THE NEW ARMENIAN MEDICAL JOURNAL
Vol.7 (2013), No 3, p.33-43

OFFICIAL PUBLICATION OF THE YEREVAN STATE MEDICAL UNIVERSITY

Review

INTERACTION OF HALOGENS WITH NUCLEIC ACIDS AND ITS CONSEQUENCE

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Received 1/27/2013; accepted in final form 8/15/2013

Abstract

The analysis of literature shows that all halogens can react with important biological macromolecules, such as DNA and RNA. The consequences of mentioned reactions can be different, i.e., both protection from further toxic stimuli and toxic (mutagenic and possibly carcinogenic) effects. All halogens are genotoxic, but induce this effect only at high doses not relevant for humans.

The biological consequences of such reactions of halogens with deoxyribonucleic and ribonucleic acids and chromosomes warrant further investigations.

Keywords: *halogens, DNA, RNA, micronuclei, sister chromatid exchanges, chromosome aberrations, Ames test, comet assay.*

Introduction

The halogens or halogen elements are a series of nonmetal elements from Group 17 (formerly: VII, VIIA) of the International Union of Pure and Applied Chemistry (IUPAC) Style periodic table, comprising fluorine (F), chlorine (Cl), bromine (Br), iodine (I) and produced by radioactive decay in nature, – astatine (At) [*Thomas G., 2000; Close F., 2004*]. Astatine due to its short half-life is found only in minute amounts. It is currently the rarest naturally-occurring chemical element with less than 30 *g* estimated to be contained in the entire Earth's crust [*Close F., 2004*]. Because of this circumstance, this element will be not considered in the present review.

Halogens are highly reactive and as such can be harmful or lethal to biological organisms in sufficient quantities. This high reactivity is due to the atoms being highly electronegative owing to their high effective nuclear charge. They can gain an electron by reacting with atoms of other elements and can easily react with biomolecules [*Thomas G., 2000*].

In drug discovery, the incorporation of halogen atoms into a lead drug candidate results in analogues that are usually more lipophilic and less water-soluble. Consequently, halogen atoms are used to improve penetration through lipid membranes and tissues. Consequently, there is a tendency for some halogenated drugs to accumulate in adipose tissue [*Thomas G., 2000*].

The chemical reactivity of halogen atoms depends on both their point of attachment to the lead and the nature of the halogen. Aromatic halogen groups are far less reactive than aliphatic halogen groups, which can exhibit considerable chemical reactivity. For aliphatic carbon-halogen bonds the C-F bond is the strongest and usually less chemically reactive than aliphatic C-H bonds. The other aliphatic-halogen bonds are weaker, their reactivity increasing down the periodic table. They are usually more chemically reactive than aliphatic C-H bonds. Consequently, the most common halogen substitutions are the less reactive aromatic fluorine and chlorine groups [*Thomas G., 2000*].

Halogenation of bases of nucleic acids (bond of halogens to bases) is a widespread method used for solving crystal structures of nucleic acids [*Feig M., Petitt B., 1999; Dugal P. et al., 2000; Ennifar E. et al., 2007*].

Under *in vitro* conditions all halogens in the first turn bond to uracil, other bases are not similarly vulnerable [*Cantero G. et al., 2006*]. Among

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all halogens, uracil at C-5 position is the most vulnerable base in the structure of deoxyribonucleic acid (DNA) [*Commerford S., 1971*].

Iodination of macromolecules: Iodination of DNA by the reaction originally described by S. Commerford is extremely sensitive to the secondary structure of DNA [*Commerford S., 1971*]. This reaction has been widely used to ribonucleic acid (RNA), but has not often been applied to label DNA. The observed preference of the unpaired cytosines reaction restricts the extent for iodine incorporation into duplex DNA, but may permit iodination to be used as a probe of DNA secondary structure. As shown, iodination of polyoma and SV40 viral DNA destabilizes DNA duplex [*Anderson D., Folk W., 1976*]. Iodocytosines in SV40 DNA are preferentially removed by S-nuclease. The mentioned authors also showed that heavily iodinated DNA does not associate normally. However, DNA with only 5-10% of its cytosines re-associate with normal kinetics. These experiments showed that iodine bonds cytosins in DNA and RNA structure.

Iodine can cleave the thioated positions, if they are not shielded by ribosomal components [*Alexeeva E. et al., 1996*]. Only minor differences in iodine cleavage of ribosome bound and non-bound mRNA were observed: the nucleotide two positions upstream of decoding codons (i.e., those codons involved in codon-anticodon interactions) showed a reduced accessibility for iodine and nucleotide immediately following the decoding codons – an enhanced accessibility in both elongating states [*Alexeeva E. et al., 1996*]. Another research allowed to find that iodine can react with Cro protein obtained from bacteriophage lambda and bond DNA analogues at positions 3,3',7 and 7' [*Brennan R. et al., 1990*].

As revealed using high-pressure liquid chromatography (HPLC) iodine reacts with DNA [*Dugal P. et al., 2000*], preferably with uracil, at C-5 position likewise other halogens [*Cantero G. et al., 2006*].

In iodine-deficient children a high level of oxidized DNA bases was recorded [*Giray B., Hinkal F., 2002*]. These findings support the theory that iodine was the first antioxidant on the Earth which played an important role in evolution [*Venturi S., Venturi M., 2009*].

It was demonstrated through earlier *in vitro* studies that DNA damage was produced by povidone-iodine as a result of an *in situ* modification of the deoxycytosine moiety of DNA to 5-iododesoxycytidine [*Speck W. et al., 1976*]. In these studies, DNA was directly isolated from diploid cells and judged for shortening or for the presence of fragments. Based on new approaches, povidon-iodide was studied for genotoxic activity using the comet assay and chromosome aberrations (CA) test in Chinese hamster ovary (CHO)-KI cells. In both tests, no genotoxic effects were observed independent of iodine concentrations [*Müller G. et al., 2006*].

Different experiments showed that iodination of uracil in DNA protected against DNA-damaging effects, CA and sister chromatid exchanges (SCE) induced by topoisomerase I and II poisons – m-AMSA and camptothecin [*Cortés F. et al., 2003; Cantero G. et al., 2006; Orta M. et al., 2008*].

Investigations of possible tumor inducing ability of iodine compounds in long-term carcinogenicity studies in rats showed a weak activity of potassium iodide [*Takegawa K. et al., 2000*]. In a 2-year carcinogenicity study in rats 10% male rats and 7.5% females had salivary gland tumors after exposure to 1000 *ppm* of KI in drinking water. The authors showed that this activity was due to epigenetic mechanism, but not to genotoxicity of KI [*Takegawa K. et al., 2000*]. The influence of molecular iodine (I_2) but not iodide (I^-) on N-methyl-N-nitrosourea-induced mammary carcinogenesis was also studied [*García-Solís P. et al., 2005*]. The researchers found 30% decrease of tumors incidence in rats treated with molecular iodine and suggested that it was due to effect of iodine on the promotion phase of carcinogenesis via hormonal mechanisms. Other authors confirmed the results of anticarcinogenic action of iodine and Lugol in rat mammary carcinogenesis study with 7,12-dimethyl-benzanthracene [*Soriano O. et al., 2011*].

Experiments allowed to reveal that iodine due to its reactivity converted into iodides, which damaged DNA via indirect mechanism (oxidative stress) [*Bürgi H. et al., 2001*]. It is noteworthy that iodine compounds induced oxidative stress in cells only at relatively high doses. At low doses they possessed antioxidant properties. The plant system (*Allium* micronucleus assay) allowed researchers to show that KI prevented chromosomal aberrations induced by methylmercuric chloride and maleic hydrazide through the antioxidant mechanism

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[*Panda B. et al., 1995*]. In CHO KI-cells even at high concentrations did not induce either micronuclei (MN) or DNA damage measured by means of the comet assay [*Poul J. et al., 2004*].

Halogenated thymidine (TdR) analogs, such as 5-iododeoxyuridine (IdUrd) have been recognized as potential radiosensitizers since 1950s-1960s [*Prusoff W., 1959; Erikson R., Szybalski W., 1963*]. Equilibrium-density-gradient patterns revealed that the mode of DNA replication in D98/AG cells is of the semi-conservative type and that DNA can undergo more than one round of duplication even at the highest levels of IdUrd labelling [*Erikson R., Szybalski W., 1963*].

Due to the short half-life of IdUrd (in the order of minutes), continuous intravenous infusions over the course of radiotherapy was required to maintain adequate exposure. An oral prodrug of IdUrd, 5-iodo-2-pyrimidin-one-20-deoxyribose (IPdR), has many advantages as compared with IdUrd, including ease of administration, a more favorable toxicity profile, and a better therapeutic index in animals [*Kummar S. et al., 2013*]. At present, this prodrug is under clinical trial at the National Cancer Institute (USA) [*Kummar S. et al., 2013*].

In 1994 it was described and proposed to use in biological and medical investigations a new method, which combines the identification of DNA replicating and apoptotic cells in a single measurement by flow cytometry [*Li X. et al., 1994*]. The detection of DNA replicating cells is based on incorporation of 5-iodo-2'-deoxyuridine followed by selective photolysis at the site of incorporation of the halogenated DNA precursors. Single-strand breaks in DNA resulting from the photolysis are subsequently labelled with digoxygenin or biotin-conjugated dUTP in a reaction catalyzed by exogenous terminal deoxynucleotidyl transferase. The double-stranded DNA breaks in apoptotic cells resulting from activation of the endonuclease can be labelled in this reaction as well. However, in contrast to the photolysed DNA, the low molecular weight fraction of apoptotic cells DNA is extractable from the cells, and the degree of DNA elution can be modulated by cross-linking with formaldehyde. Thus, apoptotic cells can be distinguished and quantified by virtue of their fractional DNA content. The method was applied to study apoptosis and proliferation of human leukemic HL-60 cells and normal, mitogen-stimulated lymphocytes. Whereas apoptosis of HL-60 cells induced by the DNA topoisomerase I inhibitor camptothecin was selective to DNA replicating cells, apoptosis induced by hyperthermia showed no such selectivity. Lymphocytes that preferentially underwent apoptosis in cultures stimulated by phytohemagglutinin did not initiate DNA replication. By offering the possibility for identification of both DNA replicating and apoptotic cells in a single measurement, the method may find an application in studies of the prognostic value of both cell proliferation and death in human tumors and the apoptotic response of DNA replicating *vs* non-replicating cells to different treatments [*Li X. et al., 1994*].

Chlorination of macromolecules: Upon inflammation the activated neutrophils secrete mieloperoxidase, an enzyme able to generate hypochlorous acid (HOCl), hydrogen peroxide and chloride ion [*Baduard C. et al., 2005*]. This acid is able to damage DNAs and RNAs. The acid induces both ribo- and 2-deoxiribonucleotide derivatives, including 8-chloroguanine, 5-chlorocytosine and 8-chloroadenine. The authors established a method to detect markers of inflammation in human organism by measuring the levels of chlorination of DNA and RNA obtained from blood by means of HPLC.

The studies showed that chlorine attacks cytosine forming 5-chlorouracil (5-ClUra) [*Halliwell B., 1999; Whiteman M. et al., 2002*]. The mentioned compound is very frequently found after the inflammation process in human organism, because neutrophils release HOCl to kill microorganisms [*Hawkins C., Davies M., 2002; Jiang Q. et al., 2003*]. The acid can also easily react with DNA forming 5-ClUra, and RNA and polynucleotides [*Hawkins C., Davies M., 2002*]. Reactive chlorine species as any radicals are probably mutagenic and carcinogenic [*Halliwell B., 1999*].

Another investigation showed that leukocytes derived peroxidases, such as myeloperoxidase, use hydrogen peroxide and halides (Cl^-) to generate hypohalous acids, halogenating intermediates [*Kawai Y. et al., 2004*]. Mentioned oxidants can react with DNA bases forming 2-deoxycytidine. Hence, chlorination of DNA may therefore constitute one of the mechanisms for oxidative DNA damage at the site of inflammation [*Kawai Y. et al., 2004*].

The alkaline elution of chlorodeoxyuridine-

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containing (CldUrd) DNA was studied in two CHO cell lines, the parental AA8 and a mutant line EM9, which has a defect in repairing DNA strand breaks and a 12-fold elevated baseline frequency of SCE [*Dillehay L. et al., 1984*]. CldUrd-DNA, which gives higher frequencies of SCE than BrdUrd-DNA, had more strand breaks than BrdUrd-DNA in AA8 cells after treatment with benzamide, while without benzamide there was no difference. The accumulation of breaks in CldUrd-DNA by benzamide was shown to occur rapidly, to reach a maximum by 90 *min*, and to be readily reversible after benzamide removal. Under all conditions, EM9 cells had more strand breaks than AA8. The observed differences in strand breaks were not due to differences in incorporation efficiencies. For different halogenated pyrimidines and cell types there was a good correlation between the number of DNA strand breaks and reduction in plating efficiencies [*Dillehay L. et al., 1984*].

Hypochlorous acid, a strong oxidant derived from myeloperoxidase in neutrophils and macrophages, can chlorinate DNA bases at the site of inflammation [*Burchum P., 1999; Kawai Y. et al., 2008*]; the specified acid preferentially damaged pyrimidine residues forming oxidation products, as well as a novel chlorinated base, 5-chlorocytosine. The latter species underwent conversion to 5-chlorouracil during DNA hydrolysis. The mechanism involved in the formation of 5-chlorocytosine was not fully clarified, although a role for labile chloramines formed upon reaction of HOCl with primary amines in DNA was suggested [*Burchum P., 1999*]. Hypochlorous acid was also shown to react with DNA of *Salmonella* and to induce gene mutations in strain TA1530 of *Salmonella* [*Burchum P., 1999*].

Because little is known about the protective role of natural antioxidants, such as polyphenols, for the myeloperoxidase-derived DNA damage, Y. Kawai and co-authors screened the inhibitory effects of various phenolic antioxidants on the chlorination of the 2′-deoxycytidine residue by HOCl *in vitro* and found that green tea catechins, especially (-)-epicatechin gallate (ECg) and (-)-epigallocatechin gallate (EGCg), significantly inhibited the chlorination. These catechins also reduced nucleoside- and taurine-chloramines, which can induce secondary oxidative damage, into their native forms. Mass spectrometric and nuclear magnetic resonance analyses showed that ECg and EGCg can effectively scavenge HOCl and/or chloramine species resulting in the formation of mono- and dichlorinated ECg and EGCg. Using the HL-60 human leukemia cell line, it was found that ECg could efficiently accumulate in the cells. Immunocytometric analyses using antihalogenated 2′-deoxycytidine antibody showed that pretreatment of cells with ECg inhibited the HOCl-induced immunofluorescence. In addition, the chlorinated ECg derivatives were detected in the HOCl-treated HL-60 cells. These results showed that green tea catechins, especially 3-galloylated catechins, may be the plausible candidates for the prevention of inflammation-derived DNA damage and perhaps carcinogenesis [*Kawai Y. et al., 2008*].

Aberrant methylation patterns have long been known to exist in the promoter regions of key regulatory genes in the DNA of tumor cells [*Valinluck V., Sowers L., 2007 b*]. However, the mechanisms, by which these methylation patterns become altered during the transformation of normal cells to tumor cells, have remained elusive. It has been recently shown through *in vitro* studies that inflammation-mediated halogenated cytosine (5-chlorocytosine) damage products can mimic 5-methylcytosine in directing enzymatic DNA methylation and in enhancing the binding of methyl-binding proteins, whereas certain oxidative damage products inhibit both. It was proposed that cytosine damage products could potentially interfere with normal epigenetic control by altering DNA-protein interactions critical for gene regulation and the heritable transmission of methylation patterns; these inflammation-mediated cytosine damage products may provide, in some cases, a mechanistic link between inflammation and cancer [*Valinluck V., Sowers L., 2007 b*].

Alterations in cytosine methylation patterns are usually observed in human tumors [*Valinluck V., Sowers L., 2007a*]. The consequences of altered cytosine methylation patterns include both inappropriate activation of transforming genes and silencing of tumor suppressor genes. Despite the biological effect of methylation changes, little is known about how such changes are caused. The heritability of cytosine methylation patterns from parent to progeny cells is attributed to the fidelity

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of the methylation-sensitive human maintenance methyltransferase DNMT1, which methylates with high specificity the unmethylated strand of a hemimethylated CpG sequence following DNA replication. DNA damage that might alter the specificity of DNMT1, either inhibiting the methylation of hemimethylated sites or triggering the inappropriate methylation of previously unmethylated sites, was studied. As demonstrated, known forms of endogenous DNA damage can cause either hypermethylation or hypomethylation. Inflammation-induced 5-halogenated cytosine damage products, including 5-chlorocytosine, mimic 5-methylcytosine and induce inappropriate DNMT1 methylation within a CpG sequence. In contrast, oxidation damage of the methyl group of 5-methylcytosine, with the formation of 5-hydroxymethylcytosine, prevents DNMT1 methylation of the target cytosine. It was proposed that reduced DNMT1 selectivity resulting from DNA damage could cause heritable changes in cytosine methylation patterns, resulting in human tumor formation [*Valinluck V., Sowers L., 2007a*]. Both sodium chlorite and sodium hypochlorite were found to be strongly positive agents in the Ames assay (*Salmonella*/microsomes assay), both with and without metabolic activation and also CA inducer in Chinese hamster fibroblasts [*Ishidate M. et al., 1984*].

Chlorine dioxide and sodium chlorite were found to induce MN after a single intraperitoneal (*i.p.*) injection in mice at relatively high doses (both routes of exposure and doses are not relevant for humans) [*Hayashi M. et al., 1988*]. However, after oral treatment no effect was observed.

Bromination of macromolecules: The investigation on interactions of bromate with L1210 mouse leukemia cells found that in DNA damage either Br or BrO radicals are involved, but not the molecular Br [*Ballmaier D., Epe B., 2006*]. Bromine containing substances react with DNA both *in vitro* and *in vivo* with guanosine. Due to this reaction 8-oxo-2-deoxyguanosine appear [*Bull R. et al., 2012*].

Other researchers showed that leukocyte-derived peroxidases, such as eosinophil peroxidase, use hydrogen peroxide and halides (Br^-) to generate HOBr, a halogenating intermediate [*Kawai Y. et al., 2004*]. Mentioned oxidants can react with DNA bases forming 2-deoxycytidine. Therefore, bromination of DNA may constitute one of the mechanisms for oxidative DNA damage at the site of inflammation [*Kawai Y. et al., 2004*]. Bromine due to its reactivity converts into bromide, which damages DNA via indirect mechanism (via the oxidative stress) [*Bürgi H. et al., 2001*].

The alkaline elution of bromodeoxyuridine-containing (BrdUrd) DNA was studied in two CHO cell lines, the parental AA8 and a mutant line, EM9, which has a defect in repairing strand breaks and a 12-fold elevated baseline frequency of SCE [*Dillehay L. et al., 1984*]. BrdUrd-DNA was found to have alkali-labile sites, as well as direct breaks, neither of which were increased significantly by prior treatment of AA8 cells with an inhibitor (benzamide) or poly(adenosine diphosphoribose) polymerase. Under all conditions, EM9 cells had more strand breaks than AA8. The observed differences in strand breaks were not due to differences in incorporation efficiencies. For different halogenated pyrimidines and cell types, there was a good correlation between the number of DNA strand breaks and reduction in plating efficiencies [*Dillehay L. et al., 1984*].

Oxidants generated by eosinophils during chronic inflammation may lead to mutagenesis in adjacent epithelial cells [*Henderson J. et al., 2001*]. Eosinophil peroxidase, a heme enzyme released by eosinophils, generates hypobromous acid that damages tissues in inflammatory conditions. As shown by J. Henderson and co-workers, human eosinophils use eosinophil peroxidase to produce 5-bromodeoxycytidine. Flow cytometric, immunohistochemical, and mass spectrometric analyses all demonstrated that 5-bromodeoxycytidine generated by eosinophil peroxidase was taken up by cultured cells and incorporated into genomic DNA as 5-bromodeoxyuridine. Although previous studies focused on oxidation of chromosomal DNA, the observations of mentioned researchers suggested another mechanism for oxidative damage of DNA. In this scenario, peroxidase-catalyzed halogenation of nucleotide precursors yields products that subsequently can be incorporated into DNA. Because the thymine analog 5-BrUra mispairs with guanine in DNA, generation of brominated pyrimidines by eosinophils might constitute a mechanism for cytotoxicity and mutagenesis at sites of inflammation [*Henderson J. et al., 2001*].

As already mentioned in this review, X. Li and

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associates (1994) described and proposed to use in biological and medical investigations a new method, which combines the identification of DNA replicating and apoptotic cells in a single measurement based on incorporation of 5-bromo-2'-deoxyuridine or 5-iodo-2'-deoxyuridine (see detailed description above). Replication of less than 1% of a genome of a cell in the presence of 5-bromo-2'-deoxyuridine (equivalent of a 5-minute 10 *microM* 5-bromo-2'-deoxyuridine pulse) can be detected by the selective photolysis method. This method might find its application in studies on the prognostic value of both cell proliferation and death in human tumors, as described in *Iodination* part of the paper [*Li X. et al., 1994*].

The *in situ* presence of numerous DNA strand breaks is a typical feature of apoptotic cells [*Li X., Darzynkiewicz Z., 1995*]. The selective DNA strand break induction by photolysis (SBIP) at sites that contain incorporated halogenated DNA precursors has been proposed as a method of analysing DNA replication. Detection of DNA strand breaks thus enables one to identify apoptotic and/or DNA replicating cells. The current methods for DNA strand break labelling rely on the use of exogenous terminal deoxynucleotidyl transferase, which either directly attaches the fluorochrome conjugated triphosphodeoxynucleotides to 3'OH ends in the breaks, or indirectly labels 3'OH ends with digoxygenin or biotin-conjugated triphosphodeoxynucleotides. A limitation of these methodologies, especially restricting their routine application in the clinic, is high cost of reagents.

In 1995, X. Li and Z. Darzynkiewicz tested, whether relatively simple compound BrdUTP, which is approximately three orders of magnitude less expensive than dUTP conjugated to digoxygenin, can be used as marker of DNA strand breaks. Apoptosis of HL-60 cells was induced by DNA topoisomerase I inhibitor camptothecin. The incorporated BrdUTP was detected by fluoresceinated anti-BrdUrd MoAb. Cellular fluorescence was measured by flow cytometry as well as by Laser Scanning Cytometer. The data showed that intensity of DNA strand break labelling with BrdUTP was nearly four- and two-fold higher than that obtained with the indirect labelling using biotin- or digoxygenin-conjugated dUTP, respectively, and over eight-fold higher than in the case of direct labelling with the fluorochrome (fluorescein or BODIPY)-conjugated deoxynucleotides. The increased labelling of DNA strand breaks with BrdUTP may reflect more efficient incorporation of this precursor by terminal transferase, compared to the nucleotides with bulky fluorochrome conjugates. Thus, DNA strand break labelling with BrdUTP offers a possibility of more sensitive (and at lower cost) detection of apoptotic or DNA replicating cells, compared to the alternative methods of DNA strand break labelling [*Li X., Darzynkiewicz Z., 1995*].

Aberrant methylation patterns have long been known to exist in the promoter regions of key regulatory genes in the DNA of tumor cells. It has been recently shown by *in vitro* studies that inflammation-mediated halogenated cytosine (5-bromocytosine) damage products can mimic 5-methylcytosine in directing enzymatic DNA methylation likewise at the earlier described 5-chlorocytosine-induced damage [*Valinluck V., Sowers L., 2007 b*].

Cytosine methylation or bromination of the DNA sequence d(GGCGCC)2 was shown to induce a novel extended and eccentric double helix, which was called E-DNA [*Vargason J. et al., 2000*]. Like B-DNA, E-DNA has a long helical rise and bases perpendicular to the helix axis. However, the 3¢-endo sugar conformation gives the characteristic deep major groove and shallow minor groove of A-DNA. Also, if allowed to crystallize for a period of time longer than that yielding E-DNA, the methylated sequence forms standard A-DNA, suggesting that E-DNA is a kinetically trapped intermediate in the transition to A-DNA. Thus, the structures presented here chart a crystallographic pathway from B-DNA to A-DNA through the E-DNA intermediate in a single sequence. The E-DNA surface is highly accessible to solvent, with waters in the major groove sitting on exposed faces of the stacked nucleotides. It is suggested that the geometry of the waters and the stacked base pairs would promote the spontaneous deamination of 5-methylcytosine in the transition mutation of dm5C-dG to dT-dA base pairs [*Vargason J. et al., 2000*].

Halogenated thymidine (TdR) analogs, such as 5-bromodeoxyuridine (BrdUrd) has been recognized as potential radiosensitizers [*Prusoff W., 1959; Erikson R., Szybalski W., 1963*].

Potassium bromated, which is used as a bleaching agent in flour, was found to be strongly positive

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agent in the Ames assay (*Salmonella*/microsomes assay) both with and without metabolic activation, as well as CA inducer in Chinese hamster fibroblasts [*Ishidate M. et al., 1984*]. It also induces MN after a single *i.p.* injection in mice. The compound was further tested by oral administration and showed a clearly positive result [*Hayashi M. et al., 1988*]. Furthermore, this compound was tested for genotoxicity in human peripheral blood lymphocytes *in vitro* by SCE, CA and MN tests and also for determining any probable genotoxic potential for humans. Cells were treated with 400, 450, 500, 550 *μg/ml* concentrations of potassium bromate for 24 and 48 hours [*Kaya F., Topaktaş M., 2007*].

The SCE frequencies showed an increase after both treatment periods, however, the differences between treated cells and the control groups were found to be statistically significant only for the 48-hour treatment. In addition, potassium bromate statistically significantly induced CA after the 24- and 48-hour treatment periods. Strikingly, it induced CA as much as the positive control, mitomycin C (MMC). Furthermore, potassium bromate decreased both the cell proliferation index and the mitotic index. Although MN formation was induced by potassium bromate during the 24-hour treatment period in a dose-dependent manner, only the doses 500 and 550 *μg/ml* yielded statistically significant results. In contrast, MN formation was significantly induced at all doses during the 48-hour treatment period [*Kaya F., Topaktaş M. et al., 2007*]. In CHO cells potassium bromate induced both MN and DNA damage measured by means of the comet assay [*Poul J. et al., 2004*].

This compound is also very potent in the MN assay in mouse and rat bone marrow and peripheral blood cells [*Nakajima M. et al., 1989; Awogi T. et al., 1992; Sai K. et al., 1992*]. The mechanisms of genotoxic action of potassium bromate were studied by D. Ballmaier and B. Epe (2006). They found that exposure of mammalian cells to bromate (BrO_3-) generates oxidative DNA modifications, in particular 7,8-dihydro-8-oxo-guanine (8-oxoG). The damaging mechanism is quite unique, since glutathione, which is protective against most oxidants and alkylating agents, mediates a metabolic activation, while bromate itself does not react directly with DNA. Neither enzymes, nor transition metals are required as catalysts in the activation. The ultimate DNA damaging species has not yet been established, but experiments under cell-free conditions suggest that neither molecular bromine nor reactive oxygen species, such as superoxide, hydrogen peroxide or singlet oxygen, are involved. Rather bromine radicals (Br*) or oxides (BrO*, BrO2*) might be responsible. Compared to hypochlorite (ClO^-), bromate is much less cytotoxic, probably because the former halite efficiently reacts with proteins and other vitally important cellular constituents. In consequence, oxidative DNA damage and the induction of mutations and MN is easily detectable at non-cytotoxic concentrations of bromate, while DNA damage by hypochlorite is observed only at cytotoxic concentrations and follows a non-linear (hockey-stick-like) dose response [*Ballmaier D., Epe B., 2006*].

Potassium bromate is genotoxic rat renal carcinogen; however, the agent is genotoxic and carcinogenic in rats only at high doses [*Yamaguchi T. et al., 2008*].

Fluorination of macromolecules: Fluorine, the 17th most abundant element in the earth's crust, is a gas and never occurs in a free state in nature. Fluorine exists only in combination with other elements as fluoride compounds, which are constituents of minerals in rocks and soil [*Dhar V., Bhatnagar M., 2009*]. The fluoride ion comes from the element fluorine. Fluoride can easily react with uracil under *in vitro* conditions [*Begue J., Bonnet-Delpon D., 2006*]. The compound obtained after this reaction and called 5-fluorouracil reacts with DNA obtained from any organism and induces severe damage. This compound has been used as a potent antitumor agent since 1950s. The compound damages DNA both directly and indirectly, but mostly indirectly inhibiting thymidylate synthase involved in DNA synthesis. Fluoride can bond with uridine *in vitro* producing 5-trifluoromethyl-2-deoxyuridine [*Begue J., Bonnet-Delpon D., 2006*], which is very effective against *herpes simplex* virus infection. It is also a potent inhibitor of thymidylate synthase involved in DNA synthesis.

Fluoride is mutagenic agent, which induces gene mutations and CA in cultured mammalian cells [*Pati P., Bhunya S., 1987*]. Recently, K. Erciyas and R. Sarikaya (2009) showed that NaF induced mutations in Somatic Mutation and Recom-

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bination Test (SMART-test) in *Drosophila melanogaster*. In lymphocytes of persons exposed to excess of fluoride in drinking water the increased levels of SCE, MN, and CA were found. At the same time, mentioned authors showed that mutagenic effects induced by fluoride were not direct, but owing to ability of fluoride to damage enzymes necessary for DNA and RNA synthesis. High levels of fluoride in food of rats induced DNA damage in brain cells measured by the comet assay only in deficiency of iodine in food and water of animals. At normal levels of iodine in food and water of rats the damage of DNA was not so pronounced [*Erciyas K., Sarikaya R., 2009*]. This is in agreement with data of many investigations that, as mentioned above, iodine was the first antioxidant, which played an important role in evolution [*Venturi S., Venturi M., 2009*].

Fluorinated analogues of DNA bases are potent antiviral and antimicrobial agents, which damage DNA/RNA of microorganisms [*Kamal A. et al., 2004*]. Fluorinated analogues of uridine, thymidine and cytosine at C-2, C-4 and C-5 positions of DNA bases are potent antiviral and antitumor agents, which damage DNA or RNA targets [*Pankiewicz K., 2000; Liu P. et al., 2008*]. Many studies examined the possible effects of fluoride on chromosome damage. While there are no published studies on the genotoxic (damage to DNA) effect of fluoride in humans, numerous studies have been done in mice [*Dhar V., Bhatnagar M., 2009*]. These studies have shown no evidence of fluoride effects on chromosomes in bone marrow or sperm cells even at fluoride levels 100 times higher than those in fluoridated water. An independent group of researchers reported a similar lack of fluoride-induced chromosomal damage to human white blood cells, which are especially sensitive to agents that cause genetic mutations. Not only did fluoride fail to damage chromosomes, but it also protected them against the effect of a known mutagen. The genotoxic effects of fluoride were also studied in hamster bone marrow cells and cultured hamster ovarian cells. Again, the results support the conclusion that fluoride does not cause chromosomal damage, and therefore, is not a genetic hazard [*Dhar V., Bhatnagar M., 2009*]. In further tests, fluoride has not caused genetic mutations in the most widely used bacterial mutagenesis assay (the Ames test) over a wide range of fluoride levels. Occasional questions arise regarding effects of fluoride on human reproduction, fertility, and birth rates. Very high levels of fluoride intake were associated with adverse effects on reproductive outcomes in many animal species. Based on these findings, it appears that fluoride concentrations associated with adverse reproductive effects in animals are far higher (100-200 *ppm*) than those, to which human populations are exposed. Consequently, there is insufficient scientific basis underlying the conclusion that ingestion of fluoride at levels found in community water fluoridation (0.7-1.2 *ppm*) would have adverse effects on human reproduction [*Dhar V., Bhatnagar M., 2009*]. The National Research Council (NRC) of the US National Academy of Sciences supports the conclusion that drinking optimally fluoridated water is not a genetic hazard. In a statement summarizing its research, the NRC states that:

1. The genotoxicity of fluoride is limited primarily to doses much higher than those, to which humans are exposed.
2. Even at high doses, genotoxic effects are not always observed.
3. The preponderance of the genotoxic effects that have been reported are of the types that probably are of no or negligible genetic significance.
4. The lowest dose of fluoride reported to cause chromosomal changes in mammalian cells was approximately 170 times higher than normally found in human cells in areas, where drinking water is fluoridated, which indicates a very large margin of safety.

Conclusion

The analysis of literature shows that all halogens can react with important biological macromolecules, such as DNA and RNA. The consequences of mentioned reactions can be different, i.e., both protective from further toxic stimuli and toxic (mutagenic and possibly carcinogenic) effects. The biological consequences of such reactions warrant further investigations.

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