EFFECT OF CHROMIUM AND ARSENIC ON GROWTH AND MORPHOLOGY OF MICE FETAL CALVARIAL CELLS (MFCC)

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Abstract

Heavy metals are naturally present in environment but due to anthropogenic activities they are becoming pollutant. Naturally chromium is present in three oxidation states and its excessive amount is toxic and carcinogenic which lead to different cancers and other abnormalities. Drinking water contaminated with arsenic is big health problem all over the world and especially to developing countries. Mice calvarial cells were isolated from 18 day old fetus. Cell line was established from calvarial cells. Effect of chromium and arsenic was tested by applying different concentration of chromium and arsenic to cells. There is change in morphology of cells at higher chromium concentration, cells become round in shape and lysis of cells was also observed. Arsenic also proved to be very toxic for calvarial cells and there was complete change in morphology of cells at higher arsenic concentration. The effect of chromium and arsenic were more drastic with longer exposure time. In conclusion we can say that both chromium and arsenic are very toxic for mouse fetal calvarial cells.

Introduction

Heavy metals naturally found in earth crusts at different levels but anthropogenic activity drastically altered their biochemical balance and now many heavy metals are acting like pollutants (Xie *et al.*, 2006; Reena *et al*, 2011). These heavy metals are also used by all the three domains of life but in trace amounts and their excess may cause toxicity (Yan *et al*., 2010; Mertz, 1981). Different types of trace elements are used by different organisms. The amount and pathways of their uptake also vary from organism to organism (Reena *et al*, 2011). In living organisms these trace elements are essential in coordination and facilitate different enzyme activities and binding of molecules to receptor sites (Yan *et al*, 2010).The major source of uptake of these heavy metals is air, water and soil for plant sand then they enter in the food chain in the form of vegetables and different crops (Islam *et al*, 2007).

Chromium is a heavy metal and found in nature in three thermodynamically stable oxidation states as Cr[0], Cr[III], Cr[VI], while later two are more commonly absorbed by living organisms (Anatoly, 2011) . Chromium is an essential trace element for different living organisms (Stout *et al*, 2009), in humans it is involved in glucose and fat metabolism and its deficiency may lead to the same symptoms as of diabetes and cardiovascular diseases (Richard, 1997). Epidemiological studies show that it acts as a carcinogenic in excess (Cohen *et al*, 1993). Its inhalation leads to the cancer of respiratory system while its ingestion leads to the cancer of digestive tract. The study in different animal models also shows that the toxicity of Cr also causes damage to DNA and an oxidative stress in liver and kidney (Anita *et al*., 2009). Its toxicity also causes female infertility.

Arsenic is a metalloid and is 20^{th} most common element of earth crust. It can exist in four oxidation states as -3, 0, +3, +5. Seven different forms of arsenic have been found in water, among them Arsenite (As^{III}) and Arsenate (As^{v}) are more common (IARC, 2004). In humans and many other animals metabolism may involves the conversion of inorganic arsenic to mono, di and tri methylated products (Zuzana *et al*, 2009). Drinking water contaminated with As is effecting a huge population all over world including both developed and developing countries (Chen *et al*, 2009; Aniruddha and Urmi, 2010; Ning *et al*, 2010). If contaminated water is used for irrigation than arsenic is also taken by crops which are later used by human population and animals (Liu *et al*, 2004). It acts as a carcinogen and causes skin, lung and bladder cancers (Rahman *et al*, 2009; Christopher *et al*, 2009). Chronic arsenic exposure is also involved in diabetes, cardiovascular diseases, liver problems and chronic lungs diseases (Christopher *et al*, 2009; Clark *et al*, 2009). Some studies revealed that it acts as a risk modifying factor and also damage nerve cells (Gavin *et al*, 2008; Ning *et al*, 2010). In females exposure may cause abortion and still births. Studies on animal models show that its exposure damaged uterine tissue and abrogate estrogen signaling pathway (Aniruddha and Urmi, 2010). In utero and postnatal exposure also causes irreversible arterial and lungs damage (Christopher *et al*., 2009; Clark *et al*, 2009). In spite of all these toxic effects arsenic had been used in different medicines to treat syphilis, pernicious anemia, trypenosomiasis and chronic myeloid leukemia etc (Indira *et al*, 2010).

In the present research work we will study the effect of chromium and arsenic on growth and morphology of mice fetal calvarial cells.

Materials and Methods

Fetal calvarial cells isolation: 18th day pregnant mice was drowned by cervical dislocation. Mousewas dissected to isolate fetus from uterus. The fetus was isolated into petridish and washed with PBS. Head region was separated from rest of body and finally removed the brain. Washed the calvarial region and finally cut it into small pieces and added into eppendorf. Incomplete medium was added and re-suspend the cells by passing it from needle several time. When cells were dissociated from each other spin the cells at 1800 rpm for 5 min. the pellet was suspended in complete medium and counted the number of cells by hemocytometer. The cells were added in culture flask and incubated at 37°C with 5% in CO_2in humid environment.

Medium: DMEM Media (Gibco) was supplemented with 10% Fetal bovine serum (FBS) (PAA), and 100 U/ml penicillin, and 100 µg/ml streptomycin (ICN). The MFCC were maintained in above culture media, with incubation at 37°C with 5% CO_2, and culture passaging after three days.

Effect of Chromium and Arsenic on Cells: Stock solution of Chromium (potassium chromate) and Arsenic (Sodium Arsenate) was prepared and filter sterilized with 0.2 µm filter (Orange Scientific).Sterilized 24 (for chromium) and 12 (for arsenic) well plastic plates (NUNC) were used for experiment and equal number of cells were added in each well, 12000 and 54000 cells were added for Chromium and Arsenic effect respectively. Cells were grown in the presence of 0.2, 0.4, 0.6, 0.8 and 1.0 µg/ml concentration of chromium and arsenic for 4 days at 37°C with 5% CO_2 in a humidified environment.
After each day of growth morphology of the cells was observed and images of cells were taken with inverted microscope (Olympus, IX51) with DP-12 camera and stored. Medium was removed and washed the cells twice with PBS. The cells were detached with Trypsin-EDTA and counted the number of cells on each day by hemocytometer.

Results

Calvarial Cell Line: Cells started attaching to plastic surface of flask after few hours. Morphologically all cell were same. The shape was intermediate between spindle (MSCs like) and flattened (fibroblasts like) (Fig. 1). After few days cells became confluent (Fig. 2). Cells were grown for several passages to develop cell line of calvarial cells.

Effect of Chromium on growth and morphology of mice fetal calvarial cells: A remarkable change was observed in the morphology of cells by increasing the concentration of chromium. Cells became round shape with increased concentration of chromium (repetition). Cell lysis with leakage of granules from the cells was also observed with increasing concentration of chromium (repetition). (Fig: 2). A marked reduction in number of mice fetal calvarial cells was also observed (Fig: 3).

Effect of Arsenic on growth and morphology of mice calvarial cells: Growth and morphology of mice calvarial cells was also badly affected by increasing the concentration of Arsenic. A marked reduction in the number of cells was observed on fourth day at 1µg/ml (Fig. 5).Morphology of the mice calvarial cells was also altered with increasing Arsenic concentration (Fig. 4).

Discussion

Studies on heavy metals in different part of the world like India, China, Bangladesh, USA, Taiwan, Germany Japan, Australia etc. proved that they are environmental pollutants (Xie *et al*, 2006; Chen *et al*, 2009). A number of *in vivo* and *in vitro* studies have been performed to check their toxicity. But it is found that it is easy to control the conditions *in vitro*. Different studies showed that chromium (Anatoly, 2011) and arsenic (Zuzana *et al*, 2009) in some of their oxidation states act as persistent environmental pollutants and are not degradable by chemical or biological means (Konstantin and Anatoly, 2008).

When we observed the effect of chromium on mice calvarial cells, it was found that in control number of cells increased with passage of time while there was reduction in number of cells with increase in concentration of chromium as well as increase time of exposure. Remarkable reduction in number of cells was observed on third and fourth day especially there was great reduction in number of cells at day four (Fig. 3). There was also change in morphology of cells and cells became round in shape. All these changes clearly indicate that chromium has a very toxic effect on the mice calvarial cells. Different in vitro studies showed cell death and toxicity in different cell lines due to chromium (Debasis *et al*, 2002; Bagchi *et al*, 2001; Neelam *et al*, 2008; Fei *et al*, 2002). It is also found that after treatment with chromium cells lost there alignment and adherence to the dish surface and turned to round shape (Mohamed and Nadia, 2009).

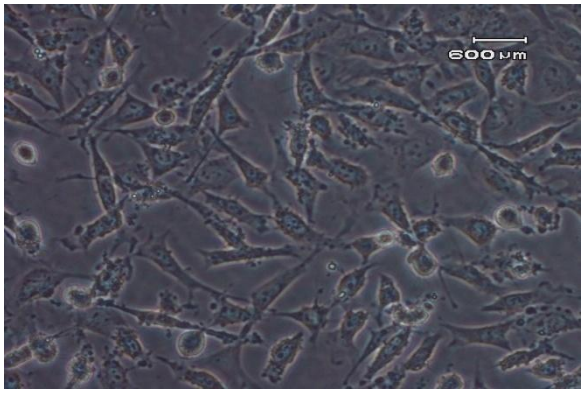

Fig. 1(a). Initial growth of mice fetal calvarial cells

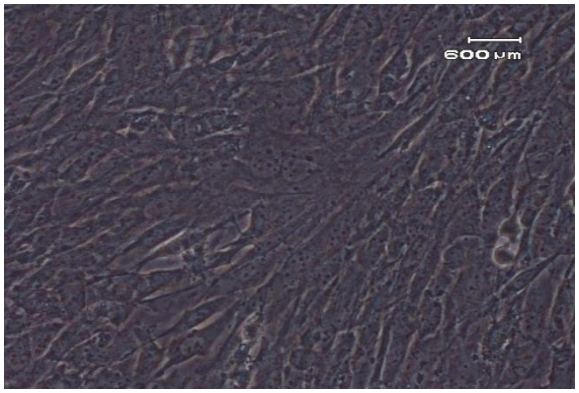

Fig. 1(b). Growth of calvarial cells at confluency

Day-4

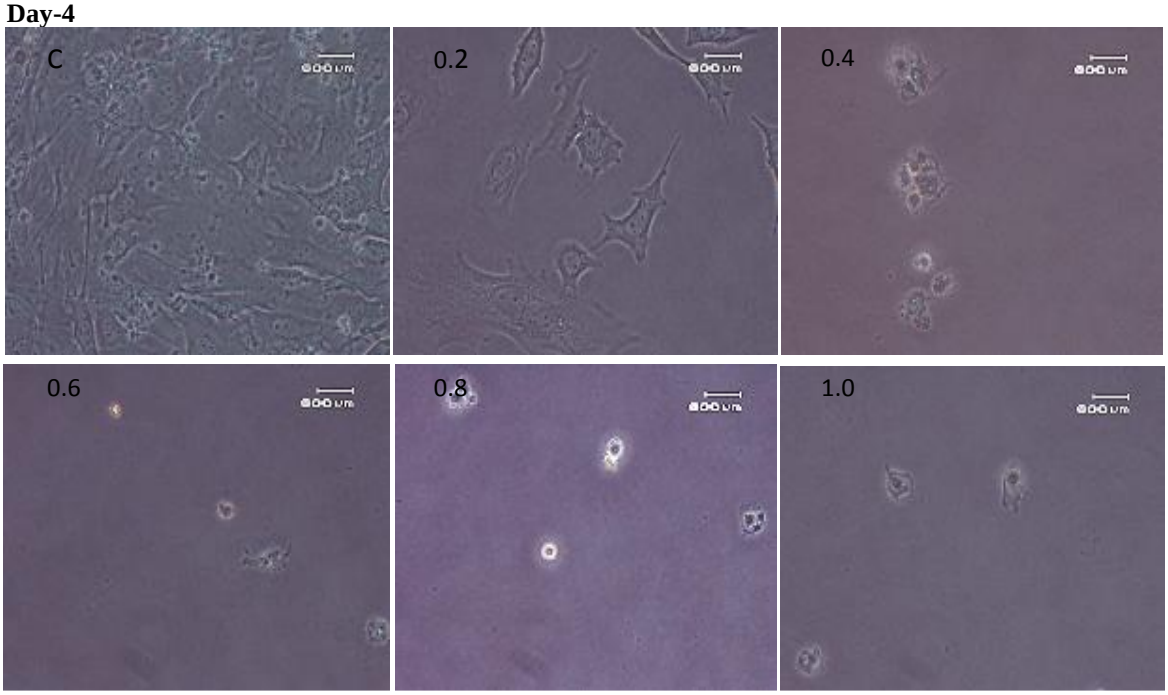

Fig. 2. Images of cells at 4th day after chromium treatment. C (Control) and 0.2, 0.4, 0.6, 0.8 and 1.0 are the concentration of chromium in µg/ml. The treated concentration of chromium is given

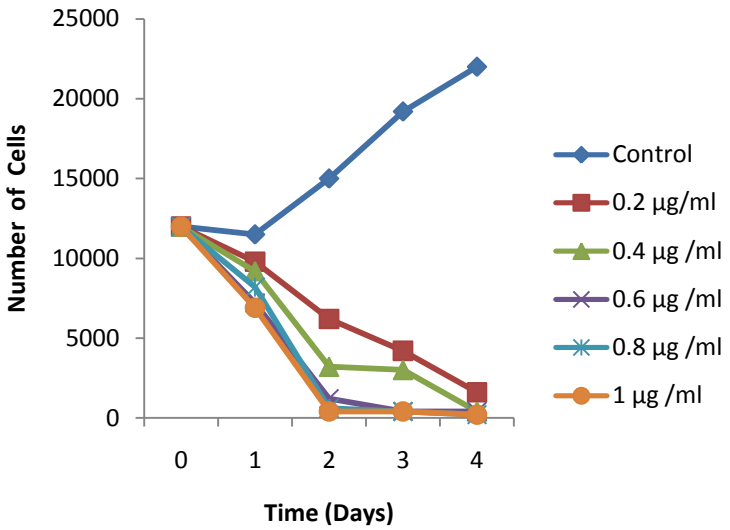

Fig. 3. Effect of heavy metal (Chromium) on growth of mice fetal calvarial cells.

Day-4

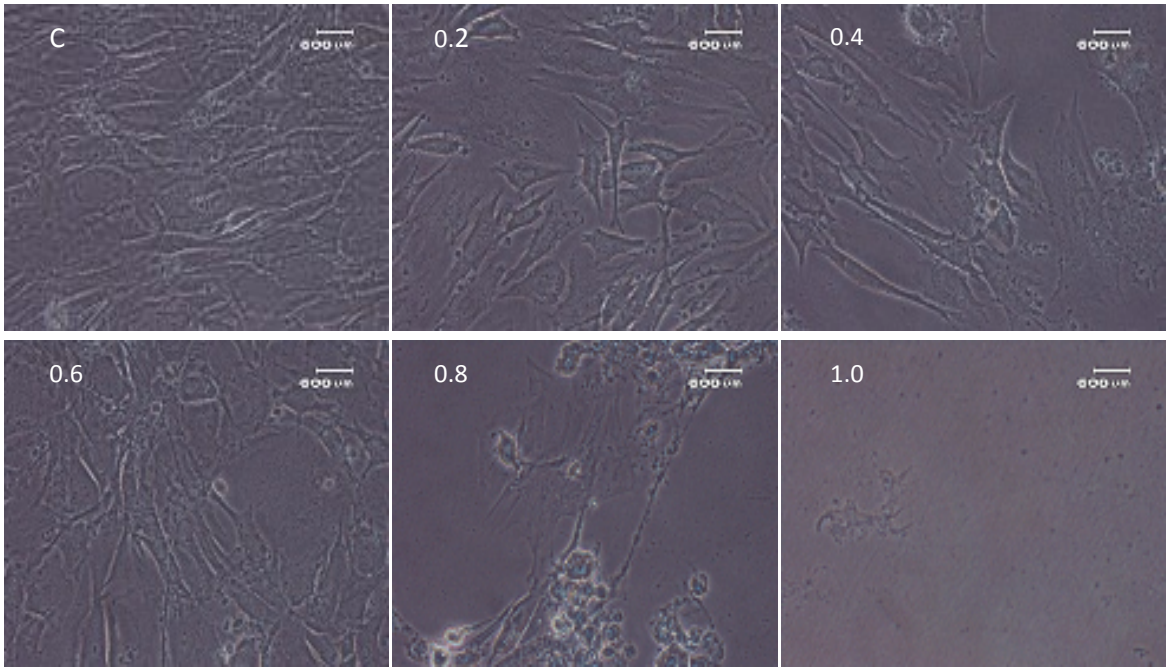

Fig. 4. Images of cells at 4th day after arsenic treatment. C (Control) and 0.2, 0.4, 0.6, 0.8 and 1.0 are the concentration of arsenic in µg/ml. The treated concentration of arsenic is given on each

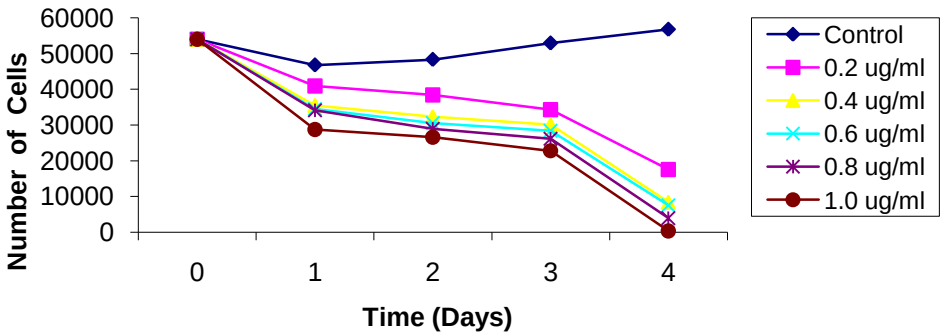

Fig. 5. Effect of heavy metal (Arsenic) on growth of mice fetal calvarial cells.

In case of arsenic, maximum reduction was observed at fourth day and 1.0µg/ml concentration. Morphology of mice calvarial cells also changed to a greater extent at 1.0µg/ml. These changes showed that high concentration of arsenic is toxic for mice calvarial cells. A number of studies confirmed the toxicity (Amal *et al*, 2007) and carcinogenicity of Arsenic but paradoxically a large number of studies are available in which arsenic induced cell death is used against acute promyelocytic leukemia (APL) and other malignancies (Yihu *et al*., 2010; Teruaki *et al*, 1998;Tun-Kyi *et al*, 2008;Indira *et al*, 2010;Geniece *et al*, 2005; Frazier *et al*, 2006). Mainly Arsenic trioxide is used as a chemotherapeutic agent, although different concentrations of As_2O_3 are effective against different cell lines. Some studies suggest that 0.5–2 µM dose of $As_2O_3$is enough to induceapoptosis (Min *et al*, 1999). It is also found that depending on the concentration it may act as cytotoxic and cytostatic (Michael *et al*, 2000). In a study a serious ventricular tachycardia was observed at the therapeutic doses of arsenic trioxide in APL patients, it was found that it causes myocardial apoptosis (Yan *et al*, 2002). So it is found that Arsenic arrest cell growth and induce cell death depending on its concentration and type, it may be highly toxic or used as a chemotherapeutic agent.

In this study results indicate that heavy metals are toxic for mice calvarial cells. Chromium is more toxic than arsenic. But high concentration of heavy metals like chromium and arsenic is enough to change the morphology, and to reduce the cell proliferation.

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