CYTOTOXIC EFFECTS OF p-CRESOL IN RENAL TUBULAR CELL

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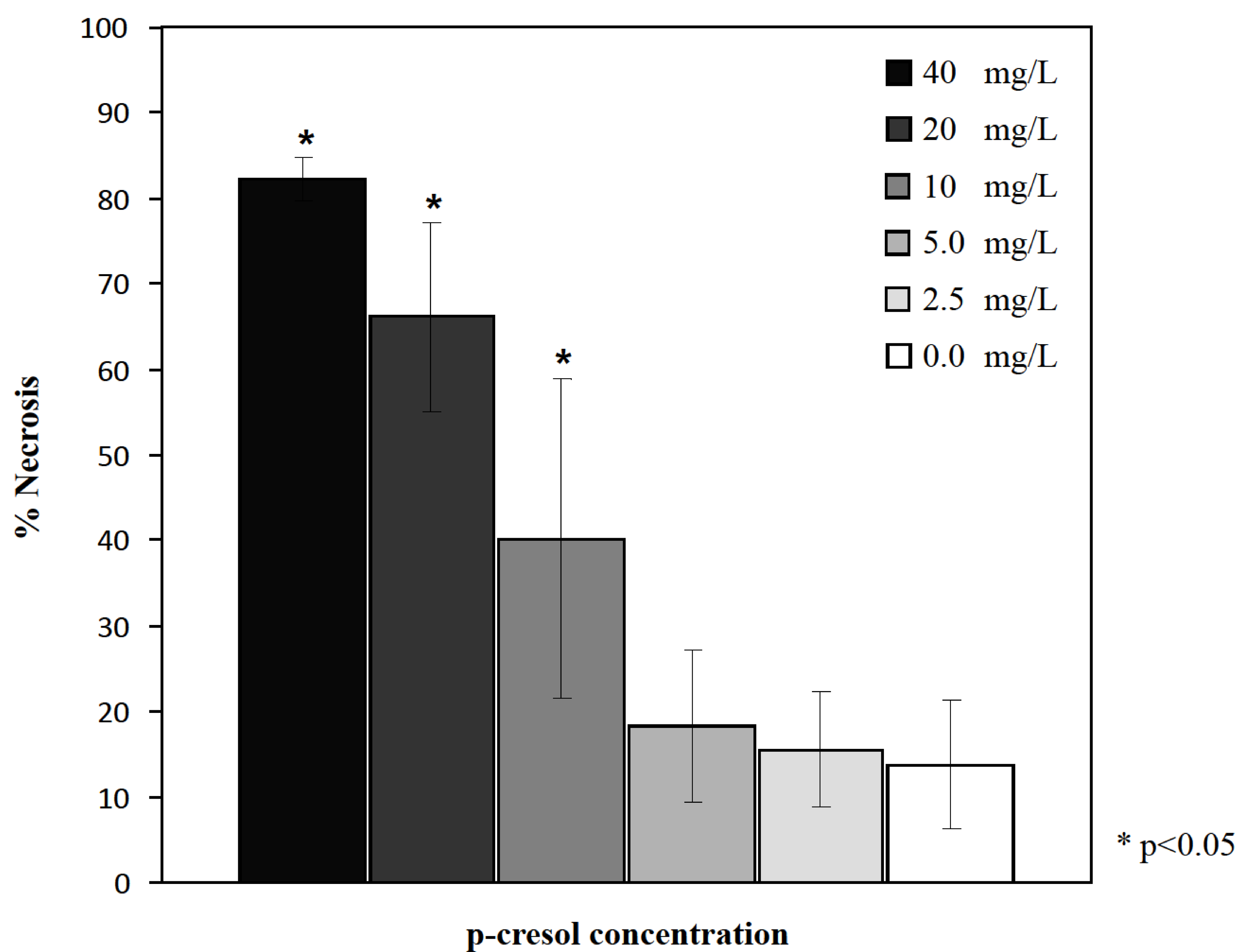
INTRODUCTION and AIMS

Uremic syndrome is characterized by a deterioration of kidney function due to the accumulation of uremic toxins. Uremic toxins are particularly difficult to remove by conventional dialysis treatments and are the major causes of mortality in CKD patients. One of the uremic toxin is p-cresol, a fenol protein-bound lipofile. The effects of p-cresol is well known in different type of cells. Many studies have revealed dual effect of uremic retention solutes on leukocyte function: blunting upon stimulation and basal activation linked to microinflammation, malnutrition, and atherosclerosis. Granulocyte function is depressed by a longer incubation with p-cresol. p-cresol inhibits both cytokine-induced expression of endothelial adhesion molecules and stimulates monocyte adhesion to endothelial cells. Moreover, p-cresol inhibits endothelial cells proliferation and wound repair in vitro, and it may be involved in increase in endothelial permeability in CKD patients. Despite the presence of many studies on p-cresol effects in different cell lines, it's still poorly understand what determines in epithelial renal cells, considering that kidney is the principal organ involved in uremic syndrome.
Our aim is to evaluate in vitro effect of p-cresol on renal tubular epithelial cell line (LOC), in terms of apoptosis and necrosis, to better understand the pathophysiological impact of this toxin.

METHODS

We incubated LOC for 24 hours in medium with scalar concentration of p-cresol, from 40 mg/L (up level in uremic patient) to 2.5 mg/l.
We used untreated LOC as negative controls.
We perform a qualitative analysis of cellular viability in treated LOC by detection of DNA ladder showing the typical apoptotic DNA fragmentation.
We perform also a quantitative analysis of cell viability in term of apoptosis and necrosis by Annexin V/Propidium Iodide Cytofluorimetric assay.
In addition, we evaluated Caspase-3 concentration by Enzyme-Linked Immuno Sorbent Assay (ELISA).
All experiments were performed 5 times.

RESULTS



We observed a strong DNA fragmentations in cells treated with low concentration of p-cresol. At increasing concentration of toxin, we noticed a decreasing DNA Ladder because the necrosis is almost the only type of cell death. We confirmed the presence of apoptotic and necrotic pathways by quantitative analysis. Cytofluorimetric determinations showed that p-cresol concentrations ≥20 mg/L cause the necrosis of >60% of cells; instead at concentration ≤5 mg/L the percentage of necrosis was comparable to control (figure).
We detected a positive trend, but no significant relationship between Caspase-3 levels and p-cresol concentrations.
We may better understand the pathophysiology of this phenomenon increasing the number of experiments.

CONCLUSIONS

In conclusion, p-cresol caused cellular death in renal tubular cells, determining necrosis in almost total cultured cells at the maximal concentration. At lower concentration, p-cresol determined cell death through apoptosis. This data was confirmed by Caspase-3 activation.
This results were consistent with the clinical studies showing a link between high concentrations of plasma-free p-cresol and haemodialysis patients outcomes, as hospitalization rates for infection disease and cardiovascular events. These results highlight the necessity of developing new therapeutic and dialytic strategies to increase p-cresol removal in CKD patients.

50ERA-EDTA 373--SP
Experimental models of CKD
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DOI: 10.3252/pso.eu.50era.2013

Poster presented at: ERA-EDTA 50° Congress Istanbul, Turkey
PosterSessionOnline.com