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Short Communication

DAMAGE TO THE ERYTHROCYTE MEMBRANE CAUSED BY CHLOROPHENOXYACETIC HERBICIDES

PIOTR DUCHNOWICZ and MARIA KOTER

Department of Biophysics of Environmental Protection, University of Łódź,
Banacha 12/16, 90-247 Łódź, Poland

Abstract: We studied the damage caused to erythrocyte membranes by chlorophenoxyacetic herbicides. An increase in haemolysis was observed. The compounds investigated caused lipid bilayer damage by lipid peroxidation, as well as an increase in membrane fluidity at the 16th carbon atom of fatty acids was observed. Metabolites caused damage to membrane proteins – the free SH group content was increased. Higher toxicity of metabolites compared to basic compounds was observed.

Key Words: Chlorophenoxyacetic Herbicides, Membrane Fluidity, –SH Groups, Hemolysis

INTRODUCTION

The herbicide properties of chlorophenoxylic compounds have been known since 1942, and their use in agriculture dates back to 1944. They are commonly used for destroying dicotyledonous weeds in crop cultures, grazing lands and lawns. They are also used for removing aquatic plants from drainage ditches, as growth regulators (of auxin-like activity), to prevent premature fruit dropping, and as substances prolonging fruit durability in storage.

Phenoxy (phenoxyacetic) herbicides are toxicity class III and IV compounds, i.e. they should exert a moderate acute toxic effect on mammals. A decrease in mobility, a decline in body mass, a drop in metabolic rate [1], the induction of various types of neoplasma [2, 3], a decrease in the activity of a series of enzymes [4], and chromosome aberrations and DNA strand breaks [5] were observed in laboratory animals.

Sixty six cases of chlorophenoxy herbicide intoxication were described in humans, of which 22 turned to be fatal [6]. Liver and kidney failure, hypotension, a decrease in enzymatic activity [7, 8] and chromosome aberrations

[9] were observed for intoxicated individuals. A higher rate of cancer mortality was observed in people frequently exposed to these herbicides [10, 11]. Insufficient information is currently available on the molecular background of the toxic properties of phenoxy herbicides for man and animals. There is an especially great need for studies on the impact of these compounds on the properties of blood, the tissue which distributes all xenobiotics to other sites in the body. In this study, we describe the damaging effects of chlorophenoxy herbicides and their metabolites on the erythrocyte membrane.

MATERIALS AND METHODS

Three herbicides: 2,4-dichlorophenoxyacetic acid (2,4-D), 4-chloro-2-methylphenoxyacetic acid (MCPA) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and their metabolites: 2,4-dichlorophenol, 4-chloro-2-methylphenol and 2,4,5-trichlorophenol were used in this study. In addition, the influence of a dimethyl-substituted compound, 2,4-dimethylphenol, was investigated.

The herbicides and their metabolite standards of 95-99% purity were purchased from Dr Ehrenstorfer GmbH, Germany.

Spin probes: 5-doxylstearic acid (5-DSA) and 16-doxylstearic acid (16-DSA) were purchased from Sigma (St. Louis, USA). All other reagents were purchased from POCh (Gliwice, Poland).

Erythrocyte haemolysis level, membrane lipid peroxidation [12], erythrocyte membrane fluidity via the spin probe method [14] and free -SH group content [15] were determined in this study. The order parameter S (for 5-DSA) and correlation times τ_B and τ_C (for 16-DSA) were calculated as previously described [13].

Erythrocytes were incubated in the presence of a 1 mM concentration of the investigated compounds for an hour at 37°C. This concentration initiated the first toxic effects as observed previously [16]. The solutions of the investigated compounds were prepared in a physiological salt solution and ethanol.

Spectrophotometric measurements were done with a SPECORD M40 spectrophotometer (Carl Zeiss Jena, Germany). ESR measurements were done with a Brücker apparatus (Germany).

The statistical significance of the results was calculated with a two-tailed paired t-Student test. All the experiments were performed at least 5 times. The confidence level was established at $\alpha = 0.05$.

RESULTS

The process of erythrocyte haemolysis strongly depended on the type of solvent. A higher rate of haemolysis was observed for compounds dissolved in ethanol, although ethanol itself did not elevate the haemolysis level in the control samples (Table 1). A higher haemolysis level was observed for the metabolites than for the basic compounds.

Lipid peroxidation did not depend on the type of solvent. A higher lipid peroxidation level was observed for the metabolites than for the basic compounds (Tab. 1).

Changes in membrane lipid bilayer fluidity were estimated based on the S orientation parameter for the 5-DSA probe and the correlation times: τ_B and τ_C for the 16-DSA probe.

Changes in the S orientation parameter value were not observed, suggesting a lack of membrane fluidity change at the 5th carbon atom of the fatty acids (Tab.1).

Tab. 1. A compilation of the investigated parameters

	Percent of erythrocyte haemolysis		Lipid peroxidation $\mu\text{mol TBARS/g Hb}$	Parameter S	-SH group $\text{mmol -SH/mg proteins}$
	in saline solution	in ethanol			
control	1.10 ± 0.10	1.25 ± 0.19	1.162 ± 0.152	0.735 ± 0.007	0.125 ± 0.003
2,4-D	2.08 $\pm 0.59^*$	–	1.578 $\pm 0.099^*$	0.733 ± 0.011	0.118 ± 0.010
2,4-dichlorophenol	2.60 $\pm 0.64^*$	4.70 $\pm 1.10^*$	2.065 $\pm 0.189^*$	0.730 ± 0.009	0.188 $\pm 0.009^*$
MCPA	1.80 $\pm 0.75^*$	–	1.834 $\pm 0.165^*$	0.741 ± 0.015	0.117 ± 0.010
4-chloro-2-methylphenol	–	5.45 $\pm 0.59^*$	1.965 $\pm 0.201^*$	0.728 ± 0.014	0.229 $\pm 0.021^*$
2,4-dimethylphenol	2.00 $\pm 0.37^*$	4.04 $\pm 0.65^*$	1.629 $\pm 0.145^*$	0.726 ± 0.012	0.135 ± 0.012
2,4,5-T	2.60 $\pm 0.38^*$	5.10 $\pm 0.81^*$	1.561 $\pm 0.106^*$	0.732 ± 0.006	0.117 ± 0.011
2,4,5-trichlorophenol	4.21 $\pm 0.35^*$	5.73 $\pm 0.98^*$	2.019 $\pm 0.231^*$	0.734 ± 0.017	0.220 $\pm 0.019^*$

* $P > 0.05$

A higher decrease in the correlation times τ_B and τ_C was observed for the metabolites than for the basic compounds, suggesting that the metabolites induced a higher level of damage in the erythrocyte membrane, and indicating an increase in its fluidity at the 16th carbon atom of the fatty acid (Fig. 1).

Damage to the membrane proteins was determined via the measurement of the amount of free -SH groups. An increase in the number of free -SH groups was observed for the metabolites, while the basic compounds did not provoke statistically significant changes (Table 1).

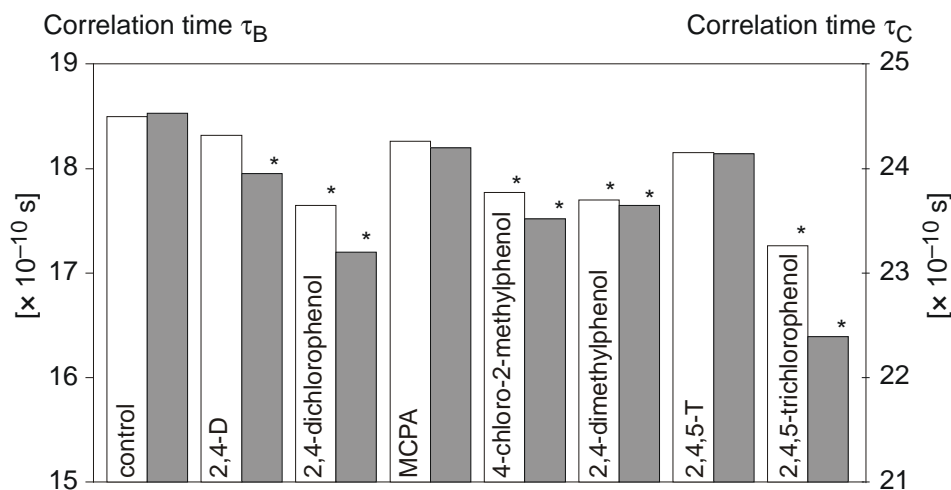


Fig. 1. Correlation time τ_B (white) and τ_C (gray); * $P > 0.05$

DISCUSSION

2,4-D may incorporate into the membrane, changing the erythrocyte shape into a spiny form – an echinocyte [17]. It causes the attenuation of the membrane structure and may cause membrane integrity loss in “older” or “weakened” erythrocytes.

The generation of oxygen free radicals by chlorinated and methylated phenol derivatives observed for ozonated water [18] may be transferred into biological systems. Lipid peroxidation may be induced by both primary and secondary free radicals generated by the investigated compounds.

Lipid peroxidation may lead to a change in membrane fluidity (long fatty acid chains are shortened), and in extreme cases to erythrocyte haemolysis (cellular death).

The measured decrease in correlation times (τ_B and τ_C) suggests that spin label molecules may move more rapidly within the bilayer. This may be due to the above-mentioned shortening of fatty acid chains and, in consequence, the increase in membrane fluidity.

Free radicals are also able to damage membrane proteins by changing their conformation or enzymatic activity. An increase in free –SH group content may be explained by conformational changes that lead to higher –SH exposure to chemical reactions, or by the breakage of the disulphide bridges. In case of the investigated compounds, it is not possible to determine which of these two processes leads to the increase in free –SH group content.

The plasma membrane is a dynamic system. Damage to one of its components may influence the others, may lead to change in cellular function, and finally to cellular death. Lipid bilayer structure disturbance by lipid peroxidation processes or by low molecular weight compound insertion may lead to perturbation in

erythrocyte deformability (especially by the action of the investigated agent simultaneously on protein conformation and function), erythrocyte shape changes, and finally to red blood cell haemolysis (erythrocyte death).

Membrane protein structure perturbations may cause i.a. erythrocyte-plasma ion exchange imbalance, increase in metHb content in the red blood cell and finally to damaged erythrocyte removal from blood circulation.

Metabolites induced higher toxic effects than basic compounds. Higher toxicity could be observed for chlorine-substituted compounds than for methylated ones.

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