HOST RESISTANCE TO INFLUENZA A VIRUS IN MICE EXPOSED TO 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN

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Introduction

Dioxin and related compounds are widespread and persistent environmental contaminants. The most toxic congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is reported to exert a variety of adverse health effects such as reproductive effects, endocrine disruption as well as immunotoxicity. A WHO meeting held in 1990 established the tolerable daily intake (TDI) of 10 pg/kg body weight/day for TCDD. This value was then reevaluated by a consultation held in 1998, organized by the WHO European Centre for Environment and Health (WHO-ECEH) in conjunction with the International Programme on Chemical Safety (IPCS) [1]. At that meeting, the consultation listed the lowest-observed-adverse-effect-levels (LOAELs) focusing on the effects seen at low doses of TCDD. The lowest value among them, 10 ng/kg of LOAEL, was quoted by Burlesson et al.[2] on the host resistance of mice against influenza A virus infection. However, this result was not utilized for the derivation of TDI, mainly due to the lack of a dose-response relationship. Thus, the TDI was established based on the LOAELs in the range of 28-73 ng/kg, which was transformed into 14-37 pg/kg body weight/day by applying a theoretical equation and dividing by an uncertainty factor of 10, leading to the TDI of 1-4 pg/kg body weight/day [1].

In the course of an immunotoxicity study of TCDD, we recognized that it is imperative to double check the sensitivity of host resistance to the influenza A virus in TCDD-treated mice. In the present study, we investigated the effects of TCDD on the mortality caused by the infection with influenza A virus (A/PR/34/8) in the same experimental protocol in terms of exposure schedule, mouse strain, sex and age, as used by Burlesson et al (1996).

Materials and methods

Animals

7-week old female B6C3F1 mice were purchased from Japan Charles River Inc. (Shiga, Japan) and subjected to acclimatization for one week before use. During the acclimatization period, the health conditions of the animals were examined daily. They were weighed at the beginning and the end of the acclimatization period and provided for the experiments.

TCDD administration

TCDD (Cambridge Isotope Laboratories, Andover, MA) was dissolved in nonane (Sigma, St. Louis, MO) at a concentration of 20 μg/ml and diluted in corn oil. Mice were dosed with either corn oil or TCDD by gavage 7 days prior to the viral infection.

Virus infection

The mouse-adapted strain of influenza virus, A/PR/34/8 (H1N1) was used for infection[3]. Mice were lightly anesthetized with ether and infected with the virus intranasally with 20 μl of the viral suspension. This procedure induces a total respiratory tract infection that causes viral shedding from the lung and leads to death from viral pneumonia.

Health condition, body weight and mortality

After TCDD administration, health conditions of each mouse maintained in a separate cage were observed twice a day until the end of the examination period for 27 days. The body weight was measured once or twice a week during the experimental period.

Results and Discussion

In the previous study, Burlesson et al[2] administered 0-100 ng TCDD/kg to 8-week old B6C3F1 female mice 7 days prior to the viral infection, infected them with the influenza A virus (A/Hong Kong/8/68) at doses capable of inducing a mortality rate of 30% or less, and observed the effect of TCDD on the host resistance. In the present study, we gave 0-500 ng TCDD/kg to mice of the same strain, sex and age and inoculated them with influenza A virus (A/PR/34/8) according to the schedule followed by Burlesson et al. The viral amount which induces a 30% mortality rate was determined in a preliminary study. Based on the results, we inoculated 200 pfu per mouse after TCDD administration. Table 1 summarizes changes in body weight of the mice. Body weight decreases were observed on Days 7 and 10 after viral inoculation in each group, thus confirming the success of the infection. The values recovered thereafter. Table 2 summarizes the survival rate of the mice. Death was observed mainly between 10 - 14 days after the viral infection. However, TCDD in dose ranges of up to 500 ng/kg did not increase the mortality of B6C3F1 female mice.

While Burlesson and coworkers[2] reported that levels of TCDD as low as 10 ng/kg increased the mortality of mice infected with influenza A virus, our results showed no augmented susceptibility to the virus by the administration of 0 - 500 ng/kg of TCDD in mice of the same strain, sex and age. The influenza virus A/PR/34/8 used in our study is a widely used strain of influenza A type virus, isolated in Puerto Rico. Influenza A/Hong Kong/8/68 used in the study by Burlesson et al. was also an A type virus isolated in Hong Kong. Both viruses can cause death by infecting the host's respiratory mucosa, leading to the development of pneumonia. The result in the present study suggests that the alteration in host resistance against influenza A virus in response to TCDD exposure is not a generally-observed phenomenon. In order to explain the discrepancy between our results and the findings reported by Burlesson et al,and to evaluate the risk of this compound and its congeners, further study of the underlying mechanism of TCDD toxicity is necessary.

References

1. Consultation on assessment of the health risk of dioxins; re-evaluation of the tolerable daily intake (TDI): Executive Summary (2000) in: Food Additives and Contaiminants, 17, 223-240.
2. Burlesson, G.R., Lebrec, H., Yang, Y.G., Ibanes, J.D., Pennington, K.N., and Birnbaum, L.S. (1996) Fundam. Appl. Toxicol. 29, 40-47.
3. Tamura, S., Iwasaki, T., Thompson, A.H., Asanuma, H., Chen, Z., Suzuki, Y., Aizawa, C. and Kurata, T. (1998) J. Gen. Virol. 79, 291-299.

Table 1. Changes in body weight after influenza A virus infection

TCDD (ng/kg)	Body weight (g) [a] Days after infection -7	0	3	7	10	13	20
0	20.0 ± 0.5	20.4 ± 0.5	21.0 ± 0.5	18.3 ± 1.7	17.6 ± 2.6	18.8 ± 3.2	22.1 ± 2.0
5	20.0 ± 0.6	20.6 ± 0.8	20.9 ± 1.0	19.4 ± 2.1	19.2 ± 2.8	19.2 ± 3.2	19.7 ± 3.1
20	20.1 ± 0.8	20.7 ± 0.8	20.8 ± 1.2	19.2 ± 2.1	18.6 ± 2.6	19.1 ± 2.6	19.9 ± 3.1
100	19.9 ± 0.7	20.9 ± 0.8	20.8 ± 0.8	20.1 ± 2.5	20.3 ± 3.1	22.8 ± 1.3	24.0 ± 0.7
500	20.3 ± 0.5	20.9 ± 0.9	21.1 ± 0.6	18.7 ± 1.2	17.9 ± 2.1	20.0 ± 2.4	21.9 ± 2.0

[a] Body weights were examined on 10 mice/group. Values were expressed as the mean ± S.D.

Table 2. Effect of TCDD on the survival rate of mice infected with influenza A virus

TCDD (ng/kg)	Surviving mice/ 25 mice [a] Days after infection 0	9	10	11	12	13	14	15	16	17	18	19	20	Survival rate
0	25	25	24	23	22	22	20	20	20	20	20	20	20	80%
5	25	25	25	23	23	23	22	22	22	22	22	22	22	88%
20	25	25	25	25	24	24	24	24	24	24	24	24	24	96%
100	25	25	25	24	24	22	21	21	21	21	21	21	21	84%
500	25	25	24	22	22	21	21	21	21	21	21	20	20	80%

[a] Survival rate was examined using 25 mice/group.