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Investigation of the accumulation of 2,4-dichlorophenoxyacetic acid (2,4-D) in rat kidneys

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Abstract

The accumulation of 2,4-dichlorophenoxyacetic acid (2,4-D) and its metabolite 2,4-dichlorophenol (2,4-DCP) in the kidneys of rats was investigated. Male and female Sprague–Dawley rats were given 2,4-D in drinking water and food for 30 days. Group A (control group) was fed a normal diet, Group B was fed 50 ppm 2,4-D in 15 g food, Group C received 100 ppm 2,4-D in 15 g food, Group D received 25 ppm 2,4-D in 15 ml drinking water and Group E was given 50 ppm 2,4-D in 15 ml of drinking water. Levels of 2,4-D and 2,4-DCP in kidneys were determined using high performance liquid chromatography (HPLC). It was observed that at low doses of 2,4-D, the metabolite, 2,4-DCP found in the kidneys.

Keywords: 2,4-Dichlorophenoxyacetic acid (2,4-D); 2,4-Dichlorophenol (2,4-DCP); HPLC; Kidney accumulation; Rat

1. Introductıon

2,4-Dichlorophenoxyacetic acid (2,4-D) is a selective herbicide of the phenoxyacetic acid group, with weak aromatic acid properties. It is used to control broad-leafed weeds. Commercial formulations of 2,4-D include the free acid, alkaline and amine salts and ester formulations. 2,4-D itself is chemically stable, but its esters are rapidly hydrolysed to the free acid. Exposure of humans and animals occurs through contaminated air, drinking water, soil and foodstuff or during production of the herbicide. It has been reported that 2,4-D may cause a health risk [1–3]. Also, *n*-butyl esters were used as a spray defoliant (a mixture of 2,4-D and 2,4,5-T) (Agent Orange) during the Vietnam War. Due to the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) contained in the mixture, poisonings characterized by porphyria and teratogenic effects occurred. Its structure resembles the specific plant hormone indolacetic acid that may play a role as a growth regulator in plants [4–6]. 2,4-D is a member of the chlorophenoxyacetic acid herbicides. Because these compounds contain chlorine, they pose a risk for dioxin formation [2].

Although it is the most highly used herbicide in the world and the third most commonly used in the United States and Canada since the 1940s, definitive data are not available concerning its carcinogenity, mutagenity and genotoxicity. Epidemiological studies have attempted to determine the relationship between 2,4-D exposure and non-Hodgkins lymphoma. In addition, its relation to soft tissue sarcomas and Hodgkins disease has not been defined. Although 2,4-D has been shown to be safe in humans and animals (oral LD_{50} 300–1000 mg/kg body weight in mammals), it has been noted that central nervous system symptoms characterized by motor incoordination, weakening of reflexes, tenseness and coma may develop with increased doses in humans, monkeys, rats, dogs, cats, pigs and rabbits. It has also been reported that it affects skeletal muscles and may produce myotony in rats, humans and cats [7–9].

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4-DCP, 2,4-dichlorophenol

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Absorption of chlorophenoxy compounds is via the gastrointestinal tract. There is minimal absorption from the lung and skin [10]. The herbicide is a lipophilic weak acid with a low pK_a. Due to its lipophilicity, biological membranes are targets. While information on its metabolism is limited, it has been reported that following oral administration, almost all of the dose is rapidly absorbed and eliminated in the urine [11–13]. The kidney is the target organ in subchronic studies of 2,4-D in rats [1,14]. In the present study, the accumulation of this herbicide in the kidneys of rats was investigated.

2. Materials and methods

Forty male and female Sprague–Dawley rats were obtained from Istanbul University, Cerrahpasa Faculty of Medicine, Experimental Animals Production and Investigation Centre. The experiment protocol was approved by the Istanbul University, Faculty of Veterinary Medicine, Experimental Animal Ethics Committee.

2.1. Procedure for experimental animals

Rats, approximately 2 months of age, weighed between 130 and 140 g. Animals were kept under observation for 1 week before experimentation under usual conditions (20 ± 2 °C and 50 ± 5% humidity) in the animal room. Food and water were prepared by the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Istanbul University.

The 40 rats were randomly divided into 5 groups of 8 animals each. Group A (control) was fed a normal diet and drinking water, Group B was given 50 ppm 2,4-D in food, Group C was given 100 ppm 2,4-D in food, Group D was given 25 ppm and Group E 50 ppm 2,4-D in drinking water for 30 days. Food and water was prepared each morning. After mixing 2,4-D into the food, 15 g of food was given to each rat.

2.2. Reagents and materials

Acetonitrile, methanol, diethyl ether (HPLC grade), hydrochloric acid, 2,4-dichlorophenoxyacetic acid and 2,4-dichlorophenol were purchased from Merck (Darmstadt, Germany). 2,4-D and 2,4-dichlorophenol (2,4-DCP) stock solutions (0.1 mg/ml) were prepared by dissolving in bidistilled water. SPE cartridges containing 500 mg of the Isolute C-18 were obtained from IST (Mid Glamorgan, UK).

2.3. High performance liquid chromatography (HPLC) procedure

Analysis used the Shimadzu LC-10A liquid chromatograph with a UV detector at a wavelength of 235 nm. Twenty microlitres were injected for all standards and final extracts of kidney samples. The mobile phase was a mixture of 4% acetic acid/acetonitrile (60:40). A Hypersil ODS (125 mm × 4 mm, 5 μm) column was used for separation. The flow rate was 1 mL/min (pressure about 2100 psi) and the temperature was ambient.

2.4. Extraction procedure

A 1-g kidney sample was homogenized in the ultra-turrax (T 25, IKA, Germany) by adding 10 ml of distilled water and 1 ml 1N HCl. To this was added 30 ml diethyl ether followed by shaking for half an hour. Each sample was centrifuged at 20 °C and 4000 rpm for 15 min, and the supernatant was removed. This procedure was repeated three times. The diethyl ether collected was completely evaporated to dryness at 40 °C under a stream of nitrogen gas. The C-18 SPE cartridge was drained with a vacuum manifold system, washed first with 3 ml methanol and then 10 ml 4% acetic acid. A 20-μl volume of acidic solution was injected into the HPLC.

2.5. Standards for calibration graphs

To produce the calibration curve, 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4 and 5 ppm concentrations of 2,4-D and 2,4-DCP standard solutions were prepared in deionised water. These standard solutions were analyzed to generate the calibration curve.

2.6. Linearity and recovery

The method was linear from 0.2 to 5 ppm ($r^2 = 0.998$). Linearity was assessed by a weighted ($1/x^2$) least squares regression analysis. The limit of detection was determined at a signal-to-noise ratio of 3 (S/N = 3), and was determined to be 0.03 ppm for 2,4-D and 0.05 ppm for 2,4-DCP. The recovery was determined by comparing peak areas after extraction with peak areas obtained by direct injection of equal amounts of 2,4-D and 2,4-DCP. The mean recoveries from kidney samples of 2,4-D and 2,4-DCP were found to be 61.11 and 63.09%, respectively, at concentrations ranging from 0.2 to 5 ppm ($n = 3$).

2.7. Statistical analysis

All data were computerized as mean ± S.D. using The GraphPad Instat software. Levels of significance were determined by one-way analysis of variance (ANOVA). For all statistical tests, differences were regarded as significant if $p < 0.05$.

3. Results

Kidney levels of 2,4-D and its metabolite 2,4-DCP (mean ± S.D.) in the control, food and water groups are shown in Table 1. 2,4-D and 2,4-DCP were found in all