TOXICITY OF DIMETHYLAMMONIUM SALT OF 2,4-DICHLORO-PHENOXYACETIC ACID IN RATS AFTER ORAL ADMINISTRATION

Dinara Kaioumova*, Farit Kaioumov** and Caner Süsal*

*Department of Transplantation Immunology, Institute of Immunology, University of Heidelberg, Heidelberg, Germany
**Department of Histology and Embryology, Bashkir State Medical University, Ufa, Russia

Introduction

Since its introduction short after the World War II, because of its low production costs and "relatively" mild toxicity, 2,4-dichlorophenoxyacetic acid (2,4-D) has become the most widely used herbicide in the world (1-3). However, although it is considered relatively non-toxic, 2,4-D in the n-butyl ester form was even included in a 1:1 mixture with 2,4,5-trichlorophenoxyacetic acid in the so-called agent orange, a powerful defoliant which was used between 1965-1970 by the U.S. Army in the Vietnam conflict (4). Due to the insolubility of the acidic form of 2,4 D, commercial herbicide formulations contain more soluble forms of 2,4-D, including alkali and amine salts, and esters.

Although the highly immunotoxic substance 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is not included in commercial 2,4-D preparations, other dioxins, including those with a 2,3,7,8-chlorine substitution were present (5). TCDD is well known for its immunotoxic effects, particularly, on the thymus which, as a T lymphocyte generation site, is extremely vulnerable to toxic agents (6-9). A number of substances have been identified so far that act in different ways on the thymus: some organotin compounds act on immature lymphoblasts in the outer cortex of the thymus; glucocorticosteroids destroy small thymocytes and 2,3,7,8-tetrachlorodibenzo-p-dioxin epithelial cells in the cortex; cyclosporin A has toxic effects on dendritic and epithelial cells in the medulla. The mechanisms of toxicity include binding to the aryl hydrocarbon receptor (dioxins), Ca^{2+}-dependent activation of an endogenous endonuclease which leads to DNA fragmentation due to the programmed cell death apoptosis (dioxins and glucocorticosteroids), and interference with cell proliferation (some organotin compounds) (10).

In the present paper, we investigated by histological, histochemical, and molecular methods the oral toxicity of a commercial herbicide formulation, which contains a dimethylammonium (DMA) salt of 2,4-D, on the rat thymus and spleen.

Material and Methods

The DMA salt of 2,4-D in a commercial formulation containing 500 g dichlorophenoxyacetic acid/l was obtained from the Chimprom Agrochemical plant in Ufa, Russia. Male 10-12 week-old Wistar rats weighting 250-300 g were kept in plastic cages at 22 ± 2^0C and 50±10% humidity. Water and food were supplied *ad libitum*. Four animals per dose group were exposed once to 0, ½ (228 mg/kg), 1/20, and 1/200 LD50 of the herbicide via an esophageal cannula. Thymus, spleen, and blood were removed 4, 8, 12, or 24 hours after treatment under ether anesthesia. Pieces of thymus and spleen were fixed in 10% formalin, processed routinely, and embedded in paraffin. Paraffin sections, cut 5-7 microns in thickness, were stained with hematoxylin and eosin (H&E).

In addition, paraffin sections of the spleen were stained for hemosiderin according to the Perls method (11). Detection of DNA fragmentation in the nuclei of thymocytes and peripheral blood lymphocytes (PBLs) was carried out as described previously (12).

Results and Discussion

Four h after exposure, migration of lymphocytes into the interlobular connective tissue was observed in the thymus of rats that were exposed to ½ LD50 of DMA salt of 2,4-D, and this became more impressive after 8, 12, and 24 h of exposure. As shown in Figure 1, in some cases, blood stagnation and perivascular edema were accompanied by hemorrhage. Cell depletion was observed in the cortex and trabecular disposition of thymocytes in the medulla. The corticomedullary junctions became indistinct. The thymic lobules were decreased in size. Similar changes were observed in all treatment groups.

Moreover, when the nuclei of thymocytes were investigated for DNA fragmentation using gel electrophoresis, with all herbicide doses, a "ladder" pattern characteristic for programmed cell death apoptosis was observed 4 h after exposure. As shown in Figure 2, apoptosis was also detected 8, 12, and 24 h after exposure to 1/20 and 1/200 LD50. However, no apoptosis was found with ½ LD50 after 8, 12, or 24 h because under these conditions the substance caused total degradation of DNA (necrosis) and a smear in the gel. In PBLs, no DNA fragmentation was detected with any of the tested doses after different exposure times (Fig. 2)

A gradual diffuse atrophy of the white pulp was found in the spleen of all rats, affecting the periarteriolar lymphocyte sheaths (PALS) and follicles, particularly already 4 h after exposure to ½ LD50. In all dose groups, this finding was more pronounced after 24 h. There was also an increase in the number of lymphocytes and macrophages in the red pulp.

After 24 h exposure to the substance, in all dose groups, we noted in the red pulp of spleen many macrophages containing hemosiderin (Fig. 3). The white pulp was inactive, however, in the 24 h exposure groups, macrophages with hemosiderin granules were found in the center of the white pulp.

We conclude from the present findings that DMA salt of 2,4-D, a widely used herbicide which is considered relatively non-toxic, affects the lymphoid system by destroying the vascular integrity in the thymus, causing cell depletion in the white pulp of spleen and in the cortex of the thymus, which in the case of thymus is at least partly due to the programmed cell death of thymocytes and/or increased cell release into the peripheral blood. It is also possible that this herbicide has hemolytic activity and causes intervascular hemolysis of erythrocytes. Also, the increase of hemosiderin in macrophages indicates phagocytosis of destructed erythrocytes.

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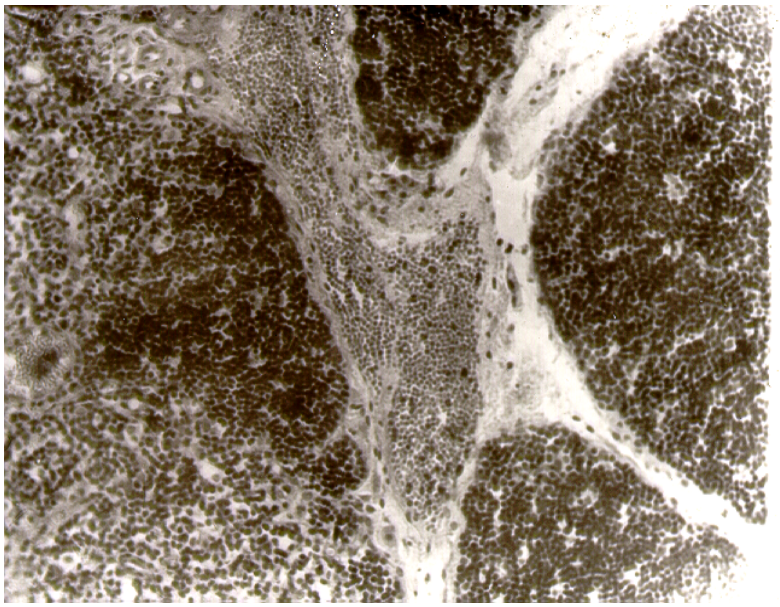

Fig. 1. Light microscopic picture of hematoxylin and eosin-stained paraffin section of thymus of a rat that was exposed 8 h to ½ LD50 of dimethylammonium salt of 2,4-dichlorophenoxyacetic acid (H&E x 20). Blood stagnation and perivascular edema of large interlobular vessels are observed.

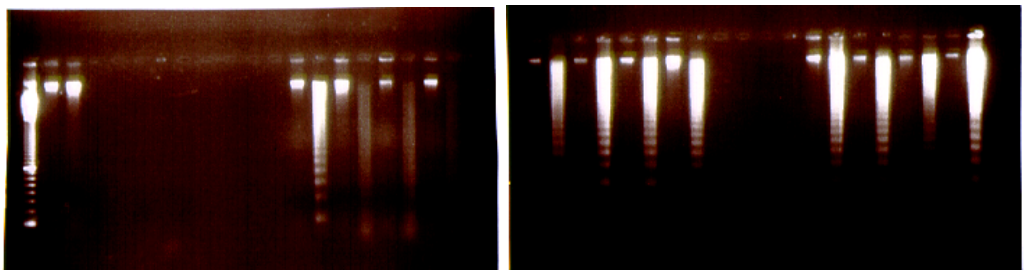

Fig. 2. Nucleosomal DNA fragmentation analysis of peripheral blood lymphocytes and thymocytes of dimethylammonium salt of 2,4-dichlorophenoxyacetic acid-exposed rats in gel electrophoresis. M: 100 bp DNA marker; C1: normal lymphocytes; C2: normal thymocytes; L: lymphocytes and T: thymocytes after 4, 8, 12, and 24 h exposure to ½, 1/20, or 1/200 LD50. after different exposure times to 1/20 and 1/200 LD50, nucleosomal DNA fragmentation was found in thymocytes, but not in lymphocytes of rats. With ½ LD50, DNA fragmentation was observed in thymocytes after 4 h exposure only because after >4 h exposure, DNA was degradated totally.

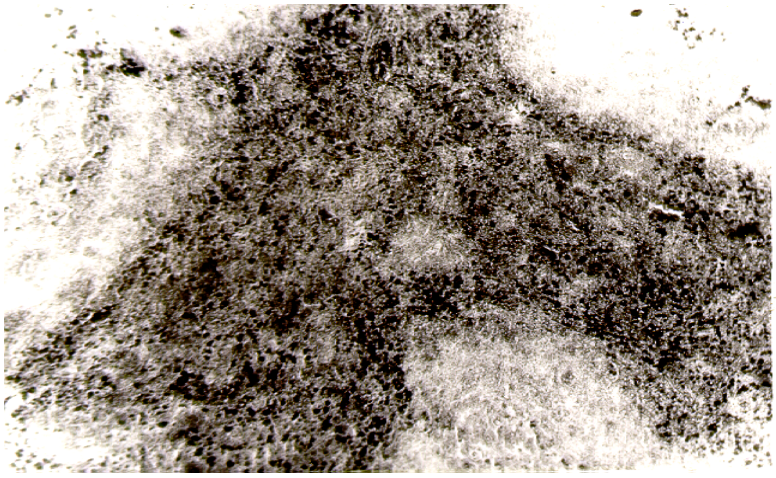

Fig. 3. Histochemical staining of the spleen for hemosiderin (Perls x 40). An extensive hemosiderin load of macrophages is observed in the red pulp of spleen after 24 h exposure of rats to ½ LD50 of dimethylammonium salt of 2,4-dichlorophenoxyacetic acid.