Short Communication

Pharmacological inhibitors of JNK and ERK kinases SP600125 and U0126 are not appropriate tools for studies of drug metabolism because they activate aryl hydrocarbon receptor

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Abstract. Mitogen-activated protein kinases (MAPKs) are important regulators of aryl hydrocarbon receptor (AhR). An immense progress in MAPKs´ biochemistry was attained with the discovery of their specific pharmacological inhibitors. Unfortunately, the inhibitors of JNK and ERK MAPKs, i.e. SP600125 and U0126, respectively, affect AhR-CYP1A signaling pathway because they are partial agonists of AhR and induce CYP1A genes. This implies that SP600125 and U0126 are inappropriate tools for studies of the role of MAPKs in AhR regulation. The results from studies using SP600125 or U126, past or future, should be interpreted with prudence regarding their stimulatory effects on AhR-CYP1A pathway.

Key words: Aryl hydrocarbon receptor — MAPK — JNK — ERK — SP600125 — U0126

Superfamily of cytochrome P450 enzymes plays an important role in the biotransformation of xenobiotics, activation of procarcinogens to ultimate carcinogens, and in the metabolism of endogenous compounds. The regulation of many P450s occurs at transcriptional level and it involves steroid receptors (glucocorticoid receptor; estrogen receptor), nuclear receptors (vitamin D receptor; retinoic acid receptor), and xenoreceptors (constitutive androstane receptor; pregnane X receptor; aryl hydrocarbon receptor (AhR)), including their co-repressors, co-activators and dimerization partners (retinoic X receptor; AhR nuclear translocator) (Pascussi et al. 2004, 2008; Dvorak et al. 2005; Pavek and Dvorak 2008). Transcriptional activity of these receptors is tightly regulated, involving ligand-dependent activation, proteasome degradation, transcriptional/translational control or covalent modification. There is growing body of evidence that mitogen-activated protein kinases (MAPKs) are important regulators of these receptors. Recently, we have published review dealing with the mutual interactions between MAPKs and AhR (Henklova et al. 2008). Hence, studying a role of MAPKs in the regulation of P450 or in the function of P450s´ regulators is of value.

MAPKs are involved in essential cellular processes, such as signal transduction, apoptosis and carcinogenesis (Dhillon et al. 2007). MAPKs family comprises basically three members: c-Jun-N-terminal kinase (JNK) (Weston and Davis 2007), p38 kinase (Bradham and McClay 2006) and extracellular-regulated protein kinase (ERK) (Meloche and Pouyssegur 2007). An immense progress in the research of MAPKs´ was the discovery of specific pharmacologic inhibitors of JNK, p38 and ERK, i.e. SP600125 (Bennett et al. 2001), SB203580 (Cuenda et al. 1995) and U0126 (Favata et al. 1998), respectively (for structures see Fig. 1). Hence, it sounds logically to use these compounds in elucidating the role of MAPKs in P450s regulation. However, the major drawback of pharmacological inhibitors is, in general, their

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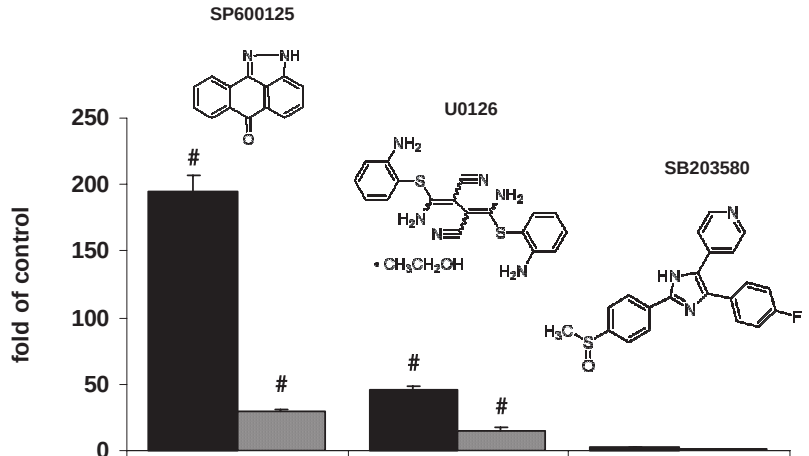

	SP600125	U0126	SB203580
CYP1A1	195	46	3
CYP1A2	29	15	1

Figure 1. Effects of MAPKs´ inhibitors on CYP1A1 and CYP1A2 mRNAs expression in primary human hepatocytes. Cells were treated for 24 h with vehicle (DMSO) and specific inhibitors of JNK (SP600125; 10 μmol/l), ERK (U0126; 10 μmol/l) and p38 (SB203580; 10 μmol/l). CYP1A1 and CYP1A2 mRNAs were determined as described in Materials and Methods section. Showed are the data from culture FT280. The data are expressed fold induction over DMSO-treated cells, normalized *per* GAPDH mRNA levels. # value is significantly different from control cells ($p < 0.05$).

non-specificity and possible interferences with other cellular targets. Indeed, this paper describes that SP600125 and U0126 stimulate AhR-CYP1A signaling pathway.

Originally, SP600125 was identified as a ligand and antagonist of AhR (Joiakim et al. 2003). Ongoing study shows that SP600125 is not antagonist but partial agonist of human AhR that significantly induces CYP1A genes in human hepatocytes and HepG2 cells (Dvorak et al. 2008). Similarly, U0126, a specific inhibitor of ERK upstream kinase MEK1, was demonstrated as a partial agonist of the AhR in Hepa-1 cells where it induced CYP1A1 protein (Tan et al. 2002). Andrieux and co-workers also showed that U0126 is a ligand and agonist of AhR, since it induced CYP1A1 mRNA and protein in primary rat hepatocytes and human hepatoma B16A2 cell line (Andrieux et al. 2004). In contrast, another authors found that U0126 is not an AhR ligand since even at high dose it did not displace [3H]-2,3,7,8-tetrachlorodibenzo-p-dioxin from binding to AhR. However, it facilitated AhR nuclear translocation and enhanced AhR/ARNT dimer binding to dioxin responsive element (Chen et al. 2005).

In the current paper, the effects of MAPKs inhibitors SP600125, SB203580 and U0126 on AhR-dependent expression of CYP1A1 and CYP1A2 genes in primary cultures of human hepatocytes were screened.

Human hepatocytes were isolated and cultured as previously described (Pichard-Garcia et al. 2002). Experiments were performed in three independent primary human hepatocytes cultures (five cultures for SP600125). RT-PCR measurements were performed in duplicates.

Isolation of total RNA, synthesis of cDNA and RT-PCR determination of CYP1A1, CYP1A2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were performed as described elsewhere (Vrzal et al. 2008). Relative expression of CYP1A1 and CYP1A2 mRNAs was normalized on GAPDH as housekeeping gene.

Primary cultures of human hepatocytes were treated for 24 h with vehicle (DMSO) and specific inhibitors of JNK (SP600125; 10 μmol/l), ERK (U0126; 10 μmol/l) and p38 (SB203580; 10 μmol/l). High concentration of MAPKs inhibitors was chosen regarding the fact that these compounds are subjected to metabolic conversion in hepatocytes (Andrieux et al. 2004; Dvorak et al. 2008). The levels of CYP1A1 and CYP1A2 mRNAs were monitored, since both genes are under transcriptional control of AhR (Kawajiri and Fujii-Kuriyama 2007). Both, SP600125 and U0125 significantly induced CYP1A1 and CYP1A2 mRNAs, the effects of SP600125 being much more pronounced. In contrast, SB203580 did not alter the levels of CYP1A1 and CYP1A2 mRNA (Fig. 1).

Numerous studies have shown that MAPKs could be involved in regulation of drug-metabolizing enzymes, probably *via* phosphorylation of the receptors involved in P450s´ regulation. Our research group recently published a comprehensive material dealing with mutual interactions between MAPKs and AhR, a prominent regulator of drug-

metabolism (Henklova et al. 2008). Hence, examination of MAPKs involvement in CYP1A1/2 regulation is of topical interest. However, it was demonstrated, in the current paper and elsewhere, that specific pharmacological inhibitors of JNK and ERK kinases SP600125 and U0126 stimulate AhR-CYP1A signaling pathway as these compounds are partial agonists of AhR that induce CYP1A genes (Joiakim et al. 2003; Andrieux et al. 2004; Dvorak et al. 2008). It can be concluded that chemical inhibition of JNK and ERK by SP600125 and U0126, respectively, is not valid approach in the studies of MAPKs role in AhR-CYP1A-dependent drug metabolism. Consistently, the data obtained from studies using SP600125 or U0126 should be interpreted with prudence with respect to the effects on AhR-CYP1A signaling pathway. While an alternative approaches such as gene silencing, reporter assays or use of mutant vectors may be applied in proliferating cells, these techniques are not compatible with majority of primary cell, e.g. hepatocytes. In this case, the use of cell-permeable small peptide inhibitors could be promising. On the other hand, pharmacological inhibitors still have irreplaceable role in clinical applications.

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