Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin upon Hemostasis and Hematologic Function in the Rat

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Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) has been identified as a toxic contaminant of chlorophenols and related hydrocarbons that are widely used in agriculture and industry. It has been implicated in various diseases, including human cases of chloracne (1) and porphyria cutanea tarda (2) and in chick edema (3). Its teratogenicity has been documented in rats (4) and mice (5). Monkeys fed toxic fat, later found to contain TCDD and other chlorinated derivatives of dibenzo-p-dioxin, were observed to undergo a decrease in the cellular elements of the bone marrow and the peripheral blood (6). In contrast, increases in hematocrit and leukocyte count were observed in rats treated with TCDD (7). Depression of blood platelets in TCDD-treated rats and guinea pigs has been recently observed (8). The present studies were undertaken in order to characterize hematologic changes and in particular platelet function alterations in rats exposed to TCDD.

Methods

Animals

Female CD rats, weighing 150–175 g, received daily oral doses of TCDD in an acetone–corn oil mixture at a level of 10 μg/kg. Control rats received an equivalent dose of acetone and corn oil. On days 10 and 14 of treatment animals were anesthetized with methoxyflurane, and blood was withdrawn by cardiac puncture.

Marrow Megakaryocyte Studies

Bone marrow preparations were made as described by Eurenius (9), and smears were prepared with a Wright-Leishman-Giemsa stain.

Platelet Function Studies

Bleeding times were determined on anesthetized animals. Lacerations were made on the ear with a blood lancet, and puncture sites were blotted with filter paper at 30-sec intervals until bleeding stopped. Clot retraction was determined as described in the literature (10). Platelet factor III activity was evaluated with the prothrombin consumption test (11) performed on blood that was allowed to clot with and without added Inosithin. Platelet aggregation was determined macroscopically in a mixture containing, in a final volume of 0.5 ml, 0.1 μmole ADP, 1.0 μmole calcium chloride,
5.0 μmole Tris HCl, pH 7.35, and platelet-rich plasma with a final platelet concentration of 200,000/mm³.

Prothrombin times were performed as described in the literature (12). Factor X assays were performed by measuring clotting time in a mixture containing test serum, 0.1 ml, factor X-deficient plasma (Dade), 0.1 ml, and Simplastin (General Diagnostics), 0.2 ml.

Fibrinogen Degradation Products

Fibrinogen degradation products were assayed in the serum of control and treated rats by using the Wellcome FDP Kit (Burroughs-Wellcome), which employs a hemagglutination-inhibition system.

Other hematologic parameters were determined by routine methods.

Statistical analysis was performed by the Student's t test.

Results

Red cell parameters of control and treated rats are given in Table 1. Packed cell volumes were elevated in TCDD-treated rats at days 10 and 14 of treatment. Similarly, erythrocyte counts were increased in the treatment as compared to control groups at days 10 and 14. Reticulocyte counts differed significantly at day 14, presumably representing lowered values in the control animals. Mean corpuscular volume and mean corpuscular hemoglobin were significantly depressed in treatment groups at day 10 but not day 14. TCDD-treated rats did not differ significantly from controls with regard to either hemoglobin or mean corpuscular hemoglobin concentration.

White cell parameters of control and treated rats are given in Table 2. Total leukocyte counts were elevated, but not significantly, in TCDD-treated animals at 10 and 14 days. Neutrophil counts were significantly increased in both treatment groups when compared to controls. Lymphocyte and monocyte counts were elevated in rats treated 10 and 14 days, and eosinophil counts were slightly depressed. These differences, however, were not statistically significant.

Table 3 demonstrates that platelet counts were significantly lowered in treated rats at days 10 and 14. Bone marrow preparations did not show a corresponding decrease in megakaryocyte numbers of animals treated 14 days. Megakaryocyte size and numbers of nuclei per megakaryocyte were also evaluated, and these were observed to be equivalent in control and treatment groups.

Bleeding times were equivalent in treated and control rats at day 14. These results are presented in Table 4. Clot retraction was diminished in both 10-day and 14-day treatment groups compared to controls. The velocity of platelet aggregation was not significantly altered in TCDD-treated rats.

Table 1. Red cell parameters in rats administered daily oral doses of TCDD of 10 μg/kg.*

|         | 10 Days |         | 14 Days |
|---------|---------|---------|---------|
|         | Control | Treated | Control | Treated |
| Hemoglobin, g/100 ml | 12.3 ± 0.7 | 13.5 ± 0.8 | 12.6 ± 1.2 | 14.8 ± 1.4 |
| Packed cell volume, % | 37.4 ± 2.3 | 43.4 ± 3.7* | 36.6 ± 2.3 | 45.0 ± 4.0* |
| Erythrocyte count × 10⁶, per mm³ | 5.0 ± 0.5 | 6.7 ± 0.4** | 5.2 ± 0.3 | 6.2 ± 0.7* |
| Reticulocyte count, % | 4.2 ± 1.2 | 4.1 ± 1.0 | 1.5 ± 0.6 | 3.3 ± 1.2* |
| Mean corpuscular volume, μm³ | 75.8 ± 4.1 | 65.0 ± 7.0* | 71.1 ± 1.8 | 73.0 ± 2.2 |
| Mean corpuscular Hb concentration, % | 32.8 ± 1.4 | 31.2 ± 1.7 | 34.5 ± 1.3 | 32.9 ± 1.4 |
| Mean corpuscular Hb, pg/cell | 24.9 ± 1.2 | 20.2 ± 1.1** | 24.5 ± 0.9 | 24.3 ± 1.6 |

* Values presented are means ± standard deviation. Four animals were tested in each group. Values marked with asterisks differ significantly from control values: *P <0.05; **P <0.01.
However, prothrombin consumption tests revealed markedly prolonged times, both with and without Inosithin, in rats from both treatment groups. This contrasted with equivalent prothrombin times observed in control and treated animals. No difference in factor X levels between control and treated rats was observed at 14 days.

Serum fibrinogen degradation products were not observed in either control or treatment groups at days 10 and 14. Four animals were tested in each group.

**Discussion**

The elevations in packed cell volumes and erythrocyte counts of TCDD-treated rats reported in the present study are consistent with dehydration and consequent hemoconcentration. These results are in agreement with earlier investigations of TCDD (7). Presumably, the previously reported depression of hematopoiesis in monkeys fed toxic fat (6) represents species variation or the effect of a different toxic contaminant.

The alterations in red cell indices and the leukocytosis with neutrophilia, lymphocytosis and eosinopenia that have been observed in this study are nonspecific hematologic changes consistent with widespread toxicity of TCDD. Of interest is the observed selective depression of blood platelets in treated rats. In view of the finding of normal marrow megakaryocytes, decreased production of platelets in treated rats is unlikely. It is possible that TCDD results in disseminated intravascular coagulation with thrombocytopenia due to the aggregation and incorporation of platelets into platelet-fibrin microthrombi. However, the absence of serum fibrinogen degradation products in treated animals argues against this possibility. Increased peripheral des-
Table 4. Platelet function and procoagulant activity in rats administered daily oral doses of TCDD of 10 μg/kg.

|                     | 10 Days          | 14 Days          |
|---------------------|------------------|------------------|
|                     | Control          | Treated          | Control          | Treated          |
| Bleeding time, min  | —                | —                | 3.6 ± 0.9        | 4.1 ± 2.7        |
| Clot retraction, %  | 73.6 ± 5.8       | 60.8 ± 4.3**     | 81.2 ± 6.0       | 69.8 ± 4.3*      |
|                     | (7)              | (6)              | (3)              | (3)              |
| Velocity of platelet aggregation, sec | 18.0 ± 1.4       | 26.0 ± 8.5       | 23.0 ± 6.4       | 51.2 ± 40.0      |
|                     | (2)              | (2)              | (3)              | (3)              |
| Prothrombin time, sec | 11.8 ± 0.5       | 11.7 ± 1.9       | 11.7 ± 1.8       | 13.5 ± 3.1       |
| Prothrombin consumption test, sec | 22.8 ± 6.7       | 58.5 ± 1.7***    | 18.9 ± 3.6       | 53.0 ± 8.2***    |
| Prothrombin consumption test with Inosithin, sec | 26.2 ± 9.3       | 60.0 ± 0.0***    | 19.8 ± 6.8       | 51.9 ± 16.2*     |
| Factor X, sec       | —                | —                | 19.0 ± 2.0       | 21.0 ± 0.0       |
|                     | (2)              | (2)              | (2)              | (2)              |

* Values presented are means ± standard deviation. Numbers in parentheses indicate number of animals tested; in all other groups four animals were tested.

Values marked with asterisks differ significantly from control values: *P <0.02; **P <0.01; ***P <0.001.

Obstruction of platelets due to an antibody response is a possible mechanism for the observed thrombocytopenia. Such a mechanism is proposed in human cases of hypersensitivity to quinidine and Sedormid (19).

The diminished clot retraction in TCDD-treated animals is consistent with the observed thrombocytopenia. Otherwise, platelet function, as assessed by bleeding time and velocity of aggregation, was not altered. Similarly, decreased platelet factor III activity, which would be reflected in a shortening of the prothrombin consumption time that was corrected by the addition of inosithin, was not observed in treated rats. On the contrary, prothrombin consumption times were consistently prolonged in the treatment groups. In view of the normal prothrombin times, this prolongation of prothrombin consumption times is not readily explainable. Factor X levels were normal in treated animals. Deficiency or inactivation of factor VII, which has been reported in human cases of liver and renal failure and after exposure to propythiouracil, salicylates and indanedione drugs (14), is a possibility. Prolongation of the prothrombin consumption time in factor VII-deficient dogs has been reported (15), but this is accompanied by prolonged prothrombin times. Similarly, deficiency or inactivation of prothrombin might prolong the prothrombin consumption test, but again, an abnormal prothrombin time would be expected. It remains to perform factor VII assays and other coagulation studies in animals exposed to TCDD.

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