# Bisphenol A Induced Podocitopathy with Proteinuria and Hypertension in Mice

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## INTRODUCTION

In recent years, humans have suffered considerable exposure to bisphenol A (BPA) which is widely used in the production of polycarbonate plastic and epoxy resins lining food and beverage containers. The USA Centers for Disease Control and Prevention found BPA present in the urine of 95% of US adults (1,2). Recently, in the National Health and Nutrition Examination Survey (NHANES) 2003–2004 and 2005–2006, it was found that higher urinary BPA concentrations were associated with various diseases including cardiovascular diseases and type 2 diabetes (3,4). More recently, exposure to BPA has been associated with low-grade albuminuria in Chinese adults (5). However, no studies have examined the potential role of BPA in the pathogenesis of renal diseases, particularly in diabetes.

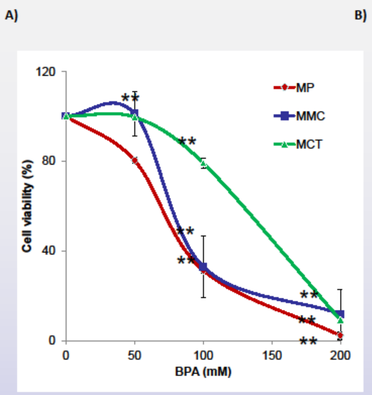
## AIMS

To assess the renal effects of BPA in renal cells as well as in the kidney of mice.

## MATERIALS AND METHODS

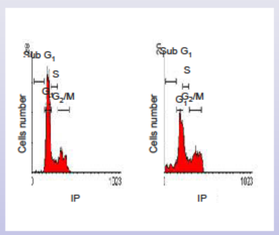
**Cell cultures:** We used three well-established mouse kidney cell lines – cortical tubule MCT cells, mesangial cells and podocytes.
**Western Blot Analysis: P**odocytes were cultured in the presence of 100 and 1000 nM of BPA and protein expression was analyzed by Western blot.
**MTT cell viability assay:** After treatments MTT was added to each well and the plates were incubated for 1 h at 37ºC. Then, thia was solubilizaded and the absorbance was measured at a test wavelength of 570 nm.
**Flow cytometry:** Flow cytometry was used to detect apoptotic cells and the distribution of cell cycle.
**Cell hypertrophy index:** Cells counted using a Neubauer haemocytometer. Equal numbers of cells were lysed and the total protein content was determined by the Bradford's method. Total protein was expressed as micrograms of protein per $10^4$ cells.
**[H3] leucine incorporation:** Cells were pulsed [H3] leucine, and then solubilized and the precipitated proteiins were centrifuged and resuspended in 0.5N NaOH. Aiiquots were counted in a scintillation counter.
**Animal model:** CD1 mice (25-30g) were used. To analyze the renal effects of BPA, mice (N=17) were intraperitoneally injected with BPA at 50mg/Kg -dissolved in oil- Monday through Friday for five weeks.
**Studies in Conscious Animals and Sample Collection:** Arterial pressure was measured in conscious animals by means of a tail-cuff sphygmomanometer. Also, mice were placed in metabolic cages and 24-hour urine was collected for creatinine and protein measurement as previously reported. Blood was taken by cardiac puncture under ether anaesthesia, for creatinine measurement. One kidney of each animal was removed, weighed and the total proteins were extracted. The remaining kidney was fixed for morphological and immunohistochemical studies. The degree of renal hypertrophy was expressed as an index, the ratio of kidney weight to total body weight.
**Statistical analysis:** All results are expressed as mean ± SEM. Statistical significance ($p<0.05$) was assessed by Kruskal-Wallis test or ANOVA, when appropriate.

## RESULTS




B)

| | C | BPA 100 µM |
|---|---|---|
| SubG1 | 2,03±0,95 | 9,19±2,72** |
| G1 | 57,37±9,63 | 48,75±9,37 |
| S | 12,08±4,96 | 12,25±3,78 |
| G2/M | 24,46±3,11 | 24,44±4,44 |

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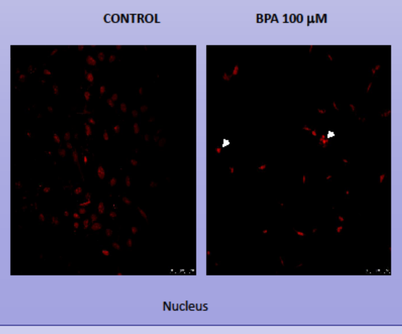



**Figure 1. A) Effect of bisphenol A (BPA) in cell viability on renal cells.** MP, MMC and MCT were incubated with different doses of BPA for 72 hours, and the cell viability was assayed by MTT. *P<0.05 and **P<0.01 using ANOVA test for the comparison between control and BPA-treated cells. **B) Changes in the cell cycle** were measured by cell cytometry. **C) Visualization of apoptotic cells** displayed "apoptotic bodies" (arrows) and dense chromatin.

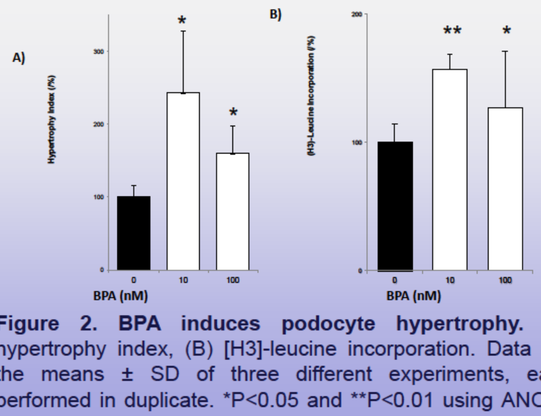
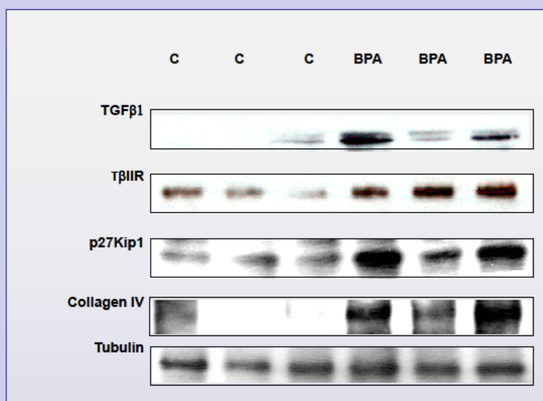



**Figure 2. BPA induces podocyte hypertrophy.** (A) hypertrophy index, (B) [H3]-leucine incorporation. Data are the means ± SD of three different experiments, each performed in duplicate. *P<0.05 and **P<0.01 using ANOVA test



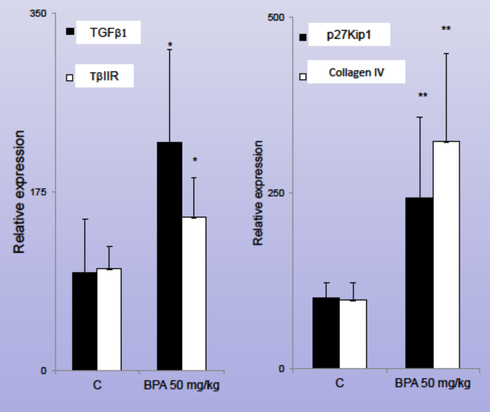
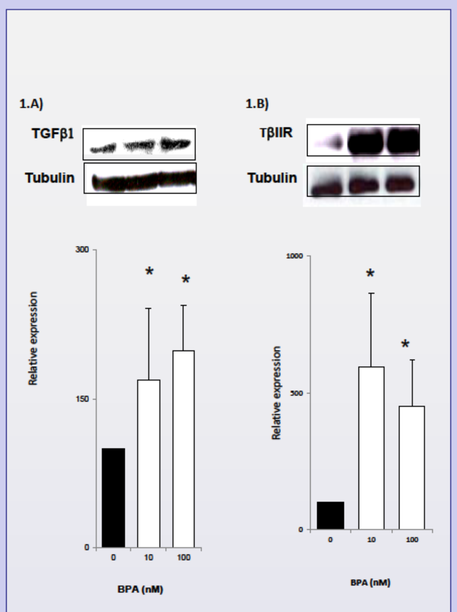
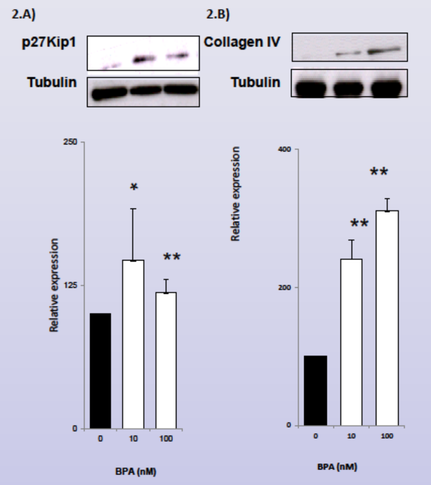




**Figure 7. Renal protein expression in BPA-injected mice.** The TGFβ1 system, p27Kip1 as well as collagen IV were analyzed by *Western blot*. Data are the means ± SD of three different experiments, each performed in duplicate. *P<0.05 and **P<0.01 using ANOVA test for the comparison between control and BPA-injected mice.



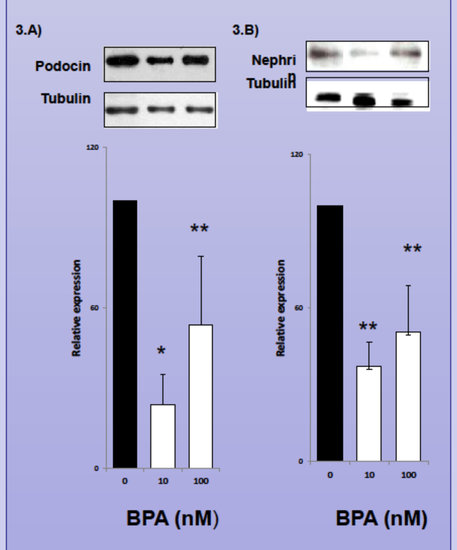




**Figure 3. Expression of TGFβ1, TβIIR, P27e$^{kip1}$, collagen IV, podocin an nephrin on podocytes cultured in the presence of Bisphenol A (BPA)**. It was analyzed by *Western blot*. Data are the means ± SD of three different experiments, each performed in duplicate. *P<0.05 and **P<0.01 using ANOVA test for the comparison between control and BPA-treated cells.

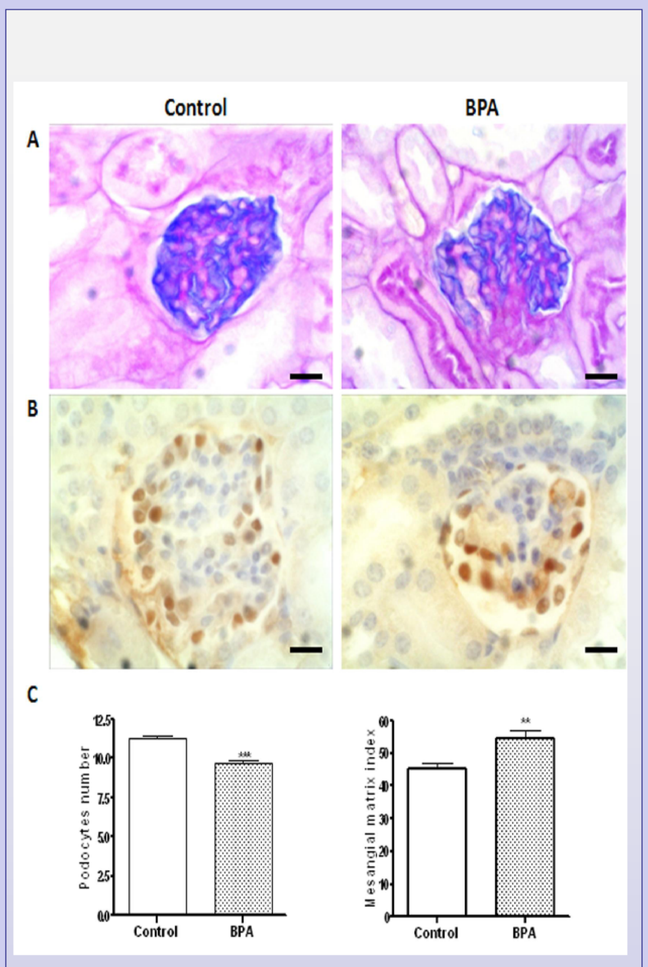
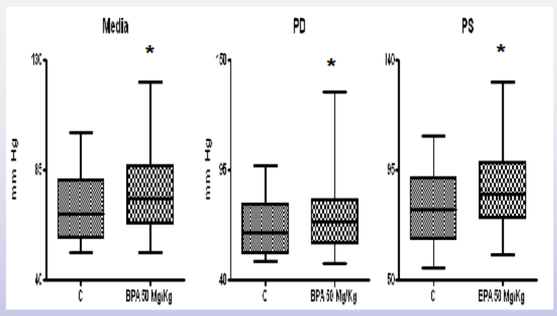



**Figure 5. Renal histology and immunohistochemistry for WT-1.** A: Sections from kidney of control and BPA-injected mice stained with PAS-Alcian blue. The renal corpuscles from BPA mice showed higher staining with PAS and alcian blue than controls (pink and blue colours). X300. B: WT-1 labelling. In BPA-injected mice the podocyte cells number (brown nuclei) was decreased with respect to control mice. X300. C: Left, graph representing the statistical analysis for the podocyte cell number. Right, mesangial matrix index. Data are means±SD. **$p$< 0.01, *** $p$<0.0001




**Figure 6. Electron micrographs of podocytes from control mice (A) and BPA-injected mice (B and C).** B. The cytoplasm of BPA-injected mice showed an enlarged cytoplasm (asterisk) as well as broadening of foot processes (white star). C. Presence of collagen fibers (star) between two podocytes (arrows) with light cytoplasm and condensed chromatin suggesting apoptotic images.




**BPA induces proteinuria, renal hypertrophy and glomerular hyperfiltration:** BPA-injected mice (N=17) display an increased urinary albumin excretion (C 2.31±1.4 mg/24h vs BPA 5.13±1.5, p<0.05); an increase in the hypertrophy index (C 0.60±0.06 mg/g vs BPA 0.68±0.04, p<0.05) as well as an increases in the glomerular filtration rate (C 0.05 ml/min vs BPA 0.08, p<0.05) as assessed by the endogenous creatinine clearance.

**Figure 4. Levels of blood pressure in mice treated with of bisphenol A (BPA).** Levels of systolic pressure (A), diastolic pressure (B) and pressure media. Data are the means±SD of three different experiments, each performed in duplicate. *P<0.05 and **P<0.01 using ANOVA test for the comparison between control and BPA-treated cells.

## CONCLUSIONS

These data demonstrate that Bisphenol-A exposure promotes a podocytopathy with proteinuria, glomerular hyperfiltration, podocytopenia as well as arterial hypertension. Further studies are needed to clarify the potential role of Bisphenol-A in the pathogenesis of renal diseases, particularly in diabetic patients.

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