Vol. 179, No. 1, 1991
August 30, 1991

2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN AS A POSSIBLE ACTIVATOR OF HIV INFECTION

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Received January 31, 1991

Summary The effects of 10-150 nM 2,3,7,8-TCDD on the HIV infection in the various lymphoid cell cultures have been studied. The level of reproduction of HIV was examined by determination of virus antigen as well as by measurement of reverse trascriptase activity. 2,3,7,8-TCDD does not exert any toxic influence in this concentration on the MT-4 cells and causes the induction of cytochrome P-450-containing monooxygenase. Furthermore, in MT-4 cell culture infected by HIV-1 virus an increase of virus production after a treatment of cells with 2,3,7,8-TCDD has been revealed.

The development of immunodeficiency in AIDS has been associated with specific destruction of T-helper lymphocytes and with decline in their number in the peripheral blood [1]. The ratio of T4/T8 lymphocyte populations has been found to decrease under an effect of 2,3,7,8-tetrachlordibenzo-p-dioxin (2,3,7,8-TCDD) [2]. This chemical agent elicits a much weaker immunodeficiency than human immunodeficiency virus (HIV) infection [2,3]. The mechanism of the immunotoxic action of 2.3,7,8-TCDD is unknown. Today a disconcerting spread of AIDS [4] and concomitantly a continuous rise in the contamination of the environment with xenobiotics such as 2,3,7,7-TCDD [5] are witnessed. The chance of the combined action of the HIV and 2,3,7,8-TCDD also appears to increase, and cooperative actions on the target immune cells may be more deleterious

0006-291X/91 $1.50

than each alone. Here we report the results of investigation of the effect of 2,3,7,8-TCDD upon the reproduction of the HIV in lymphoid cell cultures.

MATERIALS AND METHODS

The experiments were performed with the use of a cell culture chronically infected with human immunodeficiency virus type 1 (HIV-1), this cell culture, namely EVK-IRA/3 was kindly provided by Dr. E. Karamov (Institute of Virology, AMS USSR), and also of lymphoid cell line MT-4 highly susceptible to HIV-1 infection [6]. Cells were maintained in RPMI 1640 medium (GIBCO) supplemented with 20% fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). Estimates of 2,3,7,8-TCDD effects were based on determination of the activity of reverse transcriptase as described previously [7] with some modifications: 15 μl of each supernatant from infected cultures were added to 96-well microtiter plates that contained 50 μl of virus dilution buffer [50 mM Tris-HCl, pH7.8, 100 mM NaCl, 5 mM dithiothreitol (DTT), 0.1% Triton X-100]. 50 μl of the reaction mixture (100 mM Tris-HCl, 300 mM KCl, 10 mM $MgCl_2$, 0,1% Triton, poly(A), oligo(dT) and [^{3}H]TTP) was added and incubated 1h at 37°C. Following the incubation 50 μl of the mixture was dotted on nitrocellulose filters. The filters were washed successively in beakers containing: (i) 0,5 M sodium phosphate buffer pH 7.0 sixtimes; and (ii) 96% ethanol. The filtes were counted inscintillation counter.

The amount of viral protein was measuring by the direct immynoenzyme method [8]. Briefly subsequent twofold dilutions of culture supernatants (0,2 ml per test with addition of Tween 80 to a final concentration of 0.1%) were added to 96-well microtiter plates coated with human anti-HIV-1 IgG. Following a 60-min incubation at 37°C reaction mixtures were washed four times in phosphate saline solution with 0.1% Tween 80 and then human anti-HIV-1 IgG conjugated with horseradish peroxidase was added. After overnight incubation at 4°C the wells were washed and o-phenilendiamin solution was added. Samples with absorbance 3 times greater than negative control (uninfected cells) were considered as positive. Additionaly to exclude nonspecific signals positive serum was added to tested samples. Decrease of the absorbanse values in these cases indicated the presence of viral antigens.

In choice of the appropriate 2,3,7,8-TCDD concentration we took into account that 2,3,7,8-TCDD in range of 10-150 nM is devoid of marked cytotoxicity but does it stimulate the induction of the cytochrome P450IA1-containing microsomal monooxygenase system catalysing of 7-ethoxyresorufin [9].

RESULTS AND DISCUSSION

The effects of 2,3,7,8-TCDD upon reproduction of the HIV in MT-4 cell culture were analysed in two experimental

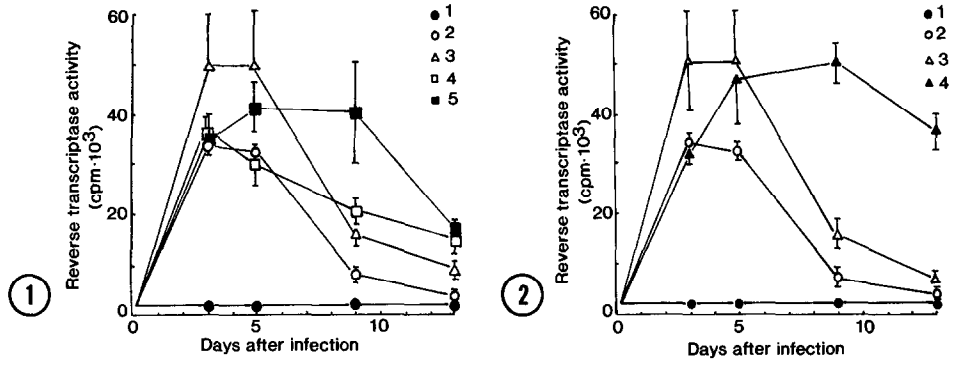

Fig. 1. Effect of different concentrations of 2,3,7,8-TCDD upon the HIV-1 reproduction by the data of reverse transcriptase activity in supernatant of MT-4 cells. 1, incubated with 150 nM 2,3,7,8-TCDD. 2, infected by HIV-1. 3, infected by HIV-1 48 hrs after incubation of cells with 10 nM 2,3,7,8-TCDD. 4, the same as that in 3 with 50 nM 2,3,7,8-TCDD. 5, the same as that in 3 with 150 nM 2,3,7,8-TCDD.

Fig. 2. Effect of prolongation of MT-4 cells incubation with 2,3,7,8-TCDD on the HIV-1 reproduction by the data of reverse transcriptase activity.
1, incubated with 150 nM 2,3,7,8-TCDD. 2, infected by HIV-1. 3, infected by HIV-1 48 hrs after incubation of MT-4 cells with 10 nM 2,3,7,8-TCDD. 4, infected by HIV-1 48 hrs later after the beginning of 1.5 hr incubation of MT-4 cells with 10 nM 2,3,7,8-TCDD.

series: (I) 48 h after the application of 2,3,7,8-TCDD (time for optimal induction of microsomal cytochrome P450IA1), the MT-4 cells were infected with the HIV-1, and (II) 1.5 h after 2,3,7,8-TCDD application, the MT-4 cells were washed off the agent (cells were precipitated by centrifugation, the culture supernatant was removed and the fresh culture medium was added), then MT-4 cells were HIV-infected 48 h later.

Figure 1 presents the results for series (I) in which the effects of different concentrations of 2,3,7,8-TCDD on HIV-1 reproduction were determined. There is 3-6 fold increase in the activity of reverse transcriptase in the cultures treated with 2,3,7,8-TCDD. Its stimulating effect most clear-cut at 2,3,7,8-TCDD concentration of 10 nM. These data correlates well with the results of Schecter et al. [10] who has determined higher PCDD/PCDF level in the blood samples of AIDS patients with opportunistic infections in comparison with HIV-positive patients without any clinical manifestation.

The data of figure 2 provide evidence for persistence in series (II), when the culture medium was replaced 1.5 h

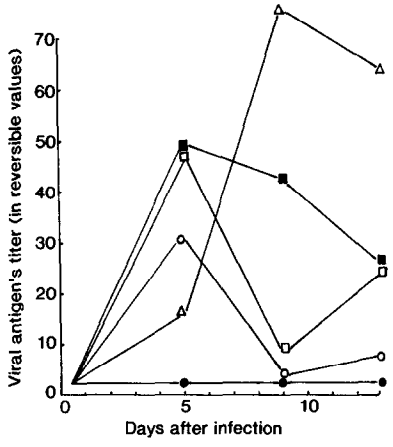

Fig. 3. Effect of 2,3,7,8-TCDD on the level of viral antigen in MT-4 cells infected by HIV-1.
1, incubated with 150 nM 2,3,7,8-TCDD. 2, infected by HIV-1. 3, infected by HIV-1 48 hrs after incubation of cells with 10 nM 2,3,7,8-TCDD. 4, the same as that in 3 with 50 nM 2,3,7,8-TCDD. 5, the same as that in 3 with 150 nM 2,3,7,8-TCDD. A multiplicity of the infection was 0.1-0.6 of infectional viral particles per cell.

after incubation, of the stimulating effect of 2,3,7,8-TCDD, too. The most striking effect following the substitution of culture medium was 9-12 days after HIV-1 infection. The stimulating influence of 2,3,7,8-TCDD added in concentration range of 50 and 150 nM was not related to the time point when it was washed off.

An increase in the activity of reverse transcriptase could not have been due to precisely its induction in the human T-cell leukemia virus type 1 used to produced MT-4 culture cells [11] or to induction of some other endogenous revertase because 2,3,7,8-TCDD treatment of uninfected MT-4 cell culture did not induce any revertase activity.

Concomitantly with determination of revertase activity, we studied the accumulation of viral proteins by immunoenzyme metod (figure 3). With aid of ELISA, we demonstrated a significant increase in viral production after treatment of cells with 2,3,7,8-TCDD. The increase was 4-8-fold being in some depending of 2,3,7,8-TCDD concentration applied.

The results obtained indicate that 2,3,7,8-TCDD has a marked stimulatory effect upon HIV-1 reproduction in primary HIV-infected culture. It is noteworthy that, in spite of the greatly elevated expression of the viral proteins and reverse transcriptase activity, the percentage of viable

cells in the 2,3,7,8-TCDD-treated culture was unaltered, when compared with untreated control culture, remaining as high as 60%.

The increase in viral product was slight (by 20-30% only) in the chronically HIV-1-infected EVK-IRA/3 cells. This cell culture was chosen as the clone showing maximum production of the virus and, perhaps, its capacity to further enhance it might have been depleted.

Activation of HIV infection has been reported for chemical compounds [12], proteins and viruses [13-15]. The effect has been in most cases accounted for by a trans-activation of the HIV that is decisive in the regulation of its reproduction. Thus, in transient expression assays, using a construct containing HIV long terminal repeats (LTR) and the gene encoding chloramphenicol acetyltransferase (CAT) within the pHIV-CAT plasmid, an approximately 10-fold promotion of CAT production by the hepatitis B virus X-protein has been achieved [16]. It is pertinent to note that it has been observed earlier that the X-protein acts as a trans-activator of the homologous and a number of heterologous enhancers of transcription [17].

Regulatory proteins of receptor type such as aromatic hydrocarbon (Ah) receptor, formed as a result of induction by 2,3,7,8-TCDD and able to trans-activate the cytochrome P450IA1 gene expression [18], may be involved in the case of 2,3,7,8-TCDD activation effect observed here. Although needing experimental verification, our assumption is that there act a similar mechanism activation effect of 2,3,7,8-TCDD, particularly a Ah-receptor-2,3,7,8-TCDD complex. The assumption appears the more plausible, when recalling that 2,3,7,8-TCDD being in complex with a highly specific cytosolic Ah-receptor is able to interact with the enhancer region of the human gene P450IA1, thereby producing a trans-activation in the construct comprising the CAT reporter gene [19].

The effect observed appears to be due to 2,3,7,8-TCDD bound to Ah-receptor because the removal of the free 2,3,7,8-TCDD does not interfere with the activation effect (figure 2).

One possibility is that the effect of 2,3,7,8-TCDD may be associated with the influence on the expression of a gene

(or genes) of the regulatory proteins of the HIV, namely tat, art or trs, determining the level of the reproduction of the virus [20]. Another possibility not to be excluded is that the activation of viral production may be the result of combined action on transcription, processing, RNA stability, translation or protein stability.

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