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THE TRANSGENIC CA-AHR MOUSE
– A MODEL FOR STUDIES OF THE CONSEQUENCES OF A CONTINUOUSLY ACTIVATED AH RECEPTOR

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Introduction

Dioxins and dioxin-like compounds (such as certain PCBs) elicit a wide spectrum of toxic effects, such as cancer, immuno suppression and reproductive and neurobehavioural effects.[1] The risk assessment of dioxins and dioxin-like PCBs is focused on their ability to specifically disturb the early (foetal and early postnatal) development. A thorough risk assessment has only been possible to perform on 2,3,7,8-TCDD, the most toxic dioxin.[1] However, since there is a common mechanism of action of these compounds the TEF-concept has been developed and is used for assessing the total risk of dioxins and dioxin-like PCBs.[2] The Ah receptor constitutes the common mechanism of action. The binding of the ligand to the Ah receptor is a first and necessary step to elicit the toxic effects of dioxin-like compounds.

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] In addition, there are limitations such as the TEF-values used are mostly based on effects other than those assessed as the critical effects, effects not even related to the critical effects.[2]

Due to these limitations and concerns there is a need for more mechanistic information on the critical effects of dioxins. Which are the critical steps between binding of the dioxin to the Ah receptor and the toxic effect(s). Do all the dioxins and dioxin-like PCBs, included in the TEF-scheme today, elicit these effects? Are the TEF-values of today valid for these effects? Do dioxin-like compounds, not included in the TEF-scheme today, also cause such effects?

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).

Materials and Methods

A mutant form of the Ah receptor (lacking the minimal PAS B motif), which is constitutively active, was defined.[3] Transgenic mice expressing this mutant AhR (CA-AhR) were created.[4,5] The mice were bred to homozygosity, compared to wild type mice (lacking the mutant receptor) and were studied at different ages. Single oral exposure of wild type animals to TCDD (in corn oil) was used for comparison. Gene expression was studied by Northern blot.

Results and Discussion

The CA-AhR mice are fertile and show a normal Mendelian 1:2:1 distribution, indicating no prenatal lethality of homozygous mutants.[4,5] The mice also show a normal sex ratio.[5]

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.[4,5,6] In addition, increased expression of the target gene CYP1A1 indicates that the mutant receptor is active in these organs.[4,5,6] The level of expression of both CA-AhR and CYP1A1 differs considerably between different 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 be able to assess the level of functional activity of the CA-AhR we compared the induction of CYP1A1 mRNA in heterozygous and homozygous mice with wild type mice exposed to TCDD (given a single dose via gavage three days prior to analysis). The results showed that the dose of TCDD, that corresponded to the CYP1A1 expression of homozygous and heterozygous mice, differed between organs.[4] In the stomach, thymus and liver of homozygous CA-AhR mice, the levels of CYP1A1 mRNA are approximately comparable to those observed in wild-type mice treated with 10, 3 and 0.3 µg TCDD/kg bw, respectively.[4] The heterozygous CA-AhR mice showed a lower CYP1A1 induction than the homozygous mice, reflecting a gene-dosage effect.

As the wasting syndrome is a classical effect of dioxin exposure we followed the body weight gain of young CA-AhR mice between the first and third month of age. However, no effect on body weight was observed in neither male nor female CA-AhR mice.[4] Other well-known effects of dioxins are thymus atrophy and liver enlargement. The relative thymus weight in CA-AhR mice is lower than in wild type mice. This effect is most pronounced in young mice and persists for six months.[4] A slight increase in relative liver weight was observed in 3, 6 and 12-months old CA-AhR mice.[4] These results on organ weights together with the level of CYP1A1 expression indicate that the activity of the CA-AhR corresponds to a relatively low dose exposure situation.

A changed distribution of single positive CD4^{+} and CD8^{+} T cells in young thymus is a sensitive effect of dioxin exposure.[1] The results of FACS analyses indicated an altered CD4+/CD8+ ratio in thymus of newborn CA-AhR mice. However, effects observed on B-cells are more marked. Consistent with previous findings in dioxin exposed mice the population of mature bone-marrow derived B cells is enlarged in CA-AhR mice.[6] In contrast, the peritoneal population of B1 cells is significantly diminished.[6] The B1 cells are important mediators of innate immunity against pathogens, such as Influenza virus and a reduced population of B1 cells may thus be involved in the decreased resistance against infections caused by dioxin exposure.

The CA-AhR mice do not show any apparent clinical signs of illness until the age of approximately six to nine months, depending on founder line. At that point they suddenly die. This mortality coincides with the presence of quite dramatic stomach tumours in the glandular part of the stomach.[4,5] These tumours originate from the glandular epithelium and penetrate all layers of the stomach wall, forming externally visible cystic lesions. It has been difficult to interpret the histopathology of the stomach tumours unambiguously. The well-organized glandular structures and low levels of cellular atypia argue for a benign phenotype. On the other hand, the reduced life

span, the aggressive expanding invasion of all stomach layers, and the adherence to surrounding organs point towards a more malignant phenotype. There is an apparent sex difference in the sensitivity to these tumours as males develop the tumours at an earlier age than females.[4,5]

Dioxins and dioxin-like compounds are endocrine disrupters shown to cause for example antiestrogenic effects in several organs.[1] We have observed expression of CA-AhR and CYP1A1 in endocrine (ovary, testis) and reproductive organs (uterus, mammary gland, epididymis, seminal vesicle). In addition, in CA-AhR female mice we have observed a decreased expression of the estrogen-responsive gene Cathepsin D in the uterus. We have also seen a reduced estrogen-induced increase in uterus weight, similarly to what is reported for dioxin-exposed animals.

Regarding effects on thyroid hormones we have observed increased expression of the thyroid hormone-metabolising enzyme UDPGT in the liver of CA-AhR mice, similarly to what has been reported for dioxin-exposed animals. However, the serum levels of free thyroxin (T4) were not changed in the CA-AhR mice that might have compensated for the increased metabolism of T4.

To conclude, we have developed a transgenic mouse model with a constitutively active Ah receptor. 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. Since the CA-AhR is continuously active at a relatively low level and from early development we believe that this model resembles the human exposure scenario and thus is suitable for studies on mechanisms of action of Ah receptor ligands. Continued studies are, however, needed to further validate the CA-AhR model.

Acknowledgement

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