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Original Article

Purinergic Receptor Antagonist A438079 Disrupts Benzo[a] pyrene-Mediated IL-1β, CYP1A and CYP1B Transcript Induction Pathway in Zebrafish: Insights into Possible Mechanisms

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Abstract

The deleterious effects of benzo[a]pyrene are mainly due to its metabolites generated by CYP1 metabolism. P2X7 is an important member of purinergic receptors involved in diverse cell signaling cascades, as the inflammasome pathway. Receptor blocking has beneficial effects in several models of inflammatory and neurological diseases. In our study, we show for the first time that the A438079, a "selective" P2X7 antagonist, promotes a downregulation in IL-1β, CYP1A1 and CYP1B1 gene transcription in *Danio rerio* gills exposed to benzo[a]pyrene. Our results show a modulation of IL-1β and CYP1 mRNAs, suggesting a possible novel mechanism involving the P2X7 receptor in benzo[a]pyrene-mediated CYP1 induction. Nevertheless, as A438079 was also proven to block the membrane ATP channel pannexin-1, the effects of this compound on downregulating CYPs transcription would also be due to a disruption of Ca^{2+} influx necessary to activate CYPs transcription by the AhR-Arnt pathway.

Keywords: P2X7, benzo[a]pyrene, zebrafish, CYP1A1, CYP1B1, gene transcription, A438079

INTRODUCTION

The P2X7 receptor is an important member of the purinergic P2X family of ATP-activated ion channels involved in a wide number of pathophysiological conditions, including activation of the NLRP3 inflammasome and neurotransmitter signaling (Burnstock, 2017; Di Virgilio *et al.*, 2017). The chemical compound A438079 (CAS 899507-36-9) is considered a P2X7-selective antagonist that virtually does not act on other purinergic receptors (McGaraughty *et al.*, 2007). Several types of cell injuries caused by different conditions related to immune system activation (e.g., fibrosis, apoptosis, and oxidative stress) can be efficiently prevented by this compound (Huang *et al.*, 2014; da Silva *et al.*, 2014; Deng *et al.*, 2021; Santos *et al.*, 2022). The scientific literature has pointed to the role of NLRP3 inflammasome on the hepatic injury process caused by acetaminophen (APAP) (Hoque *et al.*, 2012) and that the use of A438079 has shown to be protective against this deleterious process (Xie *et al.,* 2013). In this case, it was shown that the NLRP3 inflammasome blockade was not involved in this protective mechanism, but the inhibition of the activities of cytochrome P450 (CYP) enzymes was responsible for hepatic protection by decreasing the generation of APAP toxic metabolites (Xie et al., 2013).

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(Iglesias & Spray, 2012). Compounds like TCDD, BaP, and 7,12-dimethylbenz[a]anthracene, three known CYP1A1 inducers, have been reported to increase Ca^{2+} levels in the human mammary epithelial cell line MCF-10A (Tannheimer *et al.,* 1997; 1999) and in mouse testicular TM4 Sertoli cells (Zhang *et al.*, 2022). Changes in free intracellular Ca^{2+} cause alterations in the activity of several kinases involved in the phosphorylation of key signaling proteins that modulate gene transcription. Previous investigations revealed that DNA binding by human and mouse AhR-Arnt heterodimers requires phosphorylation of both proteins, whereas formation of AhR-Arnt heterodimers requires phosphorylation of Arnt only (Pongratz *et al*., 1991; Berghard *et al.*, 1993). Moreover, Long *et al.* (1999) showed that a protein kinase C-mediated event is required for the AhR to form a functional transcriptional complex that leads to trans-activation. Therefore, intracellular Ca^{2+} might be a key factor in the induction of CYP1A1 by various compounds. Thus, it can be additionally rationalized that P2X7 and pannexin-1 ATP channel blockade by A438079 disrupts the Ca^{2+} signaling process necessary for the activation of the AhR-Arnt signaling pathway, resulting in a decrease in the transcription of CYP genes (Figure 1).

CONCLUSION

The main finding of this study was the interference of A438079 on the BaP-mediated CYP1A1, CYP1B1 and IL-β gene upregulation, which could suggest the involvement of P2X7 receptors in the modulation of these genes in zebrafish. Based on these results, we hypothesize that the substantial lower upregulation of CYPs in the BaP+A438079 group was due to lower AhR activation due to diminished Ca^{2+} influx and/or reduced Nf-kβ-mediated AhR transcription in combination with a possible impairment of the p53 pathway, effects that resulted from the blockade of P2X7 receptor and pannexin-1 ATP release channel. The main implication of these results is that despite the pharmacological blockade of P2X7 may result in interesting anti-inflammatory effects, it impairs CYP-mediated biotransformation, which would reduce the capacity of organisms to metabolize xenobiotics and pharmaceutical drugs. However, these insights require further experiments to better clarify such mechanisms.

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