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Effects of Sodium 2,4-Dichlorophenoxyacetate on Renal Function in the Rat

J. Łukowicz-Ratajczak and J. Krechniak

Department of Toxicology, Medical Academy, Al. Marksa 107, 80-416 Gdańsk, Poland

Chlorophenoxy compounds, of which 2,4-dichlorophenoxyacetic acid commonly abbreviated as 2,4-D, has the biggest share, are the most familiar chemicals used in weed control in Poland. Despite their widely use as herbicides their mechanism of toxic action is poorly understood.

The salt forms of phenoxy acids are rapidly hydrolyzed to free acids (Erne 1966). Chlorophenoxy acids are not significantly metabolized in mammals and promptly excreted by active secretion into urine (Grunow and Böhme 1974).

2,4-D has been found to be concentrated in kidney slices by an active transport mechanism (Berndt and Koschier 1973).

High doses of chlorophenoxy compounds influence renal function (Koschier and Berndt 1976; Koschier and Acara 1979) wheras lower doses seem to be relatively ineffective in relation to kidney.

The present study investigates the influence of sodium 2,4-D on several renal function parameters.
The current results confirm the relatively low nephrotoxicity of this herbicide.

MATERIALS AND METHODS

Male, Wistar rats weighing 185 to 215 grams, maintained with standard food and water ad libitum, were injected intraperitoneally with a sterile solution of sodium 2,4-dichlorophenoxyacetate (2,4-D) adjusted to pH=7,4. The herbicide was administered every other day for 12

Send reprint request to Dr Jerzy Krechniak at the above address.

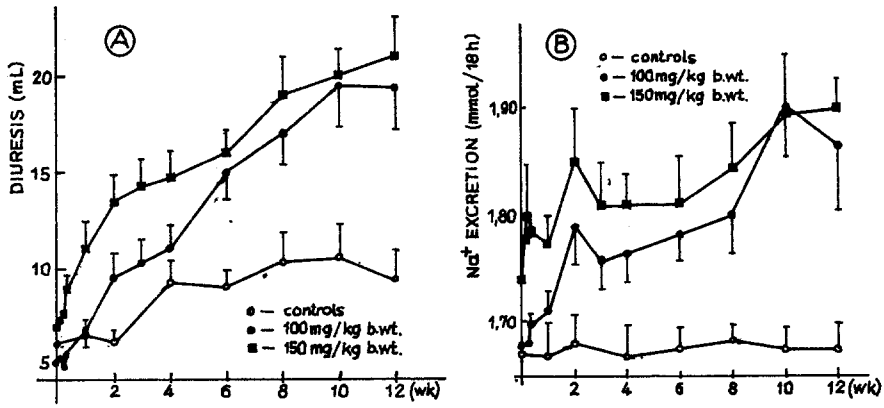

Figure 1. Urine volume (A) and urinary excretion of sodium (B) in rats treated with sodium 2,4-D. Each point represents the mean ± S.D. in 8 animals.

weeks in two doses: 100 mg/kg (group II) and 150 mg/kg (group III). Control animals (group I) were given the equivalent amount of sodium in saline.

Blood and urine were collected at the 2,3,7,14,21,28, 42,56,70 and 84 day of the experiment. Urea concentration was determined according to Walton et al. (1965) in blood obtained from a tail vein. Urine sample were collected from animals placed in glass metabolic cages for 18 h. Excretion of sodium and potassium was ascertained by flame photometry (Pye Unicam 2900 Flame Photometer). Hydrogen ions were determined in urine by a Radiometer BMS 3 pH-meter, alpha ketoacids by the Friedman and Haugen method (1943), alpha amino nitrogen by the Doi method (1981), protein by the biuret method and urea by the Walton et al. method (1965). Moreover diuresis and pH were measured.

At 3 and 8 weeks of the experiment glomerular filtration and parameters of acid-base balance in blood were determined. Clearance experiments were performed by the continuous infusion technique according to a standard procedure. The data were analyzed statistically using Student's t-test.

RESULTS AND DISCUSSION

Data illustrating the changes in diuresis during the course of the experiment are shown in figure 1 A. In both groups of animals treated with sodium 2,4-D the volume of urine is significantly increased ($p < 0,001$) when compared with controls.

Rats treated with sodium salt of 2,4-D excreted with

urine more sodium than controls ($p < 0,05$). The increase occurs in a dose and time dependent manner (figure 1B).

The glomerular filtration rates (table 1) after 3 and 8 weeks of dosing in rats given the larger dose were decreased ($p < 0,05$) to 0,76 and 0,97 ml/min. respectively. No significant changes were found in animals receiving 100 mg/kg of sodium 2,4-D.

Table 1. Glomerular filtration in rats treated with sodium 2,4-D (ml/min.)

Time / Group	3 wk	8 wk
I	1,29 ± 0,08	1,20 ± 0,06
II	1,13 ± 0,07	1,22 ± 0,08
III	0,76[a] ± 0,04	0,97[a] ± 0,06

Values presented are the mean ± standard deviation of 8 rats/group. I- controls group; II- rats treated with 100 mg/kg sodium 2,4-D; III- rats treated with 150 mg/kg sodium 2,4-D; a- significantly different from controls: $p < 0,05$

The concentrations of urea in blood (figure 2A) and urine (figure 2 B) are markedly elevated ($p < 0,001$) in animals given the larger dose of sodium 2,4-D when compared with controls. In rats receiving the 100 mg/kg dose the increase is not significant.

A decline in urinary pH was noticed in rats treated with the larger dose of sodium 2,4-D, however the decline was not significant (figure 3 A). In addition the secretion of hydrogen ions is elevated in animals given sodium 2,4-D (figure 3 B). In rats given the 150 mg/kg dose the increase is highly significant ($p < 0,001$).

The parameters of acid-base balance in blood as well as the concentration of potassium, alpha ketoacids, alpha amino nitrogen and protein (results not shown) in both groups of animals dosed with sodium 2,4-D did not differ significantly from controls.

In the present experiment rats were given intraperitoneally sodium 2,4-D and several renal function parameters were examined.

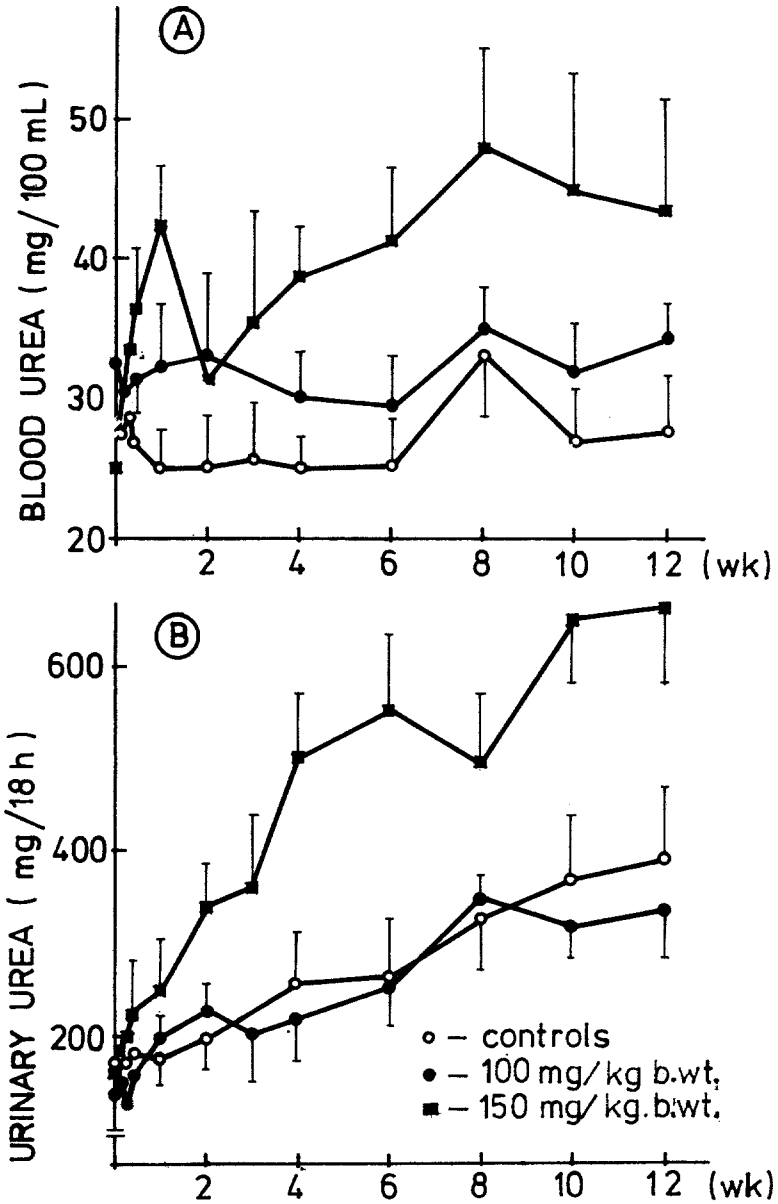

Figure 2. Urea in blood (A) and urine (B) in rats treated with sodium 2,4-D. Each point represents the mean ± S.D. in 8 animals.

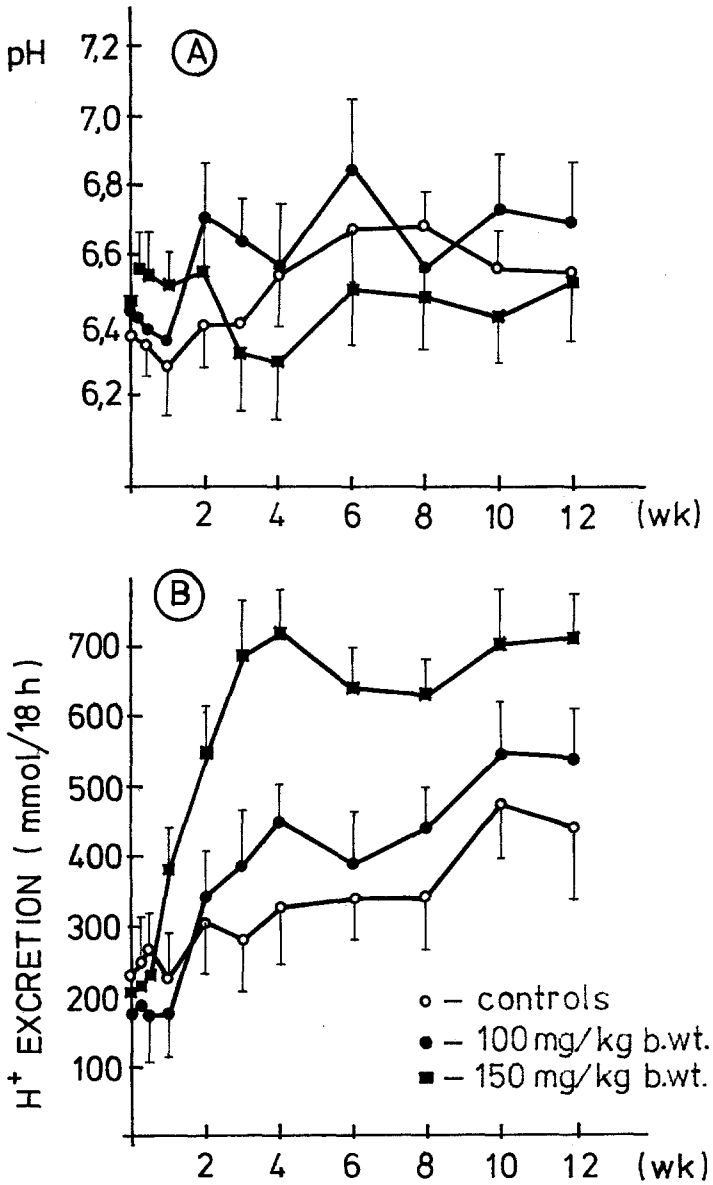

Figure 3. Urinary pH (A) and excretion of hydrogen ions (B) in rats treated with sodium 2,4-D. Each point represents the mean ± S.D. in 8 animals.

The selected doses of the herbicide (100 and 150 mg/kg) were the highest which the animals survived during the 12 weeks experiment. Repeated treatment with sodium 2,4-D at the dose of 200 mg/kg proved to be lethal for rats.

The increase in urinary output caused by 2,4-D was dose and time dependent (figure 1 A). An elevation in diuresis found also by Kociba et al. (1979) in an experiment on mice given 30 mg/kg/day of 2,4,5-T for 2 years. The increased diuresis was accompanied by slight morphological changes and an increase in weight of kidney.

In addition glomerular filtration was distinctly decreased (table 1) by the larger dose of sodium 2,4-D. Koschier and Acara (1979) demonstrated in the isolated perfused rat kidney that high doses (1 mM) of 2,4,5-T caused a decrease in GFR. The exact nature of the impairment of GFR is not understood at present.

In this study rats treated with the herbicide excreted more sodium than control animals (figure 1 B). The excretion of sodium was dose and time dependent.

A considerable increase in blood urea concurrent with an elevation of urea concentration in urine were found in rats treated with sodium 2,4-D (figure 2). Repeated administration of the larger dose caused a rise in blood urea concentration for the first 8 weeks of dosing and an increase in urea excretion throughout the whole time course of the experiment.

Decreased glomerular filtration, increased blood urea, urinary output and sodium excretion are typical syndromes of kidney impairment caused by several nephrotoxic compounds.

Concurrently in animals treated with the larger dose of sodium 2,4-D an enhanced secretion of hydrogen ions was noticed (figure 3). It seems that this is directly connected with the urinary excretion of 2,4-dichlorophenoxyacetic acid, as it salts rapidly hydrolyze to the free acid which is eliminated with urine in 90% as an unchanged compound (Erne 1966; Grunow and Böhme 1974).

In our experiment sodium 2,4-D did not alter the acid-base balance in rats. Also no significant changes in excretion of potassium ions, alpha ketoacids, alpha amino nitrogen and protein were ascertained.

In conclusion, the results of this study indicate that 2,4-D given in repeated sublethal doses caused renal

impairment by depressing glomerular filtration and increasing blood and urinary urea. The absence of ketoaciduria, aminoaciduria and proteinuria indicates that no functional signs of the damage of proximal tubules were ascertained. Increased diuresis and natriuresis may indicate an injury of other parts of the nephron. Enhanced urinary output and excretion of sodium without an impairment of proximal tubule function, as documented by micropuncture techniques, is typical for loop diuretics (Odlind 1984).

It seems that the loop of Henle is the site of action of 2,4-dichlorophenoxyacetic acid in the kidney.

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