

Effects of omeprazol and ketoconazole on aryl hydrocarbon receptor (AHR)

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Abstract

Omeprazole and ketoconazole have been shown to activate the aryl hydrocarbon receptor (AHR) signaling pathway, in spite of the fact that they bind to the receptor with low or no affinity. The aim of this study was to investigate whether ketoconazole and omeprazole can act as indirect activators of AHR. In order to evaluate the effects of ketoconazole and omeprazole on AHR signaling, we measured cytochromes p450 (CYP1A1) enzyme activity by ethoxyresorufin-O-deethylase (EROD) assay as the endpoint. 6-formylindolo [3, 2-b]carbazole (FICZ), at 1nM concentration, caused a transient elevation in the catalytic activity of CYP 1A1. Ketoconazole and omeprazole were found to induce CYP1A1 at concentrations above 50 μ M. At an early time of incubation (3hr), a dose-dependent inhibition of FICZ-induced EROD activity was seen. When omeprazole or ketoconazole were added together with FICZ, a prolonged activation of CYP1A1 was observed at a later time of incubation (24h). Taken together, our findings support our earlier observation that CYP 1A1 inhibitors can act as AHR activators though inhibition of metabolic degradation of FICZ.

Keywords: Aryl hydrocarbon receptor, Omeprazol; Ketoconazole, 6-formylindolo [3, 2-b]carbazole (FICZ), Cytochromes p450 (CYP1A1).

1. Introduction

Aryl hydrocarbon receptor (AHR) is a ligand dependent transcription factor, belonging to the basic helix loop helix super family of transcription factors, which responses to the structurally diverse groups of chemicals including synthetic and natural compounds by regulation of expression of AHR target genes (1). Among AHR target genes, those genes encoding drug metabolism enzymes, including the cytochromes p450 (CYP1A1, CYP1A2 and CYP1B1) as well as NAD (p) H:quinine oxidoreductase1, aldehyde dehydrogenase 3 and glutathione S transferase A1, are well

described (4). Beside the critical role of CYP enzymes in biotransformation of endogenous and exogenous chemicals in particular, CYP A1 and B1 have received more attention due to their role in bio-activation of pro-carcinogens into carcinogens and formation of reactive metabolites (5). AHR mediates toxicity of many environmental pollutants such as the most toxic anthropogenic compound, 2, 3, 7, 8- tetracholorodibenzo-p-dioxin (TCDD) (2).

After ligation, AHR undergoes conformational changes and moves into the nucleus, where it binds to its nuclear partner, aryl hydrocarbon receptor nuclear translocator (ARNT). Then the AHR-ARNT hetero-dimer is attached to the xenobiotic response element (XRE) and causes

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transcriptional activation of AHR target genes (3). Among all natural activators of AHR tested so far, a photoproduct of tryptophan, 6-formylindolo [3, 2-b]carbazole (FICZ), has been identified as the most potent endogenous activator of AHR (6). It has been found that FICZ can induce CYP1A1 as well as the other AHR target genes (7-10). FICZ, as an ideal substrate for CYP1A1, has been proposed as a potential endogenous ligand that is able to regulate its physiological function through AHR-CYP1A1-FICZ regulatory feedback mechanism (7, 11). Despite of its efficient metabolic clearance in the body, FICZ can be distributed in the body via the systemic circulation and induces significant but transient expression of CYP1A1 in an effective manner (6, 11).

Several classes of natural compounds and drugs with different molecular weights have been found to activate the AHR signaling pathway, leading to expression of AHR downstream target genes, in spite of the fact that they bind to the receptor with low or no affinity (6, 12-14). It has been already shown that two widely prescribed pharmaceutical drugs, omeprazole (OMP) and ketoconazole (KTZ) are able to induce CYP1A1 through activation of AHR signaling pathway, but their mode of action is not yet understood (15, 16). In our earlier study, we showed that some polyphenols, heavy metals, and oxidants are able to inhibit the metabolic degradation of FICZ and thereby indirectly cause activation of AHR signaling (6, 12, 13). In this study, we aim to determine whether KTZ and OMP can activate AHR indirectly by inhibition of metabolic clearance of FICZ.

2. Material and methods

2.1. Materials

FICZ was obtained from Syntactic AB (Sweden). Dimethyl sulfoxide (DMSO), 7-ethoxyresorufin, KTZ, and OMP were purchased from Sigma-Aldrich (Germany). All cell culture reagents and the media used in this study were purchased from Invitrogen.

2.2. Cell culture and treatments

Human HepG2 cells were grown in DMEM supplemented with 10% fetal bovine se-

rum (FBS), 100 µg/ml streptomycin, and 100 IU/ml penicillin at 37 °C in 5% CO₂ atmosphere. Treatments were started by replacing the growth medium with fresh medium without FBS, containing different concentrations of the desired tested chemicals. The treatment lengths varied and are indicated in the results section.

2.3. Cell viability assay

HepG2 cells were seeded onto 96-well plates and grown for 24 hr. The cells were treated in a medium without FBS containing FICZ and the mentioned drugs for 24 hr. After the incubation period, the medium was removed and the cells were rinsed with PBS. Then, the cells were exposed to MTT dye for 4 hr and the blue purple formazan crystal products were dissolved in DMSO for quantification of cell viability by measuring the absorption at 570 nm.

2.4. Determination of CYP1A1 enzyme activity

To examine whether KTZ and OMP are able to induce the CYP1A1-dependent 7-ethoxyresorufin O-deethylase (EROD) activity, HepG2 cells were seeded onto 96-well plates and grown for 24 hr and then treated with FICZ and drugs alone or in combination, for 3 and 24 hr. After treatment period, the medium was removed and the cells were rinsed with PBS. The EROD activity was started by adding 2 µM 7-ethoxyresorufin in sodium phosphate buffer (50 mM, pH 8.0) to each well and cells were incubated at 37 °C for 20 min. Then formation of resorufin was measured at excitation and emission wavelengths of 535 nm and 590 nm. Finally, the data were normalized according to the amount of cellular protein content.

2.5. Statistical Analysis

All measurements were performed in triplicates (n=3). Dose- and time-course studies of EROD activity were performed at least in three independent experiments. For comparison between the experimental groups, one-way ANOVA analysis following Tukey test or student t-tests was used. $p < 0.05$ was considered to be statistically significant. Data are expressed as means ± SD.

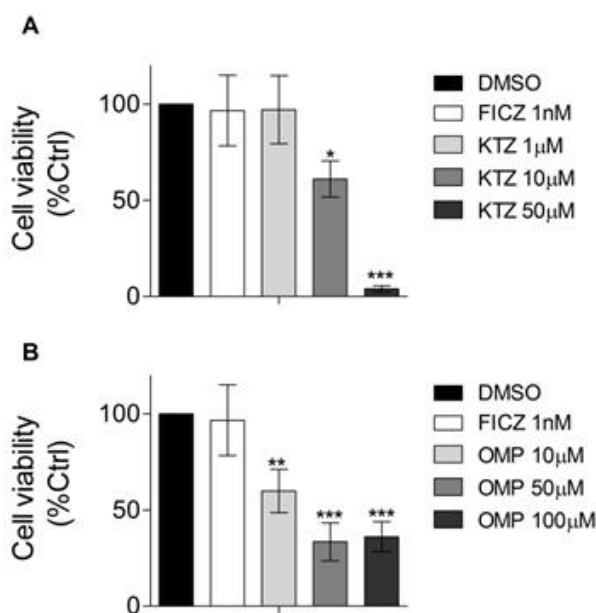


Figure 1. Effects of KTZ, OMP, and FICZ on cell viability of HepG2 cells determined by the MTT assay. (A) Cells were exposed to KTZ (1-50 μM) and FICZ (1nM) for 24 hr. (B) Cells were exposed to OMP (10-100 μM) and FICZ (1nM) for 24 hr. Results are presented as means ± S.D. from three independent experiments. Statistically significant difference with control: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

3. Results

3.1. Effect of KTZ and OMP on cell viability

HepG2 cells were treated with different concentrations of drugs or FICZ for 24 hr. After incubation periods, cell viability was measured according to the materials and methods. The results

showed that FICZ alone or drugs at low concentrations had no significant effect on cell viability (Figure A and B). KTZ and OMP at highest concentration resulted in a remarkable declining in the cell viability of HepG2 cells (Figure A and B).

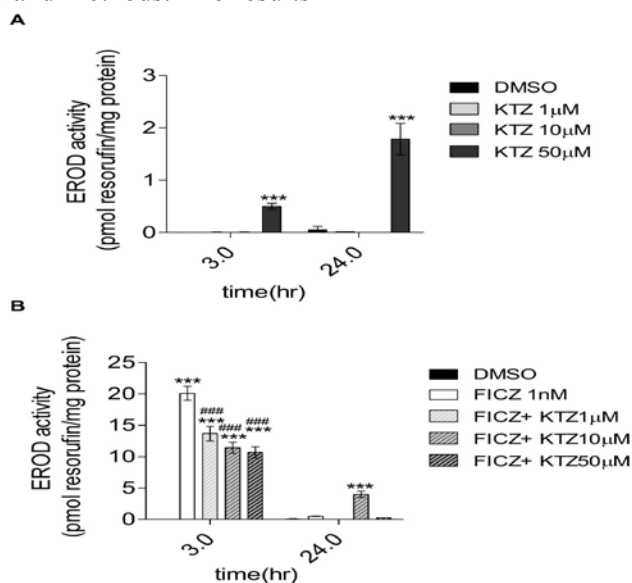


Figure 2. CYP1A1 enzyme activity in HpG2 cells treated with (A) KTZ (1, 10 or 50 μM) alone or (B) in combination with FICZ (1 nM). Treatments were terminated after 3 or 24 hr, and the EROD activity (pmol resorufin/mg protein) was measured. Data are expressed as means±SD. *** $P < 0.001$ statistically significant difference with control; #### $P < 0.001$ statistically significant difference with FICZ treated group.

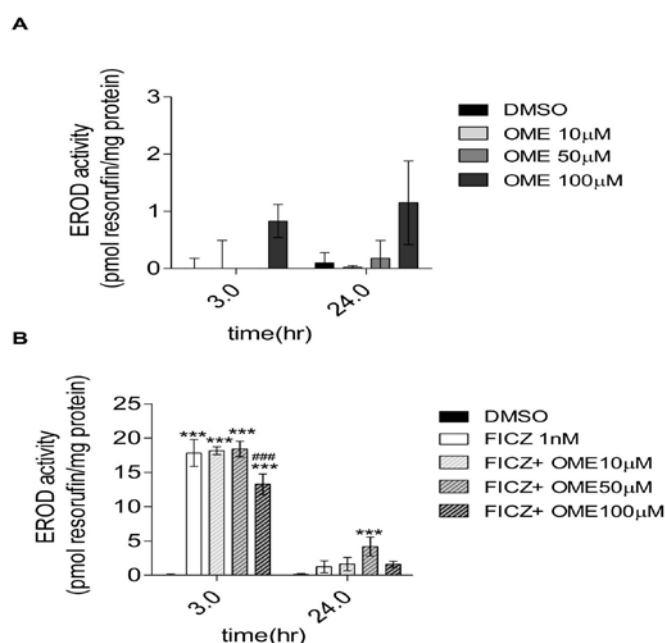


Figure 3. CYP1A1 enzyme activity in HpG2 cells treated with (A) OMP (10, 50 or 100 µM) alone or (B) in combination with FICZ (1nM). Treatments were terminated after 3 or 24 hr, and the EROD activity (pmol resorufin/mg protein) was measured. Data are expressed as means ± SD.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ statistically significant difference with control; ### $P < 0.001$ statistically significant difference with FICZ treated group.

3.2. Impact of KTZ and OMP on CYP1A1 enzyme activity

CYP1A1 enzyme activity is shown in figures 2A and 3A. Treatments of HepG2 cells with 1 and 10 µM KTZ or 10 and 50 µM OMP had no significant effects on EROD activity after 3 and 24 hr exposure. However, the enzyme activity was increased by 50 µM KTZ or 100 µM OMP after 3 hr. FICZ, at 1 nM concentration, caused a transient elevation in the catalytic activity of CYP1A1. KTZ and OMP were found to be inducer of CYP1A1 at concentrations above 50 µM. At an early time of incubation (3 hr) KTZ and OME caused inhibition of FICZ-induced EROD activity. When OMP or KTZ were added together with FICZ, a prolonged activation of CYP1A1 was observed at a latter time of incubation (Figure 2B and 3B).

4. Discussion

In this study, CYP1A1 enzyme activity was chosen to test the AHR-activating capacity of KTZ and OMP. It has already been shown that activators of AHR are inducers of CYP1A genes, so the EROD assay is an accepted method for detecting AHR activation capacity of chemicals. Our

results showed that both the tested pharmaceutical drugs were able to induce EROD activity by themselves (Fig 2A and 3A). As we expected, a transient elevation in the catalytic activity of CYP1A1 was seen by FICZ (Figure 2A and 3A). When OMP or KTZ were used in combination with FICZ, a prolonged CYP1A1 activity was observed (Figure 2B and 3B).

Our results demonstrated a transient induction of EROD activity after 3 hr treatment with FICZ. This finding is not too surprising, because FICZ is an excellent AHR ligand and it can be metabolized by the CYP1A1 rapidly and efficiently (11). In accordance with our previous results, a significant elevation in the CYP1A1 enzyme activity was shown following the exposure to OMP or KTZ (17, 18). KTZ and OMP are considered to be non-classical AHR agonists; however, their mode of action is not completely elucidated. Although it is generally accepted that ligand binding is a key step for AHR-mediated transcriptional activation, our results, as well as some other reports have shown that OMP is an inducer of CYP1A1 without binding to the AHR ligand binding pocket (16, 19, 20). Likewise, ligand binding studies revealed

that KTZ is a weak ligand of AHR and it can weakly cause AHR translocation into the nucleus and binding to the xenobiotic response element (XRE) (18). We speculated that OMP and KTZ can induce CYP1A1 transcription through a pathway other than direct activation of AHR. Our results and some other previous studies demonstrated that FICZ at very low concentrations (picomolar) can activate the AHR *in vivo* and *in vitro* (6). It has been shown that inhibition of CYP1A1 by oxidants such as UVB and H₂O₂, phytochemicals, and metals can lead to enduring presence of FICZ and sustained induction of AHR through an indirect mechanism (6, 12, 13). Since KTZ and OMP have exhibited CYP1A1-inhibiting properties (Fig 2 and 3) (21-23), it can be expected that prolonged AHR activation by these chemicals is through an indirect mechanism. AHR activation by these compounds alone can be explained by low levels of FICZ existing in the commercial DMEM medium;

and this background level of FICZ is sufficient to induce CYP1A1 enzyme (6). Taken together, these findings support our earlier finding that indicated an indirect way of AHR activation by molecules with low or no affinity for binding to the AHR through inhibition of FICZ metabolism.

Funding

This work was supported by the Research Grants from the Shiraz University of Medical Sciences, Iran (Grant numbers 93-01-05-8157; 93-01-05-8158).

Acknowledgement

The authors of this manuscript wish to express their appreciation to Shiraz University of Medical Sciences, Shiraz, Iran.

Conflict of Interest

None declared.

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