PLEIOTROPIC *trans*-ACTIVATING EFFECTS OF TCDD, B[α]A AND B[α]P ON STRUCTURAL GENES OF *CYP1A1* AND HIV-1 IN HUMAN CD4 LYMPHOID CELLS

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Dioxin-like compounds and PAHs have generated marked concern for human health, because of their widespread occurrence in the environment and their potential toxicity. Thus, TCDD produces in experimental animals and humans a diverse set of biological responses, including modulation of the microsomal monooxygenases and changes in the immunological response[1,2]. The mechanism of the immunomodulatory action of TCDD is still unknown. In contrast, the inductive effect of TCDD and PAHs on the microsomal monooxygenases, mediated by *Ah*-receptor, is a well-known phenomenon[3]. Thus, the *Ah* receptor is involved in at least two distinct responses: one response includes the structural genes of the *Cyp1* family, and the other response concerns genes not known to be related to the above functionally-linked genes[3-6].

The effects of TCDD were studied here in human CD4 lymphoid cells. Among with its dose-dependent and time-dependent effects on genetic induction of CYP1A1, the same parameters were assessed in regard to HIV-1 gene. It was done as TCDD and HIV-1 both affect the same target, i.e. CD4 lymphocytes[7,8]. More data on this suject have been published by us earlier[9,10]. The EROD and AHH activities in CD4 (MT-4 line) cells treated with TCDD, B[α]A or B[α]P were carried out according to an endpoint fluorimetric methods[11,12]. Estimates of TCDD effects were based on determination of the activity of viral reverse transcriptase[13] and the amount of viral protein[14].

SD4 cells were incubated with B[α], B[α]P or TCDD at different concentrations. As the table shows, 10 μM B[α]A or B[α]P led to increased EROD and AHH activity in the microsomal fraction of the cells. We have observed also some augmentation in the CYP1A1 mRNA content, and a higher level of CYP1A1 was detected by Western blot (data not shown). The induction effects of TCDD were more clear-cut. Thus, after incubation of 1.0 nM TCDD with CD4 cells there was a 2.1-fold increase of AHH activity and a 3.3-fold increase of EROD activity. By using Northern blot as well as inhibitory analysis with monoclonal and polyclonal antibodies raised against CYP1A1, we showed induction of CYP1A1 isoform the expression of which is mediated by the *Ah* receptor.

The effects of TCDD upon reproduction of HIV-1 in CD4 cells showed TCDD at 10 nM was the most potent activator of viral reverse transcriptase, i.e there was 3- to 6-fold increase of the enzyme activity (see Fig. 1, a). This result was similar to the effects of 10 nM TCDD on the AHH and EROD activities catalysed by CYP1A1 in CD4 cells (Table). A maximal peak of reverse transcriptase activity was observed at

Table. Effects of 1.0, 10.0 and 50.0 nM TCDD and 10 μM B[α]A and B[α]P on AHH and EROD activities in human CD4 cells

Monooxygenase activity	Control	TCDD 1.0	TCDD 10.0	TCDD 50.0	B[α]A	B[α]P
AHH activity, pmol 3-hydroxy-B[α]P formed/ min per 10^6 cells	7.5 (0.6)*	16.0 (1.2)	27.7 (3.0)	17.7 (1.9)	15.0 (1.6)	13.1 (1.1)
EROD activity, pmol resorufin formed/min per 10^6 cells	12.0 (5.5)	40.0 (3.3)	48.1 (3.2)	29.9 (1.6)	26.9 (2.2)	19.7 (1.7)

*The values represent the mean data of 5-6 experiments. Numbers in parenthesis are ± S.E.M.

days 3-5 of HIV-1 infection. As for viral protein, its content estimated by ELISA in TCDD-treated CD4 cells was 4- to 8-fold greater at day 5 than in control (Fig. 1, b). The dynamics of viral protein concentration was insignificant over the period day 5 to day 13 of HIV-1 infection.

In this study we ascertained the ability for TCDD and PAHs in CD4 human lymphoid cells to increase the cytochrome P450-mediated monooxygenase activities, the products of *Cyp1* gene family[3]. TCDD was a more potent inducer than B[α]A and B[α]P as its induction effect was observed at 1.0 nM TCDD. Considering the stimulatory effects of TCDD on HIV-1 production in CD4 cells, an increase of viral reverse transcriptase activity could not be due to induction of this activity in human CD4 cells by leukemia virus type 1 used to produce MT-4 culture cells or to induction of another endogenous RNA-dependent DNA-polymerase, because TCDD treatment of uninfected cells did not accompany induction of any reverse transcriptase activity (Fig. 1).

We are witnessing today a disconcerning spread of AIDS. The key problem of the desease is what factors are responsible for transformation of HIV-positive patients to patients with clinical manifestation and what mechanisms to participate in this transformation. If they understand those factors and mechanisms, an approach could be found to prevent or postpone the clinical phase of AIDS. Among the factors which are obviously involved, the smoking (in particular, B[α]P as a principal component of tabacco smoke) has been recently shown to increase at least twice the frequency of clinical manifestation among HIV-positive patients. The results obtained here indicate that

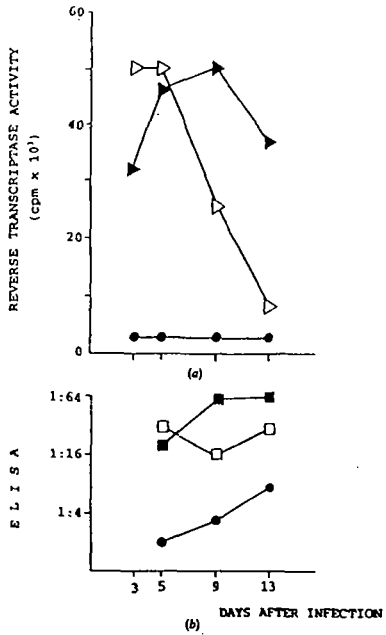

Figure 1. Dynamics of the effect of 10 nM TCDD on (*a*) reverse transcriptase activity and (*b*) viral protein reproduction in MT-4 cells infected with HIV-1.

(●), Control values; (△), reverse transcriptase activity determined after 48 h incubation of MT-4 cells with 10 nM TCDD; (▲), same after 1·5 h incubation of MT-4 cells with 10 nM TCDD; (□), amount of viral protein determined by ELISA after 48 h incubation of MT-4 cells with 10 nM TCDD; and (■), same after 1·5 h incubation of MT-4 cells with 10 nM TCDD. The multiplicity of infection was 0·1–0·6 infectional viral particles per cell.

even 1.0-10.0 nM TCDD had a marked stimulatory effect on HIV-1 production in primary HIV-infected CD4 cells. Because the same concentration of TCDD caused the *Ah*-receptor-mediated induction of CYP1A1, it makes possible to suggest that intracellular receptor-ligand complex is involved to *trans*-activate the CYP1A1 gene and, presumably, HIV-1 gene.

Activation of HIV infection may be effected by chemicals[15], and proteins and viruses[16,17]. In most cases, the effect has been accounted for by a *trans*-activation of the HIV-1 gene that is decisive in the regulation of its reproduction. The *Ah* receptor is a regulatory protein forming an active receptor-ligand complex due to induction by TCDD; the complex was shown to *trans*-activate *Cyp1a1* gene expression[1,3]. Although requiring experimental verification, the suggestion is made that there exists a similar mechanism involved in the activation by TCDD of the HIV-1 gene. If this is the case, a search for distinct drug, an effective competitor with TCDD (or B[α]P in tabacco smoke) for the *Ah*-receptor could solve the problem how to prevent transformation of latent infection in HIV-positive patients to clinical phase of AIDS. By using the model described, we now are testing some natural flavonoids, known to compete with TCDD for the *Ah*-receptor, as potential anti-HIV drugs.

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