



Institute of Environmental Medicine
Unit of Work Environmental Toxicology

Influence of Oxidative Stress on Aryl Hydrocarbon Receptor Signaling

ACADEMIC DISSERTATION

For the Degree of Doctor of Medicine at Karolinska Institutet

To be publicly defended at Atrium, Nobels väg 12

Karolinska Institutet, Stockholm

Wednesday Jun 05 2013, 1:00 p.m.

Afshin Mohammadi Bardbori

Main Supervisor:

Professor Agneta Rannug
Karolinska Institutet
Institute of Environmental Medicine

Co-Supervisor:

Professor Ulf Rannug
Stockholm University
Department of Molecular Biosciences
The Wenner-Gren Institute

Professor Ralf Morgenstern
Karolinska Institutet
Institute of Environmental Medicine

Faculty Opponent:

Dr. Thomas Haarmann-Stemann
IUF Leibniz-Institut für
Umweltmedizinische Forschung
Düsseldorf, Germany

Examination Board:

Professor Cynthia de Wit
Stockholm University
Department of Applied
Environmental Science

Docent Siamak Haghdoost
Stockholm University
Department of Molecular Biosciences
The Wenner-Gren Institute

Docent Annika Wallberg
Karolinska Institutet
Institute of Environmental Medicine

Stockholm 2013

ABSTRACT

The aryl hydrocarbon receptor (AHR), a multifunctional protein and a key regulator of drug metabolizing enzymes, belongs to the basic-helix-loop-helix (bHLH)/PAS (Per-Arnt-Sim) super-family of transcription factors. The AHR responds to exogenous and endogenous chemicals by induction or repression of a large number of genes involved in many physiological processes and normal development.

The diverse spectrum of AHR activators from well-known planar hydrophobic halogenated aromatic hydrocarbons (HAHs) to chemical compounds whose structure and physicochemical properties are very different from classical AHR ligands suggests that the AHR has a tremendously promiscuous ligand binding pocket. Due to the absence of a 3D structure of the ligand binding domain, promiscuity of the AHR has remained elusive. However, increasing experimental evidence indicate that the non-typical AHR ligands might activate the AHR signaling pathway indirectly by inhibiting the metabolic turnover of an endogenous ligand of the AHR. Therefore, the objective of this thesis was to characterize the inhibition of degradation of 6-formylindolo[3,2-*b*]carbazole (FICZ), the suggested natural high affinity AHR ligand, as a mechanism that could explain the earlier described agonistic properties of structurally very diverse AHR activators. The obtained results show that FICZ is a potent AHR agonist *in vitro* and *in vivo* which can distribute to the body through systemic circulation and induce cytochrome P450 1A1 (CYP1A1) the prototypical AHR target in various organs. The studies presented in this thesis demonstrate that if the metabolic clearance of FICZ is compromised, femtomolar concentrations of FICZ are sufficient to activate AHR signaling.

The AHR signaling pathway seems to be sensitive to oxidative stress but the redox regulation of AHR has not been well characterized. Studies on dioxin and other reactive oxygen species (ROS) producing agents have demonstrated that the AHR is a mediator of oxidative stress. Indeed, AHR works in close concert with the master regulator of antioxidant responses, nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Multiple sources of ROS appear to be involved in modulating AHR signaling and probably via three major systems, microsomes, mitochondria and NADPH oxidase enzymes (NOXs). Furthermore, it has been observed that many environmental pollutants, including metals and other NOX-activators increase the levels of the diffusible molecule hydrogen peroxide (H₂O₂) and change the cellular redox status and thereby interfere with cell growth kinetics and the endogenous functions of the AHR. To increase the understanding of downstream adaptive responses to oxidative stress, including up-regulation of antioxidant genes and modulation of AHR signaling was another objective of this work. The findings demonstrate that superoxide anion (O₂^{•-}) or H₂O₂ produced by NOXs can negatively and positively modulate the AHR signaling pathway. The importance of cellular redox levels which can influence endogenously activated AHR signaling broadens our earlier knowledge and explains why many oxidants behave both as AHR antagonists and agonists.

In summary, this thesis extends the mechanistic understanding of the promiscuity of AHR and provides important information with regard to the redox regulation of AHR endogenous signaling.

ISBN 978-91-7549-163-9