Pilot Study Investigating the Effect of TCDD on the Hamster Antibody Response

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Introduction

TCDD has been studied for its effects on the immune system of mice and rats as much as any other xenobiotic using the sheep red blood cell (SRBC) antibody response. This immune assay requires all of the cellular components of a classical immune response (e.g., B-cells, T-cells, antigen presenting cells)[1] and it is the primary assay of the U.S. EPA immunotoxicity guidelines. Literature indicates varying degrees of sensitivity amongst species to the adverse effects of TCDD.[2,3] While validated in the mouse and rat for regulatory testing, this assay has never been fully characterized for use in other species such as the hamster, a species which has demonstrated some resistance to TCDD effects. Only one report in the peer-reviewed literature has evaluated effects of TCDD on the immune system using hamsters.[4] Yellon *et al.* reported no effect on Mixed Lymphocyte Response and a variable, time of day-dependent decrease in spontaneous blastogensis by day 7. Thus, the SRBC antibody plaque-forming cell assay (PFC) was developed and optimized in the hamster, and a hamster-to-mouse comparison was used to evaluate if the hamster's immune response was as insensitive as it's response to TCDD-induced lethality and wasting syndrome.

Material and Methods

Nine week old female Golden Syrian hamsters and 7 week old female B6C3F1 mice were used in the study. During the development and optimization, hamsters were immunized with a single intraperitoneal (IP) injection of varying SRBC concentrations ($2 \times 10^8 - 2 \times 10^9$/ml) on three, four, five, six or seven days prior to sacrifice and conduct of the PFC assay. To evaluate the effectiveness of the hamster PFC assay, hamsters and mice received dexamethasone (DEX) at 0.1, 0.3 or 1.0 mg/kg IP, or cyclophosphamide (CYP) at 1, 3 and 10 mg/kg IP for 14 days. In the TCDD comparison study, mice and hamsters were gavaged with 0.3, 1.0, 3 or 10 mg/kg/day TCDD for 14 days prior to the PFC assay. A second study was performed in hamsters using IP TCDD administration. On day 11 during the DEX, CYP or TCDD dosing the hamsters and mice were immunized with a single injection of approximately 6×10^8 SRBC/ml delivered IP in 0.5 ml and approximately 5×10^8 SRBC/ml delivered IV in a 0.2 ml, respectively. The data were analyzed using a one-way analysis of variance (ANOVA), a two-tailed Dunnett's tests performed with SAS/STAT® User Guide (SAS Institute Inc. 1989).[5]

Results and Discussion

The optimal hamster PFC antibody response to SRBC occurred on day 4 after immunization using 6.5×10^8 SRBC/ml. The mean day 4 antibody response was 10-fold greater than the antibody response from spleens harvested 3 or 6 days after immunization, and 4-fold greater than in animals harvested 5 days after immunization. The body weights, spleen weights and total spleen cell counts were unaffected by varying the immunizing doses of SRBC.

The hamsters demonstrated a dose-related suppression of immune response to CYP and DEX, similar to that of B6C3F1 mice (data not shown). At 10 mg/kg CYP, the hamster PFC response was statistically different from control animals. Overall, comparable PFC suppression by DEX and CYP was seen for both hamsters and mice. The hamster, therefore, appears to be a suitable model for immunotoxicity testing as it demonstrates sensitivity to immunosuppressants comparable to proven rodent strains such as B6C3F1 mice.

The PFC responses from hamsters administered 0.3, 1, 3 or 10 mg/kg TCDD for 14 consecutive days via oral gavage or i.p. injections were not adversely affected (Figures 1 and 2). The hamsters did not experience any body

weight loss nor were the organ weights (spleen, liver or thymus) affected. Although there appears to be some PFC variability seen with TCDD gavage experiment (but not i.p.), the antibody responses of the hamsters treated with TCDD were not markedly different from those of control animals. Conversely, the antibody response data from B6C3F1 mice demonstrated a dose-related suppression with gavage TCDD which was significantly different from controls at 10 mg/kg TCDD. Although all mice gained weight, spleen weights of mice exposed to 1, 3 or 10 mg/kg dioxin were significantly decreased (18%-32% respectively) from those of control mice, as were the total spleen cell counts for mice exposed to 1 and 10 mg/kg (data not shown).

In summary, the hamster PFC response to SRBC was optimized at day 4 following immunization with a 0.5 ml of approximately 6 x 10^8 SRBC per ml. Hamsters were observed to be as sensitive to prototype immune suppressants (DEX and CYP) as are B6C3F1 mice. However, our preliminary results suggest that the hamster may be more resistant towards the immunosuppressant effects of TCDD than mice. Additional studies will be needed to more fully investigate this preliminary observation. Studies of highly exposed human populations have not demonstrated consistent or clinically relevant immune system alterations associated with exposure to TCDD, or related compounds.[6,7]

References

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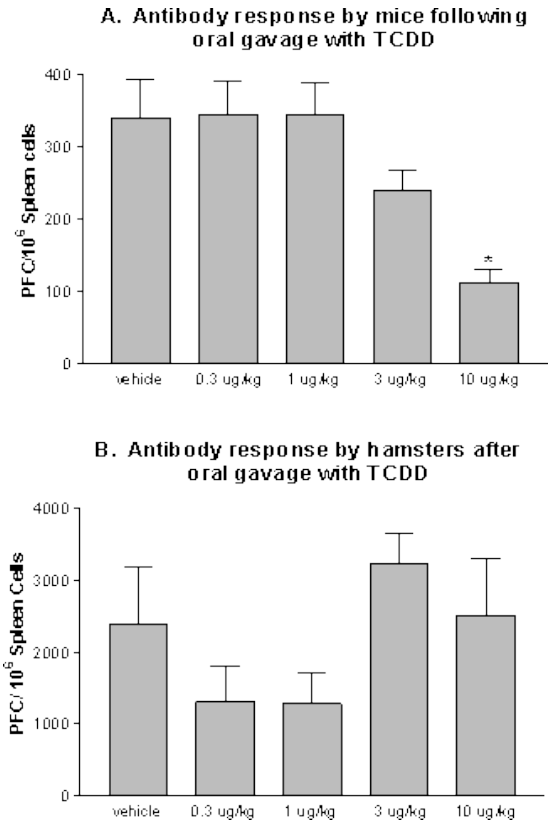
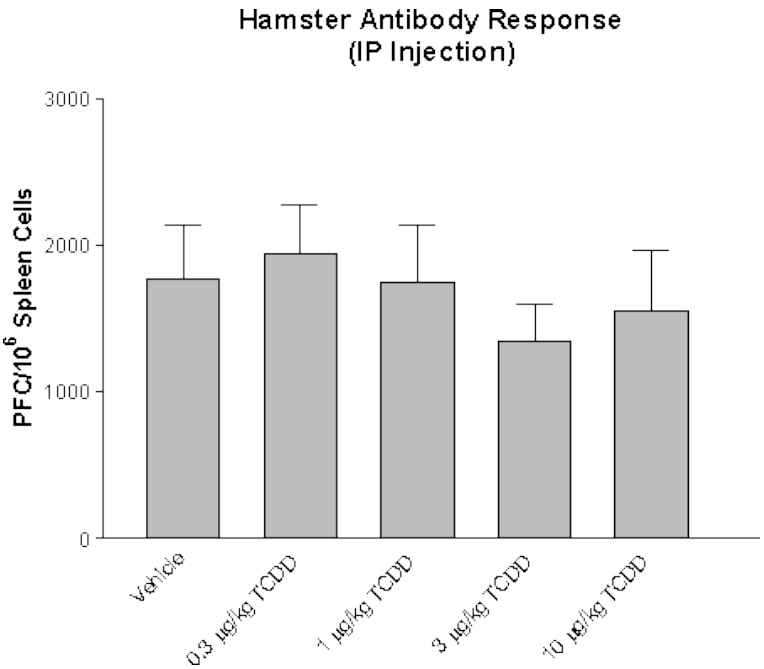
Figure 1. Antibody Response of Mice and Hamsters Exposed (Oral Gavage) to TCDD



Hamsters or mice were administered the specified dose TCDD via oral gavage for 14 days. On day 11, hamsters were immunized IP with 0.5 ml SRBC suspension at 6.5 x 10^8 cell/ml while mice received 0.2 ml IV injections containing 5.1 x 10^8 cells/ml. Spleens were harvested on day 15 and the PFC assay was conducted. Data were compared statistically using Dunnett's test and asterisks indicate statistical significance at $p < 0.05$ (*) compared to control values.

Figure 2. Antibody Response to SRBC after IP Exposure to TCDD



Hamsters were administered the specified dose TCDD via IP injection for 14 days. On day 11, hamsters were immunized IP with 0.5 ml SRBC suspension at 5.5 x 10^8 cell/ml. Spleens were harvested on day 15 and the PFC assay was conducted. Data were compared statistically using Dunnett's test.