Phorbol ester-modulation of estrogenic genomic effects triggered by the environmental contaminant benzanthracene

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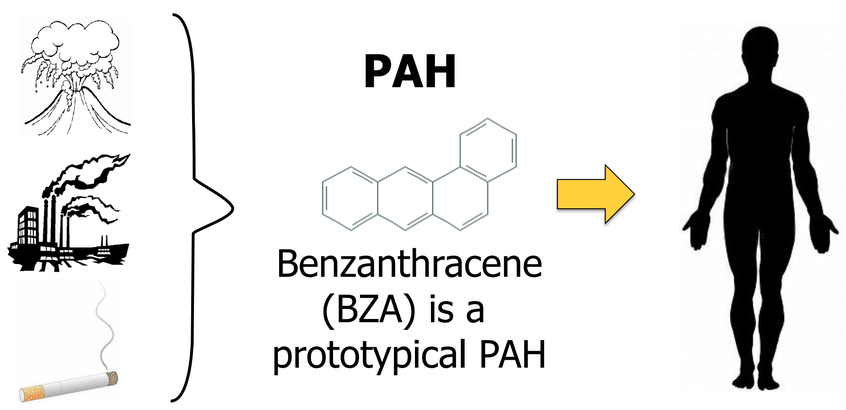
PNRPE — PROGRAMME NATIONAL DE RECHERCHE SUR LES PERTURBATEURS ENDOCRINIENS

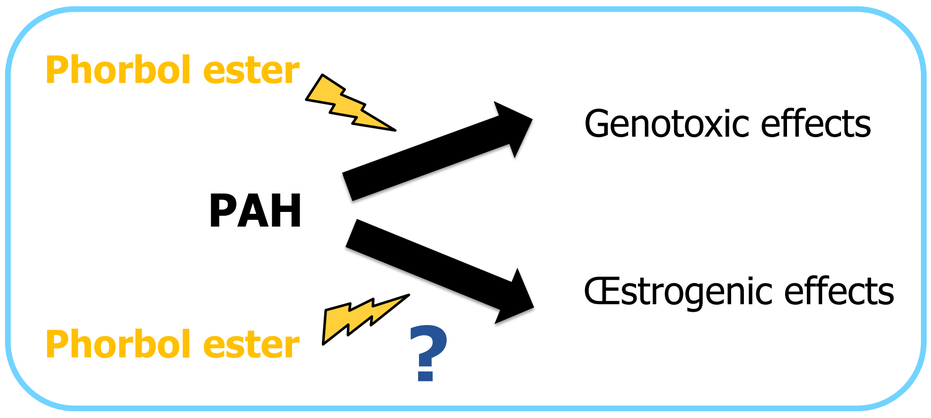
Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widely-distributed environmental contaminants, to which humans are commonly exposed. They originate from incomplete combustion of organic materials and are notably found in diet, cigarette smoke, diesel exhaust particles and some occupational atmospheres. They exert various deleterious effects towards human health, especially carcinogenic, immunosuppressive, atherogenic and inflammatory effects. They are also considered as endocrine disruptors and some PAHs such as benzanthracene (BZA) have notably been shown to display estrogenic effects.



Aim

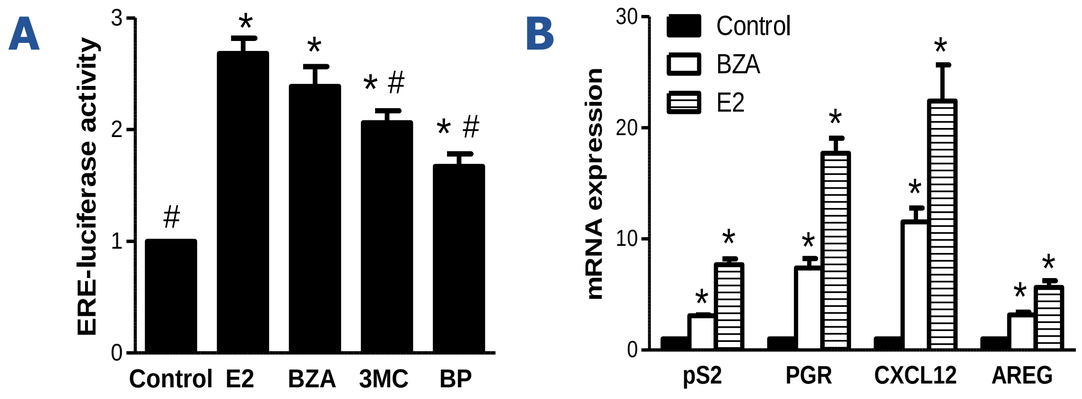
Genotoxic effects of PAH have been shown to be modulated by phorbol ester such as phorbol 12-myristate 13-acetate (PMA), wich is a potent activator of proteines kinases C (PKC).



The present study investigated whether PKC activation can also modulate estrogenic effects of BZA.

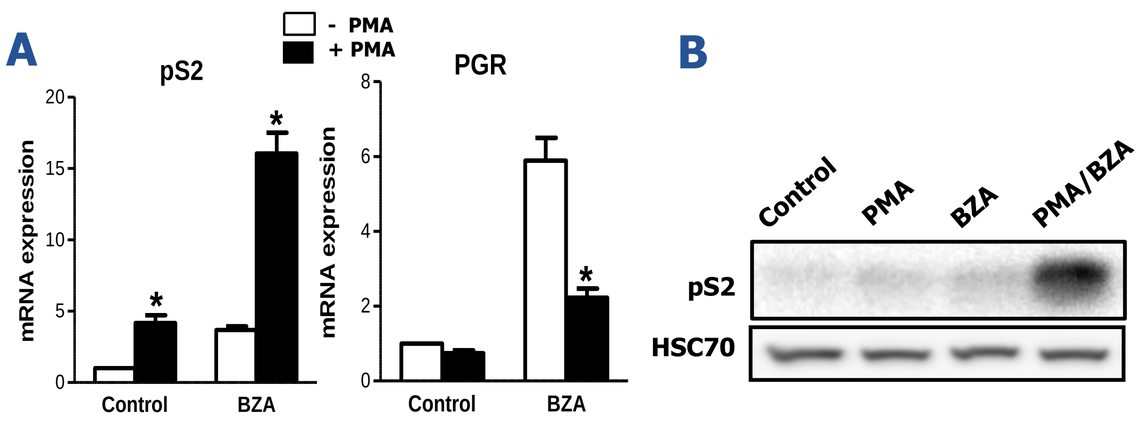
Results

BZA and others PAH exert estrogenic effects by up-regulation of various target genes



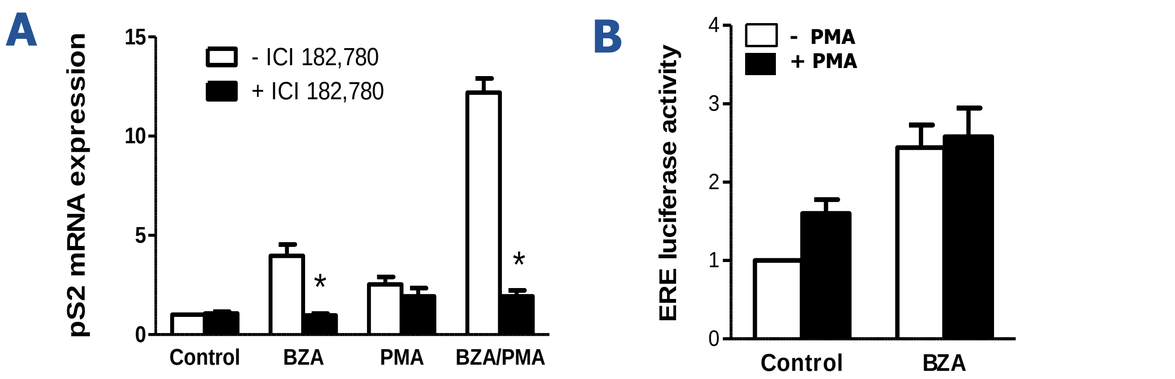
(A) MCF-7/MELN cells were untreated or exposed to 10 µM BZA, 10 µM 3-methylcholanthrene (3MC), 10 µM benzo(a)pyrene (BP) or 10 nM estradiol (E2) for 8 h. ERE-related luciferase activities were then determined. *, $p<0.05$ (when compared to control cells); #, $p<0.05$ (when compared to E2-exposed cells). **(B)** MELN cells were either untreated or exposed to 10 µM BZA or 10 nM E2 for 8 h. mRNA expression of pS2, PGR, CXCL12 and AREG was then determined by RT-qPCR. *, $p<0.05$ (when compared to control cells)

The PKC activator PMA differentially modulate estrogenic genomic effects triggered by BZA



MELN cells were either untreated or exposed to 10 µM BZA in the absence or presence of 80 nM PMA for 8 h. **(A)** mRNA expression of pS2 and PGR was then determined by RT-qPCR. *, $p<0.05$ (when compared to control cells). **(B)** pS2 protein expression was determined by Western blotting.

Estrogen receptor is implicated in PMA-mediated modulation of estrogenic genomic effects of BZA, in a non-uniform way

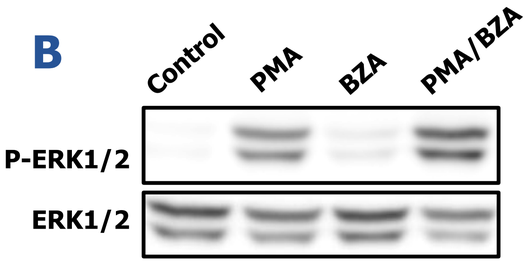


(A) MELN cells were either untreated, exposed to 10 µM BZA or 80 nM PMA or co-exposed to BZA and PMA, in the absence or presence of the estrogen receptor antagonist ICI 182,780 for 8 h. pS2 mRNA expression was then determined by RT-qPCR. *, $p<0.05$ (when compared to counterparts not exposed to ICI 182,780). **(B)** MELN cells were either untreated or exposed to 10 µM BZA in the absence or presence of 80 nM PMA for 8 h. ERE-related luciferase activity was next determined. *, $p<0.05$ (when compared to counterparts not exposed to PMA).

Modulation by PMA of estrogenic genomic effects of BZA is mediated by PKC and by ERK

A

Inhibitor	% inhibition	targets
GFX	69	PKC-α,-β,-δ,-ε
Gö6983	62	PKC-α,-β,-γ,-δ
Gö6976	23	PKC-α,-β
Rottlerin	43	PKC-δ
siPKCδ	70	PKC-δ
U0126	57	MEK1/2
PD98059	52	MEK1/2



(A) MELN cells were either untreated, exposed to 10 µM BZA or 80 nM PMA or co-exposed to BZA and PMA, in the absence or presence of various kinase inhibitors indicated in the Table for 8 h. Percentage of inhibition were calculated considering expression of pS2 mRNA determined with PMA/BZA co-treatment without inhibitor as 0 % inhibition. **(B)** MELN cells were either untreated, exposed to 10 µM BZA or 80 nM PMA or co-exposed to BZA and PMA, for 8 h. Expression of phospho-ERK and total ERK was then determined by Western blotting.

Conclusions

In summary, the phorbol ester PMA was shown to differentially modulate estrogenic genomic response triggered by the PAH BZA, in a PKC- and ERK-dependent manner. Knowing that PKC activation can be triggered by various pollutants to which humans are also exposed, such as pesticides or surfactants, such modulation could occur in realistic conditions. Non-genomic modulation of estrogenic effects of PAHs by PKC activation may have therefore to be considered with respect to the deleterious effects of these environmental contaminants towards the endocrine system.

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