Characterization of the transgenic CA-AhR mouse - cell specific expression of the CA-AhR using CYP1A1 as a marker

Sara Brunnberg[1], Maria Lindstam[1], Patrik Andersson[1], Lorenz Poellinger[2], Annika Hanberg[1]

[1]Institute of Environmental Medicine, Stockholm
[2]Department of Cell and Molecular Biology, Stockholm

Introduction

The risk assessments of dioxins and dioxin-like PCBs performed by WHO and EU lead to major concerns. The tolerable daily intake for humans has been assessed to be within the range of human exposures occurring in the general population today [1].
Dioxins are known to adversely impair reproduction and affect development of reproductive organs, as well as the early development of the immune and the nervous systems. The Aryl hydrocarbon Receptor (AhR) mediates most toxic effects of dioxins, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and PCBs.
In order to study the mechanisms of toxicity of ligands of the Ah receptor we have created a transgenic mouse model expressing a constitutively active Ah receptor (CA-AhR). The mutant Ah receptor is expressed and functionally active in most (or all) organs. Consequently, the CA-AhR mice show several of the well-known effects of dioxin exposure [2,3]. Since the CA-AhR is continuously active at a relatively low level and from early development, this model resembles the human exposure scenario and is thus suitable for studies on mechanisms of action of Ah receptor ligands.

Methods and Materials

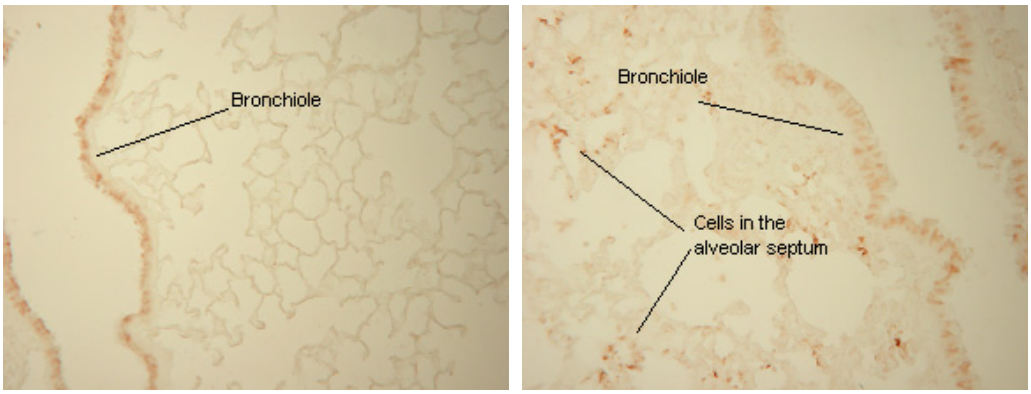
A mutant form of the Ah receptor (lacking the minimal PAS B motif), which is constitutively active, was defined [4]. Transgenic mice expressing this mutant AhR (CA-AhR) were created [2,3]. The mice were bred to homozygosity, compared to wild type mice (lacking the mutant receptor) and were studied at the age of 3 month. The animals were killed by CO_2 asphyxiation followed by cervical dislocation. The organs were dissected and weighed and frozen in liquid nitrogen for RNA analysis and/or fixed in 4% buffered formaldehyde for immunohistochemistry. All experimental procedures were approved by the local ethical committee on animal experiments. Cell specific expression of the CA-AhR was studied by immunohistochemistry with the CYP1A1 protein as a marker. Immunohistochemistry was performed according to Annas et al. with some modifications [5]. Briefly tissues which had been fixed in 4% buffered formaldehyde were dehydrated and embedded in paraffin. The embedded tissues were sectioned (5-µm) and collected on super frost

slides (Menzel-Glazer). Following rehydration the samples were heat-treated for 15 minutes in Tris-EDTA (pH 9.0) in a microwave oven. Endogenous peroxidase was blocked in 3 % H_2O_2 in methanol for 15 minutes. After washing with PBS with 0.3 % Triton X-100 (PBS-T), the sections were incubated in 1 % bovine serum albumin (BSA) in PBS-T for 30 minutes at room temperature to block non-specific binding of the antibodies. The samples were again washed with (PBS-T) before the primary antibody, CYP1A1 (AB1247, Chemicon), diluted in 1 % BSA in PBS-T (1:750) was applied to each tissue section and left overnight at + 4˚C. After washing the sections were incubated with secondary antibody, horse radish peroxidase-conjugated Envision+ (DAKO), for 30 minutes in room temperature. Negative controls were performed in all tissue by omission of the primary antibodies. The control incubation was performed simultaneously with the experiment. The negative controls did not show any staining. To develop colour, the sections were incubated in diaminobenzidine (DAB) for 5 – 10 minutes, after which they were rinsed in water, and the heart was counterstained with hematoxylin to better identify different structures. The samples were dehydrated and cover slipped with xylene substitute mountant, Neo-Mount (Merck). The sections were studied in microscope (Leitz Diaplan) and photographed using a digital camera (Nikon Coolpix 995).

Results and Discussion

The CA-AhR gene is expressed in all organs studied, i.e. thymus, spleen, lymph nodes, bone marrow, T- and B-lymphocytes, liver, lung, muscle, skin, brain, heart, kidney, glandular stomach, oesophagus, fore stomach, duodenum, jejunum, ileum, colon, uterus, ovary, mammary gland, testis, epididymis, seminal vesicle [2,3,6]. In addition, increased expression of the target gene CYP1A1 indicates that the mutant receptor is active in these organs [2,3,6]. The level of expression of both CA-AhR and CYP1A1 differs considerably between organs, as well as between CA-AhR and CYP1A1 within the same organ, indicating that the induction of CYP1A1 is not directly proportional to the level of expression of the CA-AhR. To further characterize the activity of CA-AhR we investigated in the present study the cellular localization of the CYP1A1 protein in the lung and heart with immunohistochemistry.

Within the lungs are the internal bronchi, which undergo extensive branching to give rise to the distributing bronchioles. The bronchioles have no cartilage or glands in their wall. The epithelium is low columnar to cuboidal and consist of ciliated and non-ciliated cells. One cell of major interest in the bronchioles is the non-ciliated Clara cell, which is involved in the metabolism of xenobiotics. The alveoli are where the gas exchange takes place between the blood and the inspired air. The alveolar septum of the alveoli consists mainly of alveolar epithelial cells (type I and II alveolar cells) and endothelial cells of the rich capillary network. Macrophages are also found within the interalveolar septa [7,8].



A B

Figure 1. Immunohistochemical localization of CYP1A1 in lung from 3-month-old male mice. (A) Control, wt mice (B) CA-AhR mice

In the wild-type mice, cells in the epithelium of the bronchioles expressed CYP1A1 (Fig 1A). The cells which express the enzyme look very much like Clara cells, columnar cells with a prominent apex which protrudes into the airway lumen. In the transgenic mice the expression of CYP1A1 was identified in the same cells in the bronchioles, Clara cells, as in the wild-type mice, but also in cells in the alveolar septum (Fig 1B). Since the cells of the alveolar septa are very hard to identify at the light microscopic level it is difficult to specify which cells express CYP1A1. Our results are in accordance with other studies, in which laboratory animals have been exposed to AhR ligands, such as 3-Metylcholantrene or TCDD. In these studies expression of CYP1A1 has been detected in Clara cells, type II alveolar cells and endothelial cells [9,10,11].

The heart wall is composed of three layers; a superficial epicardium, a middle myocardium, and a deep endocardium. All three layers are richly supplied with blood vessels. The epicardium consists of mesothelial cells on the outer surface of the heart and its underlying connective tissue. The blood vessels and nerves that supply the heart lie in the epicardium. The myocardium composed mainly of cardiac muscle forms the bulk of the heart. Within this layer, the branching cardiac muscle cells are coupled to each other by crisscrossing connective tissue fibres and arranged in bundles. The inner layer, endocardium, is continuous with the endothelial linings of the blood vessels leaving and entering the heart [7,8]. In the heart of wild-type mice there were no cells expressing CYP1A1 (Fig 2A). In CA-AhR mice, an even-distributed pattern of expression of CYP1A1 was detected (Fig 2B). Stained cells were found in the myocardium and epicardium and in the linings of the endocardium. Since the wall of the heart is richly supplied with blood vessels and together with the data from the lung it is most likely to believe that the cells expressing CYP1A1 are endothelial cells. The observed expression in CA-AhR mice is coherent with a previous study which reported detection of CYP1A1 in the endothelial cells of the capillaries and coronary veins in mice that have been exposed to the AhR agonist β-naphtoflavone [5].

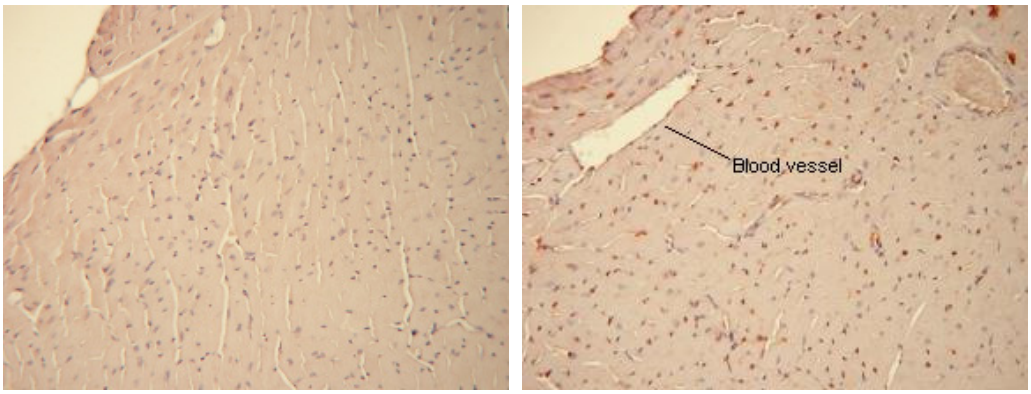


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Figure 2. Immunohistochemical localization of CYP1A1 in heart from 3-month-old male mice counterstained with hematoxylin. (A) Control, wt mice. (B) CA-AhR mice.

To conclude, we have studied the cellular localization of the CYP1A1 protein as a marker for an active CA-AhR in the lung and heart of with immunohistochemistry. We found expression of CYP1A1 in certain celltypes of the lung and heart that could correspond to endothelial cells. However, in the lung also epithelial cells expressed CYP1A1. Studies on the cellular expression of CYP1A1 in other organs are on-going

Acknowledgements

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