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Role of CYP3A4 in the regulation of the aryl hydrocarbon receptor by omeprazole sulphide

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Abstract

Cross-talk between nuclear receptors involved in the control of drug metabolism is being increasingly recognised as a source of drug side effects. Omeprazole is a well known activator of the aryl hydrocarbon receptor (AhR). We investigated the regulation of AhR by omeprazole-sulphide, a degradation metabolite of omeprazole, using *CYP1A* mRNA induction, reporter gene assay, receptor DNA binding, ligand binding, nuclear translocation, trypsin digests, and drug metabolism analysis in mouse Hepa-1c1c7, human HepG2 cells and primary human hepatocytes. Omeprazole-sulphide is a pure antagonist of AhR in Hepa-1c1c7 and HepG2 hepatoma cell lines. In Hepa-1c1c7 cells, omeprazole-sulphide is a ligand of AhR, inhibits AhR activation to a DNA-binding form, induces a specific pattern of AhR trypsin digestion and inhibits AhR nuclear translocation and subsequent degradation in response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. However, in highly differentiated primary human hepatocytes treated with rifampicin an agonist of the pregnane X receptor (PXR), omeprazole-sulphide behaves as an agonist of AhR. Inhibition of drug metabolizing enzymes by ketoconazole restores the antagonist effect of omeprazole-sulphide. Metabolic LC/MS analysis reveals that omeprazole-sulphide (AhR antagonist) is efficiently converted to omeprazole (AhR activator) by cytochrome P450 CYP3A4, a target gene of PXR, in primary human hepatocytes but not in hepatoma cells in which PXR is not expressed. This report provides the first evidence for a cross-talk between PXR/CYP3A4 and AhR. In addition, it clearly shows that conclusions drawn from experiments carried out in cell lines may lead to erroneous in vivo predictions in man.

Keywords: CYP3A4; Aryl hydrocarbon receptor; Preganae X receptor; Omeprazole-sulphide; Rifampicin; Antagonist; Agonist; Cross-talk

1. Introduction

Xenobiotic metabolism is dependent on two main groups of genes. First, those encoding xenobiotic metabolizing and transporting systems (XMTS), including notably the super-families of cytochrome P450s (CYP1-CYP3), conjugation enzymes and transporter proteins [1,2]. Second, those encoding specific receptors (so-called xenosensors) capable of controlling the coordinated and adapted expression of XMTS in response to xenobiotics, including notably the aryl hydrocarbon receptor (AhR) [3–5], the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR) [6–11].

Abbreviations: OM, omeprazole; OMS, omeprazole-sulphide; CYP, cytochrome P450; AhR, aryl hydrocarbon receptor; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; DMSO, dimethylsulfoxide; 3-MC, 3-methylcholanthrene; RIF, rifampicin; KT, ketoconazole; HPLC/UV/MS, high performance liquid chromatography analysed with a UV detector and coupled to mass spectrometry; IC_{50}, concentration producing 50% inhibition.

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The aryl hydrocarbon receptor (AhR) is a member of the PAS family of basic helix-loop-helix ligand-activated transcriptional factors [4,12,13]. In the absence of ligand, the AhR is a cytoplasmic protein characterized by association with the molecular chaperone hsp90 and co-chaperone proteins. Upon binding of an agonist, AhR protein undergoes a series of structural changes that lead to its nuclear translocation where hsp90 is released from the AhR, and heterodimerisation with ARNT, another basic helix-loop-helix protein. The heterodimer binds to specific xenobiotic responsive elements and activates a battery of genes including members of cytochrome P450 (CYP) family 1 and many other genes primarily involved in the detoxication and transport of xenobiotics and drugs. Because of the major role of the AhR in the metabolism, toxicity, teratogenicity and genotoxicity of the numerous aryl hydrocarbons present in diet and environment, an impressive number of studies have been devoted to the regulation of this receptor and its target genes [4,14,15].

PXR and CAR are members of the nuclear receptor superfamily (NR1I2 and NR1I3) and form functional heterodimers with RXRα [6–11]. Both receptors are expressed mainly in the liver, small intestine, colon as well as in the kidney but are not expressed or expressed at very low level in other tissues and not expressed in most cell lines. These receptors are activated by a wide variety of structurally diverse exogenous and endogenous compounds including drugs such as rifampicin, phenobarbital, steroid hormones and derivatives, and dietary compounds such as coumestrol and hyperforin. PXR and CAR appear to be responsible for the xenobiotic-mediated induction of a battery of genes including CYP2B, 2C and 3A families, glucuronosyltransferases, sulfotransferases, and drug transporters [16].

Omeprazole (OM), a benzimidazole-derived antiulcer drug acting as a gastric proton pump inhibitor in stomach parietal cells [17], is known to be an aryl hydrocarbon-like inducer of CYP1As and has been shown to be a potent activator of the AhR [18–22]. We have recently undertaken a structure-activity relationship study on the effect of various derivatives and analogues of OM on AhR activation. In the present study we show that OMS a degradation metabolite of OM, is a ligand and pure antagonist of AhR in mouse and human hepatoma cell lines. However, when tested in cultured highly differentiated primary human hepatocytes exhibiting significant drug metabolism activity, OMS behaves as an agonist of the AhR. Strickingly, modulations of the drug metabolism activity of the cells and notably CYP3A4 through the use of enzyme inducer (rifampicin) or inhibitor (ketoconazole) lead to corresponding modulations of the apparent properties of OMS from an antagonist to an agonist and vice versa, resulting from the conversion of OMS to OM. We conclude that CYP3A4, a target gene of PXR, play a critical role in controlling the functional interaction between OMS and the AhR. Thus OMS is expected to play a different role (antagonist versus agonist of AhR) in different tissues or cell populations depending on the expression of both PXR and CYP3A4.

2. Materials and methods

2.1. Chemicals

Dulbecco's modified Eagles, Williams E and Ham-F12 culture media, dimethylsulfoxide (DMSO), 3-methylcholanthrene (3-MC), rifampicin (RIF), ketoconazole (KT) and culture medium additives were from Sigma (Saint Louis, MO). 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) was from BCP instrument (Lyon, France), omeprazole (OM) and omeprazole sulphide (OMS) were kindly provided by Astra Hassle (Molndal, Sweden), γ-(^{32}P)d-ATP was from Amersham International (Amersham, England). Chromatography solvents (*n*-hexane, isopropanol), methanol, and chloroform were purchased from Merck (Nogent sur Marne, France).

2.2. Primary human hepatocyte and hepatoma cell cultures

Hepatocytes were prepared from lobectomy segments, resected from adult patients for medical reasons unrelated to our research program. The tissue encompassing the tumor was dissected by the surgeon and sent for anatomopathological studies, while the remaining tissue was used for hepatocyte preparation. No information on the patients was available to us, apart from age and sex and the reason for surgery. Hepatocytes were isolated as previously described [23,24]. Twenty four hours after plating (freshly plated hepatocytes) cells were treated for 16 h with 10 nM TCDD or 100 μM OM, in the absence or presence of 3–50 μM OMS, control cultures receiving DMSO only. In other experiments, the standard culture medium was discarded and replaced by a long-term (serum-free) culture medium consisting of William's E medium (Sigma) supplemented as described [23]. In some experiments, hepatocytes were pre-treated for 3 days with 10 μM rifampicin (RIF), 1 μM ketoconazole (KT) or both (RIF+KT), before treatment with TCDD and OMS as indicated above. Mouse Hepa-1c1c7 and human HepG2 hepatoma cells were cultured in DMEM supplemented with 10% FCS, non essential aminoacids, 2 mM glutamine, 1 μM sodium pyruvate (HepG2 only), 100 mg/ml penicillin and streptomycin and treated as indicated above.

2.3. RNase protection assay and Northern blot

Total RNA was isolated from cultured cells and analysed either by RNase protection assay or by Northern blot, as described [19,21].