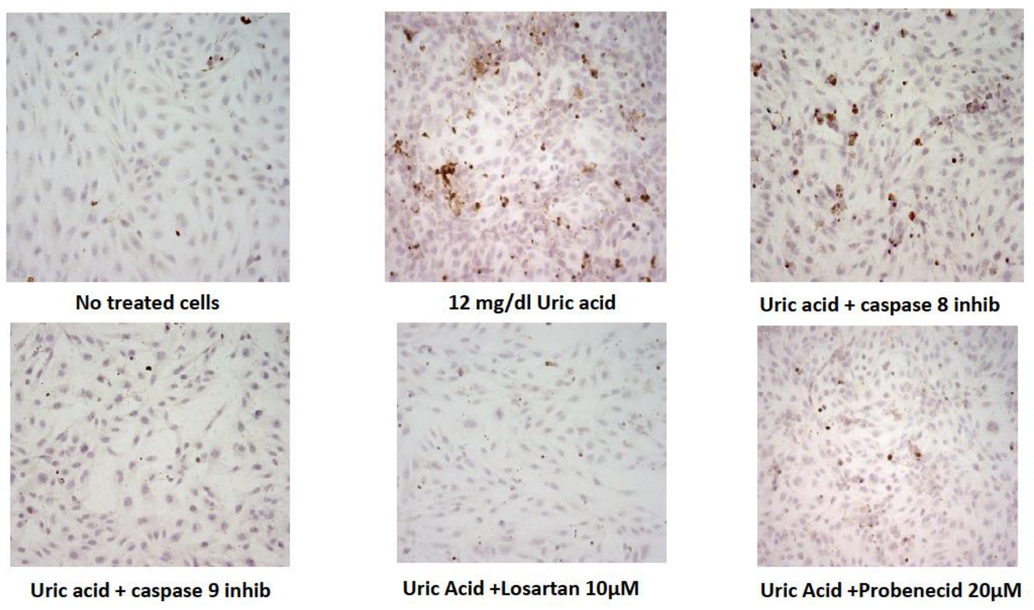
Inhibition of ROS production by NADPH Oxidase Inhibitor (Fig. 7)





When HK-2 were pre-treated by DPI (NADPH oxidase Inhibitor), apoptosis was blunted.

UA effects on apoptosis: the effect was blunted by Caspase 9 inhibitor and Losartan and Probenecid, but not by Caspase 8 inhibitor



Conclusions: We demonstrated that even moderatly elevated levels of UA can induce proapoptotic effects on human proximal tubular cells, by intrinsic pathway (Caspase 9 dependent). Elevated UA levels also induce oxidative stress. Incubation with probenecid and losartan inhibited apoptosis induced by 12 mg/dl UA exposition.

References:

(1)Mild hyperuricemia and subclinical renal damage in untreated primary hypertension; Viazzi F et al; Am J Hypertens. 2007 Dec;20(12):1276-82.

(2) Uric acid correlates with the severity of histopathological parameters in IgA nephropathy; Myllymäki J et al; Nephrol Dial Transplant. 2005 Jan;20(1):89-95.

(3)Uric acid stimulates vascular smooth muscle cell proliferation and oxidative stress via the vascular renin-angiotensin system. Corry et al; Journal of Hypertension 2008, 26:269-275

(4)Uricosuric action of losartan via the inhibition of urate transporter 1 (URAT 1) in hypertensive patients; Hamada T et al; Am J Hypertens. 2008 Oct;21(10):1157-62

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