BISPHENOL A IS A UREMIC TOXIN THAT PROMOTES MITOCHONDRIAL INJURY AND DEATH IN TUBULAR CELLS

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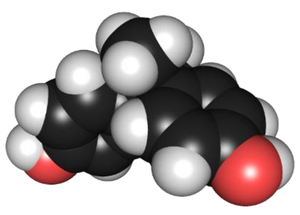
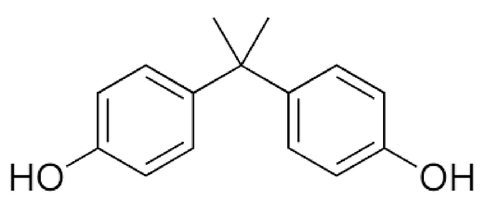
INTRODUCTION

Protein-bound uremix toxins, such a p-Cresol (pC) and metabolites, are harmful chemicals difficult to remove by hemodialysis. Bisphenol A is a ubiquitous environmental toxin, structurally related with pC, that accumulates in CKD, but is not currently considered a uremic toxin. Our aim was to characterize the nephrotoxic potential of BPA. Specifically, we addressed whether it disrupts mitochondrial function and causes cell death in energy demanding cells as tubular cells.





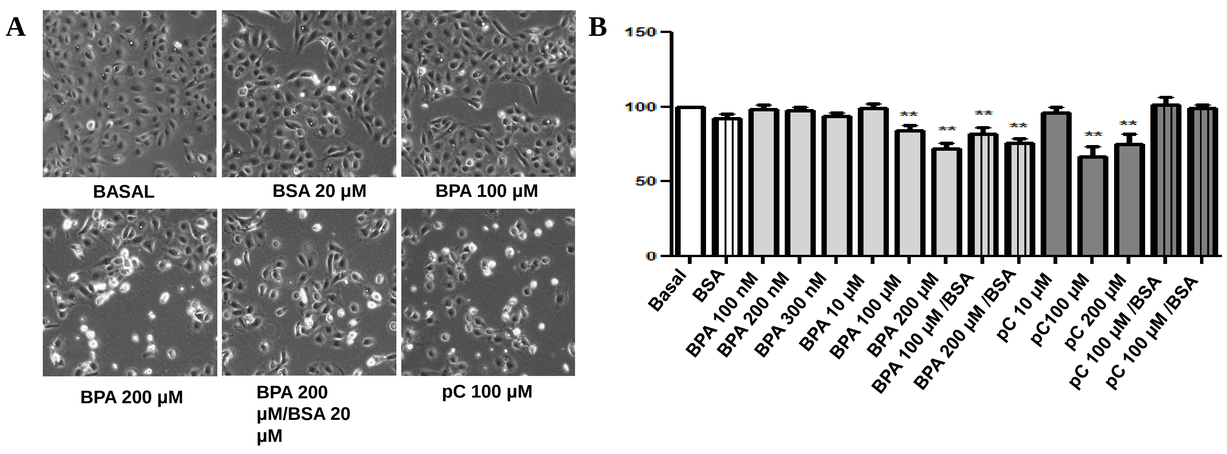
METHODS

Experiments were performed on HK-2 human proximal tubular epithelial cells.Cell death and oxidative stress were evaluated by flow cytometry and confocal microscopy in HK-2 human proximal tubular epithelial cells. Functional assays tested ATP, intracellular Ca^{2+}, mitochondrial function (TMRM), oxygen consumption, Nrf2-binding and NAPDH oxidase activity. Gene expression was assessed by qRT-PCR.

RESULTS

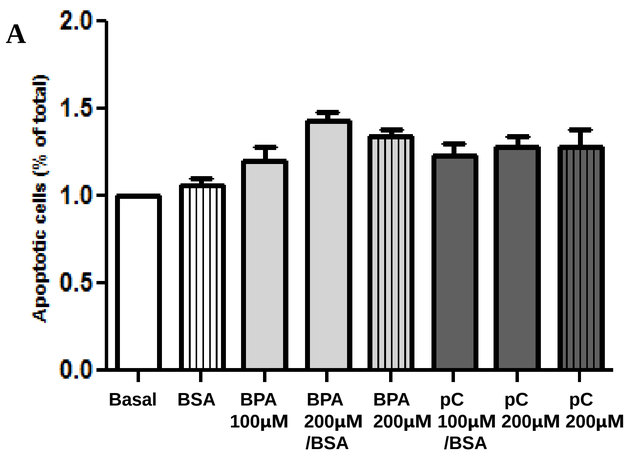
Following acute exposure (24h), proximal tubulo-epithelial cell viability is only affected by BPA or pC at concentrations higher than 100 µM. The observed mechanisms are similar for both toxins, since they both promote mitochondrial dysfunction leading to energy depletion, mitochondrial and cytoplasmic oxidative stress (MitoSOX and NAPDH oxidase) and apoptosis in a concentration-dependent manner. An antioxidant response was observed consisting of Nrf2 translocation and increased expression of the Nrf2 target genes Heme oxygenase 1 (HO-1) and NAD(P)H dehydrogenase [quinone] 1 (NQO-1).

BPA decrease proximal tubular cell viability



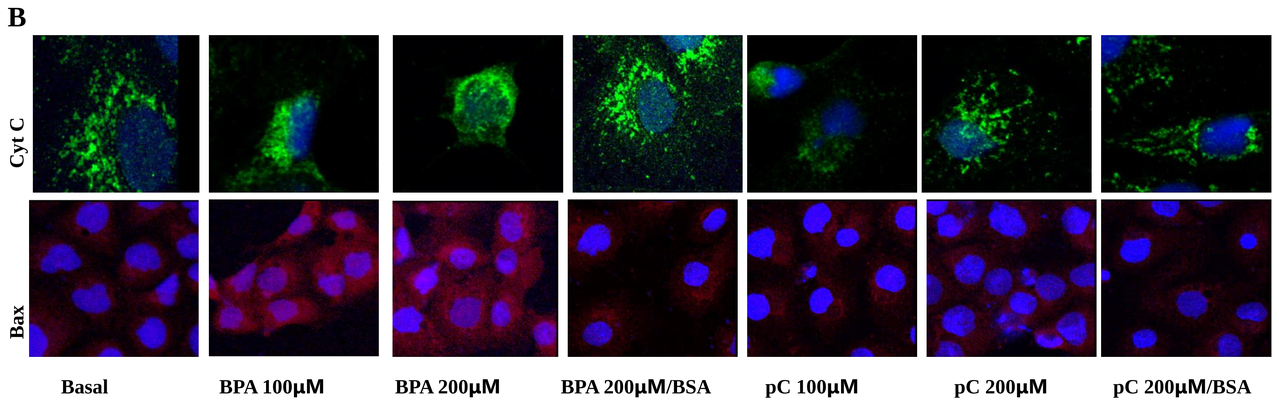
Proximal tubular cells are high energy demanding cells, sensitive to energetic disruptions and loss of proximal tubular cells contributes to CKD progression. We tested whether BPA modulates tubular cell viability. Exposure for 24 h to BPA or pC decreased cell viability at concentrations higher than 100 µM, as assessed by optical microscopy (Figure A) or MTT assay (Figure B).

BPA promotes tubular cell apoptosis



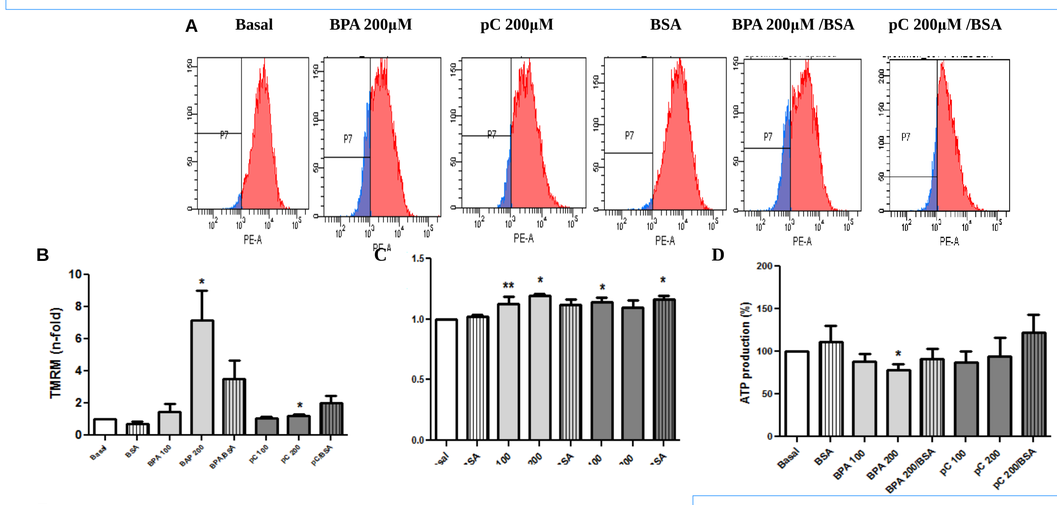
BPA and pC tubular cell toxicity was mainly due to apoptosis as assessed by propidium iodide and annexin V stainin. BPA and pC increased apoptosis dose-dependently (Figure A). Addition of albumin to culture media did not change apoptosis.

Immunofluorescence revealed a diffuse cytoplasmic Cytochrome C staining pattern in cells exposed to either toxin, consistent with release from the mitochondrial compartment (Figure B). Upon BPA or pC exposure, Bax, a critical mediator of mitochondrial injury in the signaling of apoptosis, shows redistribution to the mitochondria, forming aggregates. Albumin decreased BPA- and pC-induced Bax aggregates (Figure B) .



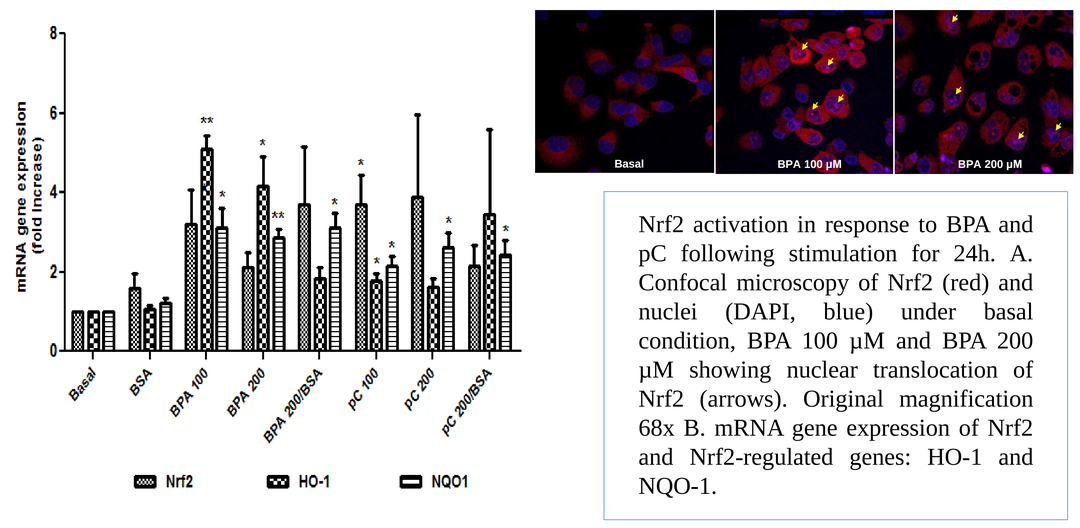
BPA promotes mitochondrial dysfunction and oxidative stress in tubular cells

Effect on mitochondrial chemiosmotic gradient expressed as fold change vs control as assessed by TMRM following stimulation for 24h. Blue represents depolarization. BPA or pC at 100 µM induced a mild increase (25-30%) in mitochondrial depolarization, while stimulation with BPA at 200 µM increased mitochondrial depolarization 6-fold. **C**. Effect on intra-mitochondrial calcium concentration expressed as fold change vs control as assessed by Fura-2. **D.** Effect on ATP synthesis expressed as % change vs control as assessed by ATP assay kit.



E. Oxidative stress was assessed following stimilation for 24h, BPA an pC concentrations expressed in µM. Intramitochondrial superoxide anion was assessed using a fluorescent probe (MitoSOX), and we also assessed cytoplasmic NADPH oxidase ROS production (F).

Adaptive antioxidant responses



Nrf2 activation in response to BPA and pC following stimulation for 24h. A. Confocal microscopy of Nrf2 (red) and nuclei (DAPI, blue) under basal condition, BPA 100 µM and BPA 200 µM showing nuclear translocation of Nrf2 (arrows). Original magnification 68x B. mRNA gene expression of Nrf2 and Nrf2-regulated genes: HO-1 and NQO-1.

CONCLUSIONS

This study demonstrates for the first time that BPA causes mitochondrial injury, oxidative stress and apoptotic death in tubular cells. These results characterize BPA as an exogenous toxin that, similar to uremic toxins, may contribute to CKD progression.

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