TYPES OF CHLORACNE IN SEVESO PEOPLE AND AHR GENETIC VARIABILITY

Elena Copreni, Cristina Dassi, Paolo Brambilla, Marcello Monti*, Pierluigi Tramacere and Paolo Mocarelli

University of Milano-Bicocca, Department of Laboratory Medicine, Hospital of Desio, Desio, Milan, Italy
* Humanitas Clinical Institute, University of Milan, Rozzano, Milan, Italy

Introduction

Aryl hydrocarbon receptor (AHR) is a ligand induced transcription factor activated by polycyclic aromatic hydrocarbons. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin) is the most biologically active and toxic member of these chemical compounds (1).
TCDD induces expression of several drug metabolizing enzymes, including CYP1A1 and CYP1B1, which are responsible for xenobiotics metabolic activation and detoxification (2).
Adverse effects of dioxin, studied in animal models, include wasting syndrome, birth defects, carcinogenesis, reproductive system impairment, endocrine disruption and immune suppression (3).
Following the accident at a chemical plant near Seveso on July 1976, people resident in the nearby area were exposed to the very high concentration of almost pure TCDD (4).
Chloracne was the only clinical alteration correlated with soil levels of dioxin (5), nevertheless some individuals developed chloracne and some others with similar or even higher TCDD serum levels did not, suggesting an individual susceptibility to this compound (4).
The genetic difference in the sensitivity of mice to the induction of CYP1A1 and adverse effects of dioxin is correlated to the polymorphism of AHR: low responders (DBA/2) mice show reduced binding affinity due to an Ala-375 to Val substitution (6).
We studied the nucleotidic sequence of the human AHR ligand binding domain in order to verify whether chloracne can be dependent on genetic variability at this domain.

Material and Methods

Blood samples were obtained after informed consent from fourteen chloracneic subjects – eight subjects with severe chloracne (type 3-4), six with mild chloracne (type 1-2) – and from four individuals with high serum TCDD levels but not affected by chloracne.
Total RNA was extracted according to the method of Chomczynski and Sacchi (7) from 1 mL of suspension of blood mononuclear cells (mean, $7x10^6$ cell /mL) isolated by gradient centrifugation on Histopaque (Pharmacia, Uppsala, Sweden).
Total RNA was retrotranscribed by using the First Strand cDNA Synthesis kit (Amersham Pharmacia Biotech, UK) and amplified by Polymerase Chain Reaction using the following primers: forward primer 5' GTGCTTCATATGTCGTCTAAG 3', reverse primer 5' AATGAGTTCACATCCTGAGGC 3'.
The sequencing was carried out with the T7 Sequenase V2.0 Sequencing kit (Amersham Life Science, Cleveland, OH) and the primer 5' CCCATATCCGAATGATTAAGAC 3', followed by polyacrylamide gel electrophoresis under denaturing conditions.

Results and Discussion

The sequencing analysis did not find any nucleotide difference in the AHR ligand binding domain among exposed subjects affected by chloracne or not. Val 381, corresponding to the murine aminoacidic residue 375, was present in all examined individuals.

This preliminary study seems to show that the interindividual difference in TCDD susceptibility, as far as chloracne is concerned, is not explained by polymorphism in the AHR ligand binding domain.

Polymorphic forms of the AH receptor are reported among several inbred strains of mice and are related to the difference in responsiveness of these animals to the polycyclic aromatic hydrocarbons.

The difference in the susceptibility of mice to the adverse effects of these environmental compounds is associated to the allelic variant in which the Ala 375 in the AHR ligand binding domain is substituted by a Val residue, determing a low AHR affinity phenotype. In humans the ligand binding domain shows Val 381 which corresponds to murine low affinity allele Val 375 (DBA/2) and this finding is common to all the chloracne cases and controls.

Similarly to mice, human populations have shown a 20-fold range of AH receptor affinity phenotype (8), but also in this case no mutations were detected in the binding domain (9).

Although some genetic variants have been reported (10,11), there is no evidence for the existence of a mutation in the ligand binding domain which confers to AHR higher affinity for ligand.

Interindividual variability in response to TCDD can be related with nucleotide variation in another AHR functional domain (i.e. transactivation domain) or could depend on the AHR molecular interaction with the receptor associated proteins HSP90, ARA9 and ARNT.

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