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TOXICITY OF DIBROMOACETONITRILE IN ISOLATED RAT COLONOCYTES

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ABSTRACT

Dibromoacetonitrile (DBAN) is a water disinfection by-product. The objective of the present work was to investigate the cytotoxic effects as well as the oxidative stress induced by DBAN in cultured rat colonocytes. Colonocytes were exposed *in-vitro* to different concentrations of DBAN (0.1-2.0 mM) for 60 min. Also, colonocytes were incubated with DBAN (1.0 mM) for different time intervals extending to 180 min. Cytotoxicity was determined by assessing cell viability and lactate dehydrogenase (LDH) release, glutathione (GSH) level and lipid peroxidation as indicated by thiobarbituric acid reactive substances (TBARS) production. Exposure of colonocytes to DBAN (1.0 mM) for 60 min caused nearly a 50% decrease in cell viability and induced a 3-fold increase of LDH leakage. In the same experiment, DBAN caused a significant decrease in cellular GSH content as well as a significant enhancement of TBARS accumulation. These toxic responses to DBAN were dependent on both concentration and duration of exposure to DBAN. Treatment of colonocytes with GSH, N-acetyl-L-cysteine (NAC) or dithiothreitol (DTT) prior to exposure to DBAN afforded different degrees of protection as indicated by significant decrease in the LDH leakage and TBARS formation as compared to DBAN alone-treated cells. Also, pretreatment of colonocytes with the antioxidant enzymes superoxide dismutase (SOD) or catalase (CAT) significantly inhibited LDH leakage and TBARS production. Preincuhation with dimethyl sulfoxide (DMSO), a hydroxyl radical scavenger or desferroxiamine (DFO), an iron chelator, diminished DBAN-induced LDH leakage and TBARS generation. Our results suggest that DBAN has a potential cytotoxic effect in rat colonocytes; and thiol group-donors, antioxidant enzymes, hydroxyl radical scavengers and iron chelators can play an important role against DBAN-induced colonotoxicity.

Key words: Dibromoacetonitrile; Colonocytes; Oxidative Stress.

INTRODUCTION

Haloacetonitriles (HAN) are by-products of drinking water chlorination (IARC, 1991). Residual chlorine in drinking water could also result in formation of HAN in vivo following consumption of chlorinated water (Mink *et al.*, 1983). HAN are used as insecticides for grains (Cotton and Walkden, 1968) and as common laboratory chemicals (Barcelo *et al.*, 1987). Several reports have indicated that levels of HAN in drinking water should be lower than those proposed by World Health Organization (1994).

Epidemiological studies indicated an association between exposure to chlorinated water and the occurrence of gastrointestinal (GI) cancers (Flaten, 1992). HAN induce adverse mutagenic and carcinogenic effects in animals (Bull *et al.*, 1985; Meier *et al.*, 1985). HAN induce sister chromatid exchange in Chinese hamster ovarian (CHO) cells and DNA strand breaks in cultured human lymphocytes (Daniel *et al.*, 1986). Animal studies indicate that HAN cause GSH depletion and inhibit glutathione-S-transferase in liver and gastrointestinal tract (GIT) (Lin and Guion, 1989).

Dibromoacetonitrile (DBAN, Br_2-CH-CN), a member of the HAN group, possesses a two-bromine substitution on the alpha carbon atom of the acetonitrile molecule. DBAN induces a mitotic chromosome loss (Zimmermann and Mohr, 1992) and primary DNA damage in *Escherichia coli* PQ37 (Le Curieux *et al.*, 1995). The potential mutagenic and carcinogenic activity of DBAN was previously reported (IARC, 1991). The GIT represents important target organs for DBAN toxicity and the mechanism of such toxicity has been suggested to involve induction of oxidative stress (Ahmed *et al.*, 1991). In addition, reactive oxygen species (ROS) generated by xanthine oxidase (XO) /xanthine (X) iron (Fe) system have been shown to activate DBAN to more toxic metabolites including cyanide (Mohamadin and Abdel-Naim, 2003).

The gastrointestinal tract is well-endowed with the enzymatic machinery necessary to form large amounts of

reactive oxygen species (Parks *et al.*, 1983; Grisham and Granger, 1988). Rat colon is particularly subject to oxidative stress induced by several factors including polymorpholeukocytes infiltration and ischemia/ reperfusion (Weiss, 1986; Bhaskar *et al.*, 1995). Generation of free radicals has been implicated in the activation of DBAN and other nitrites to the very toxic metabolite cyanide (Mohamadin *et al.*, 1996; Mohamadin and Abdel-Naim, 2003; Abdel-Naim and Mohamadin, 2004). DBAN is known to induce GSH depletion and the consequences of oxidative stress in the rat stomach (Abdel-Naim and Ahmed, 2005). Although, the colon is a potential target for DBAN toxicity, little is known about the DBAN colonotoxicity. Therefore, the objective of the present study was to investigate the potential cytotoxic effects as well as the oxidative stress induced by DBAN in cultured rat colonocytes. In addition, different thiol compounds, antioxidant enzymes, free radical scavengers and iron chelators were tested for potential protective effects.

MATERIALS AND METHODS

Animals and chemicals

Sprague-Dawley rats of either sex (130-150 g) were used in the present study. Bovine serum albumin (BSA), catalase from bovine liver (CAT), chloral hydrate, DBAN, dithiothreitol (DTT), desferrioxamine (DFO), 5,5'-dithiobis(2-nitrohenzoic acid) (DTNB), dimethyl sulfoxide (DMSO), Dulbecco's phosphate buffered saline (DPBS), ethylene diamine tetra-acetic acid disodium salt (EDTA-Na_2), N-acetyl-L-cysteine (NAC), reduced glutathione (GSH), superoxide dismutase from bovine erythrocytes (SOD), 1,1,3,3-tetramethoxypropane, thiobarbituric acid (TBA) and triton X-100 were obtained from Sigma-Aldrich Chemical Co. (St. Louis. MO, USA). All other chemicals were of analytical grade.

Isolation of colonocytes

Rat colonocytes (colonic epithelial cells) were isolated using a modification of the procedure originally described by Roediger and Truelove (1979). Briefly, overnight fasted rats (n = 3-4) were anesthetized with chloral hydrate (300 mg/kg, i.p.). Abdomen was opened and the colons were rapidly dissected out (cecum to rectum) and flushed clear of luminal contents with saline (37 °C). Then, the lumen was filled with DPBS containing 0.25% BSA and 5 mmol/L EDTA-Na_2 and both ends were ligated. The ligated colon was placed in a flask containing 100 ml oxygenated DPBS for 45 min at 37 °C in a water bath with shaking at 60 cycles/min. The luminal fluid was then drained into a polystyrene tube and the colon was averted onto a glass rod. Additional cells were isolated from the mucosa into the tube with the luminal fluid by gently stirring for a period of 30 sec. The cells were then counted and viability was determined by using trypan blue exclusion method. Cells yield was more than 90% viable.

Treatments

Cytotoxic effect of DBAN was evaluated in a dose response as well as a time course experiment. In the dose response experiment, colonocytes were exposed to different concentrations of DBAN (0.1, 0.5. 1 and 2 mM) for 60 min. DBAN was diluted in DPBS to the required concentrations. In the time course experiment, colonocytes were incubated with DBAN (1 mM) for different time intervals (0, 30, 60, 120, 180 min).

Cytotoxicity was determined by assessing cell viability and lactate dehydrogenase release (LDH). Oxidative stress induced by DBAN was determined by assessing GSH level and lipid peroxidation as indicated by formation of thiobarbituric acid reactive substances (TBARS). In the mechanistic part of our study, colonocytes were preincubated with GSH (1 mM), NAC (1 mM), DTT (1 mM), DMSO (80 mM), DFO (10 mM), SOD (500 U/ml) or CAT (1500 U/ml). All pretreatments were done 30 min before the addition of DBAN and their concentrations were consistent with those in the literature. The potential protective effects were evaluated by assessing LDH leakage and TBARS production by colonocytes after 180 min of' incubation.

Assay of LDH leakage

The activity of the cytosolic enzyme lactate dehydrogenase (LDH) was estimated according to the method described by Wroblewski and Ladue (1955) by assessing the rate of conversion of NADH (1.5 mmol/L) to NAD^+ in the presence of L(+)-lactic acid (50 mmol/L) in culture supernatants (S) as well as in the remaining cells (C) after lysis with triton X-100. The percentage of LDH leakage was calculated as follows:

$$\% \text{ leakage} = S/(S+C) \times 100$$

Assay for cellular GSH

Reduced GSH levels in colonocytes were determined by measuring total soluble-reduced sulfhydryl content (Beutler *et al.*, 1963). After incubation, cells were washed three times with phosphate buffered saline (PBS), and then, 0.7 ml of 0.2% triton X-100 and 2.5% sulfosalicvlic acid in PBS was added. Solutions were centrifuged at 3.000 *g* for 5 min. A 0.5 ml-aliquot of the acid-soluble supernatant medium was then added to 1.0 ml of 0.3 M Na_2HPO_4 solution. Spectrophotometric determinations were performed at 412 nm immediately after the addition of 0.125 ml of 5, 5'-dithiobis (2-nitrobenzoic acid) (40 mg/100 ml in 1% sodium citrate).

Lipid peroxidation assay

Lipid peroxidation was assessed by determining thiobarbituric acid reactive substances (TBARS) by the method of Uchiyama and Mihara (1978). Test solutions obtained from cultured cells were cooled to 4 °C and centrifuged for 10 min at 1000 *g*. Aliquots (750 µl) of the supernatant were combined with an equal volume of cold

12% trichloroacetic acid and centrifuged for 10 min at 1000 *g* at 4 °C to remove precipitated protein. One ml of supernatant was added to 1 ml thiobarbituric acid reagent (0.6% thiobarbituric acid in 0.1 N NaOH), and the mixture was heated at 100 °C for 20 min. The mixture was allowed to cool, and TBARS were extracted with 3 ml of 1-butanol. Appropriate blanks were concurrently made. A 1.0 mM stock solution of 1.1.3.3-tetraethoxypropane in water was diluted in various amounts of 0.01 N HCl to produce malondialdehyde (MDA). These solutions were used as TBARS standards. The 1-butanol fractions and MDA standards were determined at wavelength of 553 nm. TBARS content was expressed as nmol/mg protein. Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Data analysis

The GraphPad Prism® (version 1.02) computer program (GraphPad Software Incorporated) was used to conduct regression analysis and to plot collected data. Data are expressed as means ± SEM. Individual group comparisons were conducted using the two tailed unpaired Student's t-test. Multiple group comparisons were carried out using one way analysis of variance (ANOVA) followed by Dunnets test for post-hoc analysis. Statistical analyses were performed using software program SPSS® for Windows (version 8). The 0.05 level of probability was used as the criterion for significance.

RESULTS

Assessment of DBAN cytotoxicity

Cell survival was assessed by trypan blue exclusion method after exposing rat colonocytes to DBAN (0.1-2.0 mM). Incubation of colonocytes for 60 min with DBAN (at concentrations higher than 0.1 mM) showed significant decreases in cell viability in a concentration-related manner. Also, DBAN in a concentration of 1.0 mM reduced the viability to approximately 50% (Fig. 1 A). Therefore, subsequent time course experiments and mechanistic experiments were performed using this concentration. The data in Fig. l B illustrate the time course of viability for colonocytes exposed to 1.0 mM DBAN. The chemical caused a significant and time-dependent decrease in cell viability starting from 30 min after beginning of incubation and showed maximal cytotoxicity at 180 min (20%).

Plasma membrane damage was assessed by monitoring LDH leakage from colonocytes exposed to DBAN. Incubation of colonocytes with different concentrations of DBAN (0.1-2.0 mM) for 60 min showed significant increases in LDH leakage compared to the control incubations. This effect was concentration-dependent and in comparison to the basal LDH leakage observed in the control incubations, the enzyme leakage was progressively elevated to 3 folds (for 1.0 mM DBAN) and finally to 5.0 folds (for 2.0 mM DBAN) (Fig. 2 A). The time course effects of 1.0 mM DBAN on LDH release from colonocytes are shown in Fig. 2 B. LDH leakage was significantly elevated at all time periods. At 180 min after DBAN addition to incubations, the percentage of LDH release was maximal and mounted to approximately 4 folds that of the corresponding control value.

Assessment of oxidative stress-induced by DBAN

Incubating colonocytes in the presence of different DBAN concentrations (0.1-2.0 mM) resulted in an observable loss of cellular GSH (Fig. 3 A). However, the decrease in GSH level was significant only at DBAN concentrations higher than 0.1 mM. In the time-course experiment, exposure of colonocytes to 1.0 mM DBAN significantly reduced cellular GSH levels at time points longer than 30 min; compared to corresponding control values. GSH depletion was maximal at 180 min after DBAN addition (45% of the control value) (Fig. 3 B).

The effect of various concentrations of DBAN (0.1-2.0 mM) on lipid peroxidation, as indicated by TBARS formation, was estimated. Figure 4 A shows a significant and concentration-related increase of TBARS production in colonocytes as compared with the control value. In the time-course experiment, DBAN (1.0 mM) resulted in a significant increase in the production of TBARS in colonocytes, which occurred early at 30 min of incubation (349% of control value) and reached its maximum level after 180 min (483% of control value) (Fig. 4 B).

Assessment of potential protective effects of different antioxidants

The protective effects of different thiol-containing compounds on DBAN-induced LDH release and lipid peroxidation in colonocytes are illustrated in Table (1). All of the tested compounds (GSH, NAC and DTT) at a molar concentration equivalent to that of DBAN (1.0 mM) could significantly reduce LDH release by about 46%, 62% and 55% respectively, as compared with DBAN alone-treated incubations. Similar protective effects offered by these compounds were also observed on DBAN-induced TBARS production (35%, 57% and 46% for GSH, NAC and DTT respectively). However, pretreatment with thiol-containing compounds did not restore the basal levels of LDH release or lipid peroxidation.

The potential protective effects of antioxidant enzymes (SOD and CAT) as well as the hydroxyl radical scavenger DMSO and the iron chelator DFO against LDH leakage and lipid peroxidation in colonocytes exposed to 1.0 mM DBAN for 180 min were also evaluated (Table 2). It was found that pretreatment of colonocytes with either SOD (500 U/ml) or CAT (1500 U/ml) significantly inhibited LDH leakage by approximately 26% and 56%, respectively as compared with DBAN alone-treated cells. Also, both of the two antioxidant enzymes significantly diminished the TBARS production-induced by DBAN by 18% and 44% respectively. Pretreatment of colonocytes with either DMSO or DFO diminished DBAN-induced LDH leakage

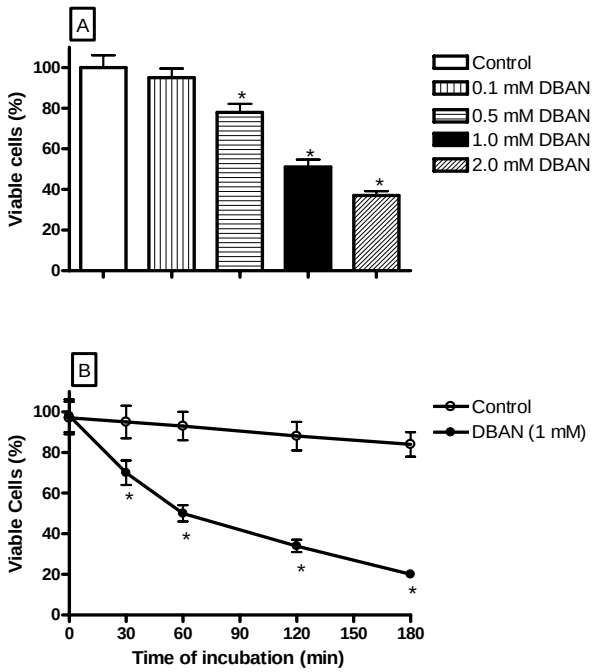

Fig. (1): (A) Dose res[pnse effect of DNAN on colonocytes viability after a 60-min incubation time; (B) Time corse effect of DBAN (0.1 mM) on colonocytes viability.
*** significantly different from corresponding control at P < 0.05**

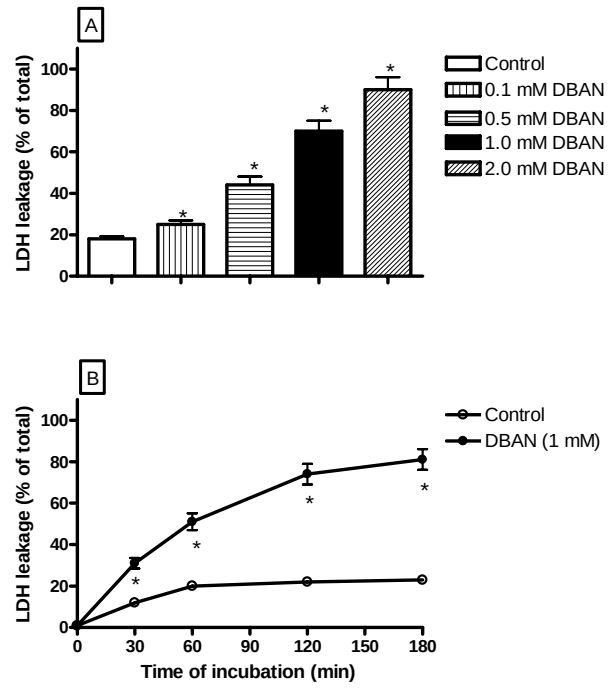

Fig. (2): (A) Dose res[pnse effect of DNAN on LDH leakage from colonocytes after a 60-min incubation time; (B) Time corse effect of DBAN (0.1 mM) on LDH leakage from colonocytes.
*** significantly different from corresponding control at P < 0.05**

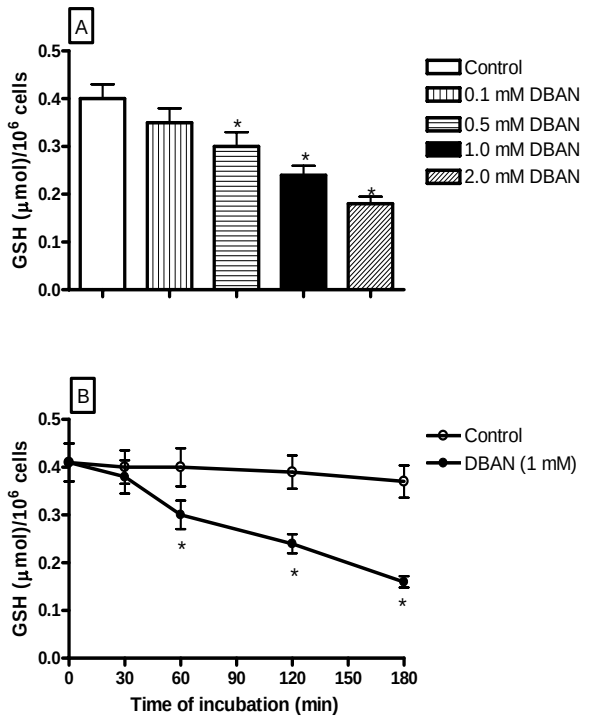

Fig. (3): (A) Dose res[pnse effect of DNAN on GSH level on colonocytes after a 60-min incubation time; (B) Time corse effect of DBAN (0.1 mM) on GSH level in colonocytes.
*** significantly different from corresponding control at P < 0.05**

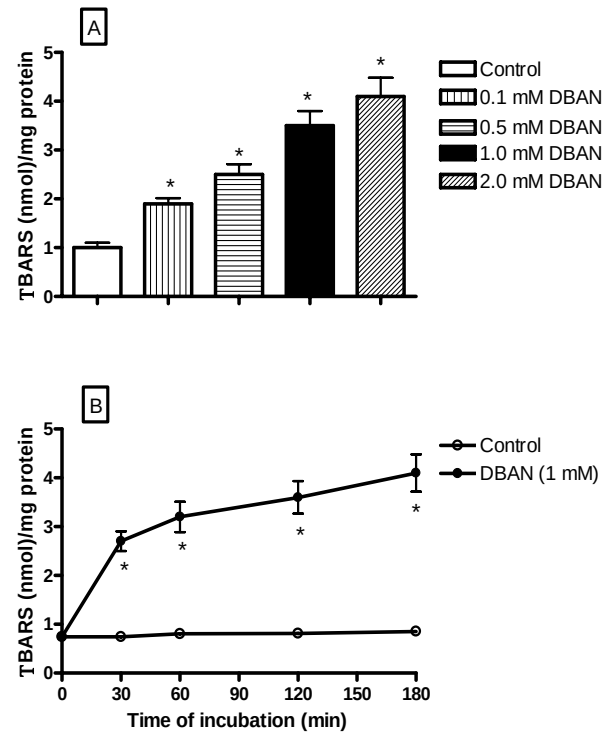

Fig. (4): (A) Dose res[pnse effect of DNAN on lipid peroxidation in colonocytes after a 60-min incubation time; (B) Time corse effect of DBAN (0.1 mM) on lipid peroxidation in colonocytes.
*** significantly different from corresponding control at P < 0.05**

by 27% and 34% of total leakage respectively, and TBARS production by 42% and 44%, respectively. However, none of the used protectors could restore LDH leakage or TBARS to control values.

Table 1: Protective effects of thiol-containing compounds on DBAN-induced LDH leakage and lipid peroxidation in colonocytes

Addition	LDH (% of total)	TBARS (nmol/mg protein)
None (Control)	19.8* ± 1.2	0.91* ± 0.3
DBAN (1.0 mM)	78.3 ± 5.5	4.4 ± 0.3
DBAN + GSH (1 mM)	41.1* ± 3.1	2.85* ± 0.01
DBAN + NAC (1 mM)	30.2* ± 2.9	1.91* ± 0.09
DBAN + DTT (1 mM)	35.5* ± 2.3	2.39* ± 0.12

Data are shown as means ± SEM of 6 incubations
All thiol compounds were added 30 min before the addition of DBAN
LDH and TBARS were determined 180 min after the addition of DBAN
* Significantly different from DBAN alone-treated group

Table 2: Protective effects of SOD, CAT, DMSO and DFO on DBAN-induced LDH leakage and lipid peroxidation in colonocytes

Addition	LDH (% of total)	TBARS (nmol/mg protein)
None (Control)	19.8* ± 1.2	0.91* ± 0.30
DBAN (1.0 mM)	78.3 ± 5.5	4.4 ± 0.33
DBAN + SOD (500/ml)	58.3* ± 5.1	3.61* ± 0.31
DBAN + CAT (1500 U/ml)	34.2* ± 3.1	2.45* ± 0.15
DBAN + DMSO (80 mM)	57.2* ± 3.3	2.56* ± 0.16
DBAN + DFO (10 mM)	51.9* ± 4.1	2.48* ± 0.12

Data are shown as means ± SEM of 6 incubations
All tested compounds were added 30 min before the addition of DBAN
LDH and TBARS were determined 180 min after the addition of DBAN
* Significantly different from DBAN alone-treated group

DISCUSSION

DBAN is an environmental chemical (IARC, 1991). Although the gastrointestinal tract is one of the most important target organs for DBAN toxicity (Ahmed *et al.*, 1991), there is a scanty of information regarding DBAN colonotoxicity. It is known that colonic epithelial cells are likely to be exposed to oxidative damage by free radicals generated within both the mucosa (from infiltrating phagocytes) and the lumen (from drugs and bacterial metabolites) (Weiss, 1986; Bhaskar *et al.*, 1995). In the present study the potential cytotoxic effects as well as the oxidative stress induced by DBAN in primary culture of rat colonocytes were evaluated.

Our data indicated that DBAN significantly decreased colonocytes viability and increased LDH leakage. In addition, assessment of the oxidative stress effects of the chemical revealed that DBAN induced GSH depletion and enhanced lipid peroxidation. The toxic response to DBAN was dependent on both concentration and duration of exposure. Indeed, the participation of oxidative stress in the cytotoxicity of DBAN has been previously reported in other target organs (Ahmed *et al.*, 1991). Moreover, oxidative stress has been implicated in the toxic insult of structurally related nitriles in the gastrointestinal cells and tissues which include chloroacetonitrile (Mohamadin and Abdel-Naim, 1999).

Lipid peroxidation and leakage of cytosolic enzymes are markers of cellular oxidative damage initiated by reactive oxygen species (ROS) (Farber *et al.*, 1990). Thus, factors interfering with the generation or effects of ROS are anticipated to protect against cell injury. The observed protective effects of GSH, DTT and NAC can be attributed to direct interaction of with reactive oxygen species and/or enhancement of cellular GSH synthesis (Maxwell, 1995; Hoffer *et al.*, 1996). Superoxide anion and hydrogen peroxide, as precursors of hydroxyl radical can exhibit similar deleterious effects (Liochev and Fridovich. 1994; Maxwell, 1995). Thus, in the present study the effectiveness of antioxidant enzymes (SOD and CAT) and the hydroxyl radical scavenger (DMSO) against DBAN-induced lipid peroxidation and enzyme leakage can be explained by their ability to remove the generated hydrogen peroxide, superoxide anions and hydroxyl radicals respectively. Superoxide anion can act as a reducing agent for ferric ions to give ferrous ions, which facilitate hydroxyl radical generation through the Fenton reaction (Ito *et al.*, 1992). Therefore, it is believed that the addition of' SOD or CAT reduces the formation of ferrous ions and hydrogen peroxide and then inhibits the production of hydroxyl radical. This is based on the known role of iron in the generation of free radicals and induction of oxidative damage (Halliwell and Gutteridge, 1990). Thus depletion of intracellular irons by DFO could indirectly prevent cell damage by inhibiting the generation of hydroxyl radical through the Fenton reaction. Collectively, the protective effects afforded by the thiol-group donors, SOD, CAT, DMSO and DFO against DBAN-induced cell injury highlights the role of ROS in DBAN-induced oxidative damage. However, despite that all the examined interventions could significantly inhibit DBAN-induced cytotoxicity, they failed to restore the normal level of LDH leakage or TBARS production. This suggests that, in addition to lipid peroxidation, other causes contribute to DBAN-induced loss of viability.

In conclusion, exposing colonocytes to DBAN inhibits cell viability, enhances LDH leakage, depletes GSH and induces lipid peroxidation. DBAN-induced oxidative stress

is at least partly responsible for its cytotoxicity. Thiol-group donors, antioxidant enzymes, hydroxyl radical scavengers and iron chelators can play an important role against DBAN-induced colonotoxicity.

REFERENCES

Abdel-Naim, A.B. and S.H. Ahmed (2005). Dibromoacetonitrile-induced gastric toxicity in rat: role of xanthine oxidase. J. Egypt. Soc. Toxicol 32, 53-60.

Abdel-Naim, A.B. and A.M. Mohamadin (2004). Myeloperoxidase-catalyzed oxidation of chloroacetonitrile to cyanide. Toxicol. Lett. 146(3), 249-257.

Ahmed, A.E.; G.I. Hussein; J.P. Loh and S.Z. Abdel-Rahman (1991). Studies on the mechanism of haloacetonitrile-induced gastrointestinal toxicity: interaction of dibromoacetonitrile with glutathione and glutathione-S-transferase in rats. J. Biochem. Toxicol. 6(2), 115-121.

Barcelo, D.; F.A. Maris; R.B.,Geerdink; R.W. Frei; G.J., de Jong and U.A. Brinkman (1987). Comparison between positive, negative and chloride-enhanced negative chemical ionization of organophosphorus pesticides in on-line liquid chromatography–mass spectrometry. J. Chromatogr. 8, 65–76.

Beutler, E.; O. Duron and B.M. Kelly (1963). Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61, 882-888.

Bhaskar, L.; M.M. Mathan and K.A. Balasubramanian (1995). Oxygen free radical induced damage during colonic ischemia/reperfusion in rats. Mol. Cell. Biochem. 151(1), 9-14.

Bull, R.J.; J.R. Meier; M. Robinson; H.P. Ringhand; R.D. Laurie and J.A. Stober (1985). Evaluation of mutagenic and carcinogenic properties of brominated and chlorinated acetonitriles: by-products of chlorination. Fundam. Appl. Toxicol. 5 (6 Pt. 1), 1065–1074.

Cotton, R.T. and H.H. Walkden (1968). The role of sorption in the fumigation of stored grain and cereal products. J. Kans. Entomol. Soc. 17, 98.

Daniel, F.B.; K.M. Schenck; J.K. Mattox; E.L. Lin; D.L. Haas and M.A. Pereira (1986). Genotoxic properties of haloacetonitriles: drinking water by-products of chlorine disinfection. Fundam. Appl. Toxicol. 6(3), 447–453.

Farber, J.L.; M.E. Kyle and J.B. Coleman (1990). Biology of disease: mechanisms of cell injury by activated oxygen species. Lab. Invest. 62, 670-679.

Flaten, T.P. (1992). Chlorination of drinking water and cancer incidence in Norway. Int. J. Epidemiol. 21, 6–15.

Grisham, M.B. and D.N. Granger (1988). Neutrophil-mediated mucosal injury. Role of reactive oxygen metabolites. Dig. Dis. Sci. 33(3 Suppl), 6S-15S.

Halliwell, B. and J.M. Gutteridge (1990). Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol. 186, 1-85

Hoffer, E.; Y. Baum; A. Tabak and U. Taitelman (1996). N-Acetylcystiene increases the glutathione content and protects rat alveolar type II cells against paraquat induced toxicity. Toxicol. Lett. 84, 7-12.

IARC (1991). Monographs on the evaluation of the carcinogenic risk to humans. Hologenated acetonitriles. International Agency for Research on Cancer, Lyon, France, 52: 269-286.

Ito, Y.; H. Hiraishi; M. Razandi; A. Terano; T. Harada and K.J. Ivey (1992). Role of cellular superoxide dismutase against reactive oxygen metabolite-induced cell damage in cultured rat hepatocytes. Hepatology 16, 247-254.

Le Curieux, F.; S. Giller; L. Gauthier; F. Erb and D. Marzin (1995). Study of the genotoxic activity of six halogenated acetonitriles, using the SOS chromotest, the Ames-fluctuation test and the newt micronucleus test. Mutat. Res. 341 (4), 289–302.

Lin, E.L. and C.W. Guion (1989). Interaction of haloacetonitriles with glutathione and glutathione-S-transferase. Biochem. Pharmacol., 38, 685-688.

Liochev, S.I. and I. Fridovich (1994). The role of $O2^-$ in the production of $HO^·$: in vitro and in vivo. Free Rad. Bio. Med., 16, 29-33.

Lowry, O.H.; N.J. Rosebrough; A.L. Forr and R.J. Randall (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-275.

Maxwell, S.R.G. (1995). Prospects for the use of antioxidant therapies. Drugs 49(3), 345-361.

Meier, J.R.; H.P. Ringhand; W.E. Coleman; J.W. Munch; R.E. Streicher; W.H. Kaylor and K.M Schenck (1985). Identification of mutagenic compounds formed during chlorination of humic acid. Mutat. Res. 157(2-3), 111-122.

Mink, D.L.; W.E. Coleman; J.W. Munch; W.H. Kaylor and H.P. Kinghand (1983). In vivo formation of halogenated reaction products following peroral sodium hypochlorite. Bull. Environ. Contam. Toxicol. 30, 394.

Mohamadin, A.M. and A.B. Abdel-Naim (1999). Chloroacetonitrile-induced oxidative stress and toxicity in rat gastric epithelial cells. Pharmacol. Res. 40(4), 377-383.

Mohamadin, A.M. and A. B. Abdel-Naim (2003). In vitro activation of dibromoacetonitrile to cyanide: role of xanthine oxidase. Arch. Toxicol. 77(2), 86-93.

Mohamadin, A.M.; M.H. El-Zahaby and A.E. Ahmed (1996). Acrylonitrile oxidation and cyanide release in cell free system catalyzed by Fenton-like reaction. Toxicologist 30(1 part 2), 238. (Abstract No. 1220).

Parks, D.A.; G.B. Bulkley and D.N. Granger (1983). Role of oxygen-derived free radicals in digestive tract diseases. Surgery 94(3), 415-422.

Roediger, W.E.W. and S.C. Truelove (1979). Method of preparing isolated colonic epithelial cells (colonocytes) for metabolic studies. Gut 20, 484-488.

Uchiyama, M. andM. Mihara (1978). Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem. 86, 271-278.

Weiss, S.J. (1986). Oxygen ischemia and inflammation. Acta. Physiol. Scand. 548, 9-37.

World Health Organization (1994). Guidelines for Drinking Water Quality, second ed., vol. 1. Recommendations. WHO, Geneva, Switzerland.

Wroblewski, F. and J. Ladue (1955). Lactic dehydrogenase activity in blood. Proc. Soc. Exp. Biol. Med. 90, 210-213.

Zimmermann, F. K. and A. Mohr (1992). Formaldehyde, glyoxal, urethane, methylcarbamate, 2,3-butandione, 2,3-hexandione, ethylacrylacte, dibromoacetonitrile and 2-hydroxypropiononitrile induce chromosome loss in Saccharomyces cerevisiae. Mutat. Res. 270: 151-166.