

Full Length Research Paper

Using irradiated human lymphocytes as a double treatment solution to assess the Genotoxicity of Arsenic (As^{3+}) and Lead (Pb^{2+}) in type and frequency of chromosome aberrations

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Chromosome aberrations (CA) induced in human lymphocytes by the combined Arsenic (As) or Lead (Pb) with gamma rays treatments in seven groups: As alone, Pb alone, gamma rays alone, As/gamma, gamma/As, Pb/gamma and gamma/Pb. were investigated. The mitotic index (MI) was not altered in the cultures treated by Pb alone in the range of concentrations from 0.05 to 2.0 $\mu\text{g}/\text{ml}$, but decreased by As alone in the range of concentrations from 0.05 $\mu\text{g}/\text{ml}$ to 0.10 $\mu\text{g}/\text{ml}$. CA frequencies induced by Pb or As alone treatments also Pb combined treatments were maintained equal, but there were changed in the As combined treatments in the same group of gamma rays dose. A change of dicentric (Di.) frequencies in the As/gamma combinations with the same gamma rays dose was clearly and it seems to decrease in dose of 0.5 Gy clearer than in dose of 0.10 Gy, it means that the influence of Arsenic action to biodosimetry in low dose was stronger than in high dose. The tendency of Di. and fragments (Fra.) in the gamma/As combinations was also the same of those in As/gamma. In fact, the Arsenic action had still inactivated the repair enzymes after 60 minutes of irradiation. The decrease of Di. frequencies and increase of Fra. frequencies in the combinations with the same dose of gamma rays and difference Arsenic concentrations were according to inactivation of repair enzymes.

Keywords: Arsenic; Lead; gamma rays; DNA damage; repair enzyme; cohesive free ends; blunt free-ends; un blunt free-ends.

INTRODUCTION

Using chromosomal aberrant analysis in biodosimetry has been recognized and recommended by the International Atomic Energy Agency (IAEA) from 1982 (IAEA, 1986). Acute biodosimetry along with chronic biodosimetry were used to estimate the aspect of radiation risks (Hartwig et al., 2003). Along with the calibration dose-effect curves, standard frequencies of dicentric per 1000 metaphases were used for biodosimetry in the cases of natural radiation or chronic irradiation (Wang et al., 2005; Hayata, 2005; Jiang et al., 2000). Arsenic was known as a poisoner in the archaic world, Arsenic and Lead were recognized as the

persistent bioaccumulative toxins (PBT) and carcinogens. The strong evidence of increasing cancer risks in the natural with high Arsenic contaminations were showed by MHDRA, 2000; ATSDR, 2003; IARC, 2004 and Arsenic contamination in water have been become worldwide problem (Deknudt and Deminatti 1987; Mahata et al 2004; Preston 1990; Smith 2005; Voisin 2004).

Arsenic is maintained in environment by inorganic and organic compounds with derivative of trivalent and pentavalent with a large levels on the world (Tapio 2005), WHO guideline for drinking water is 0.01 mg/l and almost developing countries apply in a limit to 0.05 $\mu\text{g}/\text{l}$. Arsenic drinking water of USA standard from 1962 to January 22, 2001 was 0.05 $\mu\text{g}/\text{l}$, this standard was changed to 0.01 $\mu\text{g}/\text{l}$ in 2006 (Schwerdtle et al., 2003; Smith 2005; Voisin et al.,; WHO 2001). The high residue of Arsenic

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was detected in protein and some human organs such as teeth, nail, hair, urine which have been used as the biomarkers for estimating of Arsenic contamination in the high background areas (Basu et al., 2005; Preston 1984)

Genotoxic effect

one of the most important biomarkers to assert the relationship between Arsenic and cancer was also detected in the recent cytogenetic studies. The significant high frequencies of DNA damage, chromosome aberrations, micronuclei, chromatid breaks in lymphocytes of the residents who lived in the high arsenic background contamination were observed in West Bengal, India 2003, 2004, 2005 (Basu et al., 2005; Mahata et al., 2003; Mahata et al 2004), Lukhuni gorve, Russian 2005, Antofagasta, Chile 2004, 2005 (Martinez et al.,).

For *in vitro* study, the significant difference on DNA damage frequencies, chromosome aberrations, micronuclei, and chromatid breaks induced in the cell lines and human lymphocytes between control and treatment, between low and high Arsenic concentration were observed.

The observed data of Dopp 2004, 2005 on inorganic and organic Arsenic in trivalent and pentavalent compounds with different concentrations for CHO-9 cell line, fibroblasts cells and hepatoma cells showed that frequencies of micronucleus, chromosome aberrations, and sister chromatid exchanges depended on kind of cell and types or concentrations of Arsenic.

The Arsenic compounds in the trivalent oxidation state exhibit the strongest genotoxic effects. Dopp showed that the viability was significantly decreased incubation (1h) of the cells with 1 μM As(i)(III) or 1 μM As(i)(V) but with 500 μM MMA(III) or 100 μM MMA(V) (Deknudt and Deminatti 1978; Dopp et al., 2004). In the data of Hamadedeh 2002, Schwedtle 2003, Hadi 2003, Arsenic concentrations in a range of 0 - 5 μM were not aspect to survival of cells (Hartwig et al.,2003; Jha et al.,1992; Mahata et al., 2003; Jiang et al.,). In the effort to find out the impact mechanism of Arsenic in molecular level have been conducted.

The observed data showed that Arsenic is a repair inhibited agent of DNA damages. In 1997, with the genetic evidence, Flessel suggested that Arsenic, chromium, and molybdenum compounds may be influenced to the accuracy of DNA repair processes in microorganisms (Dopp et al., 2005). Hamadeh 2002, detected in 0.005-5 μM of As(III) concentrations, gene expressions associated with DNA repair (e.g., p53 and Damage-specific DNA-binding protein 2) decreased and gene expressions indicate the cellular response to oxidative stress increased in normal human epidermal keratinocytes (Hartwig et al.,)

The environmental contamination from Lead is a big

problem on the world. Lead concentration in drinking water standard in Vietnam from 1995 was 0.05 $\mu\text{g/l}$. The high frequencies of micronuclei in V79 cell line treated with Lead chlorite 1.1 $\mu\text{M/l}$ or Lead acetate 1.1 $\mu\text{M/l}$ or Lead acetate 0.05 $\mu\text{M/l}$ were detected by Bonaker (2005) (Bonacker et al., 2004). The significant difference of micronuclei and chromatid breaks in human melanoma cells treated with Lead acetate 10 $\mu\text{M/l}$ was detected by Poma (2003) (Poma et al., 2003). Wozniak (2003) detected the single, double DNA strand breaks and DNA-protein cross-links in the cells treated with Lead acetate 100 $\mu\text{M/l}$ (Schwerdtle et al.,). Deknude (1978) showed that Lead acetate have the genotoxicity for human lymphocytes in 100 $\mu\text{M/l}$ concentration and Lead chlorite induced dicentric aberrations higher than Lead acetate 3 folds in 30 $\mu\text{M/l}$ concentration (Deknudt and Deminatti).

Jha1992, using peripheral blood lymphocytes for Sodium Arsenite (SA) treatment alone and combination by X rays showed that SA interfered with the DNA repair process, presumably by inhibiting the ligase activity, increase in the DNA replication-dependent processes, chromatid aberrations and SCEs and enhancement of the X-ray and UV-induced chromosomal damage (Martinez et al., 2005).

Hartwig 1997, treated As(III) in 2.5 μM concentration and higher on UV irradiated to human fibroblast cell lines: 2 repair-proficient and 1 repair-deficient estimated that both of the global genome repair pathway and the transcription-coupled repair pathway are affected by Arsenite (Jha et al., 1992). In a single dose of As(III) 0-5 μM in 24 hours or UV 5 J/m^2 and combination of As before 24 hours UV treated to TK6 human lymphoblastoid cells (heterozygous at TK locus). Danaee 2004, showed that pre-treatment with As(III) specifically inhibited the repair of UV-induced pyrimidine dimer-related DNA damage.

Arsenic impairs the nucleotide excision repair pathway and leads to enhance UV mutagenesis (Wang et al., 2005). Chen 2005, with observation of the apoptotic effect was normal in human keratinocytes treated with 1 μM As, UVB 50 mj/cm^2 and UVB/As, but not normal with As/UVB, sodium arsenite decreased the pro-apoptotic effects induced by UVB (Chen et al., 2005).

In the fact that, while doubt of Arsenic causing directly DNA damage is presented, the evidence of the combinative treatment between Arsenic and mutagenic agents such as X ray, UV showed that in the none-cytogenotoxic concentrations of As (III) well known as the factor inhibited cell cycle progression, genome repair pathway, transcription couple repair pathway, nucleotid excision repair pathway.

MATERIALS AND METHODS

Materials

Lymphocytes from peripheral blood of healthy donors were treated with Arsenic and gamma rays in single

Table 1a: The distribution of experimental combinations with Arsenic in the numbers from 1 to 15

Experimental parts	Arsenic concentration	Dose of gamma rays		
		0 Gy	0.5 Gy	1.0 Gy
Pre-treatment (treated samples by As in 180 mins/37°C before irradiation)	0 µg/ml	1	4	7
	0.05 µg/ml	2	5	8
	0.10 µg/ml	3	6	9
Post-treatment (keep irradiated samples in 60 mins/37°C before treated by As)	0.05 µg/ml	10	12	14
	0.10 µg/ml	11	13	15

Table 1b: The distribution of experimental combinations with Lead in the numbers from 1' to 15'

Experimental parts	Lead concentration	Dose of gamma rays		
		0 Gy	0.5 Gy	1.0 Gy
Pre-treatment (treated samples by Pb in 180 mins/37°C before irradiation)	0 µg/ml	1'	4'	7'
	0.05 µg/ml	2'	5'	8'
	2.0 µg/ml	3'	6'	9'
Post-treatment (keep irradiated samples in 60 mins/37°C before treated by Pb)	0.05 µg/ml	10'	12'	14'
	2.0 µg/ml	11'	13'	15'

concentrations and combined treatments for detecting chromosome aberrations.

The standard Arsenic solution As_2O_3 in NaOH and HCl, pH 5.5 (100 µg/ml As) was obtained from Wako Pure Chemical Industries, Ltd, Japan. Dilution from 0.025 to 0.20 µg/ml As were prepared for treatments. The standard Lead nitrate - $Pb(NO_3)_2$ was diluted in F_{10} medium. Gamma rays ^{60}Co with Phantom 1 hole in National Institute of Radiation Sciences, Japan and gamma cell – Isscledavatel, Russia in Nuclear Research Institute, Vietnam with dose rate at exposed time 12.5 mGy/s were used for irradiation.

Methods

The whole blood in Na-Heparine from healthy donors were used for investigations. Whole blood samples after treatments were cultured in RPMI 1640 (Sigma) medium supplied with 15% Fetal bovine serum (Gibco), 1% Kanamicine, 1% L-glutamine, 1.5% Phytohemagglutinin (Sigma) and Colchimid; incubated in 37° for 48 hours.

Treatments:

+ Mitotic Index (MI) in As treatments 0, 0.025, 0.05, 0.10, 0.20 µg/ml and Pb treatments 0.05, 0.10, 0.15, 0.5, 2.0, 100, 500 µg/ml were analyzed to chose As and Pb concentrations for designed combinations in table 1a and 1b.

+ All treatments were divided into two parts: Pre-treatment (As or Pb in 180 mins/37°C in medium without phytohemagglutinin and serum before irradiation) and Post-treatment (keep samples in 60 mins/37°C after irradiation in medium without phytohemagglutinin and

serum) before treated by As or Pb. The experimental combinations were designed following: - Mitotic Index (MI) and CA were analyzed for all combinations in tables 1a, 1b.

RESULTS AND DISCUSSION

Mitotic index of lymphocytes treated with As_2O_3 and $Pb(NO_3)_2$

Treated with As_2O_3 , for the single treatments, MIs of lymphocyte cultures in whole blood and separated lymphocyte were analysed for the Arsenic concentrations from 0, 0.025, 0.05, 0.10 and 0.20 µg/ml in 48 hours. MI was not detected in the cultured treatment with 0.20 µg/ml in both whole blood and separated lymphocyte cultures. The MI values (%) of the single treatments were 2.98 ± 0.25 , 2.80 ± 0.26 , 3.26 ± 0.32 , 1.01 ± 0.07 in whole blood cultures and 11.02 ± 0.72 , 10.16 ± 0.80 , 5.70 ± 0.47 , 4.54 ± 0.41 in separated lymphocyte cultures, respectively. The data was showed in figure 1.

For the combined treatment, MI values were showed in figure 2 for the As/gamma combinations 1 to 9 and gamma/As combinations 10 to 15. The MI values (%) of the As/gamma combinations were 3.36 ± 0.41 , 3.27 ± 0.39 , 1.16 ± 0.15 ; 3.12 ± 0.37 , 1.83 ± 0.14 , 1.72 ± 0.15 and 2.33 ± 0.23 , 1.57 ± 0.15 , 1.21 ± 0.14 , respectively to groups of gamma dose and As concentrations. For the gamma/As, MI values were 3.05 ± 0.35 , 1.92 ± 0.15 ; 2.76 ± 0.28 , 1.33 ± 0.14 and 1.82 ± 0.16 , 1.45 ± 0.13 , respectively.

There were decrease in MI values following increasing As concentrations in the same group of gamma rays doses 0 Gy, 0.5 Gy and 1.0 Gy. The MI values were

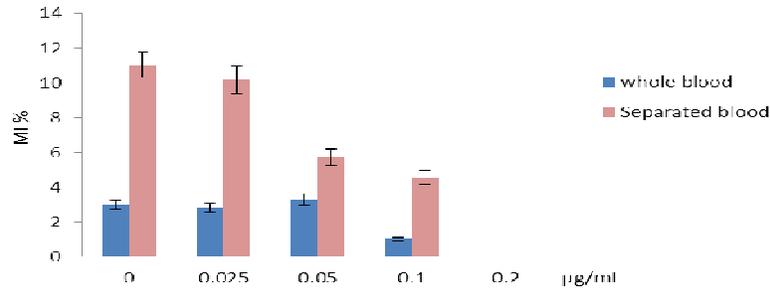


Figure 1: Mitotic index for the single treatments with Arsenic

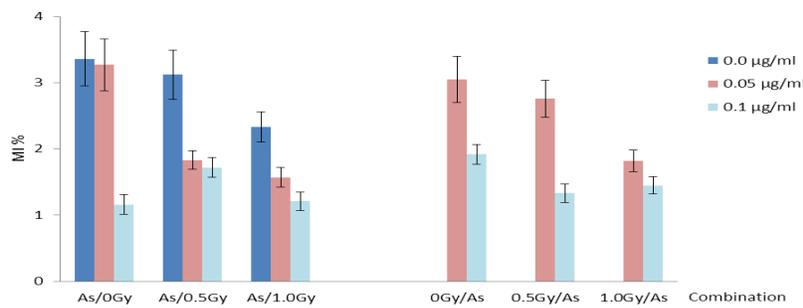


Figure 2: Mitotic index for the combinations As/gamma and gamma/As

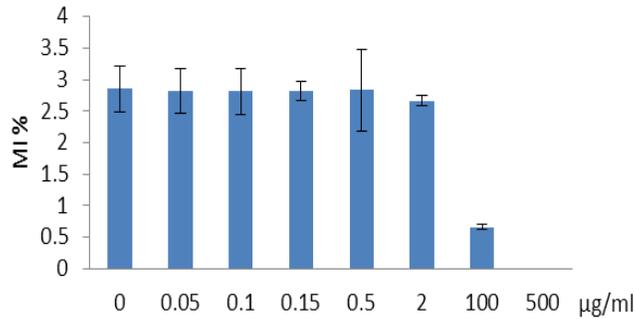


Figure 3: Mitotic index for the single treatments with Pb2+

strongly depending on Arsenic concentrations, there were decreased 1/3 with whole blood culture in 0.10 µg/ml concentration and 1/2 with separated lymphocyte culture in 0.05 µg/ml concentration. The mitotic index in all experiments was expressed a strong influence of Arsenic in single concentration also in combinations of pre and post irradiation. This result give a suggestion that the limit to 0.05 µg/l Arsenic in drinking water of WHO standard were not safety in the case of As₂O₃ contamination.

Treated with Pb(NO₃)₂, for the single treatments, MI values were not changed in the treatments from 0.05 to 2.0 µg/ml concentrations, MI value was decreased 5 folds with 100 µg/ml concentration and not detected in 500 µg/ml concentration. For the combined treatments, the average frequency and standard error of MI values among combinations were 2.59% ± 0.28%, there were not changed on MI values among the combinations with the same dose of gamma rays (figure 3 and 4).

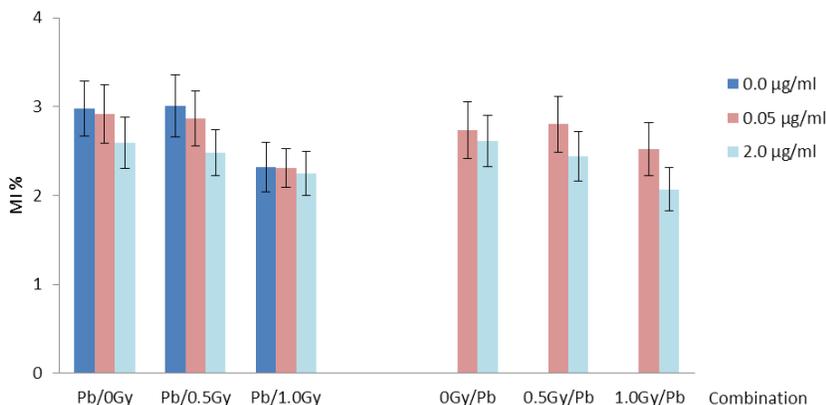


Figure 4: Mitotic index for the combinations Pb/gamma and gamma/Pb

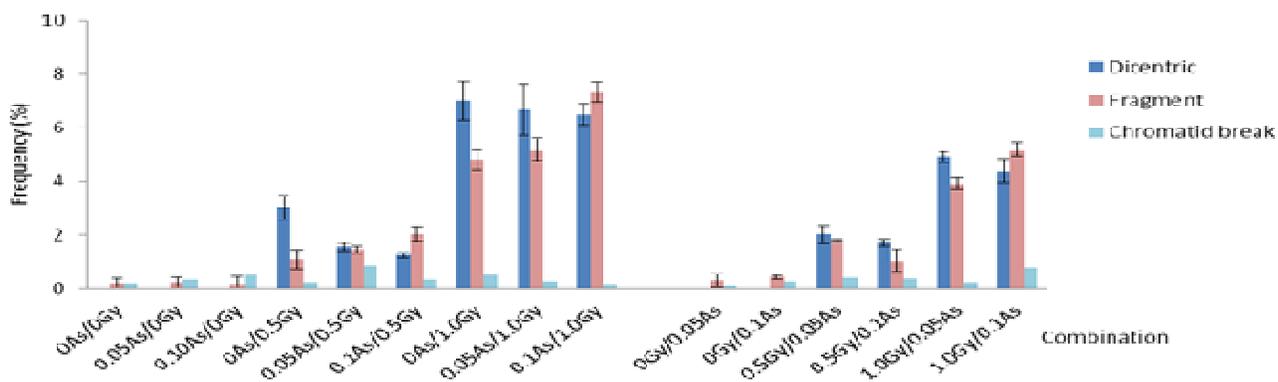


Figure 5: Frequencies of dicentric, fragments and chromatid breaks in the combined complexes Arsenic/gamma (1-15)

Chromosome aberrations after treatments with As_2O_3 and $Pb(NO_3)_2$

In types

With As_2O_3 , dicentric aberrations were not detected at the Arsenic 0.05 and 0.10 $\mu\text{g/ml}$ concentrations single after 48 hours, but fragment aberrations and chromatid breaks were detected at 0.05, 0.10 $\mu\text{g/ml}$ and 0 $\mu\text{g/ml}$ (combinations 1, 2, 3, 10, 11). Dicentrics, fragments and chromatid breaks were detected at the combinations for gamma 0.5 Gy, 1.0 Gy; As/gamma (0.05-0.10 $\mu\text{g.ml}^{-1}$ /0.05 Gy, 0.05-0.10 $\mu\text{g.ml}^{-1}$ /1.0 Gy) and gamma/As (0.5 Gy/0.05-0.10 $\mu\text{g.ml}^{-1}$, 1.0 Gy/0.05-0.10 $\mu\text{g.ml}^{-1}$). The types of chromosome aberration were not difference between those of gamma ray alone, As/gamma and gamma/As combinations. With $Pb(NO_3)_2$, dicentric aberrations were not detected at the combinations with Lead alone. Dicentric, fragment were detected at the combinations for gamma alone: 0.5 Gy, 1.0 Gy, Pb/gamma (0.05-2.0 $\mu\text{g.ml}^{-1}$ /0.05 Gy, 0.05-2.0 $\mu\text{g.ml}^{-1}$ /1.0

Gy) and gamma/Pb (0.5 Gy/0.05-2.0 $\mu\text{g.ml}^{-1}$, 1.0 Gy/0.05-2.0 $\mu\text{g.ml}^{-1}$). The chromosome aberration types were not difference between those of gamma ray alone, Pb/gamma and gamma/Pb combinations.

In frequencies

The results for frequencies were presented in figure 5 for Arsenic action and figure 6 for Lead action. For the Arsenic single treatments (1, 2, 3), there were none dicentric detected, frequencies of fragment and frequencies of chromatid breaks were also lower than 1%, mean that those lower than spontaneous frequencies. Actually, the single concentrations of Arsenic in 0.05 $\mu\text{g/ml}$ and 0.10 $\mu\text{g/ml}$ were not aspect to frequencies of chromosome aberrations, it means that the Arsenic concentrations limited to 0.10 $\mu\text{g/ml}$ concentration were none-chromosome aberrations. The Arsenic single action was not created DNA deletions lead to chromosome aberrations, For the combinations in the

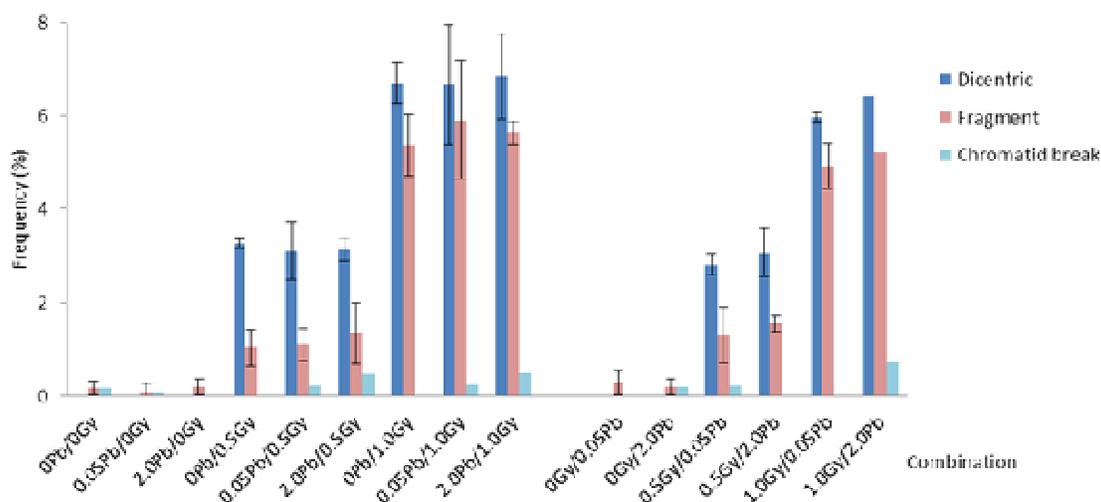


Figure 6: Frequencies of dicentric, fragments and chromatid breaks in the combined complexes Lead/gamma

same group gamma rays dose, there were abnormal in frequencies of chromosome aberration types, in those frequencies of dicentric was changed $\pm 0.95\%$ in the same dose group 0.5 Gy (combinations 4, 5 and 6) and $\pm 0.26\%$ in 1.0 Gy (combinations 7, 8 and 9), it means that the personal biodose in the case of the victims who related to environmental toxins such as Arsenic contamination will be changed.

In the combinations with pre-treatments, instead of none chromosome aberrations in the single treatments, they were detected in the combined treatments with changing of frequencies in each group of the same gamma rays dose but difference of Arsenic concentrations. In all combined treatments from point 2 to point 9, the chromatid breaks frequencies were not over 1% and not clear difference.

The dicentric frequencies was not arranged in order of Arsenic concentrations in the same dose of gamma rays, but the evidence of identical dicentric frequencies in the group 4, 5, 6 (0.5 Gy) and group 7, 8, 9 (1.0 Gy) were confirmed by Arsenic action. Alternatively, the fragment frequencies were distinguished clearly among the point 4 and 5, 6 (0.05 $\mu\text{g/ml}$), and among the point 7 and 8, 9 (0.10 $\mu\text{g/ml}$). In those, for Arsenic 0.05 $\mu\text{g/ml}$ concentration, the difference of fragment frequencies between point 4 and 5, 7 and 8 was not so high, it mean that there was no chromosome aberration effects caused by Arsenic in this concentration, this result agreed with supports of Arsenic Standard in Drinking Water of WHO. The clear difference of fragment frequencies between point 4 and 6, point 7 and 9 showed that Arsenic aspect strongly to increasing of these effects in 0.10 $\mu\text{g/ml}$ concentration.

In the combinations with post-treatment, the changing of chromatid breaks, dicentric, fragment was considered

according to groups of same As concentration/difference of gamma rays dose and same dose of gamma rays dose/difference of As concentration. The data showed that frequencies of chromatid breaks were not depended on As concentrations, but the frequencies of dicentric and fragment were still depended on As concentrations in same gamma rays dose group. The situation of type and frequency of the combinations with post-treatments showed that Arsenic in 0.05 to 0.10 $\mu\text{g/ml}$ concentration still aspect to the induction of chromosome aberrations post irradiation 60 minutes. There were correspondence with some others, who detected that 210 minutes was the time needed to complete the information of chromosome aberration after irradiation. For the single treatments with Lead, the frequencies of fragments and chromatid breaks were not showed the significant difference with those in control.

For the combined treatments, in spite of chromosome and chromatid aberration types were detected in the combinations, but the frequencies of these were difference with the results from As treatments. There were not changing of dicentric and fragment frequencies in each group of the same gamma rays dose but difference of Lead concentrations, it means that Lead 0.05 to 2.0 $\mu\text{g/ml}$ concentration did not cause the changing of types and frequencies of chromosome aberrations induced by radiation.

The actions of Arsenic (3^+) in 0.05 to 0.10 $\mu\text{g/ml}$ concentration to types and frequencies of chromosome aberrations were analysed from the combinations of each group of the same gamma rays dose. The presentation of high fragment frequencies depended on two pathways for maintaining fragments, first was the mis-repair of DNA damages induced by gamma rays lead to increase double strand breaks, second was the un-join of free

ends from the new double strand breaks. There were clear that Arsenic caused a increasing of double strand breaks, this evidence corresponded to the studies of Jha1992, Hartwig 1997, Chen 2005, in those Arsenic inhibited the repair systems of DNA damages.

The bounding of Arsenic to DNA and protein molecules, also DNA damages was explained such as the main factors creased the increasing of abnormal chromosome. The evidence in table 3 that the presenting in low levels of dicentric but high levels of fragment in the point 5 and 6 were explained by the maintenance of inactive cohensive free ends of double strand breaks. The increasing of fragment in point 5, 6, 8, 9 can be also explained that the free ends from new double strand breaks were not blunt free ends and the unblunt free ends of those will be loss join to create dicentric or joint fragments.

Conclusions

Arsenic (As_2O_3) in concentration from 0.05 to 0.10 $\mu\text{g/ml}$ and Lead ($Pb(NO_3)_2$) in concentration from 0.05 to 2.0 $\mu\text{g/ml}$ caused decrease MI in human lymphocytes. The toxicity of As for MI were different between whole blood culture and lymphocyte's separated culture, MI LD_{50} for separated lymphocytes was 0.05 $\mu\text{g/ml}$ and for whole blood culture was 0.10 $\mu\text{g/ml}$, it mean that serum in whole blood due to decrease of As toxicity for MI. Arsenic in concentrations limited to 0.10 $\mu\text{g/ml}$ and Lead in concentrations limited to 2.0 $\mu\text{g/ml}$ itself were not induced chromosome aberrations in the first cycle of human lymphocytes *in vitro*.

The frequency of dicentric aberrations decreased with increasing Arsenic concentration in the combinations exposed to the same dose of gamma rays, but frequencies of fragment aberrations increased with increasing Arsenic concentrations in these combinations. The frequency of dicentric and fragment aberrations was not changed with increasing Lead concentration in the combinations exposed to the same dose of gamma rays.

Change in frequency of dicentric and fragment aberrations was not so difference between pre-irradiation and post-irradiation combinations exposed to the same dose of gamma rays. These showed that 60 minutes after irradiation was not full time to complete the DNA damage repair progress. We suggest that Arsenic and Lead do not directly cause chromosome aberrations but Arsenic affects the DNA damage repair of lymphocytes. The Arsenic action can lead to decreasing dicentrics and increasing fragments (inactive cohensive free ends) by binding the blunt free-end of double strand breaks.

For biological dosimetry and for the dangerous level of radiations, because dicentric are used as a biomarker for dose assessments, the presence of Arsenic will cause the dosimetric value to be incorrectly recorded. The prevention of the relinking of strand breaks of DNA will

increase the danger level of exposure to ionizing radiation.

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