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Colorectal cancers and chlorinated water

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Abstract

Published reports have revealed increased risk of colorectal cancers in people exposed to chlorinated drinking water or chemical derivatives of chlorination. Oestrogen plays a dual positive functions for diminishing the possibilities of such risk by reducing the entrance, and increasing the excretion, of these chemicals. In addition, there are supplementary measures that could be employed in order to reduce this risk further, such as boiling the drinking water, revising the standard concentrations of calcium, magnesium and iron in the public drinking water and prescribing oestrogen in susceptible individuals. Hypo-methylation of genomic DNA could be used as a biological marker for screening for the potential development of colorectal cancers.

Key words: Chlorinated drinking water; Oestrogen; Sex hormones; Gender; Colorectal cancers; Trihalomethanes; Carcinogenesis; DNA hypo-methylation

Core tip: Oestrogen inhibits the absorption and increases the excretion of xenobiotics and their metabolites *via* the bile. Oestrogen has anti-hypo methylation activity on the genomic DNA by reducing the plasma levels of homocysteine. Colorectal carcinomas are the third most common tumour in both sexes across the globe. The hazard to develop tumours in different specific sites including colon and rectum in association with the long-term exposure to water disinfectants in drinking water is well established. The risk to develop tumours in the large intestines is dependent on the concentrations and frequency of exposure to the trihalomethanes in the used water for drinking. The risk to develop malignant tumours due to water pollution is higher amongst user of swimming pools and is also dependent on the frequency of showering. Indeed, this risk is much higher in those who are avid consumers of fatty foods and/or their meals lacks vegetables and fruits in this susceptible group amongst those who are users of swimming pools. Yet, this risk could be reduced by adding calcium, magnesium and removing iron from the drinking water. Boiling of drinking water is another effective measure for reducing such risk. Colorectal carcinomas arising from long exposure to trihalomethanes in drinking water are characterised an aggressive courses of development and are rarely diagnosed in early stages. Accordingly, it is quite necessary to screen for their occurrence amongst the susceptible persons. Global DNA hypomethylation is most common amongst all subjects who are susceptible to develop malignant tumours and the levels of hypo methylation increase with the prognosis of the disease. Thus screening for the hypo methylation of the relevant genomic DNA and the plasma concentration of homocysteine would be useful criterion for identifying those at risk.

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INTRODUCTION

Carcinomas of the large intestines are remarkably common across the globe with around 1.4 million new cases diagnosed in 2012[1]. The risk of developing colorectal cancer increases with age and is higher in men than in women[2].

An association between water chlorination and the development of colorectal carcinoma is well established[3-13].

The different frequencies of cancers in different genders, the recurring diagnosis of malignant tumors of the large intestines in women with breast cancer, the prophylactic effect of gravidity, together with the decreased risk among women on pills after menopause suggest that female hormones may play a role[14].

In animal models, for example, male rats that were exposed to dimethyl hydrazine (a carcinogenic mediator), had twice the risk of developing colonic tumors and noticeably shorter survival times than their female peers[14].

Estrogen mainly exerts its actions through its receptors (ERs) that exist on the target cells. ERs have been found in several malignant tumors including those of the alimentary tract[15].

Yet, whether estrogen contributes in the carcinogenic activity of the trihalomethanes that leads to the development of colorectal carcinoma is still unknown. The objective of this review is to explore the possible mechanism played by oestrogen in the cancer development process.

METABOLISM AND EXCRETION OF XENOBIOTICS

Poisons, cancer initiators and medicines are examples of xenobiotics. Many metabolizing enzymes are involved in their detoxification. The cluster of cytochrome P450 isoenzymes is one of two known classes that are responsible for the metabolism of these compounds. It consists of many isoforms[16] leading to the oxidation (mainly hydroxylation) of the particles[17]. Isoforms in the clusters 1, 2 and 3 accelerate metabolism of many exogenous compounds. Many poisons and cancer promoters usually necessitate activation by cytochrome P450 isoenzymes in order to be expelled outside the body. In general, the enzymatic activity of cytochrome P450 isoenzymes has three specific and consecutive phases: Phase I metabolism, is largely for catalysing the first step of biotransformation. However, if this catalysing activity follows a variety of conjugation steps it is entitled phase Ⅱ metabolism. This stage is usually accelerated by a variety of enzymatic systems, of which UDP Glucuronosyltransferases is the most important[18]. The purpose for conjugating the xenobiotics to the glucuronic acid is to make them soluble in water and consequently fit for excretion in bile[19,20]. Other models of phase Ⅱ systems include sulfotransferases, acetyltransferases and glutathione-s-transferase.

Later reports[21] have added the transport steps and considered them as part of the detoxification process. The role of the export pumps is to reduce the cellular concentrations of these poisons and thereby decreasing the associated harm.

These transport proteins mainly exist in the apical membrane of epithelial cells, such as enterocytes, which lie directly opposite to these exogenous toxins. The protein transporters can limit the entrance of the harmful compounds and facilitate the excretion of their metabolites as well. The last step is entitled "phase Ⅲ metabolism" in order to link it to the oxidation and conjugation steps. Yet, directing the poisons to enter into the enterocytes has been considered as first line of defence and accordingly has been entitled "phase 0 metabolism".

These protein transporters exert their activities in an active ATP dependent manner against the concentration gradients. The two most common models of these transporters are the multidrug resistance transporter 1 (MDR1) and multidrug resistance associated protein 2 (MRP2). These transporters play a remarkable role in phase 0 and phase Ⅲ defence against xenobiotics. They are present in both the intestine and liver and thence can reduce the oral bioavailability by direct inhibition of gut absorption and speedy excretion of xenobiotics and their metabolites *via* bile[19,20].

MDR1 is active against xenobiotics in both the blood-brain barrier and in the gut[22]. MDR1 are expressed in the small and large intestines but they are mostly manifested in the large intestine[23,24].

MRP2 was first identified in the apical membrane domain of hepatocytes[25]. The main function of MRP2 is to facilitate the transport of a variety of organic anions, especially the conjugated formulas, into bile and thence out of the body[26]. This means that MRP2 are capable of restricting whole body load of endogenous and exogenous toxins. MRP2 are also expressed in the kidney, in the epithelial cells of the intestine[27], in the placenta[28], and in the blood-brain barrier[29]. In the rat intestine, MRP2 expression is highest in the duodenum but few are found in the colon[30] MRP2 actively ejects the xenobiotics after hydroxylating them in the form of glucuronide, sulfate or glutathione conjugates.

REGULATION OF EXPRESSION AND ACTIVITY OF MULTIDRUG RESISTANCE PROTEINS MRP2 AND MDR1 BY ESTROGENIC COMPOUNDS IN CACO-2 CELLS

The influence of pharmacological levels of ethynylestradiol (EE) on the expression and activity of MRP2, MDR1

and breast cancer resistance proteins (BCRP) in *in vitro* models of drug transport, such as CaCo2 cells has been examined[31].

Cells treated with either 0.5 or 5 pmol/L EE for 48 h showed an increase in MRP2 (+75% and +88%) and MDR1 (+158% and +162%) protein expression, with respect to control cells. Yet, no effects were observed when cells were treated with all other concentrations tested[29]. In additional experiments performed to determine protein expression of MRP2 and MDR1in total cellular membranes as well as their mRNA levels in cells treated with 5 pmol/L EE, an increase in MRP2 (+56%) and MDR1 (+128%) protein expression, with respect to control cells was noted.

When MRP2 activity was evaluated using DNP-SG as a model substrate treatment with 5 pmol/L EE increased the ratio of protein expression by 39% when compared to control cells[28]. To the contrary, when MDR1 activity was evaluated using Rh123 as a model substrate the intracellular accumulation of Rh123 correlated inversely with MDR1 extrusion activity. Treatment with 5 pmol/L EE decreased substrate accumulation by 19% when compared to control cells[31]. Such decreased accumulation will lead to reduction in the length and concentrations of exposure and accordingly a reduction in the risk of the associated hypomethylation of repetitive DNA elements[32].

Further, when the protective effect of EE against CDNB and PQ cytotoxicity was evaluated through determination of the cell survival IC50 value related to CDNB, IC50 values were higher in cells treated with 5 pmol/L EE (33.3 ± 0.5) than in control cells (25.5 ± 0.5)[31]. Likewise, the IC50 value related to PQ cytotoxicity was higher in cells treated with 5 pmol/L EE (8.8 ± 0.8) *vs* control cells (6.8 ± 0.5 mmol/L)[31].

The same researchers also tried to evaluate whether ER mediates transporter modulation by EE. For achieving that goal, they measured MRP2 and MDR1 protein expression after treatment with 5 pmol/L EE for 48 h, in the presence or absence of ICI 182/780. The findings were that MRP2 and MDR1 protein up-regulation was abolished by the ER antagonist (EE *vs* EE + ICI)[31].

DNA HYPO-METHYLATION IS A SIGNIFICANT INDICATOR OF THE DEVELOPMENT OF CANCERS

The first reported epigenetic changes in human cancer of losses of DNA methylation (methylated 5C component was replaced by non-methylated C component) was published in 1983[33]. In their study, Gama-Sosa *et al*[33] noticed this change in DNA methylation thru the genome in a variety of carcinomas against a broad diversity of ordinary tissues. Then in further published work, Feinberg *et al*[34,35] reported hypomethylation of unrelated gene areas to cancer in colon adenocarcinomas compared with normal controls.

DNA hypo-methylation appears much more extensive in metastases. Many subsequent reports have confirmed the recurring global genomic hypo-methylation in cancers when compared with normal tissues[36-42]. This conclusion has been recently bolstered[43]. In a 2014 report by Kaz *et al*[43] genetic alterations in the methylation of genes known for their participation in the colonic carcinogenic process of the normal colon, where no colonic tumour existed suggest that these genetic alterations precede any changes in the colonic tissues and could be potentially used as predictors for the development of colorectal cancers[43].

Phases of DNA Hypo-methylation status is a significant feature during the early stages of the development of tumours or in other abnormal growths, such as hyperplasia[42,44-46]. This conclusion was confirmed further by the findings of DNA hypo-methylation prior to the identification of aneuploidy in gastrointestinal cancers[47]. Hypo-methylation of DNA, in general, increases with the tumour progression or grade of malignancy[48-51]. Yet, cancers arising from long exposure to chlorinated water are not exceptional[52]. In a study on an animal model, Coffin *et al*[52] examined the influence of the exposure to trihalomethanes in the used water for drinking on the tumour progression and DNA methylation of female B6 C3 F1 mouse liver cell line. The main finding of this study was that the trihalomethanes administered by gavage enhanced the multiplicity of the hepatocytes and decreased the methylation of the *c-myc* gene[52].

ER-α GENE HYPER-METHYLATION IS A POTENTIAL INDICATOR FOR COLORECTAL CARCINOMA

Oestrogen has anti-cancer activity and plays a significant role in suppressing the development of colorectal cancer[53]. This clearly appears from the frequently reported hypermethylation of the *ER-α* gene in malignant tumours of the large intestines[54,55], suggesting that *ER-α* gene hypermethylation could be used as a predictor for the development of large bowel cancers. ER-α is a transcription factor that, upon binding to oestrogen transfers to the nucleus to activate various genes including those involved in the inhibition of cell multiplicity[56]. The insertion of *ER-α* gene into ER-negative colon cancer cells suppressed cell proliferation[57]. Retrieval of an epigenetically inactivated *ER* gene resulted in suppression of large bowel cancer cells development *in vitro* and *in vivo*[58]. Experimental work have shown that *ER-α* gene is also hypermethylated in azoxymethane (AOM)-induced carcinoma of the large intestines in rats, lending support to a pragmatic approach to cancer suppression[59].

THE INFLUENCE OF LONG-EXPOSURE TO TRIHALOMETHANES IN THE USED WATER FOR DRINKING AND THE DEVELOPMENT OF CANCERS

Chloroform, Bromodichloromethane, Chlorodibromome-

thane, and Bromoform are common contaminants in chlorinated water.

Chloroform is considered a facilitator for the development of cancers in humans based on data from animal studies.

Oral contact to chloroform initiated tumours in two kinds of rats and at two different places. Direct gastric administration of chloroform by stomach tube caused hepatocellular carcinoma in mice of both sexes[60] and renal epithelial tumours in male mice and rats[61,62].

Benign hepatic adenomas were observed in female rats drank contaminated water with chloroform[63,64] and in female mice breathed contaminated air with Chloroform[64]. Renal tubular-cell adenomas, carcinomas, or adenocarcinoma were observed in male rats drank contaminated water with chloroform[62,63], in male mice breathed contaminated air with chloroform[65], and in male rats following combined exposure to chloroform *via* breathing and drinking contaminated suppliers[66].

No cause-effect relationship has been established between human cancer and exposure specifically to chloroform. However, an association between exposure to contaminated water and development of specific kinds of cancers has been established by community-based, cohort and case control studies[67,68], but a causal relationship could not be inferred[69-77].

Similarly, bromodichloromethane is also considered a facilitator for the development of cancers in humans based on data from animal studies. Drinking contaminated water with bromodichloromethane caused tumours at several different places in mice and rats. Direct gut administration of bromodichloromethane by a stomach tube caused renal tubular-cell adenomas and adenocarcinomas in male mice and in rats of both sexes, hepatocellular adenomas and carcinomas in female mice, and colonic adenomatous polyps and adenocarcinomas in rats of both sexes[71,77-79].

Drinking contaminated water with bromodichloromethane increased the frequencies of hepatocellular adenomas and carcinomas in males[80] and caused hepatocellular adenomas in females[81].

The data available from epidemiological studies are not conclusive to confirm on a possible relationship between the development of cancers in humans and the exposure specifically to bromodichloromethane. Several epidemiological studies indicated a possible association between drinking chlorinated water and increased risk of cancer, but these studies could not provide information on whether any observed effects were specifically related to bromodichloromethane[78].

When the risk to develop cancers due to long-term exposure to trihalomethanes *via* drinking, breathing and dermal contact from supply water of five water suppliers were analysed chloroform was the major component that caused cancer risk through both oral and dermal routes whereas bromodichloromethane was the major component through inhalation[82]. The main risk factors that enhance the development of cancers are the existence of Chloroform in the contaminated water, body weight and then the long-term exposure to chlorinated water[82,83].

Evidence exists to prove that low concentrations of calcium (Ca) and/or magnesium (Mg) in the used water for drinking increase the carcinogenic effect of TTHM and thence the development of cancers of oesophagus[83], kidney[84], rectum[85] and pancreas[86].

On the other hand, it was found that the presence of Fe^{3+} increases the carcinogenic activity of THMs in humans[87]. It was estimated that the risk to develop cancer from long-term skin exposure to trihalomethanes while swimming is as high as 94%[88].

THE EFFECT OF OESTROGEN ADMINISTRATION ON THE DNA METHYLATION

In a randomized double-blind, placebo-controlled, cross-over study consisting of two different stages, placebo and conjugated horsey oestrogen, Friso *et al*[89] investigated the effect of administration of oestrogen in thirteen volunteer postmenopausal women on the genomic and promoter DNA methylation in peripheral mononuclear cells and on the plasma concentrations of homocysteine, folate, vitamins B6 and B12. In this study, oestrogen was prescribed as oral pills containing 0.625 mg CEE while placebo consisted of twin pills but lacking the active constituent. Each course lasted 8 wk and these two courses were separated by a 4-wk period[90]. At week 8 of each stage, blood samples were taken for measuring plasma homocysteine, plasma pyridoxal-50-phosphate, serum folate and vitamin B12 levels. DNA was extracted from peripheral blood mononuclear cells in order to estimate genomic and promoter DNA methylation status.

The findings of this study were that: (1) plasma homocysteine levels were markedly decreased during the CEE phase compared with the placebo; (2) mean homocysteine levels during the placebo phase were 9.29 mmol/L (it was within the normal reported range by Stabler *et al*[91] in 2004; (3) the oestrogen treatment reduced the mean concentration of homocysteine to 8.08 mmol/L; (4) the extent of genomic DNA methylation in peripheral mononuclear cells was noticeably increased after the oestrogen treatment as opposed to the placebo; (5) there was no significant difference in the promoter DNA methylation of the *ERa*, *ERb* and *p16* genes between the oestrogen and placebo; and (6) there were no significant differences in serum folic acid, vitamin B12 and plasma vitamin B6 levels between the two treatment arms. These findings indicate that oestrogen administration could increase the methylation of the genomic DNA. Together with the well-documented data proving that a decreased level of genomic DNA methylation is a common feature of tumorigenesis, that it appears early prior to the DNA mutation that takes place later in the evolution of neoplasm[45] this means that oestrogen administration has a prophylactic function against the development of cancers by enhancing genomic DNA methylation.

Table 1 Effect of heating and boiling water on trihalomethane content

Compound	Level (μg/L)				
	Original tap water	80 ℃ 1 min	100 ℃ 0 min	Boiling 1 min	Boiling 5 min
Chloroform	45.6	23.2	12.3	9.4	4.1
Bromodichloromethane	44.6	24.1	13.5	10.8	4.6
Chlorodibromomethane	42.3	24.1	14.4	12.3	5.5
Bromoform	35.9	21.3	13.9	13.5	6.8

Available from: URL: http://monographs.iarc.fr/ENG/Monographs/vol52/mono52-6.pdf.

DISCUSSION AND CONCLUSION

Chlorine is commonly used as a chemical disinfectant in water supplies, in the prevention of algal, bacterial and general slime growths in treatment plants and pipe works, in the control of tastes and odours, and in the removal of iron, manganese and colouring additives[92].

Trihalomethanes are derivatives of the outcome of the reaction between chlorine/chloride, with contaminants in water supplies, such as organic compounds, bromide and iron.

The associated health threats including colorectal cancers are dependent on the frequency of exposure to and the levels of trihalomethanes in the used water for drinking. These threats could be reduced by restricting the use and contamination by trihalomethanes of public drinking water[83,93], or by boiling the water[94] (Table 1), or by adjusting the concentrations of calcium, magnesium and iron[83-87].

Genomic DNA hypo-methylation could be used as a reliable biomarker for identifying susceptible cases and oestrogen replacement therapy could be used for reversing detected hypo-methylation and consequently reducing the risk of the carciogenesis[86,89,90].

However, in cancers of the colon and rectum, like other ER-linked cancers, the ablation of the sex hormones would be necessary, once the disease occurs, for delaying the progress of the disease. It is well-documented that once the disease manifests the role of oestrogen would be altered in that it will enhance global DNA hypo-methylation[95] and thereby restricting of its availability would be beneficial.

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