RESEARCH COMMUNICATION

Modulation of Arsenic Induced Cytotoxicity by Tea

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Abstract

Arsenic, a naturally ocurring chemical element, is considered hazardous to human health. Inorganic arsenic compounds were found to induce cytotoxicity in Chinese hamster V-79 cells in culture. The arsenite form was more toxic than arsenate. Extracts of green and two varieties of black tea, as well as their principal polyphenols, (-)-epigallocatechingallate and theaflavin, efficiently counteracted the cytotoxic effects of arsenic compounds. On the basis of the amount of tea extract that afforded 50% protection to the cells from arsenic induced cytotoxicity, black tea was found to be as effective as green tea. The protective effect was attributable to the contents of not only (-)-epigallocatechingallate but also of theaflavin, the latter being a predominant polyphenol present in black tea.

Key Words: Arsenic - cytotoxicity - modulation - green tea - black tea - (-)-epigallocatechingallate - theaflavin

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Introduction

Arsenic, a naturally occurring metalloid, is present in the environment as a potentially hazardous substance and normally gets into the food chain through ground water. Epidemiological evidences have demonstrated that arsenic is a potential human carcinogen (IARC, 1980; WHO,1981; Chen et al., 1988; Chen et al., 1992). In the environment arsenic occurs mainly as compounds of arsenite (As^{3+}) and arsenate (As^{5+}). In mammals, including humans, inorganic arsenicals undergo methylation leading to the formation of compounds such as dimethyl arsinic acid (DMA) (Sakurai et al, 2002). Arsenic compounds are cytotoxic and these have been shown to induce gene amplification (Lee et al, 1988), chromosome aberrations (Datta et al, 1986; Jha et al, 1992; Gurr et al, 1993), aneuploidy (Vega et al, 1995; Moore et al, 1996; Moore et al, 1997), mitotic arrest of cells(Endo et al, 1992, Kashiwada et al, 1998), inhibit DNA repair (Okui and Fujiwara, 1986) and act as promoters and co-mutagens for a variety of agents (Rossman, 1981; Lee et al, 1985; Okui and Fujiwara, 1986; Jha et al, 1992). Although arsenic is a human carcinogen, the underlying carcinogenic mechanism is not known (Kessel et al, 2002).

The major source of arsenic contamination in humans is through ingestion of food and water and to some extent due to inhalation. In West Bengal as well as in neighboring Bangladesh arsenic contamination in drinking water is regarded as the biggest natural calamity in the world (Das et al, 1995; Mandal et al, 1996, Khan et al, 2003). It is considered important to find some way out to prevent the toxicity of arsenic. The aim of this study is to evaluate the potential of natural factors in counteracting the cytotoxicity of arsenic compounds. Phenolic compounds with cancer chemopreventive property are preferred natural factors in this regard. Tea, the most popular beverage in the world, is a rich source of phenolic compounds. Both green and black tea contain different forms of catechins and their oxidized products (Jain, 1999). The present study shows the efficacy of tea - both green and black - in modulating the cytotoxic effect of arsenic in a mammalian cell culture system.

Materials and Methods

Chemicals

MEM and fetal bovine serum (FBS) were purchased from GIBCO-BRL. Gentamycin, penicillin, streptomycin, MTT[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide], doxorubicin (adriamycin), dimethylsulfoxide (DMSO), (-)-epicatechingallate (ECG), (-)-epigallocatechin (EGC), (-)-epigallocatechingallate

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(EGCG) and theaflavin (TF) were purchased from Sigma-Aldrich. Other reagents were of analytical grade and procured locally.

Cell Cultures

Chinese hamster male lung fibroblast cells (CH V-79) routinely maintained in our department were used in these studies. Cells were cultured in MEM supplemented with 10% heat inactivated FBS and gentamycin (40 µg/ml), penicillin (100 units/ml) and streptomycin (10 µg/ml) and were grown at 37°C in a humidified atmosphere of 5% CO_2/95% air.

Analysis of Tea Polyphenols by HPLC

Tea polyphenols, mainly catechins and theaflavin from popular brands of green tea and black tea (Darjeeling tea , Assam tea) purchased from local market were estimated quantitatively by HPLC essentially according to the procedure of Khokhar et al. (1997). Tea extract was prepared by brewing 2.5g of dry matter with 100 ml of freshly boiled water (100° C) for 5 min. The HPLC system consisted of a Rheodyne injector, a 515 lb dual pump with a pump control module (Waters, USA), a Novapak RP C-18 column (150 x 3.9 mm, 5 µm) attached with a guard column set at 30° C and a 996 PDA detector (Waters, USA) set at 278 nm. The separation of major catechins was carried out by using a gradient of 5-25% acetonitrile in 0.025 M KH_2PO_4, pH 2.4. TF was separated in isocratic mode where mobile phase used was acetic acid:acetone:water (1:60:39) and detected at 365 nm. Standard mixture of catechins and TF was integrated and calibrated using a Millenium 32 upgraded software system. Unknown samples from tea were quantitated against the standard.

Cytotoxicity

Cytotoxicity was assessed by the MTT assay (Roy et al., 2002). Exponentially growing cells ($1x10^4$) were plated in 96-well plates and after 24 h of growth treated with a series of concentrations of each arsenic derivative, alone or with various concentrations of tea polyphenols/extracts. Doxorubicin was used as the positive control. Incubation was carried out at 37°C for 24 h. MTT solution was added to each well (1.2 mg/ml) and incubated for 4h. The reaction results in the reduction of MTT by the mitochondrial dehydrogenases of viable cells to a purple formazan product. The MTT-formazan product dissolved in DMSO was estimated by measuring absorbance at 570 nm in an ELISA plate reader.

Results

Treatment of exponentially growing cells with sodium arsenite, sodium arsenate and dimethyl arsinic acid (DMA) for 24 h shows that at the dose range tested sodium arsenite and sodium arsenate were cytotoxic to the CH V-79 cells. Dose response of these cells to arsenic compounds as assessed by MTT assay is depicted in Fig 1. It is to be noted that DMA failed to induce any cytotoxicity in these cells.

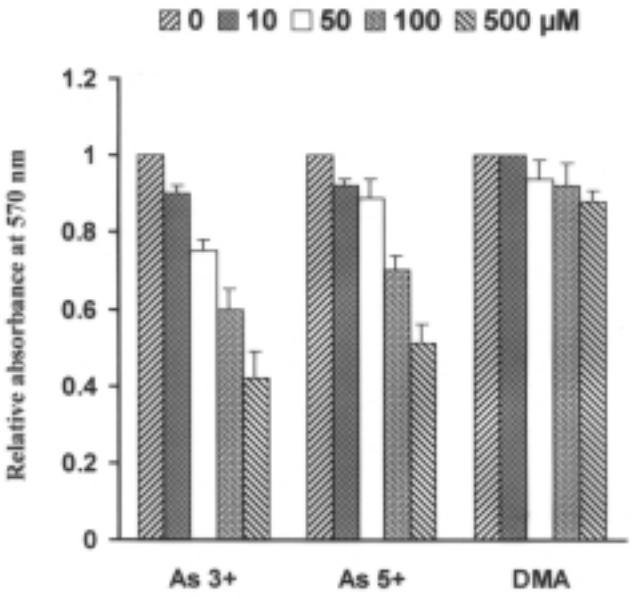

Figure 1. Arsenic Induced Cytotoxicity in Chinese Hamster V-79 Cells. V-79 Cells were Treated with Sodium Arsenite, Sodium Arsenate and Dimethyl Arsinic Acid at Different Concentrations for 24 h. The Cytotoxicity was Assessed by MTT Assay as Described in the Text.

Modulation of arsenic induced cytotoxicity was examined in detail using tea polyphenols EGCG and TF, as well as tea extracts : green tea (GT), black tea – both Darjeeling (DT) and Assam (AT). Arsenic induced cytotoxicity was significantly counteracted by pure tea polyphenols EGCG and TF. Extracts of green (GT) as well as black (DT, AT) tea were also found to be efficient in protecting cells from cytotoxicity induced by arsenic compounds. These results are shown in Fig. 2. Based on the results of MTT assay effective concentration of each tea compound required to afford 50% protection (EC_{50} values) was calculated and the results are given in Table 1. Value for GT was found to be comparable with that observed for either DT or AT, particularly in combating cytotoxicity of potent arsenite. As DMA was weakly cytotoxic it was not included in these studies.

Polyphenol contents of tea extracts were analyzed by HPLC, and the values are shown in Table 2. As expected,

Table 1. Concentrations of Tea Compounds to bring about 50% Protection (EC_{50}) in Arsenic induced Cytotoxicity

Arsenic compound (500 µM)	EC_{50} value by				
	EGCG (µM)	GT (µg/ml)	AT (µg/ml)	DT (µg/ml)	TF (µM)
As^{3+}	1.06	50	50	65	10
As^{5+}	0.32	30	65	65	0.55

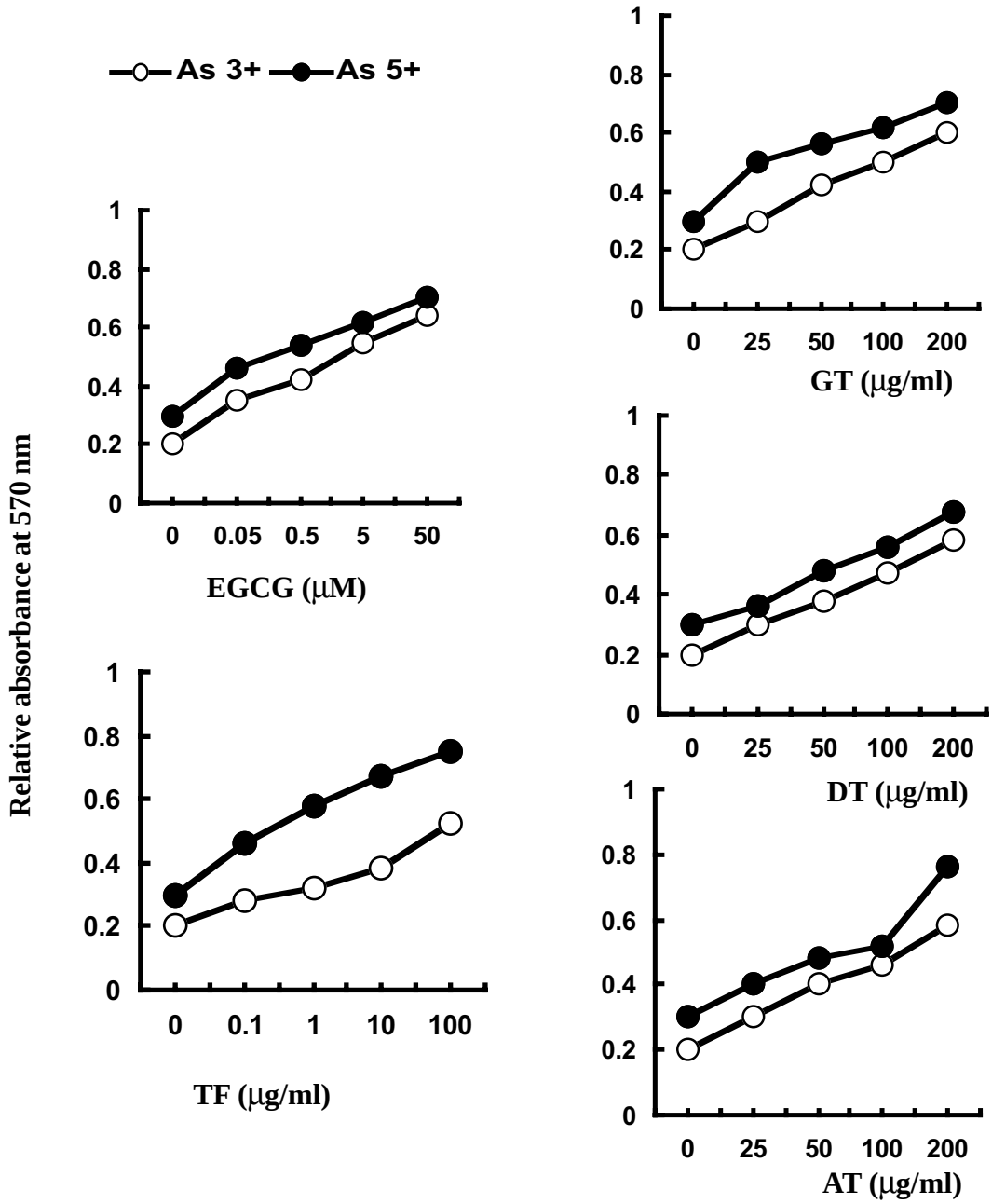

Figure 2. Reversal of Arsenic induced Cytotoxicity by Tea Polyphenols and Tea Extracts. V-79 Cells were Simultaneously Exposed to Tea Compounds and Arsenic at different Concentrations for a Period of 24 h. The Cytotoxicity was Assessed by MTT Assay as Described in the Text.

Table 2. Polyphenol (catechin) Contents of Tea Extracts (2.5%)

Polyphenol (catechin)	Polyphenol content (mg/ml) in		
	GT	DT	AT
EGC	0.91	0.29	0.15
EGCG	2.46	1.37	0.39
ECG	0.37	0.45	0.22
TF	0.20	0.50	0.96

concentration of catechin derivatives was more in green tea than in black tea. On the other hand, theaflavin is more abundantly present in black tea. Table 3 shows contents of EGCG and TF present in the effective concentration of green and black tea that bring about 50% protection against arsenic induced cytotoxicity (EC_{50}). The combined amount of EGCG and TF to afford 50% protection against arsenite amounts to 5.32 μg for GT, 4.59 μg for DT and 2.71 μg for AT. These values against arsenate are 3.19 μg for GT, 4.86 for DT and 3.51 for AT. AT thus is more efficient than DT or GT in counteracting the cytotoxic effect of arsenic compounds.

Table 3. Contents of EGCG and TF in EC_{50} Level of Tea Extracts

Arsenic compounds (500 μM)	Polyphenol	GT	DT	AT
		(μg)		
As^{3+}	EGCG	4.92	3.56	0.78
	TF	0.4	1.30	1.93
As^{5+}	EGCG	2.95	3.56	1.01
	TF	0.24	1.30	2.50

Discussion

Inorganic arsenic compounds are regarded as environmental toxicants and carcinogens for humans (IARC, 1980; WHO,1981; Chen et al., 1988; Chen et al., 1992). In mammals as well as in human species inorganic forms undergo methylation leading to the formation of DMA which is a potent human carcinogen (Sakurai et al, 2002). DMA, though an active carcinogen, has been reported to be nearly 3 orders of magnitude less cytotoxic (Sakurai et al, 2002). Both arsenate and arsenite are genotoxic, capable of inducing chromosome aberrations and sister chromatid exchange in rodent as well as in human cells (Datta et al, 1986; Jha et al, 1992; Gurr et al, 1993; Vega et al, 1995; Moore et al, 1996; Moore et al, 1997), and in this regard arsenite is more potent than arsenate (Wan et al, 1982; Jacobson-Kram and Montailbano, 1985; Kochhar et al, 1996; Moore et al,1997). Arsenite is also generally considered more acutely toxic than arsenate (Naqvi et al, 1994). In the present study the cytotoxic effect of arsenic compounds has been demonstrated in Chinese hamster V-79 cells. It is seen that inorganic forms of arsenic namely, sodium arsenite and sodium arsenate, show cytotoxicity as evident from MTT assay, while the organic form, which is the carcinogenic form, failed to impart appreciable toxicity in this cell line. It is also observed that the cytotoxicity due to sodium arsenite is more than that due to sodium arsenate. The cytotoxicity might be due to cytogenetic aberrations or molecular derangement that led to necrosis or apoptosis. Easy and cheap means to counteract the cytotoxic effect of this environmentally hazardous compound will indeed be beneficial for the society. Tea is a popular beverage, next only to water. Tannins present in tea comprise a variety of polyphenolic compounds which are endowed with interesting pharmacological properties. Green tea, which is non-fermented, primarily contains polyphenolic catechins such as (-)-epicatechin (EC), (-)-epigallocatechin (EGC) and their gallates (ECG and EGCG). These become oxidized and polymerized during fermentation process that gives black tea with a rich colour and aroma. The forms present in black tea are theaflavin and its derivatives (Jain, 1999). Tea extract and its polyphenols are regarded as promising cancer chemopreventive agents (Ahmad et al.,1998; Siddiqi and Das, 1999) as a result of specific cellular and molecular responses (Roy et al., 2001), and because of their antioxidant function (Rice-Evans, 1999) and antimutagenic and antigenotoxiic properties (Kuroda, 1996; Kuroda and Hara, 1999). The catechin EGCG is the most active among the polyphenols. It has been well documented that phenolic compounds from different natural sources having similar properties elicit considerable chemopreventive activity against experimental carcinogenesis induced by a variety of chemicals (Huang and Ferraro, 1992; Ferguson, 1994; Starvic, 1994; Surh, 1998).

EGCG, the most abundant polyphenol in green tea, has been found in the present study to be very efficient in preventing the cytotoxicity of inorganic arsenic compounds. TF, the black tea polyphenol, is also effective in blocking the cytotoxic effect of arsenic compounds. The highlight of the present study is such that black tea has been found to be equally effective as green tea in counteracting the toxic effect of inorganic arsenic compounds. Among the two varieties of black tea DT contains more EGCG than AT. However, AT is more efficient in preventing toxicity of arsenite, the most toxic arsenical. This is attributed to the presence of significant TF content in AT. This is clearly evident from the individual catechin contents present in the effective concentration (EC_{50}) of each tea extract to combat arsenic cytotoxicity. Other studies have also shown that TF present in black tea possesses the antioxidant potency which is comparable with the antioxidant activity of green tea (Leung et al., 2002). Black tea also has been found to be more effective than green tea and its individual catechin constituents in proportionate amounts in abrogating production of NO and O^{2-} in activated murine macrophages (Sarkar and Bhaduri, 2001). The results obtained in this study have relevance in emphasizing the beneficial value of tea consumption in preventing the adverse effects of arsenic exposure in man.

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