Role of Integrin Linked Kinase (ILK) in endothelial dysfunction associated with uremia in Chronic Kidney Disease

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INTRODUCTION AND AIM:

Patients with chronic kidney disease (CKD) have a much higher risk of cardiovascular diseases than the general population. According to the most accepted hypothesis, endothelial dysfunction and damage that is present in almost all patients with CKD, seems to be the start element in the cascade of events that leads to cardiovascular disease. The endothelium of patients with CKD is permanently exposed to uremic toxins. Several uremic toxins, mostly protein- bound compounds such as indoxyl sulfate and p-cresyl sulfate that have poor clearance by conventional dialysis, induces specific endothelial toxicity. However, the molecular mechanism by which the uremic toxins regulate the early stages of endothelial dysfunction remains unclear. Recent studies have demonstrated the important role of integrin-linked kinase (ILK), a multidomain signaling protein that localizes to focal adhesions, in the maintenance of endothelial integrity.

AIM: In the present study we investigate the involvement of ILK in the mechanism that lead to vascular endothelial dysfunction that occurs in uremia

MATERIALS AND METHODS

Confluent EA.hy926 endothelial cells were incubated for different concentrations and times with indoxyl sulfate (IS) and/or p-cresol (pc), in the presence of 2,5% of human serum. The protein level of ILK and phospho-serine 9-glycogen synthase kinase 3β (P-GSK-3β) was determined by Western blot. ILK kinase activity was also determined by immunoprecipitation in vitro kinase assay. Cell number was assessed using the MTT assay and endothelial cell proliferation was also determined by 5-bromo-2-deoxy-uridine (BrdU) incorporation into cellular DNA. The effect of uremic toxins on cell viability was measured by Tripan blue exclusion. The apoptosis was determined by Anexin-V, flow cytometric analysis of cells with sub-G1 DNA content and by morphology

RESULTS

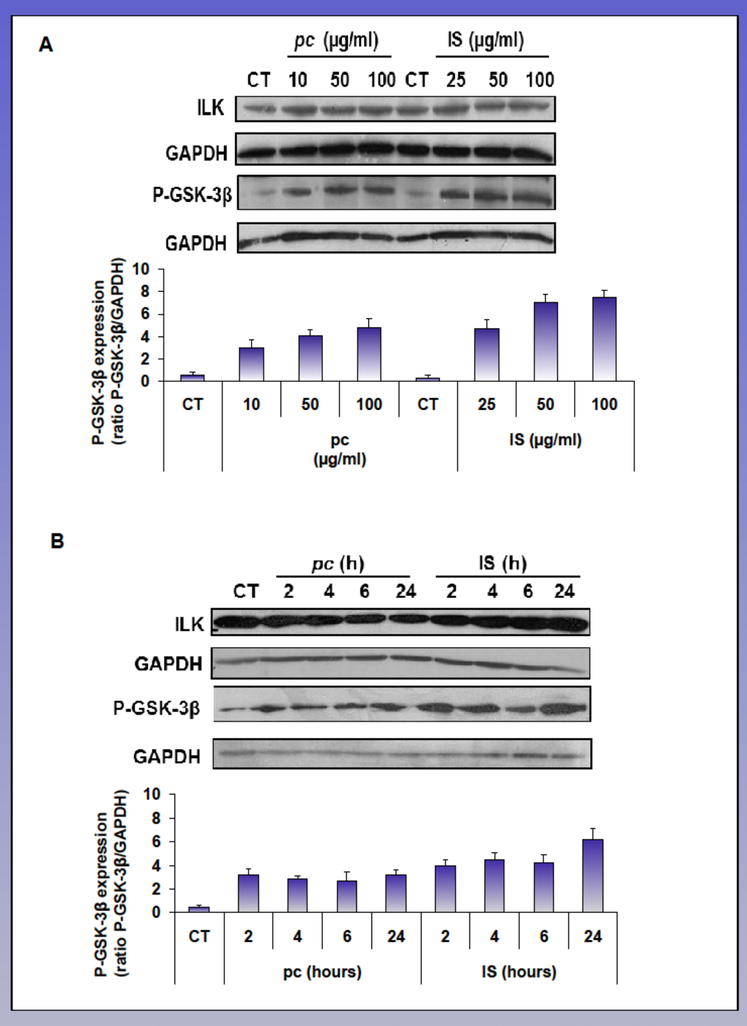

Figure 1. Uremic toxins increases ILK activity in EA.hy926 cells: Cells were incubated in medium supplemented with 2,5% normal serum (NS) plus p-cresol (pc; 10, 50 or 100 µg/ml) or indoxyl sulfate (IS; 25, 50 and 100 µg/ml) for 24 hours (A) or with 2,5% NS plus pc (10 µg/ml) or IS (25 µg/ml) for different times (B). Representative western blots of ILK or phosphorylated GSK-3β in the serine-9 residue (P-GSK-3β) are shown. Total GAPDH levels were determined as their respective endogenous control. Bars represent the normalized densitometric analysis of the blots against the endogenous control values. All the values are represented as mean ± SEM of 5 independent experiments. *= p<0.05 vs control (CT; 2,5% NS, 24 hours).

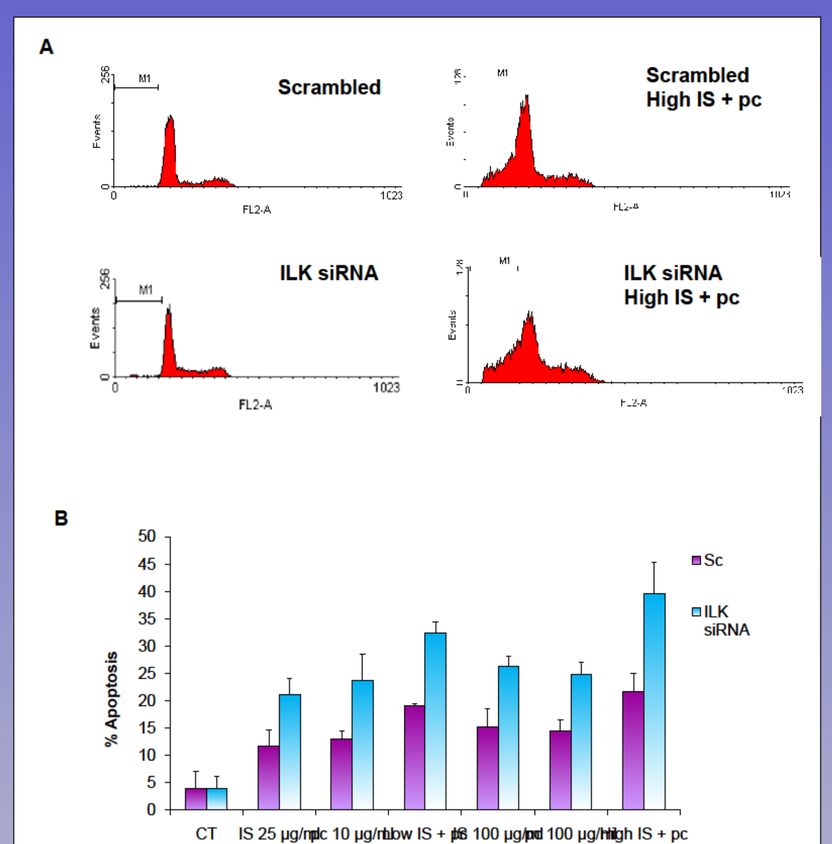

Figure 3. Role of ILK in the EA.hy926 cells uremic toxins-induced apoptosis production: Cells were depleted of ILK with specific siRNA (100nM) and afterwards were incubated in medium supplemented with 2,5% NS plus indoxyl sulfate (IS; 25 or 100 µg/ml), p-cresol (pc; 10 or 100 µg/ml), combination of low concentrations of IS (25 µg/ml) and pc (10 µg/ml) (Low IS + pc) or plus combination of high concentrations of IS (100 µg/ml) and pc (100 µg/ml) (High IS + pc) for 24 hours. Scrambled RNA (Sc) was used as control. After incubation, apoptosis was determined in PI-stained cells and analyzed by flow cytometry. Data are expressed as mean SEM of 7 independent experiments. *= p<0.05 vs control (CT); # =p<0.05 vs Sc (CT; 2,5% NS, 24 hours).

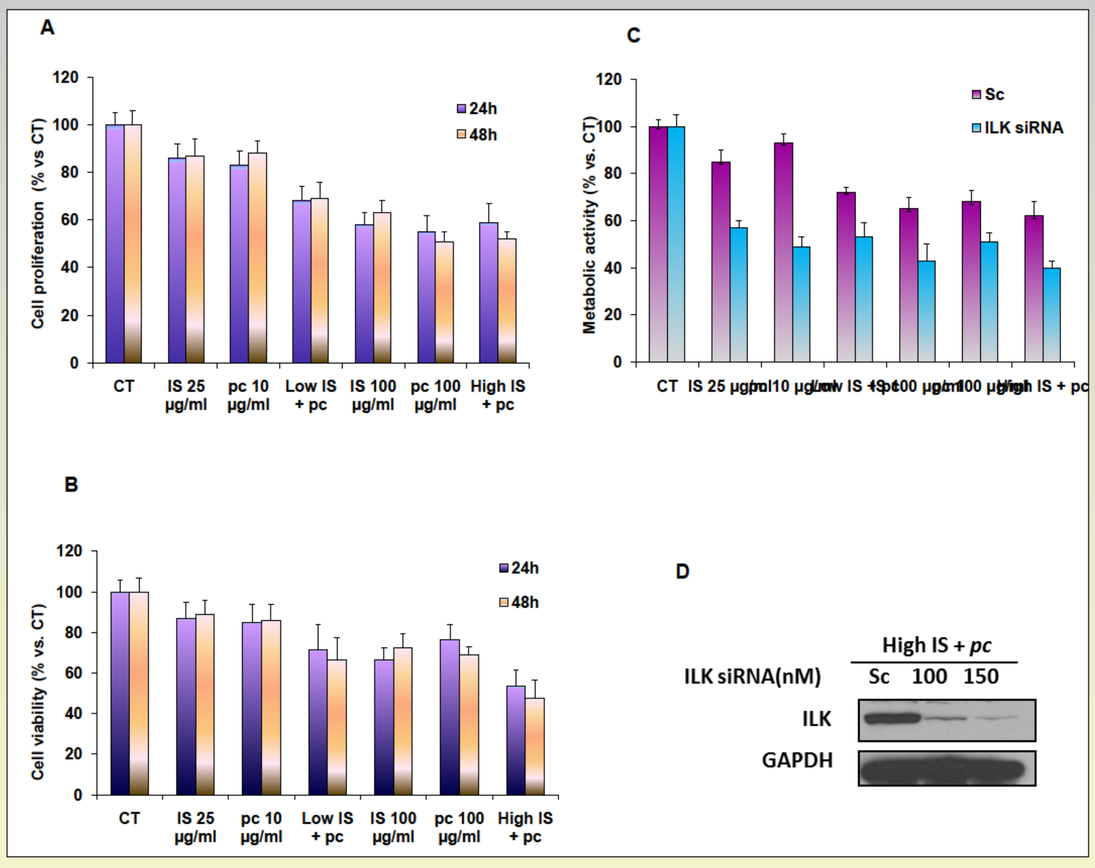

Figure 2: Role of ILK in the EA.hy926 cells proliferation and viability decrease induced by uremic toxins: (A and B) Cells were incubated in medium supplemented with 2,5% NS plus indoxyl sulfate (IS; 25 or 100 µg/ml), p-cresol (pc; 10 or 100 µg/ml), combination of low concentrations of IS (25 µg/ml) and pc (10 µg/ml) (Low IS + pc) or plus combination of high concentrations of IS (100 µg/ml) and pc (100 µg/ml) (High IS + pc) for 24 and 48 hours. After incubation, endothelial cell proliferation was measured by cytometric analysis of 5-bromo-2-deoxy-uridine (BrdU) incorporation (A) or endothelial cell viability was determined by Trypan Blue exclusion (B). (C) Cells were depleted of ILK with specific siRNA (100nM) and treated afterwards as in A, for 24 hours. Scrambled RNA (Sc) was used as control. Endothelial cell viability was measured by MTT assay. Data are expressed as mean ± SEM of 6 independent experiments. *=p<0.05 vs control (CT); # =p<0.05 vs Sc (CT; 2,5% NS, 24 or 48 hours). (D) Representative western blot of total ILK levels expression to check its depletion and GAPDH levels as endogenous control.

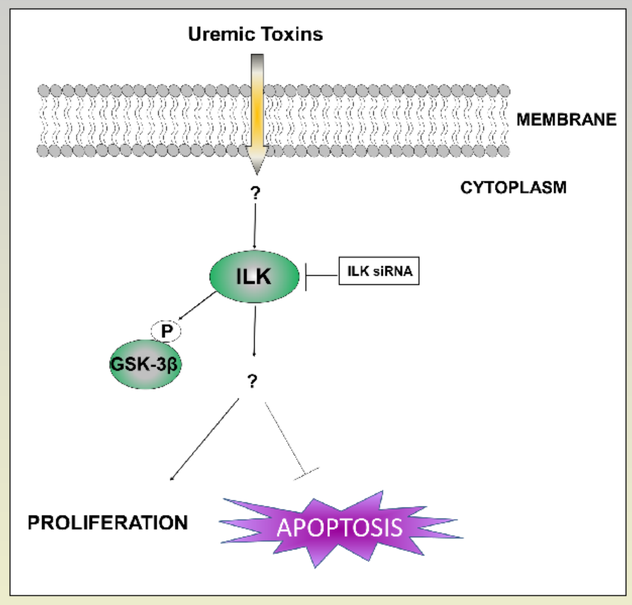

Figure 4: Schematic representation of the mechanisms involved in the protective effect of ILK/AKT pathway in uremic toxins-induced apoptosis of EA.hy926 cells. The added uremic toxins up-regulates ILK pathway, that provides a survival signal which protects the endothelial cells from the decreases proliferation and increases apoptosis induces by uremic toxins.

CONCLUSIONS:

-This study demonstrate for the first time the implication of ILK in the endothelial cells apoptotic process induced by uremic toxins, that are difficult to remove by standard dialysis strategies.

-These results identify a molecular mechanism that could play a protective role in early stages of endothelial dysfunction observed in uremic patients

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