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Effects of 2-Methyl-4-chlorophenoxyacetic Acid on the Catalase Liver Activity of Chicken Embryos

R. Maci,[1] M. Colleoni,[2] P. Fumagalli,[2] and A. Santagostino[2]

[1]Biology Dept., Zoology and Comparative Anatomy Section, University of Milan, 20133 Milano, Italia and [2]Institute of Pharmacology, Faculty of Sciences, University of Milan, 20129 Milano, Italia

Although the herbicide 4-chloro-2-methylphenoxyacetic acid (MCPA) is used widely as an agricultural chemical, few studies have been made of its biological effects in animals. Epidemiological research has suggested it may be carcinogenic (Hardell and Sandstrom 1979) and recent studies have substantiated the hypothesis that phenoxyacetic herbicides exert their carcinogenic action indirectly via peroxisome proliferation (Vainio et al. 1983; Hietanen et al. 1985).

Recently in our laboratory we have used chicken embryos as models for mechanistic and descriptive toxicology of various pesticides and indirectly to monitor environmental quality (Maci and Arias 1987).

The purpose of this study was to investigate the effect on hepatocyte catalase (the marker enzyme for peroxisomes) of chicken embryos treated with a commercial form of MCPA (Erbitox E30); the effects of a pure form of MCPA sodium-potassium salt were studied as a comparison.

MATERIALS AND METHODS

Fertile White Leghorn hen eggs obtained from a local hatchery were used. Eggs were stored for no more than one week before incubation.

MCPA, as a sodium-potassium salt (Aldrich) and as Erbitox E30 (Siapa, Roma, Italy) containing 28% MCPA sodium-potassium salt, was dissolved in distilled water and was injected in single doses of 2 mg or 0.4 mg/egg, respectively 1/2 and 1/10 of the LD_{50} in the chicken embryo (Maci and Arias 1983), into the egg air chambers on day 0 of their incubation period. All solutions were sterilized through a 0.45 μm Millex-Ha

Send reprint requests to Dr. A. Santagostino Institute of Pharmacology, Faculty of Sciences, Via Vanvitelli 32/A, 20129 Milano, Italia.

filter.

The eggs were then incubated for 19 days in a forced-draught incubator with automatic hourly rotation of eggs at 37.8°C and 85 % relative humidity.

On the 19th day the embryos were weighed and the livers were removed and rinsed in ice-cold 0.01 M phosphate buffer solution at pH 7.4. The livers were homogenized in four volumes of ice-cold 0.01 M phosphate buffer, pH 7.4, with an Ultra Turrax (Ika Werk); the homogenates were subsequently sonicated for 30 min with an Ultrasonic (Sti. Min.) and then centrifuged at 20.000xg for 20 min at 0°C. The supernatants were diluted with 0.01 M phosphate buffer solution (PBS), at pH 7.4 in a 1:1 (v:v) ratio and 1% by volume of ethyl alcohol was then added, to prevent formation of catalase compound II.

Catalase activity was analyzed with a Clark oxygen electrode essentially using a method developed by Del Rio et al. (1977). An oxygen monitor (Yellow Springs) was used, connected to an ultrathermostat (Lauda).Homogenates were added to 3 ml 0.01 M PBS at pH 7.4, de-aerated with nitrogen and containing 0.6 mM H_2O_2 at 25°C. The enzymatic activity was calculated from the initial rate of O_2 liberation. Catalase activity was expressed as μmol O_2 /min/ mg of protein.

Proteins were measured by the method of Lowry et al.(1951) with bovine serum albumin as standard.

Analysis of variance (ANOVA) and Duncan's test (Steel and Torrie 1960) were used for statistical analysis.

RESULTS AND DISCUSSION

Treatment with MCPA as the pure sodium-potassium salt or as Erbitox E30 at 2 mg/egg, significantly reduced the size and weight of the chicken embryo body (Table 1).Although the weights of the livers of treated embryos and controls were not significantly different, the liver/body weight ratios of the treated embryos were significantly higher.
Treatment with MCPA seemed to affect only the livers. In fact in all the embryos treated with MCPA as the pure form and with 2 mg Erbitox E30, and in 55% of those given 0.4 mg/egg, the livers turned a greenish colour.
Histological examinations of liver showed few changes, consisting of vacuolization of the hepatocytes and occasional bile thrombi. In the 2 mg/egg treated embryos the gallbladder was empty , suggesting that bile efflux from the liver may be affected.

Table 1. Effects of Erbitox E30 and pure MCPA on body weight, liver weight and on the ratio of liver weight/100 g body weight of the chicken embryo (mean±SEM ; n=9)

Treatment	Body weight (g)	Liver weight (g)	Liver weight/ Body weight (g/100g)
Control	34.79±0.59a*	0.56±0.03a	1.61±0.08a
Erbitox 0.4mg/egg	32.61±0.63a	0.63±0.02a	1.93±0.04b
Erbitox 2mg/egg	29.16±0.50b	0.58±0.02a	1.97±0.08b
MCPA 2mg/egg	26.87±0.73b	0.60±0.04a	2.20±0.12b

*Data followed by the same letter in the same column are not significantly different at 5% by Duncan's New Multiple Range Test.

As far as catalase activity is concerned, only the dose of 2mg/egg of Erbitox E30 caused a significant increase compared to controls . MCPA, as the pure sodium-potassium salt,induced an identical increase ,showing that the effect of Erbitox E 30 on catalase activity can be attributed to the MCPA in the herbicide(Table 2)

Table 2. Effects of Erbitox E30 and pure MCPA on hepatic catalase activity in chicken embryo (mean± SEM ; n=9)

Treatment	Catalase activity μmol O_2/min/mg	% Δ from Control
Control	4.85±0.24a*	-
Erbitox 0.4mg/egg	5.39±0.39a	+11.13
Erbitox 2mg/egg	7.17±0.43b	+47.83
MCPA 2mg/egg	7.50±0.45b	+51.21

*Data followed by the same letter are not significantly different at 5% by Duncan's New Multiple Range Test.

The significant increase of catalase activity in the livers of chicken embryos treated with MCPA may be evidence of peroxisome proliferation, as already reported by Vainio et al. (1983) in rats, an the

relative hepatomegalia (Table 1) seems to confirm. However further histological studies and measurement of another "marker" such as ß-oxidation of fatty acids will be useful to confirm this peroxisomal proliferation.

There is much debate at present on whether the carcinogenicity and peroxisomal proliferative activity induced in rats by reasonable doses of certain widely used compounds is in fact likely to constitute a problem for man when exposed to these substances. The question is still wide open whether these compounds act through similar mechanisms in primates and other species. However, in initial attempts to investigate this, chicken embryos could well offer a useful alternative to the large-scale use of mammals.
The data obtained in this study and other recent work in chicken embryos concerning the interference of phenoxy-herbicides on hepatic metabolizing enzyme activities (Santagostino et al. 1986) are very similar to the results reported by others in rats (Vainio et al. 1983; Hietanen et al. 1983).This model is easy and cheap, and offers high sensitivity and selectivity. It could lend itself not only to prescreening of embryotoxicity, but also to toxicity studies on hepatocytes.

REFERENCES

Del Rio L, Ortega MG, Lopez AL, Gorge' JL (1977) A more sensitive modification of the catalase assay with the Clark oxygen electrode. Anal Biochem 80:409- 415

Hardell L, Sandstrom A (1979) Case-control study: Soft-tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. Br J Cancer 39:711-717

Hietanen E, Ahotupa M, Heinonen T, Hamalainen H, Kunnas T,Linnainmaa K, Mantyla E, Vainio H (1985) Enhanced peroxisomal ß-oxidation of fatty acids and glutathione metabolism in rats exposed to phenoxyacetic acid. Toxicology 34:103-111

Hietanen E,Linnainmaa K,Vainio H (1983) Effects of phenoxyherbicides and glyphosate on the hepatic and intestinal biotransformation activities in the rat. Acta Pharmacol Toxicol 53:103-112

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurements with the Folin phenol reagent . J Biol Chem 193:265-275

Maci R, Arias E (1983) Toxicity of 2-methyl-4-Chlorophenoxyacetic acid (MCPA) in chick embryos. Acta Embryol Morphol Exp 4:201

Maci R, Arias E (1987) Teratogenic effects of the fungicide Maneb on chick embryos. Ecotoxicol Environ Safety 13:169-173

Santagostino A, Geoni L, Leone MP, Maci R, Marabini L (1986) Effect of MCPA on the hepatic microsomal

enzymes of chicken embryo. VII Congresso Nazionale della Societa' Italiana di Tossicologia, Bari 1-4 october 1986, Abstracts, p 68

Steel RGD, Torrie JH (1960) Principle and procedures of statistic. McGraw-Hill Book Company,Inc.,New York

Vainio H, Linnainmaa K, Kahonen M, Nickels J, Hietanen E, Marniemi J, Peltonen P (1983) Hypolipidemia and peroxisome proliferation induced by phenoxyacetic acid herbicides in rats. Biochem Pharmacol 32:2775-2779

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