IMMUNOTOXICITY OF DIOXIN: AN OVERVIEW

J.G.Vos, H.Van Loveren and H.J.Schuurman

National Institute of Public Health and Environmental Protection

P.O.Box 1, 3720 BA BILTHOVEN, The Netherlands

Abstract

Sublethal exposure to dioxin produces thymic atrophy and as a result, thymus-dependent immunity is suppressed. Consequently, the effects of dioxin also comprise impaired resistance to various infectious agents. This report compares data from laboratory animal studies with human data. From the various studies dealing with the mechanism of dioxin-induced thymic atrophy, it is concluded that thymic epithelium is the most likely target of dioxin.

Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (dioxin) causes diverse toxic effects, with a remarkable interspecies variation. The lethality of dioxin for different species differs also greatly. Despite this variation, dioxin causes atrophy of the thymus in all species investigated (McConnell and Moore, 1979). The atrophy occurs at sublethal doses of TCDD, and is reflected microscopically by lymphocyte depletion in the cortex. This finding suggests that dioxin may alter immune responses, and has initiated immune function studies in various species (reviewed by Exon et al., 1987; Vos and Luster, 1989). This paper discusses the effect of dioxin on thymus-dependent immune responses and on host resistance parameters. In addition, the mechanism of dioxin-induced thymic atrophy is discussed.

Immunotoxicity in Laboratory Animals

Immune Function Studies

Results of immune function studies in mice, rats and guinea pigs indicate that TCDD suppresses cell-mediated immunity in a dose related fashion (see Exon et al.,1987; Vos and Luster,1989). Parameters investigated include delayed-type hypersensitivity responses, rejection of skin, graft versus host activity, and *in vitro* mitogen reponses of lymphoid cells. This suppression appears to be an age-related phenomenon as dioxin causes more severe immunotoxic effects after perinatal administration than after administration in adulthood. This may be associated with the thymus activity especially early in life. In the rat, perinatal exposure seems a prerequisite to produce immune suppression. Besides

suppression of the cell-mediated immunity, dioxin can also impair humoral immunity. The effects on antibody synthesis after primary and secondary immunisation are variable and require higher dose levels. Effects on classical macrophage functions have not been observed.

Resistance to Infectious Agents

Effects of dioxin have also been noted on resistance to infectious agents (Table I).

Table I Effect of Dioxin on Host Resistance in Mice

Strain	Effect	Exposure	References
C57Bl/6	no effect on resistance to Herpes suis	20 µg/kg b.w. per os, 4 times	Thigpen et al., 1975
C57Bl/6	reduced resistance to Salmonella bern	1 µg/kg b.w. per os. 4 times	Thigpen et al., 1975
Swiss	reduced resistance to endotoxin	5 µg/kg b.w. per os, 4 times	Vos et al., 1978
Swiss	reduced resistance to endotoxin	1 µg/kg diet pre/postnatal	Thomas and Hinsdill, 1979
Swiss	no effect on resistance to Listeria monocytogenes	10 µg/kg diet pre/postnatal	Thomas and Hinsdill, 1979
Swiss	reduced resistance to Salmonella and Listeria	50 µg/kg diet 5 weeks	Hinsdill et al., 1980
B6C3F1	Peduced resistance to Listeria	5 µg/kg b.w. per os, 4 times pre/postnatal	Luster et al., 1980
C57Bl/6	reduced resistance to Herpes Type II	0.01 µg/kg b.w. i.p. 4 times	Clark et al., 1983
B6C3F1	reduced resistance to Plasmodium yoelli	5 µg/kg b.w. per os, once	Tucker et al., 1986
B6C3F1	reduced resistance to Streptococcus pneumoniae	1 µg/kg b.w. per os 14 times	White et al., 1986

Host resistance assays are very valuable for risk assessment, and comprise more than one aspect of the specific and non-specific defence mechanisms. Using different mouse strains and different treatment schedules dioxin suppressed the resistance to Salmonella bern. Salmonella typhimurium. Streptococcus pneumoniae, Herpes 11, and Plasmodium yoelli (malaria) Variable effects have been reported for the resistance to Listeria monocytogenes. Dioxin had no effect on mortality in Herpes suis (pseudorabies)-infected mice. Further studies showed that dioxin-exposed mice revealed a marked susceptibility to endotoxin from Gram-negative bacteria, which could explain the decreased resistance to

infection with Salmonella bacteria. Similarly, suppressed serum complement levels (total hemolytic complement activity and complement component C3) following low dose dioxin treatment of mice could explain the decreased resistance to Streptococcus pneumoniae (White et al.,1986)

Immunotoxicity in Man

Regarding the study of the effects of dioxin on the human immune system there are few published reports available (see Vos and Luster 1989). In children in the dioxin contaminated area of Seveso no abnormalities in serum immunoglobulin concentrations and mitogen responses of T and B cells were revealed. Immune alterations (delayed-type hypersensitivity skin reactions to standard antigens) have been reported in persons from a dioxin-contaminated area of Missouri. This suggests that long-term dioxin exposure is associated with suppressed cell-mediated immunity. In the follow-up of individuals for whom an immunologic anergy was shown, none was anergic any more. The two most likely explanations for this phenomenon are the weak potency of the skin test applied in the first study or the sensitization to the antigen by the first test (Evans et al., 1988). Unequivocal immune alterations have been observed in Taiwanese residents exposed to dioxin-related polyhalogenated aromatic hydrocarbons. Consumption of rice oil contaminated with polychlorinated biphenyls and dibenzofurans caused acneiform skin lesions, pigmentation of skin and nails, liver damage and abnormal immune function. Serum IgM and IgA concentrations and the percentage of T lymphocytes in the peripheral blood were decreased. The cell-mediated immune system was investigated by delayed-type hypersensitivity responses. The percentage of patients showing a positive skin test to streptokinase and streptodornase was significantly lower compared to controls. This suppression of cell-mediated immunity was reproduced in a follow-up study (skin test to tuberculin). Thus, clinical observations of immune alterations, particularly of the thymus-dependent immunity, in individuals exposed to these chemicals demonstrate the relevance of studies in experimental animals.

Mechanism of Immunotoxicity

Many studies have been performed to investigate the mechanism of dioxin induced thymic atrophy, which is a dominant characteristic of dioxin toxicity (see Vos and Luster 1989). Different possible indirect mechanisms could be excluded such as a contribution of stress hormones, because adrenalectomy or hypophysectomy did not influence the dioxin effect. Also a role of growth hormone, of reduced food intake, or of zinc deficiencey was excluded. In mice it was established that the susceptibility to dioxin treatment is genetically determined. Susceptibility segregates with the locus encoding a cytosolic protein mediating aryl hydrocarbon hydroxylase activity. This receptor (Ah, aromatic hydrocarbon receptor) has a high affinity for dioxin: the ligand-receptor complex can associate with nuclear sites, thereby influencing gene expression. High levels of the receptor are found in the mouse and rat thymus: particularly in the epithelial cells. Thus, the effect of dioxin on the thymus may be through an action on epithelial cells. This has been substantiated in in vitro studies on cultured mouse and human epithelial cells. The enhanced

lymphoproliferative capacity of thymocytes after coculture with epithelial cells is reduced when the latter are pretreated with dioxin. A second argument comes from mouse radiation chimera's, where dioxin-induced suppression of cytotoxic T-lymphocyte activity is determined by the host (epithelium) and not the donor (bone marrow, subsequently thymocytes). Thirdly, recent ultrastructural studies of dioxin-exposed rats indicate that dioxin acts on the thymic epithelium, reflected by the formation of epithelial cell aggregates and a more differentiated state of the cortical epithelium (De Waal et al., In press). Finally, investigations of persons exposed to dioxin in Missouri suggest an association between dioxin exposure and the function of thymic epithelium, as the mean serum level of the thymic peptide thymosin-α1 was significantly lower when compared to a control group (Stehr-Green et al., 1989). Thus, the dioxin-induced thymic atrophy may be explained by the inability of epithelial cells to provide the support needed to induce T cell maturation and differentation. In line with this explanation are the altered homing patterns of T cells from dioxin treated mice, which feature is characteristic of undifferentiated T cells.

Apart from the effect on epithelium, a direct action of dioxin on rat thymocytes has been shown in *in vitro* studies, resulting in a Ca^{++}-dependent endonuclease-mediated DNA-fragmentation and cell death (McConkey et al., 1988). This latter finding contrasts to earlier studies showing no cytotoxicity of dioxin when added to cultures of several cell types, including lymphocytes and monocytes. But, in these latter studies lower TCDD concentrations of dioxin were investigated. In addition to an effect on the thymus and hence on cell-mediated immunity, dioxin also affects B lymphocytes, as manifested by suppressed (thymus-independent) antibody responses. This suppression that occurs at higher concentrations, also appears to be Ah-receptor-mediated. Impaired B cell maturation is thought to be due to direct inhibition of cell maturation by dioxin and unlike the effects on cell-mediated immunity, is less dependent on the age of the animals.

Conclusion

Dioxin is a potent immunotoxicant. Perinatal exposure results in more severe effects than if the chemical is administered during adult life. This seems particularly true for cell-mediated immune functions. Humoral immunity can also be impaired, usually at higher dose levels. Because of the vulnerability of the developing immune system, breast-fed infants may represent a high risk group for the immune effects of dioxin and related chemicals. Dioxin-induced thymic atrophy and suppression of thymus-dependent immunity can be explained by an effect of dioxin on thymic epithelial cells resulting in a decreased capacity of these cells to support intrathymic maturation and differentation.

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