Investigating the Physiological Role of Human Aryl Hydrocarbon Receptor

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Motivation

Dioxins are a group of chlorinated organic chemicals, and the term usually includes polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzo-furans (PCDFs). Some of them have harmful characteristics depending on the number and structural position of chlorine atoms. 2, 3, 7, 8-tetrachlorodibenzop-dioxin (2, 3, 7, 8-TCDD, TCDD or dioxin), is considered one of the most toxic compounds released into the environment. TCDD produces a broad spectrum of effects on the human health, at very low concentrations. At non-lethal doses, reproductive and developmental effects, hepatocarcinogenesis, tumor promotion, and immune suppression are observed. Its toxicity and clinical effects are mediated by AhR (Aryl hydrocarbon Receptor). AhR is a cytosolic transcription factor that, in its latent state, forms a multiprotein complex that includes HSP90 (90KDa heat shock protein). Upon ligand binding, the AhR-HSP90 complex translocates to the nucleus, where AhR complexes with its heterodimerization partner, the AhR Nuclear Translocator (Arnt), to modulate expression of AhR target genes containing specific DNA enhancer sequences, known as AhR responsive elements. AhR belongs to the bHLH-PAS protein family, whose members are considered as biological sensors for a variety of stimuli, controlling neurogenesis, vascularization, circadian rhythms, metabolism and stress responses to hypoxia, among others. AhR is highly conserved in evolution and is present in many cell types. The selective forces that led to the high degree of conservation of the AhR amino acid sequence are unknown and its physiological function(s) are still being elucidated. The search for ligands that regulate AhR transcriptional activity under physiological conditions has had limited success. There is a long list of candidate AhR agonists including sterols, indigoids, heme metabolites, tetrapyrroles such as bilirubin, arachidonic acid metabolites and dietary components. Therefore, we investigated the interaction of AhR with dioxin or other ligands either physiologically present in the human body or introduced by food intake, with the aim of obtaining insights about the AhR physiological role and, at the same time, to investigate the dioxin toxic activity.

Methods

Molecular modelling of AhR has been applied according to comparative modelling procedures, which include the use of public software for search of sequence similarity and sequence alignments (BLAST, CLUSTALW), secondary structure predictions (PSIPRED),
protein modelling (MODELLER), analysis and comparison of structural properties at the different levels of structural organization (DSSP, NACCESS, PROCHECK, PROSA). Protein-ligand docking simulations were conducted using Autodock Tool version 4.0. CASTp server was also used to analyze putative ligand binding pockets.

**Results**

Our work has included three steps: (i) the modelling of the 3D structure of hAHR-LBD, by comparative modelling; (ii) the characterization of the dioxin binding activity, by docking simulations, and the recognition of amino acids involved in the binding pocket and in the interaction; and (iii) the docking simulation for other ligands, with a similar analysis of the binding pocket in order to compare with dioxin binding features. The presence of different putative binding sites for the ligands was highlighted by our investigations. The hypothesis to be further investigated will be the putative competition and/or interaction of these ligands to bind these sites under physiological conditions, as well as the dioxin role, with the aim of suggesting a possible mechanism to explain its toxic activity.

**Acknowledge**

MIUR FIRB ITALBIONET (RBPR05ZK2Z and RBINo64YAT_003) (for A.M., L.M.)

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