BISPHENOL A INDUCES ENDOPLASMIC RETICULUM STRESS, APOPTOSIS AND A PROINFLAMMATORY RESPONSE ON ENDOTHELIAL CELLS

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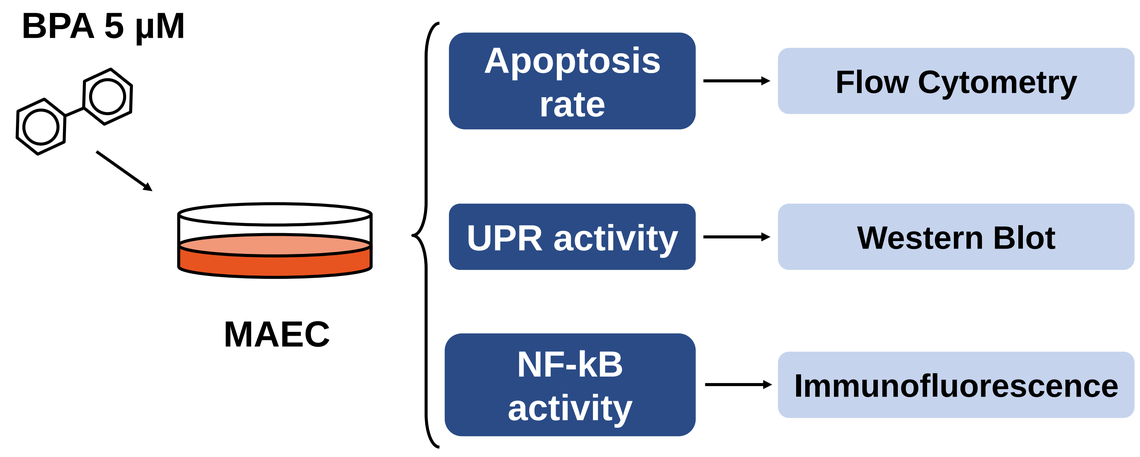
INTRODUCTION

Bisphenol A (BPA; 2,2-*bis*-(4-hydroxyphenyl) propane) is a synthetic monomer used in the production of polycarbonate plastics and epoxy resins. BPA exposure has been associated with cardiovascular diseases. In previous studies, we observed that BPA exposure causes endothelial dysfunction and hypertension in mice by uncoupling eNOS activity, resulting in oxidative and nytrosative stress through accumulation of reactive oxygen species (ROS) (Saura, 2014). Moreover, recent studies suggest that BPA may enhance atherosclerosis, despite the underlying molecular mechanism remains unidentified (Fang, 2015). We analyzed the activation of the unfolded protein response (UPR), which is triggered when there is an excessive accumulation of unfolded proteins within the Endoplasmic Reticulum (ER) This process is activated to resist the stress, and if this is not achieved, to promote apoptosis to avoid tissue damage. One of the consequences of the UPR-related survival process is the activation of NF-κB, which is known to be a pro-inflammatory factor.

The goal of this study was to determine if BPA activates the UPR and if BPA triggers the apoptotic and/or the inflammatory pathway.

EXPERIMENTAL DESIGN

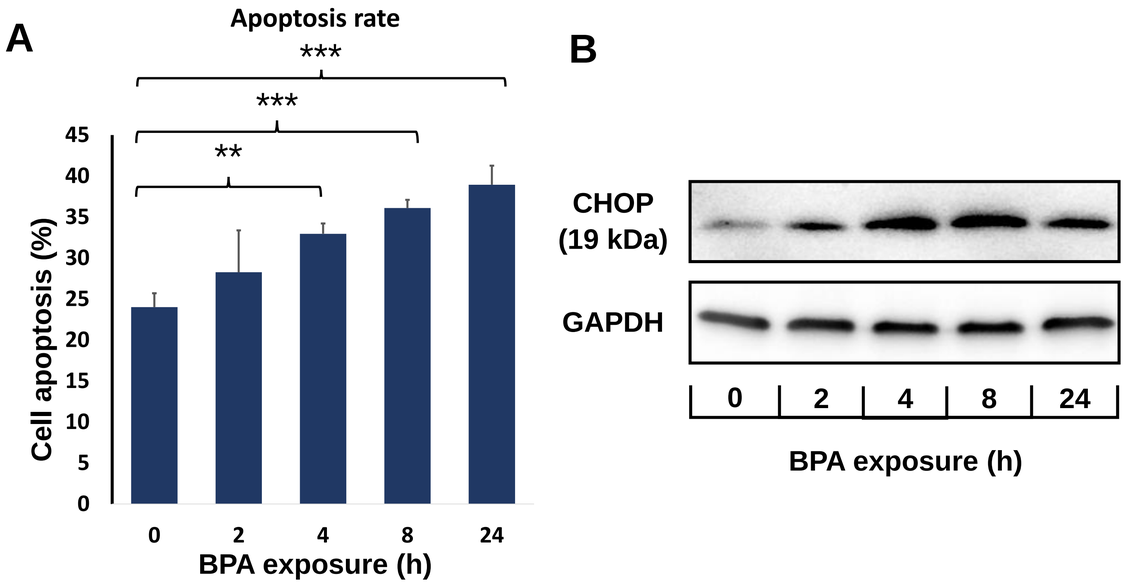


METHODS

Mouse Aortic Endothelial Cells (MAEC) were exposed to BPA 5 µM for 0-24 hours. Apoptotic rate was determined by flow cytometry, and protein expression was analyzed by Western Blot and Immunofluorescence.

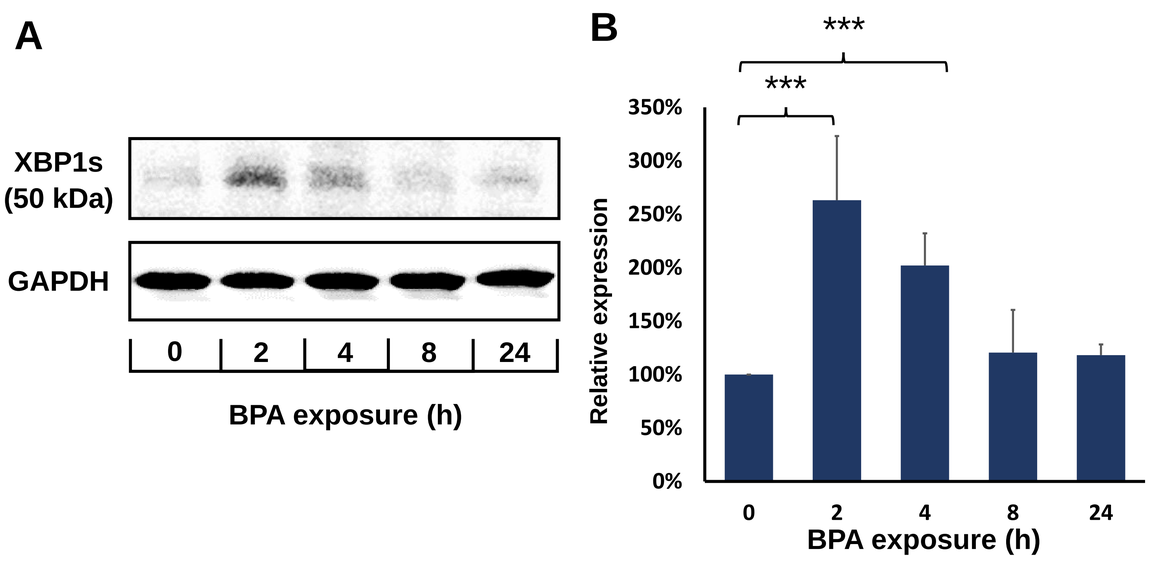
RESULTS

Figure 1.- Apoptosis induction in BPA-exposed MAEC.



A) The DNA fragmentation assay performed by flow cytometry in a time course (0-24 h) of BPA exposure showed a positive relation between apoptotic rate and BPA increasing exposure time.**p<0.01, ***p<0.001 (n=3). **B)** Also, this correlated with the expression of the proapoptotic UPR protein CHOP, determined by Western blot at the same times, using GAPDH as loading control.

Figure 2.- BPA exposure induces XBP1 mRNA splicing.

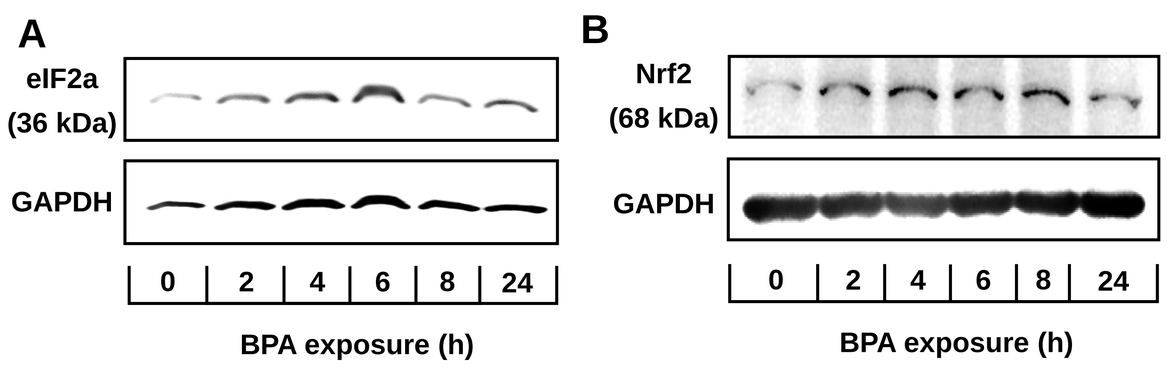


IRE1 is one of the activating proteins of the UPR, and it promotes the XBP1 mRNA splicing. The resulting protein, XBP1s (50 kDa), triggers pro-survival responses. **A)** Western blotting of protein extracts from BPA-treated MAEC showed that BPA induces the XBP1s expression at short times (2 and 4h). **B)** Densitometry analysis of the XBP1s expression from 3 independent experiments. ***p<0.001.

CONCLUSIONS

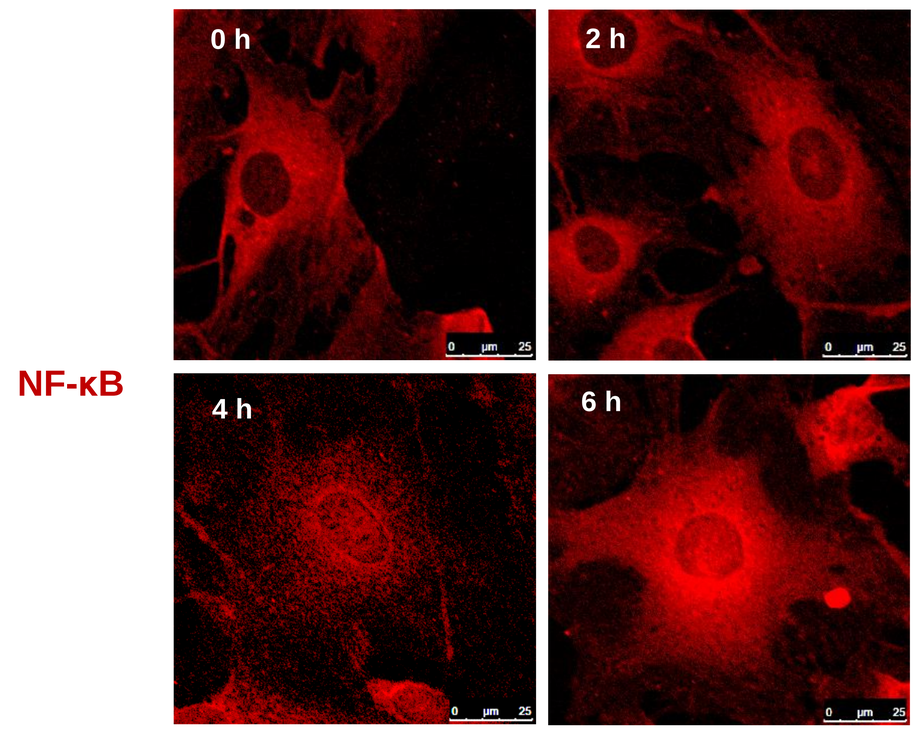
Under a BPA concentration of 5µM, both IRE1 and PERK pathways of the Unfolded Protein Response are activated, promoting an early antioxidant and pro-inflammatory response and a latter apoptotic response induced by the activity of CHOP.

Figure 3.- BPA induces the eIF2α expression and antioxidant responses.



PERK is another starting protein of the UPR. It activates eiF2a, which can triggers pro-survival or pro-apoptotic responses. The pro-apoptotic response will end with the expression of CHOP, and the pro-survival response will promote the activity of Nrf2, an antioxidant protein. **A)** Immunoblot showed that BPA activates the PERK pathway by inducing eIF2α expression at early times (2, 4 and 6 hours). **B)** Also, Nrf2 is expressed the same way, although at 24 hours the antioxidant response returns to a basal state. In both blots GAPDH was used as loading control.

Figure 4.- NF-κB translocation to the nucleus is promoted in BPA-exposed MAEC.



NF-kB, known to be a protein related with the UPR and inflammatory processes, translocates to the nucleus in MAEC under BPA 5 µM exposure mainly at 4 and 6 hours.

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