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Environmental pollutants parathion, paraquat and bisphenol A show distinct effects towards nuclear receptors-mediated induction of xenobiotics-metabolizing cytochromes P450 in human hepatocytes

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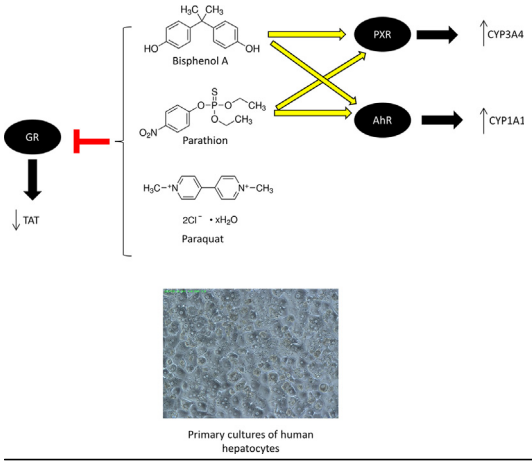
HIGHLIGHTS

- Parathion and bisphenol A activate AhR and induce CYP1A1 mRNA in HepG2 cells.
- Parathion and bisphenol A induce CYP3A4 mRNA and protein in human hepatocytes.
- Paraquat antagonizes glucocorticoid receptor action in human hepatocytes

GRAPHICAL ABSTRACT



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ABSTRACT

Environmental pollutants parathion, bisphenol A and paraquat were not systematically studied towards the effects on the expression of phase I xenobiotics-metabolizing cytochromes P450 (CYPs). We monitored their effects on the expression of selected CYPs in primary cultures of human hepatocytes. Moreover, we investigated their effects on the receptors regulating these CYPs, particularly arylhydrocarbon receptor (AhR), pregnane X receptor (PXR) and glucocorticoid receptor (GR) by gene reporter assays. We found that parathion and bisphenol A are the activators of AhR. Moreover, they are the inducers of CYP1A1 mRNA in hepatoma cells HepG2 as well as in human hepatocytes by AhR-dependent mechanism via formation of AhR-DNA-binding complex, as revealed by gel shift assay. All three compounds possessed anti-glucocorticoid action as revealed by GR-dependent gene reporter assay and a decline in tyrosine aminotransferase (TAT) gene expression in human hepatocytes. Moreover, parathion and bisphenol A are the activators of PXR and inducers of CYP3A4 mRNA and protein in the primary cultures of human hepatocytes.

In conclusion, the studied compounds displayed distinct activities towards nuclear receptors involved in many biological processes and these findings may help us to better understand their adverse actions in pathological states followed after their exposure.

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1. Introduction

The transcriptional regulation of major xenobiotics-metabolizing human cytochromes P450 occurs through xenoreceptors including aryl hydrocarbon receptor (AhR), pregnane X receptor (PXR) and constitutive androstane receptor (CAR), and also through steroid receptors (e.g. glucocorticoid receptor GR) and nuclear receptors (e.g. vitamin D receptor VDR) (Pascussi et al., 2000, 2001, 2003). Xenoreceptors act as sensing factors that detect drugs, food additives or environmental pollutants and in turn they up-regulate CYPs but also phase II enzymes and transporters in order to eliminate xenobiotics out of the body. Thus, any compounds affecting activity or function of these receptors may have significant impact on metabolism and disposition of xenobiotics and eubiotics, such as lipids, saccharides or hormones (Spruiell et al., 2014; Rysa et al., 2013; Moreau et al., 2008).

Environmental pollutants and food contaminants produced by industry, e.g. pesticides, flame retardants, plasticizers or dyes may have adverse effects on human health, as revealed by numerous epidemiological studies (Gascon et al., 2014, 2015; Valvi et al., 2014; Shim et al., 2009; Dahlgren et al., 2003). There is a great number of data in the literature describing induction of xenobiotics-metabolizing human cytochromes P450 by food contaminants and environmental pollutants (Novotna and Dvorak, 2014; Vrzal et al., 2013; Kamenickova et al., 2013; Ayed-Boussema et al., 2012; Lee et al., 2006; Nekvindova et al., 2006). Interestingly, the effects of notoriously known and common environmental and food contaminants including parathion (PTH), bisphenol A (BPA) and paraquat (PRQ) (Fig. 1A), on the expression of xenobiotics-metabolizing human cytochromes P450, were studied less systematically.

Parathion (PTH) is an organophosphate used as a potent insecticide with highly toxic action to non-target organisms, including humans. Poisoning by this compound was common in agricultural workers especially in the third world (Rastogi et al., 2010; Eskenazi et al., 2004). The plasma level depends on the route of exposure and true toxicokinetics were obtained from accidental or suicidal poisonings (Eyer et al., 2003). Maximal plasma levels of PTH reached 20–35 μM, and the elimination half-life varied among 7.5–30 h (Eyer et al., 2003).

Bisphenol A (BPA) is a monomer of polycarbonate plastics widely used in consumer products. Significant amount of BPA is released into food and human saliva from inner coating of food cans, bottle tops, water pipes and dental composites (Brotons et al.,

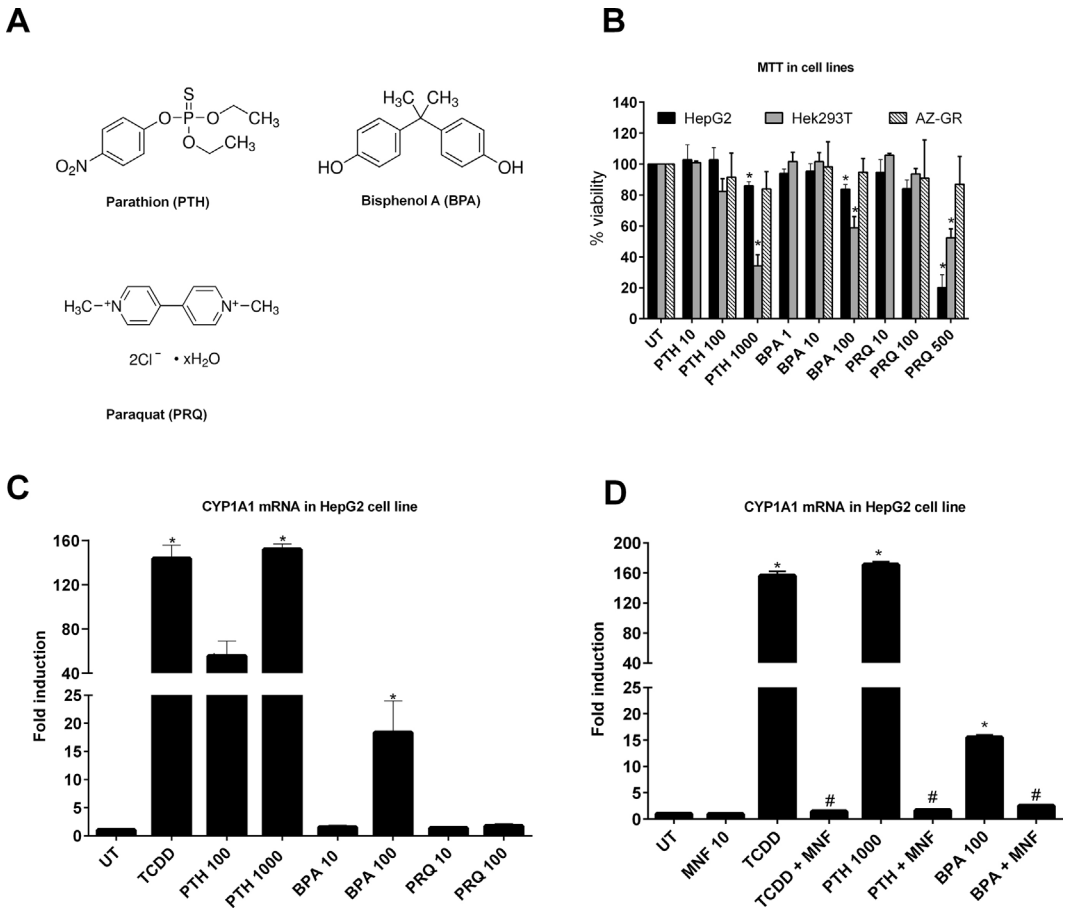

Fig. 1. A, Structures of tested chemicals; B, viability of HepG2, HEK293 and AZ-GR cells by tested compounds–Cell lines HepG2, Hek293T and AZ-GR were seeded into 96-well plates at a concentration 25 000 cells /well and stabilized for 16 h. Thereafter, the cells were treated with parathion (PTH; 10, 100, 1000 μM), bisphenol A (BPA; 1, 10, 100 μM), paraquat (PRQ; 10, 100, 500 μM) and/or DMSO (UT; 0.1% v/v) for 24 h. MTT assay was performed as described in Section 2. Results are expressed as percentage of viability as compared to control cells ± SD and represent the means of three consecutive passages. *–values significantly different from DMSO-treated cells ($p < 0.05$); C, Cells were treated with parathion (PTH; 100 μM, 1000 μM), bisphenol A (BPA; 10 μM, 100 μM), paraquat (PRQ; 10 μM, 100 μM), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; 5 nM) and/or DMSO (UT; 0,1% v/v) for 24 h. Thereafter CYP1A1 mRNA was determined as described in Section 2. Results are expressed as fold induction over DMSO-treated cells ± SD. The data are mean from 3 consecutive cell passages. The data were normalized per GAPDH mRNA levels. *–value is significantly different from DMSO-treated cells ($p < 0.05$); D; HepG2 cells were treated with TCDD (5 nM), parathion (PTH; 1000 μM), bisphenol A (BPA; 100 μM), DMSO (UT; 0,1% v/v) in the absence or presence of 3′-methoxy-4′-nitroflavone (MNF; 10 μM) for 24 h. Thereafter CYP1A1 mRNA was determined as described in Section 2. Results are expressed as fold induction over DMSO-treated cells ± SEM. The data are mean from 3 consecutive cell passages and were normalized per GAPDH mRNA levels. *–value is significantly different from DMSO-treated cells ($p < 0.05$). #–value is significantly different from MNF-untreated counterparts ($p < 0.05$).