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Effects of Chloroform on the Activity of Na^+-K^+ Atpase, Catalase, Glutathione and Malondialdehyde Levels in Human Erythrocytes

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The objectives of this study is to assess the effects of the chloroform on erythrocytes by measuring the Na^+-K^+ATPase (EC.3.6.1.39) and catalase (EC 1.11.1.6) activities and also the amount of reduced glutathione and malondialdehyde. *In vitro* exposure of intact human erythrocytes or freshly prepared erythrocyte membranes to chloroform for different contact times (0, 6, 12, 24, 48 h) resulted in the inhibition of erythrocyte membrane Na^+-K^+ATPase activity in a time dependent manner. The results also indicated that catalase activity, glutathione and malondialdehyde levels were sensitive parameters for chloroform.

Key Words: Chloroform, Catalase, Glutathione, Malondialdehyde, Erythrocytes.

INTRODUCTION

Chlorination of drinking water has been one of the most effective public health measures ever undertaken[1], but the widespread application of chlorine as a drinking water disinfecting agent has raised concerns about the risk of human exposure to chlorine[2]. Disinfection of water through chlorine treatment can lead to the formation of disinfection by-products (DBPs), which were found to pose risks to health and to the environment[3].

Trihalomethanes (THMs) are the most prevalent disinfection by-products identified in chlorinated drinking water. Once THMs formed, they are difficult to remove. Among the THMs, chloroform generally occurs at the highest concentration in finished water[4]. Most of THMs (trichloroacetic acid, dichloroacetic acid, various haloacetonitriles and chlorophenols) have been shown to be toxic, carcinogenic and mutagenic as well as chloroform[5-7]. In 1976 the US National Cancer Institute published results showed that high doses of chloroform could cause cancer in rats[8]. On the basis of these results, US EPA (1998) has regulated to "maximum contaminant level" of 0.1 ppm for trihalomethanes in the drinking water.

It has been shown *in vitro* that phospholipids are the major site of chloroform induced damage in experimental conditions resembling the physiological status of the liver[8]. Additional health effects from chlorine have also been revealed in human studies[9,10]. At present, it is generally accepted that there is a relationship between rectal, colon and bladder cancer and water quality[11]. However, little haematoxicological data are available. In studies, *in vitro* erythrocytes have been used as a well-characterized model cell for the human studies[12,13]. Na^+-K^+ ATPase is responsible for the ion gradient, osmotic pressure and transport of nutrients such as carbohydrates and amino acids in cell[14,15] and measurement of Na^+-K^+ ATPase activity is a tool used to investigate the biochemical alterations affecting the cation transport system. Antioxidant defense system such as catalase (CAT) and glutathione (GSH) are necessary to protect the cell against the intracellular or extracellular effects of many lipophilic xenobiotics such as chloroform which may produce reactive oxygen species during its biotransformation in the cell. The present study was undertaken to determine if chloroform formed during the chlorination of drinking water affects on human erythrocytes by means of enzyme activities as Na^+-K^+ATPase activity, CAT activity and non-enzymatic defense system components as GSH and lipid peroxidation (LP).

EXPERIMENTAL

All reagents were of highest purity available and obtained from BDH (England), Merck (Germany) or Sigma Chemical Company (USA). Blood samples were obtained from the Blood Bank of Cukurova University Medical Faculty. All biochemical variables were assayed ATI Unicam UV/vis spectrometer.

Intact erythrocytes were treated with chloroform and then membranes were prepared. In other type of preparation, membranes were prepared from intact erythrocytes then treated with chloroform.

Preparation of intact erythrocytes and exposure to chloroform: Erythrocytes were washed four times with 10 mM potassium phosphate buffer, (pH 7.4) containing 135 mM NaCl. The cells were suspended to 10 % by volume in the same buffer solution. Cell suspension was exposed to chloroform with a final concentration of 25 µg/deciliter of cell suspension for different contact times (0, 6, 12, 24 and 48 h). The membranes of chloroform exposed erythrocytes were prepared according to the procedure described by Hanahan and Eckholm[16].

Preparation of membranes and then exposure to chloroform: The membranes of washed intact erythrocytes were prepared according to the procedure described by Hanahan and Eckholm[16]. A small portion of these membranes were separated to determine the Na^+-K^+ ATPase activity and malondialdehyde (MDA) level as control value. Another portion of the membrane preparation was immediately exposed to chloroform for different contact times as mentioned above.

Evaluation of biochemical parameters: In both type of preparations Na^+-K^+ ATPases were assayed as the release of inorganic phosphate (Pi) from ATP according to the method described by Luly *et al.*[17]. Assays were carried out in a final volume of 2.5 mL containing 0.3 mg membrane protein as the enzyme source, 120 mM NaCl, 10 mM KCl, 35 mM tris HCl buffer (pH 7.4), 3 mM Na_2ATP and the presence or absence of 0.2 mM ouabain. Membranes were preincubated in the mixture for 10 min at 37 ºC before starting the reaction by adding the substrate, Na_2ATP. After 0.5 h of incubation with substrate the reaction was stopped by adding 0.5 mL of 30 % trichloroacetic acid. The released inorganic phosphate was assayed by the method of Atkinson *et al.*[18] and protein was measured by the method of Lowry[19]. Na^+-K^+ ATPase activity was calculated by subtracting the activity in the presence of ouabain from the activity in its absence. Na^+-K^+ ATPase activity was expressed as µmol Pi/mg prot./h.

Catalase activities were expressed as the first-order kinetic constant of the rate of disappearance of H_2O_2 as measured by absorbance at 240 nm[20], CAT activity was expressed as one unitx $l0^2$/g of hemoglobin (Hb).

Glutathione was determined by measuring a highly coloured yellow anion formed by the reduction of DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] with non-protein sulfhydryl compounds of erythrocytes by the method of Beutler *et al.*[21]. The optical density of formed yellow substance was measured at 412 nm within the first 10 min of colour development. Entire procedure was carried out at room temperature. The concentration of GSH was calculated as mmol GSH/g of hemoglobin.

Measurement of TBA-reactive substances relative to MDA: Lipid peroxidation level in the erythrocytes and membranes were expressed in MDA. Malondialdehyde was measured according to procedure of Moore *et al.*[22], which was a modification of the procedure of Bidlack and Tappel[23]. 0.85 mL of 0.47 % 2-TBA and 0.25 mL of 100 % trichloroacetic acid were added to each 0.4 mL of membrane suspension or standard. Each tube was placed in a boiling water bath for 15 min to develop the pink colour of the reaction product and then cooled to room temperature. Samples were centrifuged to remove the small amount of denatured protein and the absorbance of each sample was measured at 532 nm. Standard MDA was prepared by acid hydrolysis of 1,1,3,3-tetramethoxy propane followed by neutralization with NaOH. The molar extinction coefficient for MDA was 2.56×10^5 and MDA levels were expressed relative to that of MDA standard in nanomoles per milligram of protein.

All numerical data are given as means ± standard deviation (SD). In the enzyme activities (Na^+-K^+ ATPase, CAT) and GSH, MDA measurements, the difference among five experiments were never in excess of 5 %.

RESULTS AND DISCUSSION

The Na^+-K^+ ATPase activities of the membranes prepared from the chloroform exposed intact erythrocytes and of the membranes directly exposed to chloroform for 0-48 h contact times are presented in Fig. 1. Na^+-K^+ ATPase activity of normal intact erythrocytes was 0.55 ± 0.03 µmol inorganic phosphate mg $prot^{-1}$ h^{-1}.

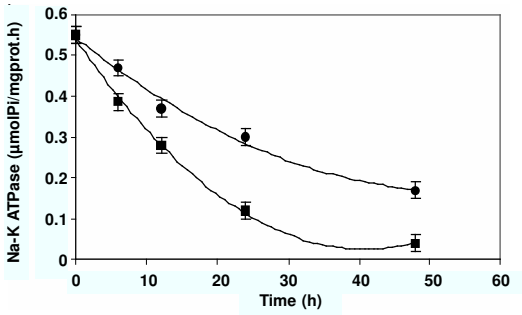

Fig. 1. Na^+-K^+ ATPase activities of erythrocytes (■) and membranes (●) after exposure to chloroform

As chloroform exposure time increased from 0-48 h at 6 h intervals, Na^+-K^+ATPase activity of the membranes directly exposed to chloroform and the Na^+-K^+ATPase activity of the membranes prepared from the chloroform exposed intact erythrocytes were inhibited in a time dependent manner. The degree of enzyme inhibition in the membranes directly exposed to chloroform was about 74 %, whereas the degree of enzyme inhibition in the chloroform exposed intact erythrocytes was about 69 % after 48 h chloroform exposure.

The malondialdehyde values in the normal untreated membranes and intact erythrocytes were measured as 0.97 ± 0.05 nmol MDA mg $prot^{-1}$ and 0.12 ± 0.01 nmol MDA mg $prot^{-1}$, respectively.

However, chloroform treatment caused striking increases in MDA values of both types of preparations (Fig. 2). The highest MDA value as1.71 ± 0.06 nmol MDA mg $prot^{-1}$ was recorded for the membranes directly exposed to chloroform which was 1.76 times greater than that of the control MDA value after 48 h chloroform exposure. The accumulation of MDA upon chloroform exposure indicates the peroxidation of membrane phospholipids and this may cause the loss of Na^+-K^+ATPase activity since the maintenance of the tertiary structures of cellular membrane proteins is dependent on their associated lipids. Alterations in lipids surrounding the embedded proteins may results in structural alterations and changes in membrane function[24]. Ptafferott *et al.*[25] showed that the addition of malondialdehyde to erythrocytes even in the absence of lipid peroxidation resulted in decreased cellular deformability.

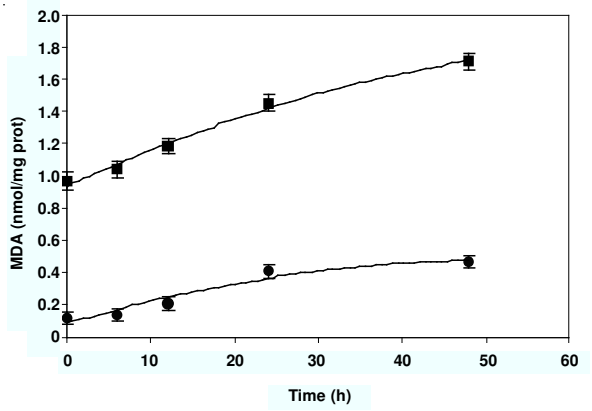

Fig. 2. Malondialdehyde levels of erythrocytes (■) and membranes (●) after exposure to chloroform

Present results clearly demonstrate that intact erythrocytes are less affected from the treatment with chloroform than the membranes directly trated with chloroform. Such a result may be due to the protective effects of cellular defense system already existing in intact erythrocytes. It was established many years ago that the disrupted tissues undergo peroxidation more quickly than healthy ones[26,27].

Mean (± SD) CAT activity in untreated (control) erythrocytes was $1.17 \times 10^2 \pm 15$ units g Hb^{-1}. A significant elevation in CAT activity (55.5 %) was found in the 6 h chloroform contact time in intact erythrocytes (Fig. 3). It may be surmised that enhanced H_2O_2 production in erythrocytes is due to oxidative effect of chloroform. However, longer contacts of chloroform with erythrocytes showed a decreasing profile of CAT activity upon exposure timesas seen in Fig. 3. The longest contact time (48 h) of chloroform with erythrocytes was caused a highest reduction (75.2 %) in CAT activity. These results indicated that chloroform and/or its possible by-products are capable of acting on CAT enzyme inactivation.

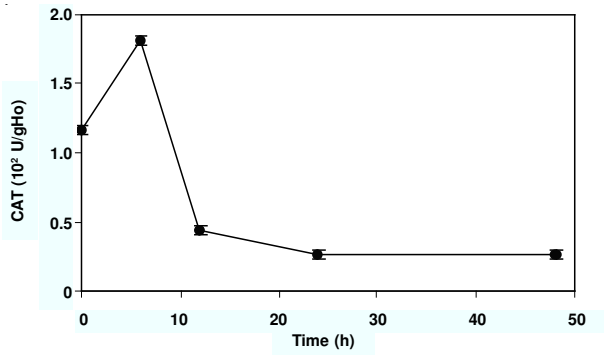

Fig. 3. Catalase activities of erythrocytes after exposure to chloroform

Mean GSH concentration in the untreated (control) erythrocytes was 0.270 ± 0.003 mmol g Hb^{-1}. As seen in Fig. 4, in short term (6 h) treatment of chloroform with erythrocytes, there was 45.9 % decrease in GSH concentration. The decrease in GSH observed in erythrocytes is probably associated with detoxification of chloroform. In longer term chloroform treatments (12, 24 and 48 h); there was a similar pattern in GSH response of erythrocytes (Fig. 4). Like an adaptive response, GSH levels were in comparable value with that of control value in longer contact times with chloroform.

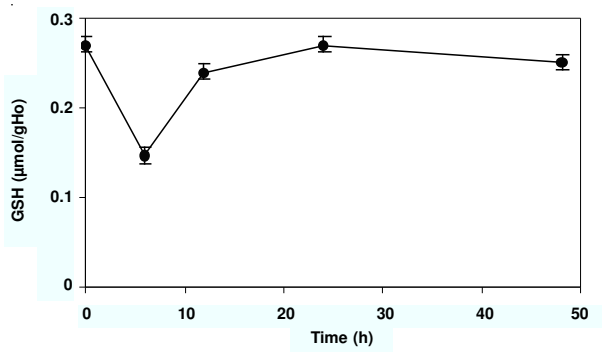

Fig. 4. Glutathione levels of erythrocytes after exposure to chloroform

The results indicated that antioxidant defense components namely, CAT, GSH and also Na^+-K^+ ATPase are sensitive parameters that could be useful biomarkers for the evaluation of xenebiotics. The relationship between the degree of inhibition of Na^+-K^+ATPase and lipid peroxidation suggests that these parameters could also be biomarkers for toxicity. All these results furnish useful data in order to provide a database relating the presence of pollutants to biological effects at a molecular level.

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REFERENCES

1. R.J. Bull, L.S. Birnbaum, K.P. Cantor, J.B. Rose, B.E. Butterworth, R.J. Pegrom and J. Tuomisto, *Fundam. Appl. Toxicol*., **28**, 155 (1995).
2. F.B. Daniel, J.R. Meier and A.B. Deangel, *Ann. Ist. Super Sanita*, **29**, 279 (1993).
3. U.S. EPA, National Primary Drinking Water Regulations: Disinfectants and Disinfectant Byproducts, Fed. Reg., 63 (241), 69389 (1998).
4. R.A. Pegram, M.E. Andersen, S.H. Warren, T.M. Ross and L.D. Claxton, *Toxicol. Appl. Pharmacol*., **144**, 183 (1997).
5. P.D. Lilly, J.E. Simmons and R.A. Pegram, *Toxicol. Lett*., **87**, 93 (1996).
6. C.L. Potter, L.W. Chang, A.B. DeAngelo and F.B. Daniel, *Cancer Lett*., **106**, 235 (1996).
7. T.E. Keegan, J.E. Simmon and R.A. Pegram, *J. Toxicol. Environ. Health*, **11**, 65 (1998).
8. R.R. Trussel and M.D. Umphres, *J. AWWA*, **70**, 604 (1978).
9. T. Cresteil, P.J. Beaune, P. Leroux, M. Lange and D. Mansuy, *Chem. Biol. Interact*., 24, 153 (1979).
10. W.D. King and D. Marrett, *Cancer Causes Control*, **7**, 596 (1996).
11. K.P. Cantor, *Environ. Health. Perspect.*, **46**, 187 (1982).
12. S.S. Tükel, R. Bilgin and S. Gül, *Biochem. Mol. Biol*., **33**, 1033 (1994).
13. R. Bilgin, S. Gül and S.S. Tükel, *Biochem. Mol. Biol. Int*., **47**, 227 (1999).
14. A.E. Koontz and R.L. Heath, *Arc Biochem. Biophys*., **198**, 493 (1979).
15. A. Askari, *Mol. Cell. Biochem.*, **43**, 129 (1982).
16. U.J. Hanahan and J.E. Eckholm, Methods Enzymology, Academic Press Inc., London (1974).
17. P. Luly, P. Baldini, S. Incerpi and E. Tria, *Experienta*, **37**, 431 (1981).
18. A. Atkinson, A.D. Gatenby and A.G. Lowe, *Biochem. Biophys. Acta*, **320**, 195 (1973).
19. O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randal, *J. Biol. Chem*., **193**, 265 (1951).
20. S. Lartillot, A. Kedziora and A. Athias, *Prep. Biochem*., **18**, 241 (1988).
21. E. Beutler, D. Duron and B.M. Kelly, *J. Lab. Clin. Med*., **61**, 882 (1963).
22. R.B. Moore, L.M. Brummitt and V.N. Mankad, *Arch. Biochem. Biophys*., **273**, 527 (1989).
23. W.R. Bidlack and A.L. Tappel, *Lipids*, **8**, 203 (1973).
24. C. Borek and M.A. Mehlman, *Environ. Res*., **42**, 36 (1987).
25. C.H. Pfafferott, J. Meiselman and P. Hochstein, *Blood*, **59**, 5 (1982).
26. A.A. Barber, *Radiat. Res*., **3**, 33 (1963).
27. J. Stock, Studies of the Antioxidant Component of Human Serum and Red Cells, Ph.D. Thesis, University of London, London, UK (1982).