Pakistan Journal of Biological Sciences 12 (7): 554-564, 2009
ISSN 1028-8880

Effect of DNA Hypomethylation on Genotoxicity and Apoptogenicity of Sodium Arsenite in Laboratory Mice

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Abstract: The present study aims to evaluate the effect of DNA hypomethylation state on genotoxicity and apoptogenicity induced by sodium arsenite ($NaAsO_2$) in normal adult male SWR/J mouse bone marrow cells. Animals were treated with intraperitoneally (i.p.) injected with (2.25, 4.50 or 9 mg kg^{-1} b.wt. of $NaAsO_2$ which represent 0.25, 0.50 or 1 of LD_5, respectively) and killed 24 h later. Another different group of male mice was treated with three doses of 5-Azacitidine (5-AzaC), 5 mg kg^{-1} b.wt. each dose and 3 h intervals between them. $NaAsO_2$ administered after 6 days of the last dose. The three single doses of sodium arsenite alone significantly ($p<0.05$) increased the rate of total structural Chromosomal Aberrations (CAs), rate of Sister Chromatid Exchanges (SCEs), micronucleus (MNs) formation, PARP and Lamia-A degradation and apoptosis as compared with the negative control. The combined treatment with hypomethylation agent 5-AzaC significantly increased the rate of SCEs induced by $NaAsO_2$ at low dose. Moreover, this treatment significantly increased the rate of polyploidy at all combined used doses. Furthermore, this treatment induced apoptosis at all used doses. The present study has shown that DNA hypomethylation had a negative effects represented in rate of (CAs), polyploidy, PARP degradation and apoptosis induced by ($NaAsO_2$). On the other hand, DNA hypomethylation had positive effects represented in decreas rate of pulverized chromosomes, centromeric attenuations, (SCEs), (MNs) formation, prevent Lamina-A degradation and apoptosis.

Key words: Methylation, genotoxicity, sodium arsenite, CAs, SCEs, Mns PARP, Lamina-A, mice

INTRODUCTION

Arsenic compound considered one of the most toxic compound in the nature. The risk of arsenic compounds increased after exposure to deferent sources and of course that was because of increasing human activities such mining, melting and pesticides production, so for long period arsenic has led to gradually accumulated in the soil (Rahman *et al*., 2001). Arsenic is released into the atmosphere from both natural and anthropogenic sources (Reichard *et al*., 2007) to contaminate air, water, food and soil, with different degrees of arsenic toxicity (Toribio and Romanya, 2005; Chowdhury *et al*., 2008) and has become a threat to all living organism including the human race (Manna *et al*., 2007). In spite of various risk several organic arsenic are still use against some cancer diseases (Chowdhury *et al*., 2008; Florea and Büsselberg, 2008). The biological effects of one metal can be modified considerably by interaction with other metals (Biswas *et al*., 1999). Studies showed that trivalent arsenic (As^{3+}) was found to be greater toxic than pentavalent arsenic (As^{5+}) (Chowdhury *et al*., 2008). We used in this study sodium arsenite which is classified by the International Agency for Research on Cancer (IARC) as a human carcinogen and its mechanism have been subject of extensive research but could still not be elucidated (Brink *et al*., 2006; Florea and Büsselberg, 2008).

Methylation of DNA plays an important role in organizing the genome and also DNA methylation levels somewhat changed following metal treatment (Lee *et al*., 1998; Reichard *et al*., 2007; Klein *et al*., 2007). Despite the large number of studies on arsenic toxicity but the data about its effects is not fully known (Dopp *et al*., 2004; Florea and Büsselberg, 2008). But several assays performed *in vivo* and *in vitro* on mammalian cells have shown that exposure to arsenical induces chromosomal aberrations and formation of micronuclei (Biswas *et al*., 1999; Bhattacharya *et al*., 2005; Klein *et al*., 2007).

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The present investigation was undertaken in an effort to determine the effects of hypomethylation state on the genotoxicity effects and apoptosis of sodium arsenite.

MATERIALS AND METHODS

All of the experimental procedures were conducted in the Genetic Lab. and Molecular Biology Lab. of the King Saud University between 2006 and 2008.

Experimental animals: Normal SWR/J laboratory male mice, 8-10 weeks old and weighing 23-36 g were used throughout the study. Animals were maintained and bred under standard laboratory at a temperature of 22±1°C, a relative humidity of 45±5% and photoperiod cycle of 10/14 h. Mouse food (commercially available in Saudi Arabia) and water were offered *ad libitum*.

Treatments: A total of 40 males were used and divided into 8 groups each group contained 5 males. Group 1 was treated with intraperitoneal (i.p.) injection of (0.2 mL/10 g b.wt.) of sterile normal saline as a negative control. Groups 2, 3 and 4 were treated with i.p. injection of $NaAsO_2$ in single various dose levels 2.25 or 4.50 or 9 mg kg^{-1} b.wt. (0.25, 0.50, 1 of LD_5, respectively). Groups 5, 6 and 7 were treated with the same doses in groups 2, 3 and 4 plus three doses of 5 mg kg^{-1}. Group 8 treated with only three doses of 5-AzaC with 5 mg kg^{-1} b.wt. each and 3 h intervals between them. Sodium arsenite was then administered after 6 days of the last dose (Plumb *et al*., 2000).

Test chemicals: Sodium arsenite was obtained from Hannover, Germany. 5-Azacytidine (5-AzaC), 50 mg of 5-Bromo-2-deoxyuridine (BrdU) tablet, Hoechest and Acridine Orange (AO) were obtained from (Sigma, UAS). BrdU was transplanted subcutaneously (Allen *et al*., 1978). The method of Preston *et al*. (1987) was used for chromosome preparations. And the method of Latt *et al*. (1981) were used for staining.

Scoring: The slides were used to simultaneously detected Chromosomal Aberrations (CAs) and Sister Chromatid Exchanges (SCEs) in the same time.

Chromosomal Aberrations (CAs): One hundred well-spread and clear metaphase from each slide (giving 100×5 = 500 $group^{-1}$) were examined for the monitoring of (CAs). Each selected metaphase was examined using the light microscope (Nikon, Eclipse E600W, Japan) by 10x and then 100x oil lenses (Preston *et al*., 1987; Scappaticci *et al*., 2000).

Sister Chromatid Exchanges (SCEs): Fifty well-spread and clear metaphase from each slide (giving 50×5 = 250 $group^{-1}$) were examined to detect (SCEs) (Allen *et al*., 1978).

Micronucleus test

Slide preparation: Femoral bone marrow cells flushed out from femur by syringe with Foetal Calf Serum (FCS) and smeared on clean glass slides. Thereafter, these cells are fixed with absolute methyl alcohol for 15 min.

Staining: Slides were stained by immersion in phosphate buffer solution and followed by treatment with Acridine Orange (AO) for 1 min. Slides were then treated with phosphate buffer solution for 10 min followed by an additional treatment with fresh phosphate buffer solution for 15 min. Slides were embedded with DPX, covered and then immediately examined using an FL EPI-Fluorescence microscope (Nikon, Eclipse E600W, Japan) at 530 wavelength.

Scoring: One thousand polychromatic erythrocytes (PCEs)-oil reddish-from each slide, (giving 1000×5 = 5000 $group^{-1}$) were examined in this study to evaluate the number of micronucleated polychromatic erythrocytes (MNPCEs) and micronucleated normochromatic erythrocytes (MNNCEs) in normochromatic erythrocytes (NCEs)-bright reddish. The ratio of MNPCEs to MNNCEs was used as an indicator of chromosomal changes, while %PCEs was used as an indicator of apoptogenicity (Garcia *et al*., 2001).

Primary antibodies (Anti-PARP and Anti-Lamina-A): Both primary antibodies were obtained from Cell Signaling, USA. Primary anti-PARP was used to detect the intact PARP (116 kDa) enzyme, as well as the large (89 kDa) and small (24 kDa) fragments produced following hydrolysis of intact PARP with caspase-3. Primary anti-Lamina-A was used to detect intact Lamina-A (70 kDa) protein, as well as the small (28 kDa), but not the large (45 kDa), fragment following hydrolysis of intact Lamina-A with caspase-6. Both polyclonal antibodies were produced by immunizing rabbits and diluted with skimmed milk (1:1000).

Secondary antibodies (Anti-rabbit IgG) HRP-linked antibodies: Secondary antibodies were obtained from cell signaling, USA. Antibodies were labeled with peroxidase and assayed using enhanced chemiluminescence (ECL) Western Blotting Detection reagents obtained from Amersham, RPN2106PC, USA.

Protein extraction: Protein extraction from mice liver was as follows:

Ten grams of mice liver was homogenized in a cold homogenizer tube containing 2 mL of homogenization buffer. The concentration of total protein in each sample was estimated spectrophotometrically (GeneQuant pro, Amersham, USA) at 595 nm. Equal volumes of 2X sample buffer and protein (30 μg μL^{-1}) were mixed in an Eppendorf tube and heated to 95°C for 5 min before loading (Hossain *et al*., 2000; Mathas *et al*., 2003).

SDS-PAGE and immunoblotting: The mix of protein and 2X sample buffer was electrophoresed through a 30% polyacrylamide gel using a PowerPac Basic system (S.N 37S/7159, Italy) at 50 V for 1 h and then at 100 V near the end of the electrophoresis. Protein was then transferred onto nitrocellulose membrane. The nitrocellulose membrane was washed several times with Phosphate Buffered Saline (PBS), incubated in 5% skimmed milk, followed by primary antibodies (Anti- PARP or Anti-Lamina-A) overnight at 4°C and then with secondary antibodies for 3 h. Protein bands were visualized using ECL according to the manufacturer's instructions. The molecular size of the visualized protein bands was determined by comparison with markers.

Statistical analysis: The data obtained in this study were statistically analyzed with SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA) using the Mann-Whitney U-test.

RESULTS

Genotoxicity

Chromosomal Aberrations (CAs): A number of structural and numerical chromosomal aberrations were scored in bone marrow cells of treated mice, in additional to some of aberration refer to chromosomal instability (Table 1).

The screened types of structural aberrations were included chromatid breakage contained (breaks, deletions, fragments, end to end association, centric fusion and ring chromosome). Whereas, the numerical changes were only polyploidy cells. On the other hand, pulverized chromosome and centromeric attenuation were scored as indicator to chromosomal instability.

A single treatment with each of the three administrated doses (2.25, 4.5, 9 mg kg^{-1}) of sodium arsenite induced a significant ($p<0.05$) increase in the total structural chromosomal aberrations and centromeric attenuation compared with the negative control (Table 1). A single treatment with the medium dose (4.50 mg kg^{-1}) of sodium arsenite induced a significant ($p<0.05$) increase

Table 1: Frequency of chromosomal aberrations induced in bone marrow cells of mice treated with sodium arsenite ($AsNaO_2$) alone and in combination with 5-Azacytidine (5-AzaC)

				No. of structural chromosomal aberrations									
				Mean animal±SD					Percentage				
Groups	Treatment (dose mg kg^{-1})	No. of examine mice	No. of dexamined cells	Chromatid breakage	End to end association	Centric fusion	Ring chromosome	Total	Total No. of cells with structural aberrations	Total No. of cells with one aberration	No. of cells with more with one aberration	No. of chromosome pulverization (Mean ±SD)	No. of centro-mercially attenuated cells (Mean±SD)	Numerical aberrations No. of polyploid cells (Mean±SD)
1	-ve control	5	500	1 (0.20±0.45)	0.00	0.00	0.00	1 (0.20±0.45)	1 (0.20)	1 (0.20)	0.00	2 (0.40±0.55)	13 (2.60±1.34)	0
2	$AsNaO_2$ (2.25)	5	500	7 (1.40±1.34)	7a (1.40±1.14)	1 (0.20±0.45)	2 (0.40±0.55)	17a (3.40±1.83)	16a 3.20	15a 3.00	1 0.20	6 (1.20±0.45)	30a (6.00±1.73)	2 (0.40±0.89)
3	$AsNaO_2$ (4.5)	5	500	9a (1.80±1.48)	4 (0.80±0.84)	1 (0.20±0.45)	5 (1.00±0.70)	19a (3.80±2.28)	16a 3.20	13a 2.60	3 0.60	10a (2.00±1.00)	42a (8.40±1.95)	3 (0.60±0.55)
4	$AsNaO_2$ (9)	5	500	9a .80±0.83)	4a (0.80±0.45)	4 (0.80±0.84)	1 (0.20±0.45)	18a (3.60±1.52)	16a 3.20	14a 2.80	2 0.40	6 (1.20±0.84)	46a (9.20±2.39)	2 (0.40±0.55)
5	$AsNaO_2$ +5-AzaC (2.25+3×5)	5	500	0a (2.00±1.58)	7 1.40±1.34)	6a (1.20±0.84)	5 (1.00±1.22)	23a (5.60±2.70)	24a 4.80	20a 4.00	4 0.80	4 (0.80±0.84)	33a,f (6.60 0.89)	17a,b (3.40 2.30)
6	$AsNaO_2$ +5-AzaC (4.5+3×5)	5	500	8 (1.60±1.52)	6 (1.20±1.30)	9a,c (1.80±0.84)	3 (0.60±1.34)	26a (5.20±4.60)	21a 4.20	16a 3.20	5 1.00	5 (1.00±0.70)	24c,f (4.80±1.79)	15a,c (3.00±1.00)
7	$AsNaO_2$ +5 -AzaC (9+3×5)	5	500	16a (3.20±1.92)	4 0.80±0.45)	10 (2.00±1.58)	2 (0.40±0.58)	32a (6.40±3.13)	24a 4.80	16a 3.20	8a,b 1.60	7 (1.40± 89)	28a,d,f (5.60±1.82)	53a,d,f (10.60±4.56)
8	5-AzaC (3×5)	5	500	10a (2.00±1.87)	2 (0.40±0.55)	1 (0.2±0.45)	0.00	13a (2.60±1.82)	13a 2.60	13a 2.60	0.00	5 (1.00±0.70)	74a (14.80±3.35)	9 (1.80±2.49)

a: Significant difference from group 1 at $p<0.0$, b: Significant difference from group 2 at $p<0.05$, c: Significant difference from group 3 at $p<0.05$, d: Significant difference from group 4 at $p<0.05$, e: Significant difference from group 8 at $p<0.05$

the number of cells with pulverized chromosomes compared with the negative control.

The combined treatment with hypomethylation reagent (5-AzaC) induced a significant ($p<0.05$) decrease in the centromeric attenuation induced by the medium and high doses of sodium arsenite compared with single doses. Also Table 1 shows (5-AzaC) induced a significant ($p<0.05$) increase in the number of polyploidy cells at all used doses compared with single doses. However, the treatment with (5-AzaC) alone wasn't induced a significant increase on this phenomenon.

Sister Chromatid Exchanges (SCEs): Table 2 shows SCEs following single treatment with three doses of sodium arsenite alone or combined with (5-AzaC). Data show that the rate of SCEs induced a significant ($p<0.05$) increase in all treatment groups with single sodium arsenite compared with the negative control. Also, the data show that the treatment with (5-AzaC) induced a significant ($p<0.05$) decrease in the rate of sister chromatid exchanges at the low dose only compared with single dose and negative control.

Micronucleus: Table 3 shows that single treatment with medium and high doses of sodium arsenite induced a significant ($p<0.05$) increase in the number of (MNPCEs) compared with the negative control. And it's clear that the combined treatment with (5-AzaC) at three doses of sodium arsenite led to high decreased in the rates of (MNPCEs) compared with single doses, but no-significant deferent compared with the negative control.

Apoptogenicity

Poly (ADP-ribose) polymerase: As shown in Fig. 1, the single treatment with three doses of sodium arsenite induced apoptosis and yielded positive results (B, C and D) in terms of the degradation of intact PARP molecules (116 kDa) to generate the large (89 kDa)

Table 2: Sister chromatid exchange frequency in bone marrow cells of mice treated with sodium arsenite ($NaAsO_2$) alone and in combination with 5-Azacytidine (5-AzaC)

				Sister chromatid exchange (SCEs)					
				Mean/group ± SD					
Groups	Treatments (Dose mg kg^{-1})	No. of examined mice	No. of examined cells	I	II	III	IV	V	Mean/animal±SD
1	-ve control	5	250	5.96±2.70	5.14±2.40	4.72±1.52	4.60±1.80	4.86±1.64	5.05±0.54
2	$AsNaO_2$ (2.25)	5	250	8.14±3.42	8.34±2.92	8.60±4.22	8.50±4.34	9.36±3.00	8.58±0.46a
3	$AsNaO_2$ (4.5)	5	250	5.16±2.00	6.12±2.32	10.86±3.80	9.90±3.70	11.60±4.76	8.73±2.90a
4	$AsNaO_2$ (9)	5	250	12.48±3.88	11.00±4.07	8.40±3.25	10.84±3.55	9.90±3.42	10.53±1.50a
5	$AsNaO_2$ + 5-AzaC (2.25+3×5)	5	250	6.24±3.48	4.80±2.72	5.50±2.20	6.82±2.91	6.06±3.18	5.88±0.77b
6	$AsNaO_2$ + 5-AzaC (4.5+3×5)	5	250	6.98±2.73	7.24±3.39	7.58±3.19	7.74±3.38	4.84±2.62	6.88±1.17
7	$AsNaO_2$ + 5-AzaC (9+3×5)	5	250	10.10±4.68	9.70±3.94	10.00±4.47	11.56±4.15	10.64±3.78	10.40±0.73a,f
8	5-AzaC (3×5)	5	250	6.96±3.10	7.76±3.30	4.72±2.00	4.90±1.68	5.40±2.47	5.94±1.34

a: Significant difference from group 1 at $p<0.05$; b: Significant difference from group 2 at $p<0.05$; f: Significant difference from group 8 at $p<0.05$

Table 3: Effect of sodium arsenite ($NaAsO_2$) alone and in combination with 5-Azacytidine (5-AzaC) on micronucleus induction in bone marrow cells of SWR/J mice

						Micronucleated cells per 1000	
Groups	Test substance	Dose (mg kg^{-1})	Sampling time (h)	No. of examined PCEs	PCEs (%)	NCEs	PCEs
1	-ve control	0	24	5000	67.87	0.60	0.24
2	$AsNaO_2$ (2.25)	2.25	24	5000	53.85	2.60	0.47
3	$AsNaO_2$ (4.5)	4.5	24	5000	47.40a	4.60a	1.26
4	$AsNaO_2$ (9)	9	24	5000	44.12a	8.00a	3.00a
5	$AsNaO_2$ +5-AzaC (2.25+3×5)	2.25+3×5	24	5000	60.37	1.20	0.30
6	$AsNaO_2$ +5-AzaC (4.5+3×5)	4.5+3×5	24	5000	53.06a	2.20	0.45
7	$AsNaO_2$ +5-AzaC (9+3×5)	9+3×5	24	5000	47.18a,d	3.40	1.25
8	5-AzaC (3×5)	3×5	168	5000	53.60	1.20	0.92

PCEs: Polychromatic erythrocytes; NCEs: Normochromatic erythrocytes; BHT: Butylated hydroxy toluene; a: Significant difference from group 1 at $p<0.05$; d: Significant difference from group 4 at $p<0.05$

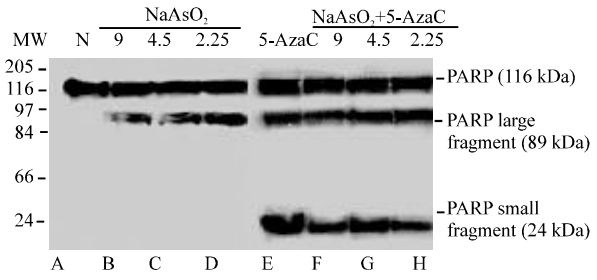

Fig. 1: Western Blot analysis of PARP from mice livers treated with sodium arsenite alone (B-D) or in combination with 5-AzaC (F-H). N: Untreated (A); MW: Marker; 5-AzaC (E)

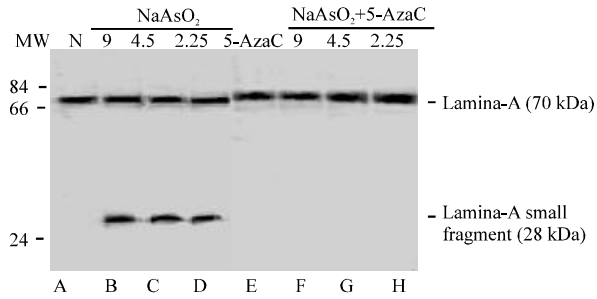

Fig. 2: Western Blot analysis of lamina-A from mice livers treated with sodium arsenite alone (B-D) or in combination with 5-AzaC (F-H). N: Untreated (A); MW: Marker; 5-AzaC (E)

fragments. Figure 1 is also shown that single treatments produced different bands which increased with low dose, while no degradation observed in negative control panel (A). The companied treatments using 5-AzaC (F, G and H) at three doses yielded positive results compared to negative control. Furthermore, treatments with 5-AzaC alone induced PARP fragmentation and let to apoptosis.

Lamina-A: Figure 2 indicated that single treatments with three doses of sodium arsenite induced apoptosis and had a positive effect (B, C and D) in terms of the degradation of intact Lamia-A molecules (70 kDa) to generate small (28 kDa) fragments, however no degradation was observed in the negative control panel (A). Companied treatments with 5-AzaC (F, G and H) at three doses had no effect to induce apoptosis.

DISCUSSION

The genotoxic effect of arsenic compounds on CAs has been reported *in vitro* and *in vivo* in several publications (Martínes *et al*., 2005; Patlolla and Tchounwou, 2005; Florea and Büsselberg, 2008). Many studies have been pointed to genotoxic effects of investigated sodium arsenite ($NaAsO_2$) (Brink *et al*., 2006; Hagiwara *et al*., 2006; Florea and Büsselberg, 2008).

The results of present study showed that the single treatment with $NaAsO_2$ at all used doses significantly increased the structural CAs. This structural CAs were included chromatid breakage contained (breaks, deletions, fragments and few of chromosome-types structural aberrations such as centric fusion and ring chromosome). The results of present study were corresponded with earlier studies used the live mice to detect genotoxicity of arsenic compounds (Ochi *et al*., 2008; Touriguine *et al*., 2008). As well as corresponded with Rahman *et al*. (2001) results on people have been exposed to high levels of arsenic in drinking water.

Pulverized chromosomes were significantly increased after treatment with only single medium dose compared with the negative control. Various mechanisms has been suggested to explain Pulverized chromosomes formation, from these: cell fusion, failure of cytokinesis following normal nuclear division (Tsutsui *et al*., 2000; Ochi *et al*., 2008). Its known that $NaAsO_2$ has potent to form genetically instability cells-multi or micronucleus cells-led to pulverized chromosomes formation in Chinese hamster (Seok *et al*., 2007). Furthermore, the genomic instability phenomenon can result from telomerase inhibition which observed in treated NB4 cell line with arsenic trioxide (As_2O_3) because of low transcription which attributed to direct affect of arsenic on transcription factors (Chou *et al*., 2001; Miller *et al*., 2002; Shen *et al*., 2008).

Also in present study, increasing in centromeric attenuation after treatment with $NaAsO_2$ was observed. And spindle fibers disorder has been suggested as a reason for centromeric disruption, followed with chromatid attenuation. As Pati and Bhunya (1989) study was pointed to that present of chromatid attenuation maybe represent important noticed related to aneuploidy, while DeHondt *et al*. (1984) considered that as early stage of endomitosis which maybe led to polyploidy. Cytoskeleton has been mentioned as a potential cellular target for arsenic because it's major constituent, tubulin, which has a relatively high sulfhydryl (SH) content (Bishayi and Sengupta, 2006; Seok *et al*., 2007; Chowdhury *et al*., 2008).

Arsenic considered a toxic and carcinogenic compound for both human and animal and produce free radicals in the cells during metabolism. Consequently, cell damage throughout activation of oxidative signals pathways (Valko *et al*., 2006; Piga *et al*., 2007). Experiments on myeloid leukemia cells showed arsenic

inhabit tubulin polymerization and disrupt microtubule formation (Li and Broome, 1999; Seok *et al*., 2007). Furthermore, kinetochore contain tubulin, so observed chromatid attenuation in this study after treatment with $NaAsO_2$ maybe as a result of $NaAsO_2$ effect on tubulin. The effect of arsenic on tubulin can be let to a number of abnormal mechanisms such as failure of cytokinesis, or random aggregation of metaphase chromosomes and these are the suggested mechanisms as reasons for pulverized chromosomes formation. Experimental evidence pointed to that the genotoxici effects of arsenic include inhibition a number of implicated enzyme in DNA repairing mechanism, DNA replication and cause structural changes. And this is confirm arsenic potential to spindle fiber disruption and induction of Reactive Oxygen Species (ROS) (Wang *et al*., 2004; Chou *et al*., 2008).

The results of SCEs test showed significantly increased in the rate of SCEs after treatment with single three doses of $NaAsO_2$ compared with negative control. This results confirm the few earlier studies which used SCEs assay to evaluate the genotoxicity effects of arsenic in human and animals tissue culture (Lee *et al*., 1985; Bernstam and Nriagu, 2000; Chou *et al*., 2008; Han *et al*. 2008). Despite what observed in this study of significantly increased in SCEs after treatment with $NaAsO_2$, but this increasing less than CAs. If we compare between increasing rate of cells with structural chromosomal aberrations and increasing rate of cells with SCEs, we will find out the structural CAs duplicated 16 times after treatment with three doses of $NaAsO_2$. This study confirmed results have been obtained from studying the effect of arsenic-contaminated water in human lymphocytes for people drank this water (Mahata *et al*., 2003).

The MNs test is consider one of the important assays used in genotoxicity to detect the effect of examined agent on chromosomes or spindle fibers damage. Present study show that the single treatment with the medium and high doses of sodium arsenite induced a significantly increase in the number of MNPCEs and caused genotoxic effects in mice bone marrow cells (Adler, 1984; Hayashi *et al*., 1994; Jagetia and Reddy, 2002). Data obtained agreed with earlier studies which has showed increased micronuclei in bladder epithelial cells for people exposed to arsenic in drinking water and cells cultured of chaises ovary hamster (Rahman *et al*., 2001; Martínez *et al*., 2005).

The obtained data showed PARP degradation after treatment with three single doses of $NaAsO_2$, this elucidate its potential to induce cytotoxicity. Many studies published on PARP sensitivity and its response to apoptosis (Qin *et al*., 2008; Ochi *et al*., 2008). Exposure of T-cells to arsenic *in vitro* results in activation of caspase 3 and 8, together with PARP degradation, DNA inhibiting repair by reduction the activation signals of DNA repair enzymes (Mathas *et al*., 2003; Qin *et al*., 2008). Furthermore, several intranucleolus changes produced from activation of caspases enzymes such as active DNase, PARP and Lamina-A degradation as apoptosis markers (Kang *et al*., 2006; Mclaren *et al*., 2006; Yu *et al*., 2008). As s result of PARP activation which resulted from early DNA damage response, NAD^+ levels may rapidly decline, which may affect the activity of the enzymes involved in glycolysis and the Krebs cycle. In an attempt to restore NAD^+ pools cell resynthesized NAD^+ by combining nicotinamide with 2ATP and as a consequence cellular ATP levels become depleted and a cellular energy crisis may arise leading to cell death. Cell that are replicating and growing and utilizing almost exclusively glucose die from NAD^+ and ATP depletion as a consequence of PARP activation (Brock *et al*., 2004; Shi *et al*., 2004; Wijk and Hageman, 2005).

Studies showed $NaAsO_2$ induced apoptosis signals from the cell surface to the nucleus of lymphocytes through fragmentation of DNA, activation of caspase and PARP degradation. Arsenic play a dual roles as anti-cancer and inducing of gentotoxicity and cytotoxicity, its these two apparently opposite effects on human life may share a common molecular mechanism.

When DNA is moderately damaged, PARP participates in the DNA repair process and the survives. However, in the case extensive DNA damage, PARP overactiveness induces a decrease of NAD^+ and ATP levels, leading to cell dysfunction or even to necrotic cell death and pathogenesis of several diseases. Spite important role of PARP to detect apoptosis, some evidence suggests the involvement of PARP in necrosis and during apoptosis PARP activity is suppress and cell is forced to die because the mild effect of toxic and this mechanism need enough energy to support apoptosis. But the necrosis is more several than apoptosis and due to severe genotoxic stimuli and this kind of cell death takes place in a tissue or organ. There are several biochemical and morphological differences between apoptosis and necrosis (Nguewa *et al*., 2003). Cell exposed to DNA-damaging agents may undergo three pathways depending on the degree of DNA damage. Thus, a mild DNA damage activates PARP, which subsequently interacts with several proteins involved in DNA repair such as polymerase II and DNA ligase III. If DNA repair proceeds successfully, then the cell survives.

If DNA damage is too severe to be repairable apoptosis take place, so that the caspase cleaves PARP. A third pathway may be induced by extensive DNA breakage in which overactiveness of PARP cleaves NAD^+ into NAD and ADP-ribose moieties and polymerized the later onto nuclear acceptor proteins and decrease of NAD^+ levels inhibits production of ATP through oxidative phosphorylation, leading to ATP depletion and necrotic cell death. As mentioned that within a population of tumor cells, necrosis and apoptosis may take place together in response to cytotoxic drugs. And this may attribute to that drug concentration reach different cancer cells; low concentration induce apoptosis and higher concentration produce necrosis (Nguewa *et al*., 2003).

The results pointed to Lamina-A degradation at three single doses of $NaAsO_2$. Earlier studies demonstrated that the activity of caspase that cleavage Lamina is required for the disintegration of nuclei in the late stages of apoptosis. The Lamina-cleavage caspase-6 is sufficient to drive nuclear events to shutting down nuclear processes followed by apoptotic execution because of lamina proteins bind specifically to most nuclear envelope proteins, histones, transcriptional regulators, gene expression regulators. Furthermore, lamina filaments interfere with chromosome segregation during mitosis. Mostly the lamina cleavage links in the apoptotic pathway and precedes DNA fragmentation (Takahashi *et al*., 1997; Chen *et al*., 2000; Cohen *et al*., 2001; Bjerke and Roller, 2006).

Anyway, the relationship between arsenic dose-response and its toxic effects still unclear because of different cell types, various biological endpoints studied, experimental scatter (Gebel, 2001). But most studies showed that the dose-response depend on exposure protocol, time exposure, dose (Yih and Lee, 1999).

5-Azacytidine was used in present study to decrease DNA methylation status compared with normal level methylation (Laird *et al*., 1995). The all combined treatment led to increasing in the rate of structural CAs, and total number of cells with structural aberrations compared with the negative control and single treatments of $NaAsO_2$. These high rates may be indicating to that hypomethylation led to increase DNA sensitivity to toxic effect by arsenic. As mentioned in few studies that hypomethylation of DNA could cause changes in specific regions of chromatin led to genome instability throughout increase sensitivity of some DNA sequences for DNA damage agents (Keshet *et al*., 1986; Lewis and Brid, 1991; Klose and Bird, 2006). These increasing in the numbers of cells with chromosomal changes after combined treatments or 5-AzaC alone reflect genome instability (pulverized chromosome and centromeric attenuation). This is support what suggested before that DNA hypomethylation which occurs away from CpG islands led to chromosomal instability which appear in different chromosomal changes (Schulz *et al*., 2002). The results of present study refer to a clear association between genome hypomethylation and chromosomal instability.

A high rate in the number of cells with centromeric attenuation were observed after a single treatment with 5-AzaC can be explain depend on that 5-AzaC could be interact with kinetochore protein synthesis (VanHummelen *et al*., 1992) which may led to centromere disruption, followed by chromatid attenuation (Dolara *et al*., 1994). On the other hand, no much affect in the rate of SCEs after treatment with 5-AzaC, the rates in treatment group with 5-AzaC alone were very close to negative control. But the combined treatment with low dose of $NaAsO_2$ induce significant decrease in the rate of SCEs compared with single treatment of $NaAsO_2$ at low dose. This is clear different between DNA lesions in CAs and SCEs production elucidate more than one mechanism is involved (Grandberg *et al*., 1980; Kaina, 2004).

In MNs test, there was non significant decrease at all combined doses compared with single doses. This can be explain based on the treatment with 5-AzaC led to delayed migration of nuclei, suggestion that cell cycle arrest might occur. And some studies as well revealed that 5-AzaC was developed employing low-dose schedules for the treatment of myelodysplasia by virtue of their favorable non-hematologic toxicity profile and beneficial effects on hematopoiesis (Haas *et al*., 2006; Ueno *et al*., 2006).

The role of combined treatment is not clear to decrease apoptogenicity, but it is induced PARP degradation. And treatment with 5-AzaC alone induced apoptogenicity, many studies which have been done on cell lines and fetal mice which led to DNA damage, disturbance of DNA methylation and gene expression and subsequently organogenesis (Gopisetty *et al*., 2005; Ueno *et al*., 2006; Seok *et al*., 2007). The treatment with 5-AzaC alone or combined with $NaAsO_2$ are not led to Lamina-A degradation and this may be attribute to that DNA hypomethylation inhibited cell sensitivity to $NaAsO_2$ toxic effect (Davis *et al*., 2000).

The apoptosis detection tests may be reveals different results, such as PARP and Lamina-A or another test, because some of specific targets are affected by different factors like caspases enzymes and another targets are not affected (Bruguera *et al*., 1978).

Several studies have been showed in different cancer diseases that DNA methylation affect on genes

throughout different cellular pathways involve apoptosis pathways, the defect of apoptosis pathways in cancer cells arrest cells death. Anyway there are a currently examination on 5-AzaC alone or combined with another compounds such as phenyl butyrate or amifostine as a clinical attempting to treat some diseases like β- thalassemia, lymphoma, lung and prostate cancer (ClinicalTrials. Gov., 2001).

CONCLUSION

Effect of DNA hypomethylation on genotoxicity and apoptogenicity of sodium arsenite in laboratory Mmice was clear but with unclear dose-response relationship. Sodium arsenite induce genotocxicity and apoptogenicity according to direct or indirect mechanism and had different potential cellular targets. Cells exposed to DNA-damaging agents may undergo three pathways depending on the degree of DNA damage, mild DNA damage activates PARP, which subsequently interacts with several proteins involved in DNA repair such as polymerase II and DNA ligase III. DNA repair proceeds successfully and the cell survives. Low concentrations induce apoptosis, while higher concentrations result in necrosis. There are several biochemical and morphological cellular differences between apoptosis and necrosis. Finally, the hypomethylation led to increase DNA sensitivity to toxic effect by arsenic and could cause changes in specific regions of chromatin led to genome instability throughout increase sensitivity of some DNA sequences for DNA damage agents.

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