Current Topics in Biophysics 2000, 24(2), 35–38

3-DIMETHYLAMINOPHENOL-INDUCED OXIDATIVE CHANGES IN HUMAN RED BLOOD CELLS

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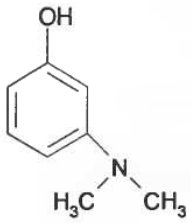
The effects of exposure of human erythrocytes to different concentrations of 3-dimethylaminophenol (DMAP) were studied, with particular attention to lipid peroxidation, hemolysis, catalase and glutathine peroxidase activities, glutathione (GSH) content and hemoglobin oxidation.

Human erythrocytes were incubated with DMAP at concentrations from 10 to 300 ppm = 72–363 nmol/ml erythrocytes of 5% hematocrit, for 1 to 5 hours. The results show that the 3-dimethylaminophenol increases of the level of methemoglobin, products of lipid peroxidation and decreases GSH concentration and glutathione peroxidase activity. All these results indicate that DMAP induces oxidative stress in the cell.

INTRODUCTION

The occurrence of phenol and its derivatives in the environment is mainly caused by human activity. Many investigations show that these compounds accumulate in living organisms in unchanged or in substituted forms associated with amino acids, carbohydrates and other molecules. Accumulation of phenolic substances often causes damages of the biological structures and disturbs the functions of cells. 3-Dimethylaminophenol (DMAP) is the metabolite of pesticides, expecially aniline and acetanilide.



3-Dimethylaminophenol (DMAP)

MATERIALS AND METHODS

Human erythrocytes and hemoglobin were obtained from whole blood, taken from donors in the Blood Bank of Łódź.

Erythrocytes were centrifuged and washed twice with 155 mM NaCl and once with phosphate-buffered saline (PBS), pH 7.4.

3-DMAP treatment of erythrocytes

Isolated erythrocytes at a hematocrit of 5% were treated at a temperature of 25°C for one hour with 10–500 ppm DMAP.

Hemolysis

The extent of hemolysis was calculated from the equation:

$$H\% = \frac{(A_{pb} - A_0)}{(A_{water} - A_0)} \times 100$$

where: H% – hemolysis of erythrocytes incubated with DMAP, A_{pb} – absorbance of sample, A_{water} – absorbance after complete hemolysis, A_0 – absorbance of the sample incubated in 155 mM NaCl.

Lipid peroxidation

The peroxidation of human erythrocytes and of their membranes lipids induced by DMAP was investigated. Malonyldialdehyde (MDA), the final product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to form a coloured complex. The level of TBA-reactive substances (TBARS) was measured on the basis of the absorbance at the wavelength of 532 nm (Stock & Dormandy, 1971).

Glutathione peroxidase activity

Glutathione peroxidase activity was measured according to the method of Rice-Evans, Diplock and Symons (1991). One unit of activity was de-

fined as nmol of NADPH oxidized/(min × mg Hb), monitored at 340 nm at 25°C.

Glutathione

Erytrocyte reduced glutathione (GSH) was determined by the modified Ellman method (Ellman, 1959). The rate of formation of 5-thio-2-nitrobenzoic acid, which is proportional to total glutathione concentration, was monitored at 412 nm at 25°C. The absorbance was read at 412 nm in a Specol-11 spectrophotometer (Carl Zeiss, Jena, Germany) against blanks.

Hemoglobin

The concentration of hemoglobin was measured by the Drabkin method (1946). Absorption spectra of hemoglobin were obtained in the wavelength range 440 nm to 700 nm using an automatic spectrophotometer (Specord M40) connected with computer. Samples consisting of 1% aqueous solutions of oxyhemoglobin were incubated at 37°C in air with continuous mixing with doses of 10–300 ppm of DMAP. The percentage of met-Hb in the total Hb content was calculated from absorbance at 630 and 700 nm both for control sample and samples with DMAP:

$$\%\text{metHb} = \frac{(A_{pb630} - A_{pb700})}{(A_{100\%met630} - A_{100\%met700})} \times 100\%$$

Statistics

The data were given as arithmetic means ± SEM. Statistical evaluation of the data was performed by the paired "t" test. The level of significance was chosen as $P < 0.05$.

RESULTS AND DISCUSSION

The results show that hemolysis of erythrocytes increases with concentration of DMAP (up to 7.43% ± 0.43 for 300 ppm; Fig.1).

The level of thiobarbituric acid-reactive substances was higher in erythrocytes incubated with DMAP in comparison with the control (Fig. 2).

Incubation of hemoglobin with DMAP (10, 50 and 300 ppm) decreased oxyhemoglobin concentration and increased the level of met-Hb. A significant decrease of α and β peaks was observed with a simultaneous increase of the height of the 630 nm peak which is typical for methemoglobin (Table 1).

Table 1. Percent of met-Hb in erythrocytes incubated with DMAP

	n	control	DMAP [ppm]
10	6	1.05 ± 0.63	1.55 ± 1.27
50	6	1.21 ± 0.85	3.19 ± 1.5*
100	6	1.14 ± 0.69	4.45 ± 2.1*
300	6	1.69 ± 0.99	7.43 ± 0.43*

Methemoglobin formation was also observed when erythrocytes were incubated with xenobiotics such as hydroxylamines or phenols. It has been suggested that the reactive intermediates were reactive free radicals derived from the xenobiotic, and a ferryl-heme oxo-complex (Nohl & Stolze, 1998). Eyer sugested that DMAP binds covalently to hemoglobin and catalytically transfers electrons from ferrohemoglobin to oxygen (Eyer, Lierheimer

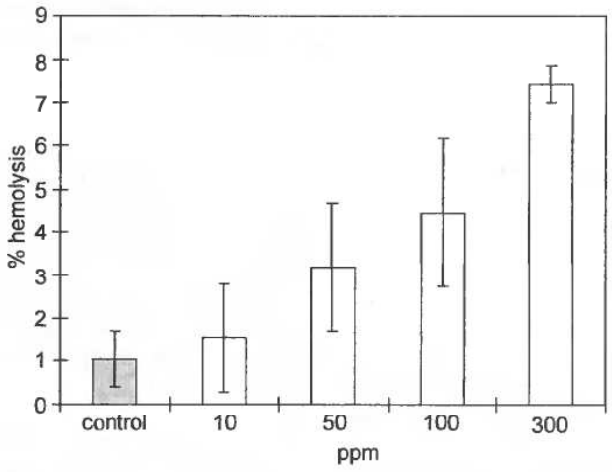


Fig. 1. Effect of DMAP dose on hemolysis in human erythrocytes

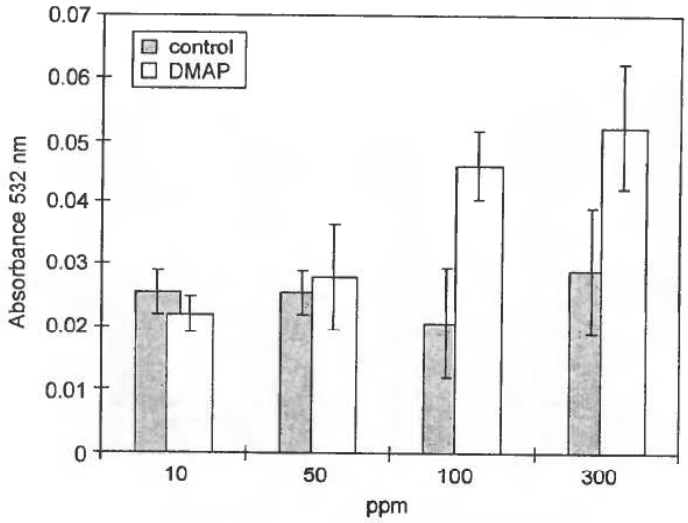


Fig. 2. The lipid peroxidation in erythrocytes incubated with DMAP

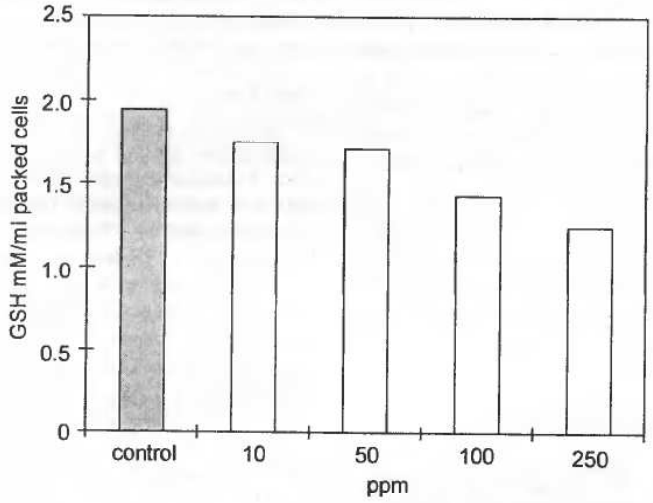


Fig. 3. The level of GSH in human erytrocythes incubated with DMAP, 1 h incubation

& Strosar, 1983; Coleman, Hayes & Jacobus, 1998).

DMAP decreased the level of GSH in the erythrocytes by only 20–30% in comparison with the control (Fig. 3). The activity of GSH-PX in human erythrocytes decreased under the influence of DMAP (Fig. 4). We show that levels of antioxiodants (GSH-PX and GSH) affect cellular cytotoxic responses to DMAP, suggesting the involvement of oxidative stress. In studies of lipid peroxidation we observed increased damage to membrane lipids when the antioxidant defenses, GSH and GSH-PX were inhibited. The differing effects of antioxidant inhibitors on DMAP-induced cytotoxicity and membrane lipid damage (hemolysis and increase lipid peroxidation, formation of methemoglobin) suggest that different mechanisms are involved in these processes.

We suggest that benzene metabolites such as DMAP inhibit antioxidant mechanisms; thus accumulation of free radicals occurs in exposed cells, predisposing them to oxidative damage. While it is known that most phenols generated by man seem to be toxic, Selasse, DeSoya, Rosario, Gao and Hansch (1998) suggests that this is related to two properties of this compound, one being the formation of free radicals, and the second being a direct attack of the phenoxyl radicals

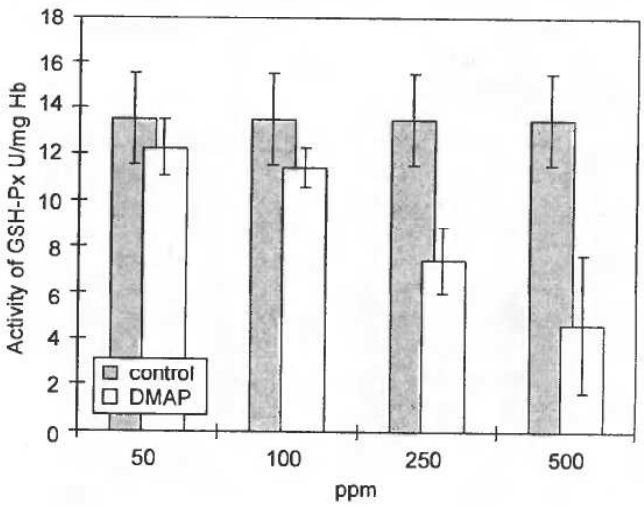


Fig. 4. Activity of GSH-PX incubated with DMAP, 1 h incubation

on a number of sensitive pathways of metabolism. It was reported that phenol, chlorophenol (Nimmagudda & Snyder,, 1995), and particularly pentachlorophenol (Yousri & Hanke, 1995) as well as hydroxylamine (Stolze, Dadak, Yang & Nohl, 1996) and 4-aminophenol (Coleman *et al.*, 1998) have these characteristics and exert similar effects.

The results of our work show that the 3-dimethylaminophenol increases of the level of methemoglobin and of TBARS, and decreases GSH concentration and glutathione peroxidase activity. All these results indicate that DMAP induces oxidative stress in the cell.

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