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Comparative assessment of growth and morphological responses reveals resilience of HeLa cells over Vero cells on exposure to Arsenic (III)

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ABSTRACT

Heavy metals, responsible for a wide range of diseases starting from asthma to cancers, are often found in surface water because of uncontrolled release of wastewater and sometimes in groundwater as well. Number of studies suggest the possible relationship between the increased heavy metal concentration in industrial proximity and the increased cases of melanoma and carcinoma patients. Unfortunately, it is not clear yet how cells respond to these exposure and what kind of changes cells go through before initiation of cancer. This study aims to evaluate cytotoxicity and morphological changes of mammalian cells in presence of arsenic (III) solution of different concentrations. HeLa as cancer cell and Vero as non-cancer cell were analyzed to evaluate different cellular growth and migration parameters. 65 µM and 8 µM As(III) concentrations were found as LC_{50} for cancer and non-cancer cells, respectively. It was found that, severity of cytotoxicity was acute for the doses of As(III) higher than 1 µM and the effect was negligible below 0.25 µM for regular mammalian cells. Cancer cells, being robust and tolerant, showed noticeable effect only beyond 1 µM and the effect was severe above 2 µM. In addition, wound healing behavior was affected significantly by the As(III) dosage. Quantitative assessment of the altered mammalian cell behaviour in different As(III)concentrations divulge the changes during arsenicosis at cellular level, which paves a momentous pathway for future battle against arsenic-mediated diseases.

INTRODUCTION

Environmental pollution and pollutant-mediated diseases have been a major problem in developing countries. Rapid industrial growth without proper implementation of environmental regulations is making this situation worse. People working in industries dealing with heavy metals and even people living near the industrial areas have been identified with different skin disorders and cancers[13]. Arsenic, chromium, mercury and lead are the prime heavy metals responsible for different skin diseases in developing countries. Bangladesh is one of the most affected countries in the world with significantly high number of arsenic poisoned (arsenicosis) patients[47]. As arsenic is not frequently found in consumable water in developed countries, arsenicosis and relevant issues have not gained as much attention as other diseases had received. Arsenic has been classified as a class-I human carcinogen by the International Agency of Research on Cancer (IARC), meaning that there is sufficient evidence of carcinogenicity to humans[8, 9]. Different clinical, epidemiological and laboratory based studies proved that arsenicosis is associated with cancers in skin, liver, stomach, lungs, hematological cells and urinary bladder [10, 14]. Unfortunately, it is not clear yet how cells respond to these heavy metal exposure and what kind of changes cells go through before initiation of cancer[8].

Though the exact mechanism of the action of arsenic on living organisms is not known yet, several hypotheses have been proposed so far[15, 16]. The metabolism of arsenic has an important role in its toxicity which involves reduction to arsenic (III) and oxidative methylation to arsenic (V). Arsenic (III) has more potent toxic properties than the arsenic (V)[17]. Most of the mammalian species methylate inorganic arsenic and such type of metabolism plays a vital role in the toxicity of arsenic[18].

Metabolism of inorganic arsenic follows a sequential process involving a two-electron reduction of arsenic (V) to arsenic (III). Then oxidative methylation converts it to organic arsenic (V) [19]. This two-electron reduction of arsenic (V) to arsenic (III) process can occur in the presence of a thiol such as glutathione (GSH) without the help of any enzyme[20, 21]. For many years, it has been proposed that, in metabolism process of arsenic monomethylarsonous acid and dimethylarsinous acid are produced as intermediates [22]. Recently, several researchers have found monomethylarsonous acid and dimethylarsinous acid in the urine of people who are chronically exposed to inorganic arsenic in their drinking water [22, 23]. Another research on rats strengthens the idea of formation of such type of intermediates. In case of rats, monomethylarsonous acid and dimethylarsinous acid were found in the bile of rats[24]. In case of pentavalent arsenic toxicity mechanism, arsenic (V) can replace phosphate in many biochemical reactions due to the similarities of their structure and properties [25]. An in vitro experiment showed that, arsenic (V) reacts with glucose and gluconate to form glucose-6-arsenate and 6-arsenogluconate, respectively [26, 27]. Moreover, arsenic (V) can also replace phosphate in the sodium pump and the anion exchange transport system of the human RBC (Red Blood Cell) causing different complications [28].

In case of trivalent arsenic toxicity mechanism, some specific functional groups within enzymes, receptors or coenzymes, such as thiols have a major role in the activity of these molecules. Arsenic (III) readily reacts in vitro with cysteine. It hampers Glutathione (GSH) protein production as GSH is made of cysteine, glycine and glutamic acid. As an important antioxidant, GSH is capable of preventing Reactive Oxidative Stress (ROS) [20, 21]. Arsenic (III) binding at closely spaced and accessible thiol groups (R-SH) may inhibit important biochemical events [29], which could lead to toxicity unless arsenic (III) binds nonessential sites in proteins [30]. Again, Pyruvate dehydrogenase (PDH), a multi sub-unit complex is disturbed by arsenic (III). To perform enzymatic activity, PDH requires the cofactor lipoic acid, a dithiol [31,33]. It is assumed that, arsenic (III) inhibits lipoic acid molecule and thus leads to hamper PDH [17]. PDH oxidizes pyruvate to acetyl Coenzyme A, which is a precursor to intermediates of the Krebs cycle. By hampering Krebs cycle, arsenic (III) may ultimately lead to decrease in production of ATP which is an energy molecule. Besides, some other studies showed that, depletion of carbohydrates was very common in arsenic dosed rats with the inhibition of PDH [33, 34].

Again, arsenic is not only a toxic substance, it is an established carcinogen [8]. Arsenic may act as a carcinogen by inducing oxygen stress, oxidative damage on DNA, inhibiting several DNA repair mechanisms, influencing signal transduction and changing gene expression[35, 36]. Animal trials showed that ingestion of dimethylarsinic acid can cause urinary bladder cancer in rats but not in hamsters and monomethylarsinic acid had no carcinogenic effects on rats. In human beings inorganic arsenic in drinking water can induce cancer of the urinary bladder, skin, lung, kidney, liver and prostate[10, 14].

In addition to industrial wastewater, arsenic in groundwater has been the prime cause of several skin disorders for years and that is suspected to be responsible for more severe diseases in the long run [4, 37, 40]. Arsenic enters into the human body through ingestion, inhalation, or skin absorption and gets distributed in a large number of organs including the lungs, liver, kidney and skin. Then Lung, kidney, pharynx, bone, large intestine, and rectum of a person may be inflicted with cancer due to the long-term frequent exposure in arsenic[41, 42]. Acute arsenic toxicity in humans involves from discomfort in gastrointestinal tract to death. It also creates vomiting, diarrhea, bloody urine, anuria, coma, shock and so on [17]. For an adult person, the lethal dose of inorganic arsenic is estimated of 13 mg arsenic/kg mass of that person [24]. Even trace arsenic exposure appears to have led to dramatic increases in cancers ranging from skin to liver to lung [43], in cardiovascular disease, and in developmental and cognitive problems for children. The bulletin of the World Health Organization (WHO) estimates that the invisible taint of arsenic in the well water could now be responsible for as many as 43,000 deaths per year in Bangladesh [43]. Chronic exposure to inorganic arsenic is also dangerous like acute exposure. Toxic effect of chronic exposure to arsenic could lead to diseases like skin lesions, blackfoot disease, peripheral neuropathy, encephalopathy, hepatomegaly, liver cirrhosis, altered heme metabolism, bone marrow depression and diabetes [44, 45].

Despite high occurrence and massive impact of arsenic toxicity in developing countries, it often failed to get enough attention like other heavy metals. Moreover, detailed cellular cytotoxicity and morphological studies need to be performed to understand the mechanism of cellular changes and dose responses in exposure of the arsenic. Unfortunately, countries with lesser healthcare innovation are primarily affected by arsenicosis, and therefore, any insight on how arsenic initiates cellular alteration should be very important for development of drugs and treatment for the affected countries. Although the generalized cytotoxic effects of different heavy metals have been reported earlier, this study solely focuses on the parametric differences between the cellular responses of two different cell lines in presence of varying dosage of Arsenic (III).

MATERIALS AND METHODS

Cell culture and other reagents.

Vero cell line and HeLa, a human cervical carcinoma cell line were cultured in DMEM containing 1% penicillin and streptomycin (1:1), 0.2% gentamycin and 10% FBS at 37°C, 5% CO_2 in a culture flask inside an incubator (Nuaire, USA). Cell culture media and other reagents were obtained from Invitrogen, USA. Cell lines used during this study were within the passage number 40. As_2O_3 (MERCK, Germany) solution in deionized water was used as the source of As^{3+}. The sample was sterilized using an autoclave (Labtech, UK). Serial dilution method was adopted to prepare solution of different concentrations. All cell culture solutions were prepared under a Biological Safety Cabinet (Model: NU-400E, Nuaire, USA).

Cell seeding and growth measurement

Cell passaging was done using the standard protocol including PBS rinsing. Cells were counted using hemocytometer. For, Vero cells, 15×10^4 cells per ml were seeded into 96-well plate and incubated at 37°C, 5% CO_2 inside an incubator. After 24 hours, media was removed and 80 µl fresh media with 20 µL of As^{3+} sample were added into each well. Cytotoxicity was examined after 48 hours using a colorimetric assay based on (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, or MTT (Cell Titerassay, Promega, USA). For HeLa cell, similar procedure was followed with 25×10^4 cells per ml media. For inhibition experiments, 100 µl of cell solution and 25 µl of arsenic sample (for control, 25 µl of sterilized water) were mixed in each wellbefore incubation. Dead cells were removed after incubation of specific duration and washing was done with PBS. Cells were

trypsinized and counted with hemocytometer under a Trinocular Microscope with camera (Olympus, Japan).

Cell migration assay

Cell migration studies were performed according to the method discussed elsewhere [46]. Specific number of cells were seeded in 3 ml culture dishes and incubated for 24 hours to reach around 70% confluency. Old media was aspirated and replaced by fresh media with the dosing of different concentrations of arsenic (III) sample. After 6 hours, 300 µm (case I) and 1000 µm (case II) scars were created on the cell monolayer. Images were taken after every 24 hours for 7 to 8 days. Cell monolayer migration rates were calculated quantitatively by measuring the movement of the cell monolayer boundary and wound healing rate were evaluated qualitatively by observing the cell confluency as well as the reduction of the scar area.

Determination of cell growth parameters

To determine LC_{50}, absorbance data of each well at 570 nm wavelength were taken with a spectrophotometer (Biotek, USA). Based on the viable cells in control dish, percentages of viable cells were calculated for other wells that were treated with different As^{3+} concentrations. Peak cell concentrations were the concentrations of viable cells determined from apexes of growth curves and specific growth rate constants (μ) were calculated by fitting the growth curves exponentially using the following formula.

$$x = x_o \exp(\mu t)$$

Where, x_o is the initial cell concentration and x is the cell concentration at time t. Cell doubling time was calculated with the help of following relationship,

$$t_d = ln2/\mu$$

Where, t_d is the cell doubling time.

All of these growth parameters were averaged from at least three sets of distinct experiments and standard deviation is indicated with error bars.

RESULTS AND DISCUSSION

50% lethal concentration (LC_{50})

MTT based colorimetric cell proliferation assay was used to determine LC_{50} of As^{3+} sample for both the Vero cells and HeLa cells. Cell growth was assessed in terms of light absorbance at 570 nm wave length. Absorbance values for Vero cells and HeLa cells incubated with different concentrations of As^{3+} solutions are shown here in Table S1 and Table S2, respectively. As expected, with the increase in As^{3+} concentrations, viable cell concentration decreased. As the higher absorbance value infers the higher number of viable cells, considering the 100% viable cells for the absorbance of well that was kept in control (no arsenic), percentages of viable cells were calculated for other wells that were affected with different As^{3+} concentrations. From the viable cells count, 8 µM and 65 µM As^{3+} concentrations were found as LC_{50} for Vero cells and HeLa cells, respectively. Smaller value of LC_{50} for non-cancer cell compared to that of cancer cell indicates the domination and robust survival of cancer cell over non-cancer cells. This is one of characteristics of cancer cells causing severe metastasis. The results comply with the previous observations that healthy cells are more vulnerable to the adverse environment than the cancer affected cells [47].

Effects of arsenic on different cell growth parameters

So, effects of arsenic exposure were studied at cellular level to assess different cell growth characteristics. Once LC_{50} was determined, different growth parameters such as peak cell concentration, time to reach peak concentration, specific growth rate constant and cell doubling time were evaluated for Vero, as regular cells and HeLa, as cancer cells in presence of different As^{3+} concentrations below LC_{50}.

Initially, 6×10^4 cells/ml cells were seeded in 96-well plate for both type of cell lines with five different concentrations of arsenic (III) solution and fresh media was replenished when needed

Table 1 : Observation of wound healing assays qualitatively of Vero cells incubated with different As^{3+} solution of concentrations below LC_{50}.

Concentration of As^{3+}	Case I		Case II	
	Duration (day)	Observation	Duration (day)	Observation
Control	1-2	Fully healed	3	Fully healed
1 µM	1-2	Fully healed	3	Partially healed with lower cell confluency
2 µM	3	Partially healed with lower cell confluency	4	Around 40% area was healed
4 µM	6	Around 20% area was healed	7	Around 10% area was healed

Table 2 : Observation of wound healing assays qualitatively of HeLa cells incubated with different As^{3+} solution of concentrations below LC_{50}.

Concentration of As^{3+}	Case I		Case II	
	Duration (day)	Observation	Duration (day)	Observation
Control	2	Fully healed	3	Fully healed
4 µM	2	Around 90% area was healed	3	Healed with lower cell confluency
8 µM	3	Around 40% area was healed	4	Around 50% area was healed
16 µM	6	No healing	7	Around 10% area was healed

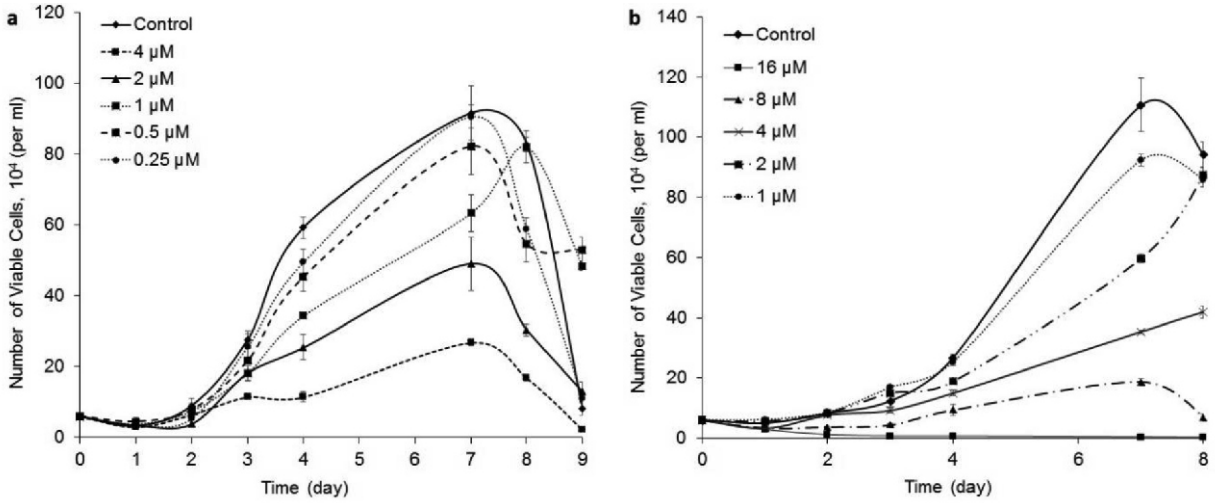

Fig. 1 : Viable cells incubated with different concentrations of As^{3+} solution below LC_{50} (a) Vero Cells; (b) HeLa cells.

during the experiment to make up for the evaporation loss as the experimental time was longer; 9 and 8 days for Vero cells and HeLa cells, respectively. Fig.1a shows the average cell counting of Vero cells in different wells with 4 µM, 2 µM, 1µM, 0.5 µM and 0.25 µM As^{3+} solution and Fig.1b shows that for HeLa cells with 16 µM, 8 µM, 4 µM, 2 µM and 1 µM As^{3+} solution. Highest growth of cells was observed for control (no arsenic) whereas growth behaviour was gradually started to be negatively affected with the increased arsenic concentration for both types of cells. Similar behaviour was reported before for other heavy metals such as copper, lead, zinc, and cadmium[48].

As seen in Fig.2, the highest peak cell concentration was found for the control and that was 92×10^4 cells/ml, and the peak cell concentrations were not significantly affected up to 1 µM arsenic (III) dosage (Fig.2a). From statistical analysis, p values were calculated as 0.84, 0.22, 0.13 for 0.25 µM, 0.5 µM As^3 and 1 µM As^{3+}, respectively, when compared with control. p values greater than 0.05 confirms the insignificant impact for lower concentrations. Arsenic (III) dosing above 1 µM caused the rapid drop in viable cells ($p<0.05$). In Vero cells, specific growth rate constant gradually decreased with the increase of arsenic (III) dosage (Fig.2c).For this growth parameter insignificant impact was observed for 0.25 µM As^{3+} ($p>0.05$), and the adverse cytotoxic effect was found for the remaining concentrations (p values < 0.05). Cell doubling time was calculated and found the lowest for the cells that were kept as control (Fig.2e). Minimal effect was observed at 0.25 µM concentrations ($p> 0.05$). Cell doubling time was found to increase for higher arsenic (III) concentrations, and for 4 µM it was almost twice compared to the control (p values <0.05). This was because of arsenic poisoning caused by higher dosage of arsenic (III) and cells took longer time to proliferate compared to regular healthy cells.

For HeLa cells, similar gradual changing phenomena of peak cell concentrationwas observed (Fig. 2b). Decrease in peak cell

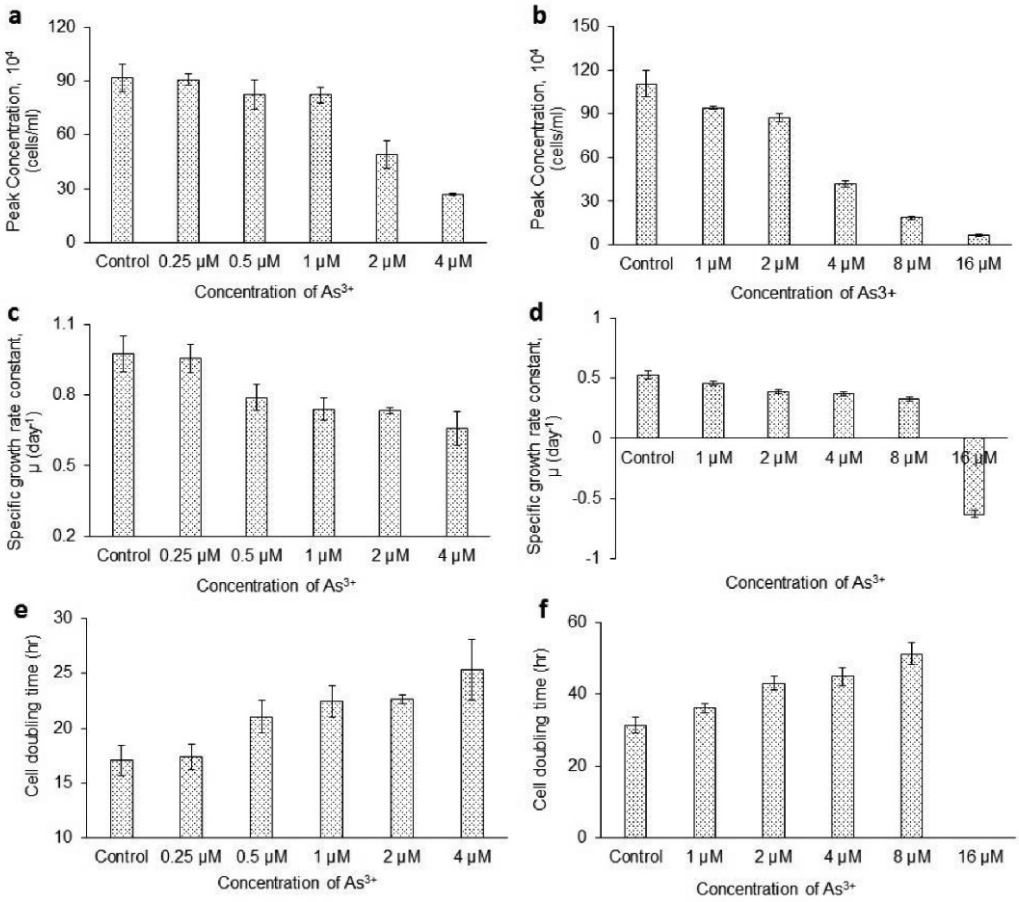

Fig. 2 : Bar chart showing peak cell numbers under different As^{3+} concentrations in case of (a) Vero cells (*p*- values were 0.84, 0.22, 0.13, 0.0024, and 0.00013, respectively when compared to control); and (b) HeLa cells (*p*- values were 0.033, 0.013, 0.00021, 0.00006, and 0.00004, respectively when compared to control). Specific growth rate constant of (c) Vero cells (*p*- values were 0.72, 0.03, 0.01, 0.006, and 0.007, respectively when compared to control); and (d) HeLa cells (*p*- values were 0.034, 0.0028, 0.0024, 0.00085, and 0.000002, respectively when compared to control). Cell doubling time of (e) Vero cells (*p*- values were 0.74, 0.031, 0.009, 0.003, and 0.0099, respectively when compared to control); and (f) HeLa cells, (*p*- values were calculated as 0.031, 0.0019, 0.0023, 0.00073, and 0.000003, respectively when compared to control).

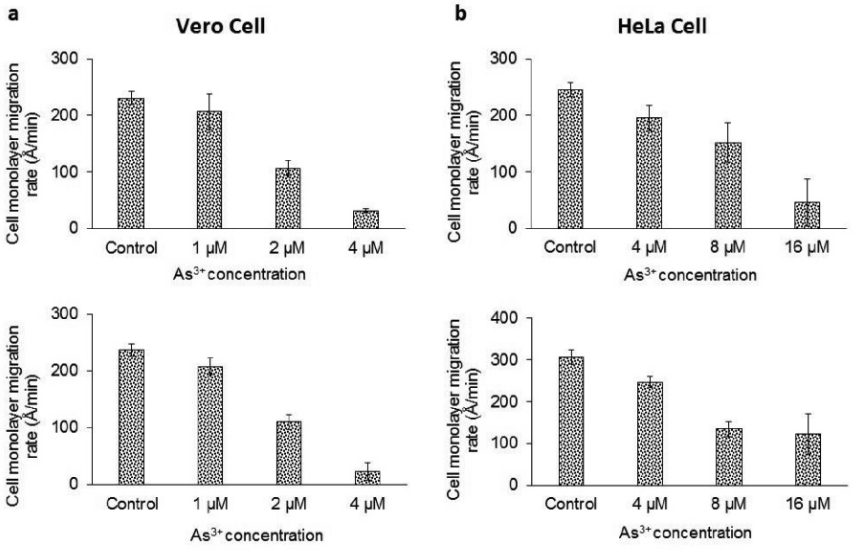

Fig. 3 : Cell monolayer migration rate of (a) Vero cells and (b) HeLa cells incubated with different As^{3+} concentrations below LC_{50}; (top) short distance and (bottom) long distance movement of cells. When compared to control, Vero cell *p*- values were 0.055, 0.00022, and 0.00004, respectively for longer distance and 0.28, 0.00025, and 0.000009, respectively for shorter distance. When compared to the control, Hela cell *p*- values were 0.0089, 0.00026, and 0.0035, respectively for longer distance; and for 0.028, 0.012, and 0.0014, respectively for shorter distance.

concentrations were statistically significant compared to the control ($p<0.05$). Specific growth rate indicated similar trend. For 16 μM arsenic (III) concentration specific growth rate constant was negative indicating significant poisoning from the very first day causing cell death (Fig.2d). For all three parameters (peak cell concentration, specific growth rate constant, and cell doubling time), effect of arsenic was evident beyond 1 μM($p << 0.05$). For both types of cells, the peak cell concentrations were reached in 7 to 8 days. Tables S3 and S4 show peak concentration, time to reach to peak concentration, specific growth rate constant and cell doubling time of Vero cells and Hela cells respectively, incubated with different As^{3+} solution of concentrations below $LC_{50.}$

Cell growth was negatively affected by the As^{3+} dosage when compared with control. Minimum cytotoxic effect was found at 0.25 μM and 1 μM arsenic (III) concentrations for Vero and HeLa cell lines, respectively. In addition, beyond 1 μM and 2 μM arsenic (III) dosage, level of cytotoxicity appeared to be significant for Vero and HeLa cell lines, respectively($p << 0.05$).

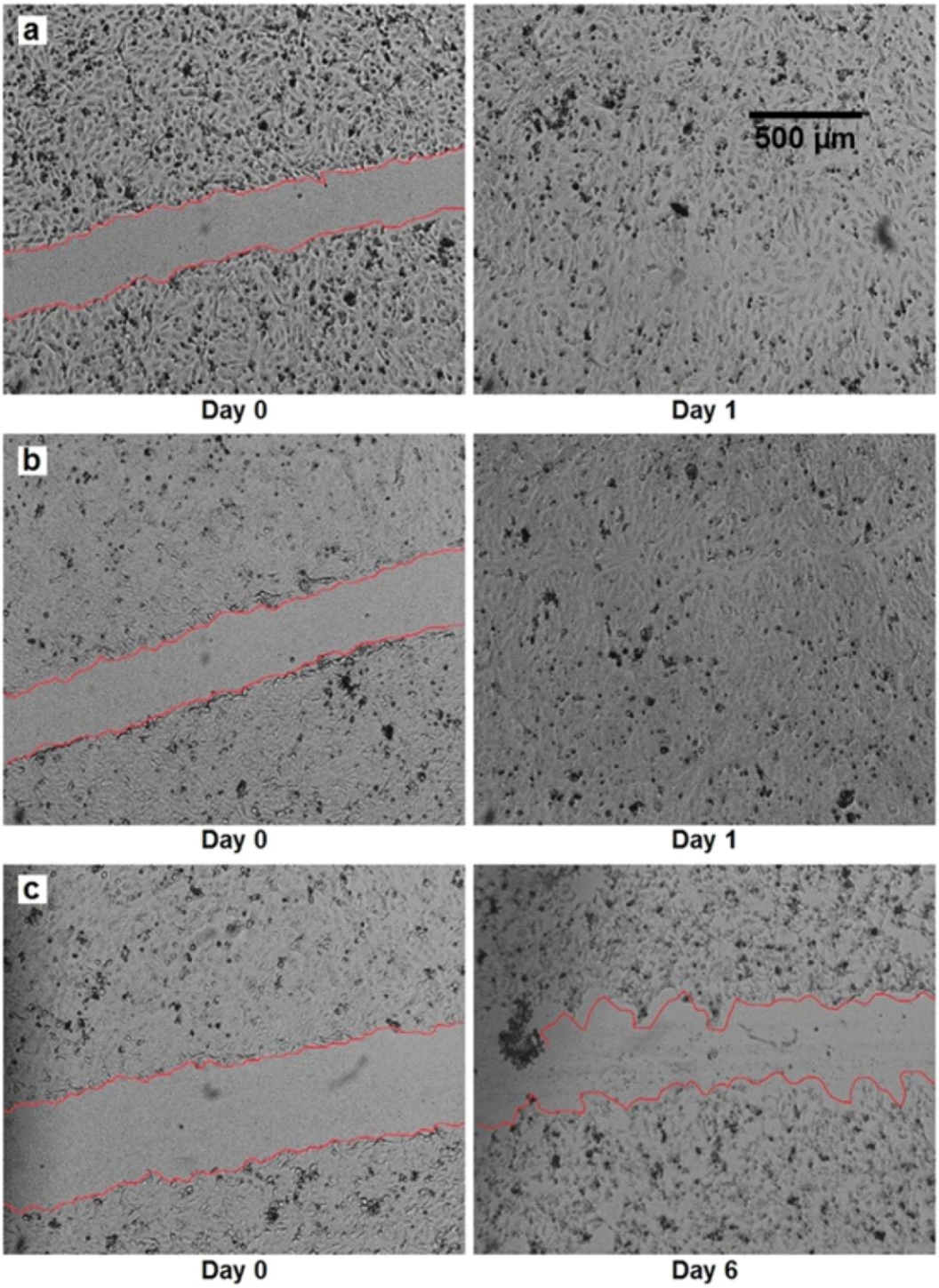

Fig. 4 : Observation of wound healing of Vero cells incubated with (a) control, (b) 1 μM and (c) 4 μM Arsenic (III) sample (Case I).

Cell migration rate and wound healing characteristics

Migration rate

Cell monolayer migration rates of Vero cells and HeLa cells were evaluated for each type of cells at two different conditions. This was performed to assess whether there were any differences in the migration behaviour for longer and relatively shorter distance scenario. Figure 3 shows the average cell monolayer migration rates, for relatively longer distance (1000 µm) and shorter distance (300 µm). As expected, cell monolayer migration rates were the highest for the control cells and the rate decreased with the increase in As^{3+} concentration. The migration rate dropped rapidly with the increment of arsenic (III) dosage for both cases for both cell lines. When compared with the control, the rate of migration for Vero cells dropped significantly for 2 and 4 µM As^{3+} concentration ($p<0.05$). p values greater than 0.05 for 1 µM As^{3+} indicates the insignificant impact on the migration rate when treated with 1 µM As^{3+}. In case of HeLa cells, the migration rate dropped significantly for all three concentrations during both long and short distance migration ($p<0.05$).

Again, it was found that cell migration rate was higher for HeLa cells than that of Vero cells. Comparison was evaluated statistically for 4 µM As^{3+} dose for both the Vero and HeLa cell line. Here,p values for Vero cells vs. HeLa cells were found as 0.00005 and 0.00025 for longer initial distance and shorter initial distance, respectively. This is most likely because of the fact that HeLa cells, being typical cancer cell line was robust and moved faster than the normal cell line despite arsenic poisoning[49]. In addition, Vero cells tend to die out quickly compared to HeLa cells at the same arsenic concentrations (observable from LC_{50} data), which ultimately lead to poorer healing procedure for Vero cells. Interestingly, Vero cell line migration behavior was found not to be affected by the travel distance (Fig.3a and 3c), whereas, slight difference was observed for HeLa cells. The latter observation could be because of inherently different migration rates of HeLa cells due to lesser impact of contact inhibition, which was more discernible at longer time.

Wound healing assay

Vero cell

Wound healing assays were performed to observe the healing process (function of both growth and migration) of mammalian cells with different As^{3+} dosages below LC_{50}. Though there are other factors involved in physiological wound healing, wound

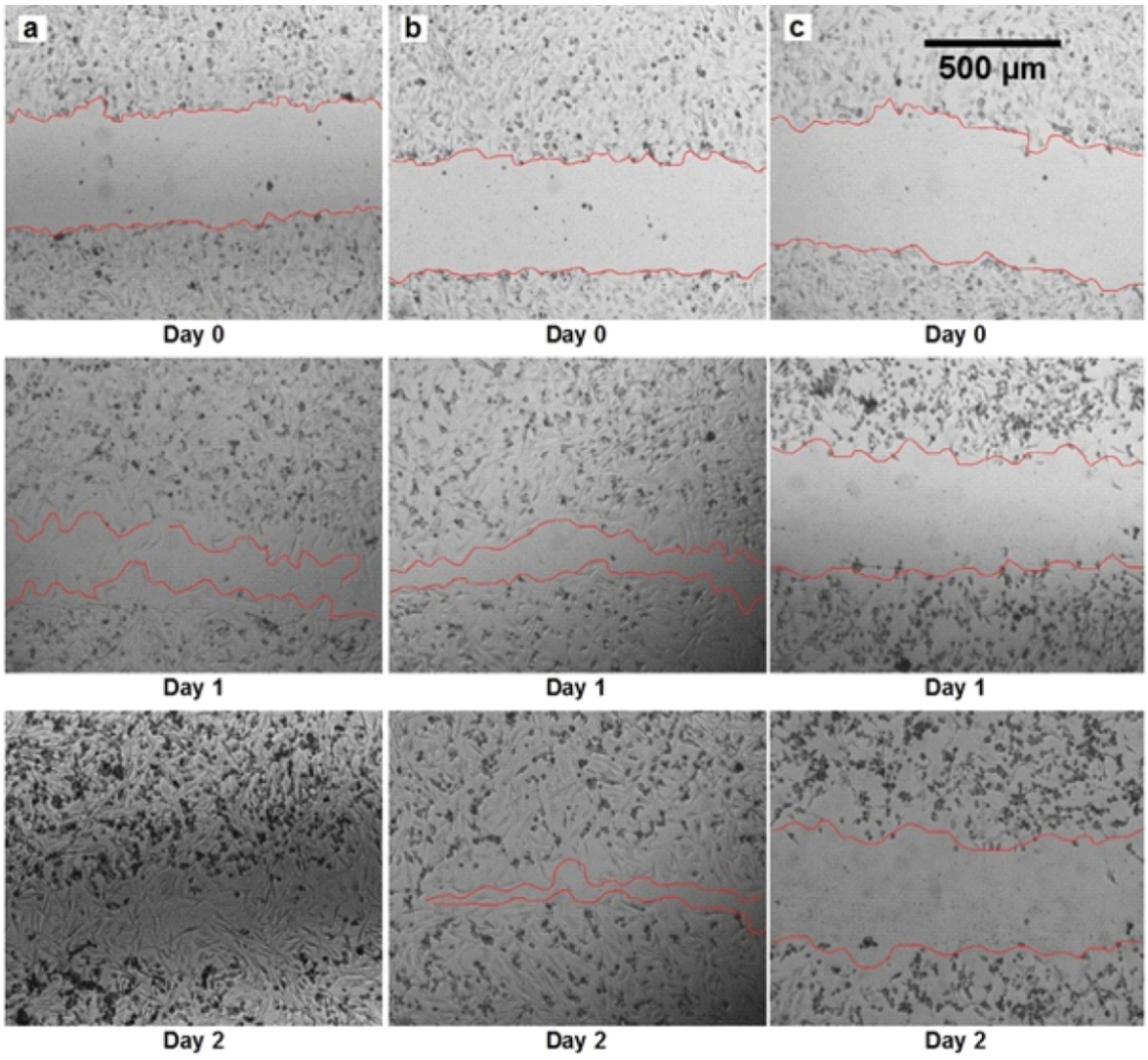

Fig. 5 : Observation of wound healing of HeLa cells incubated with (a) control, (b) 4 µM and (c) 16 µM arsenic (III) sample. (Case I)

healing assays typically provides a combined behavior governed by cell growth and cell migration rates. Qualitative results of wound healing assays of Vero cells are summarized in Table 1.Wound healing assays were done with Vero cell line in two conditions. Curing was observed from a 300 µm wide scar initially. Same qualitative results were found for control and 1 µM As^{3+} concentration. With the increment of arsenic dosing, wound healing behavior were started to be affected severely (Fig.4 and Fig.S1)

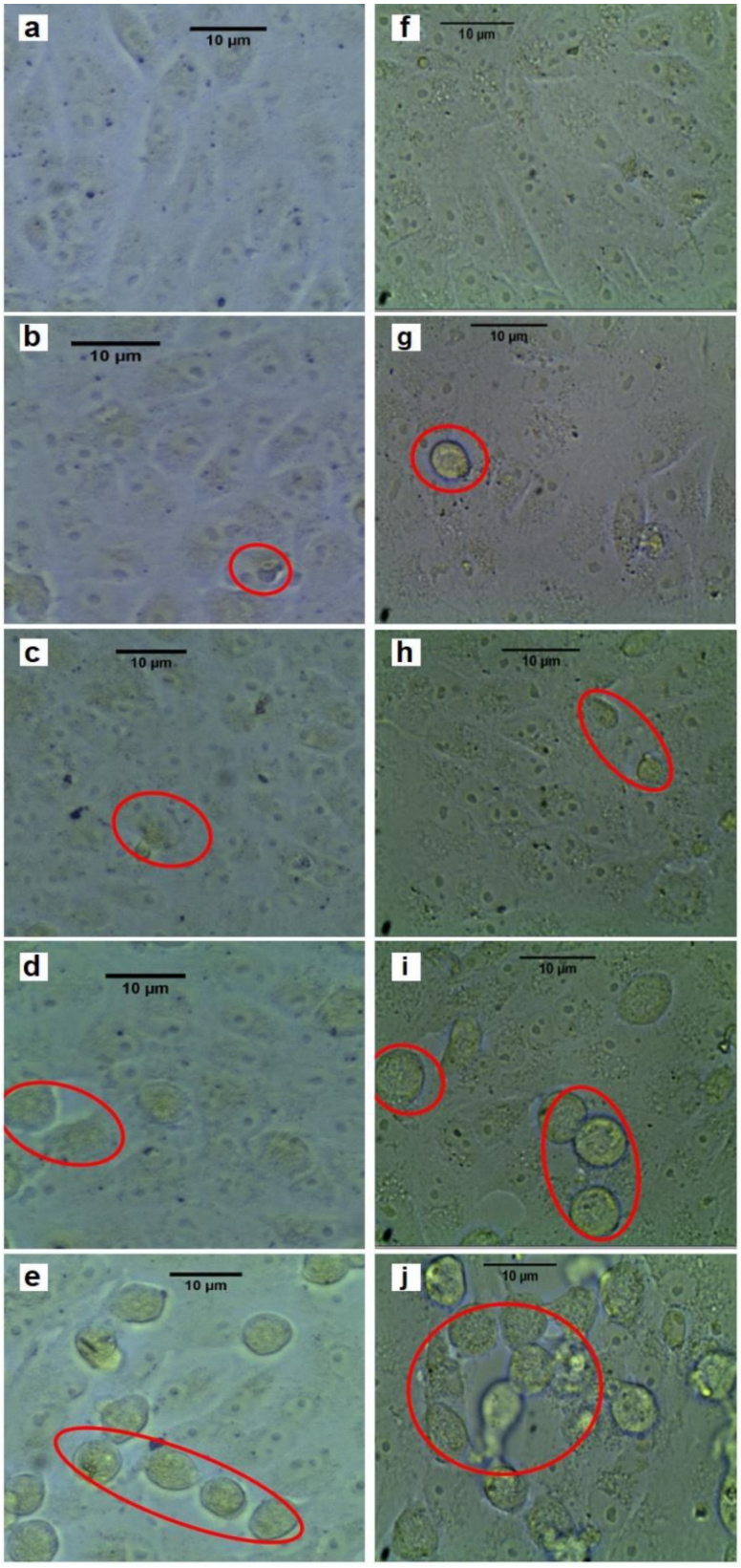

Fig. 6 : Vero cells incubated with different (control, 1, 2, 4 and 8 µM) As^{3+} solution of concentrations below LC_{50} (a-e) 20X magnification and (f-j) 40X magnification.

In another condition, healing was observed from a 1000 µm wide scar initially. Here, similar outcomes were observed with a slight variation from earlier observations (Fig. S2 and S3). Although, for case I, it was found that, wound healing was not disturbed at 1 µM As^{3+}, the effect of arsenic dosage on wound healing was noticeable at the lowest arsenic (III) dosing as well (*i.e.,* at 1 µM concentration of As^{3+}) for the latter condition. This can be because of the fact that cell growth can be hampered at very low initial confluency as caused by the larger wound.

HeLa cell

Wound healing assays were also performed for HeLa cell line with arsenic (III) concentrations of 16 µM, 8 µM, 4 µM and control for two similar wound conditions. For the first case, the scar width was 400 µm approximately and observations were made for 7 days. It took only two days for the wound to be healed completely for the control, however, for the arsenic (III) concentrations of 16 µM, no healing was done actually up to 6 days. Around 40% area was healed for the arsenic (III) concentrations of 8 µM and approximately 90% area was healed for the arsenic (III) concentrations of 4 µM. These observations are summarized in Table 2 and snapshots are shown in Fig.5 and Fig.S4. Although, the observations for 8 and 16 µM dosage were made for longer time, there was no positive change after day 2. Cells started to die afterwards and therefore, images up to the highest level of wound healing (day 2) have been shown.In case II, observations were made for 8 days. Here, a relatively bigger scar was made by 1 ml pipette tip that was around 1000 µm. For

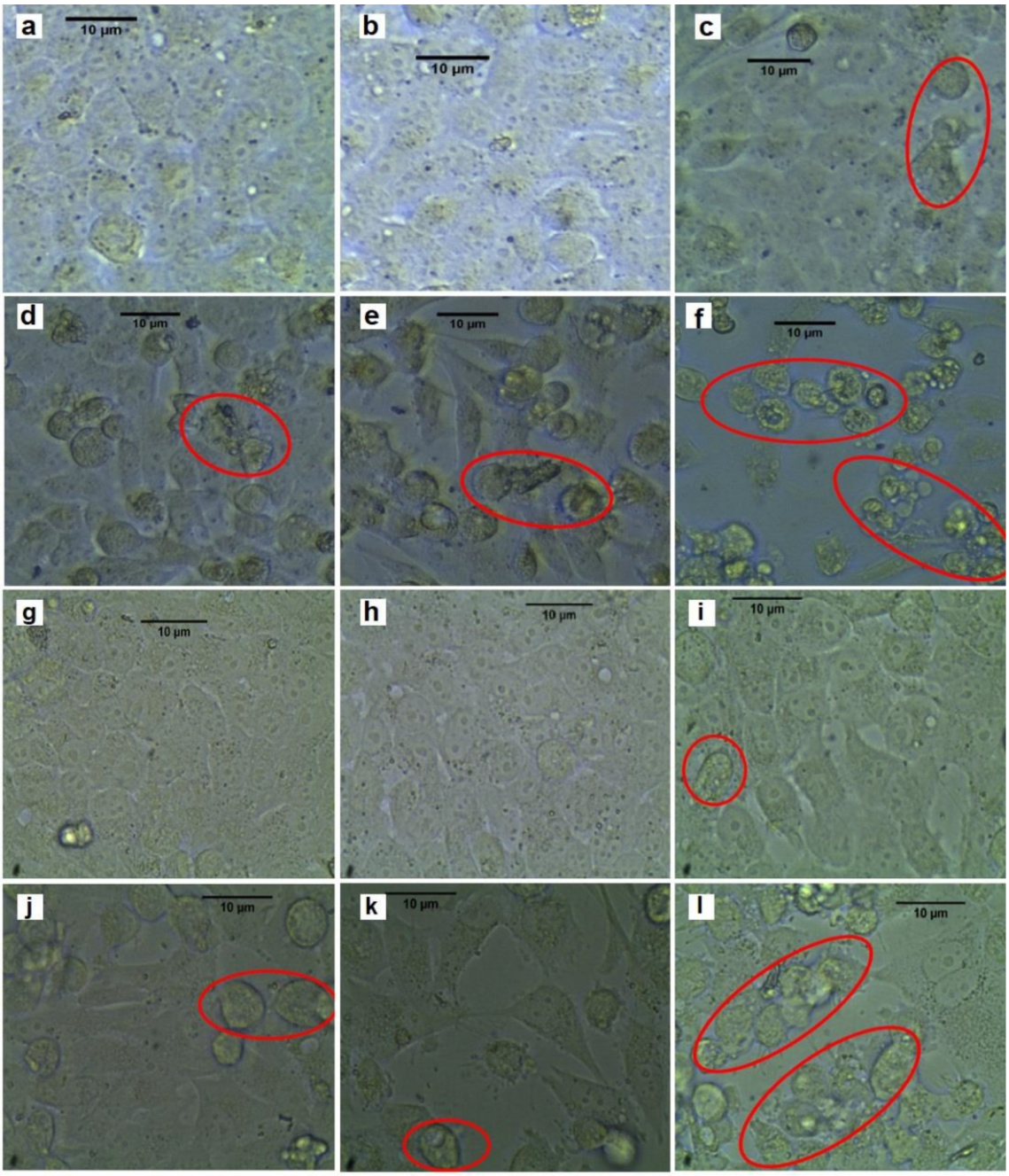

Fig. 7 : HeLa cells incubated with different (control, 2, 4, 8, 16 and 32 µM) As^{3+} solution of concentrations below LC_{50} (a-f) 20X magnification and (g-l) 40X magnification.

control, healing was finished within three days. Wound healing behavior was found to be slightly different for the different wound sizes as observed in case of Vero cells earlier (Fig.S5 and S6). Like case I, cells started to die afterwards and therefore, images up to the highest level of wound healing (day 3) have been shown. From Table 2, it can be inferred that the wound healing behavior was affected heavily with the exposure of arsenic (III).

Observation of cell damage in presence of arsenic (III)

To observe the morphological changes of mammalian cells in exposure to arsenic (III), cell images were taken after 24 hours of incubation with different As^{3+} concentrations below LC_{50} using a phase contrast microscope. The photos were taken at four different magnifications. Fig.6 shows the morphological changes of the Vero cells incubated with different As^{3+} concentrations at 20X and 40X magnifications.

Similarly, Fig.7 shows the images of HeLa cells incubated with different As^{3+} concentrations at 20X and 40X magnification.

In the figures, cells encircled by red line are the affected cells exposed to arsenic (III). Here, all affected cells were not encircled in the photos. For all the cases, it was noticed that, the higher the arsenic (III) concentrations, the more the dead and affected cells.In exposure to arsenic (III), cells started to be affected most likely because the inhibition of the lipoic acid molecule, leading to hindrance in Pyruvate dehydrogenase activity [17]. Cell morphology typically starts to change and elongated morphology with distinct cell membrane outlines turns into circular morphology. Elongated cells are the indication of healthy active cells and their protrusions during movement causes non circular morphology. Affected cells are often identified from damaged nucleus without distinct nuclear boundary. Eventually, the dead cells become round and detach from the surface, float on the liquid medium.

CONCLUSION

This study establishes the altered cell growth phenomena due to low arsenic poisoning and divulges the morphological changes at cellular level initiated by this slow poison. This would be helpful to unmask the actual biological mechanisms account for arsenic-induced toxicity and chronic effects on 'lungs, liver, skin'. Arsenic exposure was found to have very significant impact on the cytotoxic behaviour of both regular (Vero) and cancer (HeLa) cell lines. 8 µM and 65 µM As^{3+} concentrations were found as LC_{50} for Vero cells and HeLa cells, respectively. This indicates that, healthy cells are more vulnerable to the adverse environment than the cancer affected cells, which worsens the situation of cancer-patients dramatically. Growth of mammalian cells was negatively affected by the As^{3+} dosage when compared with control, both during short term and long-term observations. Minimum cytotoxic effect was found at 0.25 µM and 1 µM arsenic (III) concentrations for Vero and HeLa cell lines, respectively in terms of Peak cell concentration, specific growth rate constant, and cell doubling time. In addition, beyond 1 µM and 2 µM arsenic (III) dosage, severity of cytotoxicity was noticed for Vero and HeLa cell lines, respectively. The migration rate drops drastically with the increment of arsenic (III) dosage for both short distance and long distance travelling of both types of cell lines. Time required to heal an artificial wound was found to be longer for higher arsenic dosage. These behaviors are indicative of cellular responses during wound healing, and therefore, suggest that wound healing process could be affected heavily with the exposure of arsenic (III). Cell morphology typically starts to change and elongated morphology with distinct cell membrane outlines turns into circular morphology due to arsenic (III) dosing. Affected cells are often identified from damaged nucleus without distinct nuclear boundary. This study establishes the distinct thresholds of cytotoxicity and dose-dependent responses for cancer and non-cancer cells exposed to Arsenic (III), which enriches the perceptions and understandings of arsenicosis in terms of cellular level damage and morphological deformation.

COMPETING INTERESTS

There is no competing interest declared by any of the authors.

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REFERENCES

1. Baudouin C, Charveron M, Tarroux R, Gall Y (2002) Environmental pollutants and skin cancer. Cell Biol Toxicol 18:341348
2. Gowd SS, Govil PK (2008) Distribution of heavy metals in surface water of Ranipet industrial area in Tamil Nadu, India. Env Monit Assess 136:197207
3. Saladi RN, Persaud AN (2005) The causes of skin cancer: A comprehensive review. Drugs of Today 41:3
4. Nabi AH, Rahman MM, Islam LN (2005) Evaluation of Biochemical Changes in Chronic Arsenic Poisoning among Bangladeshi Patients. Int J Environ Res Public Health 2:385393
5. Uddin R, Huda NH (2001) Arsenic Poisoning in Bangladesh. Oman Med J 26:207
6. Alam MG, Allinson G, Stagnitti F, et al (2002) Arsenic contamination in Bangladesh groundwater: a major environmental and social disaster. Int J Environ Health Res 12:235253
7. Ahmad SA, Khan MH, Haque M (2018) Arsenic contamination in groundwater in bangladesh: Implications and challenges for healthcare policy. Risk Manag Healthc Policy 11:251261. https://doi.org/10.2147/RMHP.S153188
8. IARC (2004) Some drinking-water disinfectants and contaminants, including arsenic. Monographs on chloramine, chloral and chloral hydrate, dichloroacetic acid, trichloroacetic acid and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone. IARC Monogr Eval Carcinog Risks Hum 84:269477
9. D'Ippoliti D, Santelli E, De Sario M, et al (2015) Arsenic in Drinking Water and Mortality for Cancer and Chronic Diseases in Central Italy, 1990-2010. PLoS One 10:e0138182
10. Smith AH, Goycolea M, Haaque R, Biggs ML (1998) Marked increase in bladder and lung cancer mortality in a region of northern Chile due to arsenic in drinking water. Am J Epidemiol 147:660669
11. Chen CJ, Wang CJ (1990) Ecological correlation between arsenic level in well water and age-adjusted mortality from malignant neoplasms. Cancer Res 50:54705474

12. Hopenhayn-Rich C, Biggs ML, Fuchs A, et al (1996) Bladder cancer mortality associated with arsenic in drinking water in Argentina. Epidemiology 7:117124

13. Morales KH, Ryan L, Kuo TL, et al (2000) Risk of internal cancers from arsenic in drinking water. Environ Health Perspect 108:655661

14. Ferreccio C, Gonzále C, Milosavjlevic V, et al (2000) Lung cancer and arsenic concentrations in drinking water in Chile. Epidemiology 11:673679

15. Hei TK, Waldren C (1998) Mutagenicity of arsenic in mammalian cells: Role of reactive oxygen species. PNAS 95:81038107

16. Hussain A, Raveendran VA, Kundu S, et al (2018) Mechanisms of Arsenic-Induced Toxicity with Special Emphasis on Arsenic-Binding Proteins. In: Stoytcheva M, Zlatev R (eds) Arsenic - Analytical and Toxicological Studies. Intech Open

17. Hughes MF (2002) Arsenic toxicity and potential mechanisms of action. Toxicol Lett 133:116

18. Vahter M (1994) Species differences in the metabolism of arsenic compounds. Appl Organomet Chem 8:175182

19. Thomas DJ, Styblo M, Lin S (2001) The cellular metabolism and systemic toxicity of arsenic. Toxicol Appl Pharmacol 176:127144

20. Scott N, Hatlelid KM, MacKenzie NE, Carter DE (1993) Reactions of arsenic (III) and arsenic(V) species with glutathione. Chem Res Toxicol 6:102106

21. Delnomdedieu M, Basti MM, Otvos JD, Thomas DJ (1994) Reduction and binding of arsenate and dimethylarsinate by glutathione: a magnetic resonance study. Chem-Biol Interact 90:139155

22. Del-Razo LM, Styblo M, Cullen WR, Thomas DJ (2001) Determination of trivalent methylated arsenicals in biological matrices,. Toxicol Appl Pharmacol 174:282 293

23. Aposhian HV, Zheng B, Aposhian MM, et al (2000) DMPS-arsenic challenge test II. Modulation of arsenic species, including monomethylarsonous acid (MMAIII), excreted in human urine. Toxicol Appl Pharmacol 165:7483

24. Gregus Z, Gyurasics A, Csanaky I (2000) Biliary and urinary excretion of inorganic arsenic: monomethyl arsonous acid as a major biliary metabolite in rats. Toxicol Sci 56:1825

25. Dixon HBF (1997) The biochemical action of arsonic acids especially as phosphate analogues. Adv Inorg Chem 44:191227

26. Lagunas R (1980) Sugar-arsenate esters: thermodynamics and biochemical behavior. Arch Biochem Biophys 205:67 75

27. Gresser MJ (1981) ADP-arsenate. J Biol Chem 256:5981 5983

28. Kenney LJ, Kaplan JH (1988) Arsenate substitutes for phosphate in the human red cell sodium pump and anion exchanger. J Biol Chem, 263:79547960

29. Jr. Miller WH, Schipper HM, Lee JS, et al (2002) Mechanisms of Action of Arsenic Trioxide. Cancer Res 62:

30. Aposhian HV, Hodgson E, Bend JR, Philpot RM (1989) Biochemical toxicology of arsenic. Rev Biochem Toxicol 265299

31. Hu Su, L. and Snow, E.T. Y (1998) Arsenic toxicity is enzyme specific and its affects on ligation are not caused by the direct inhibition of DNA repair enzymes. Mutat Res 408:203218

32. Peters RA (1995) Biochemistry of some toxic agents. I. Present state of knowledge of biochemical lesions induced by trivalent arsenical poisoning. Bull John Hopkins Hosp 97:120

33. Szinicz W. L and F (1988) Effects of As2O3 on gluconeogenesis. Arch Toxicol 61:444449

34. Reichl Szinicz, L., Kreppel, H. and Forth, W. F-X (1988) Effect of arsenic on carbohydrate metabolism after single or repeated injection in guinea pigs. Arch Toxicol 62:473 475

35. European Food Safety Authority (2009) Arsenic in Food. Scientific Opinion of the Panel on Contaminants in the Food Chain. EFSA J 7:1199

36. Shankar S, Shanker U, Shikha (2014) Arsenic Contamination of Groundwater: A Review of Sources, Prevalence, Health Risks, and Strategies for Mitigation. Sci World J 2014:. https://doi.org/ http://dx.doi.org/10.1155/2014/304524

37. Chen Y, Ahsan H (2004) Cancer Burden from Arsenic in Drinking Water in Bangladesh. Am J Public Heal 94:741744

38. Mazumder GD (2007) Arsenic and non-malignant lung disease. J Env Sci Heal A Tox Hazard Subst Env Eng 42:18591867

39. Meliker JR, Wahl RL, Cameron LL, Nriagu JO (2007) Arsenic in drinking water and cerebrovascular disease, diabetes mellitus, and kidney disease in Michigan: a standardized mortality ratio analysis. Environ Heal 6:

40. Tseng CH, Chong CK, Tseng CP, et al (2003) Long-term arsenic exposure and ischemic heart disease in arseniasis-hyperendemic villages in Taiwan. Toxicol Lett 137:1521

41. Enterline PE, Day R, Marsh GM (1995) Cancer related to exposure to arsenic at a copper smelter. Occup Environ Med 52:2832

42. Janga Y-C, Somannaa Y, Kim H (2016) Source, Distribution, Toxicity and Remediation of Arsenic in the Environment A review. Int J Appl Environ Sci 11:559581

43. Loewenberg S (2017) The Poisoning of Bangladesh: How Arsenic Is Ravaging a Nation

44. Yeh S, How SW, Lin CS (1968) Arsenical cancer of skinhistological study with special reference to Bowen's disease. Cancer 21:312339

45. Cebrian ME, Albores A, Aguilar M, Blakely E (1983) Chronic arsenic poisoning in the north of Mexico. 2:121133

46. Grada A, Otero-Vinas M, Prieto-Castrillo F, et al (2017) Research Techniques Made Simple: Analysis of Collective Cell Migration Using the Wound Healing Assay. J Invest Dermatol 137:e11e16. https://doi.org/ https://doi.org/10.1016/j.jid.2016.11.020

47. Alfarouk KO, Stock C-M, Taylor S, et al (2015) Resistance

to cancer chemotherapy: failure in drug response from ADME to P-gp. Cancer Cell Int 15:71. https://doi.org/10.1186/s12935-015-0221-1

48. Pudpong S, Chantangsi C (2015) Effects of Four Heavy Metals on Cell Morphology and Survival Rate of the Ciliate Bresslauides sp. Trop Nat Hist 15:117125

49. Caccavale J, Fiumara D, Stapf M, et al (2017) A simple and accurate rule-based modeling framework for simulation of autocrine/paracrine stimulation of glioblastoma cell motility and proliferation by L1CAM in 2-D culture. BMC Syst Biol 11: