

Anti-Proliferative Effect of Arsenic, Cadmium and Lead on Human Placental Cells

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ABSTRACT

Heavy metals are ubiquitously distributed in the environment and can effect human health. Some of these heavy metals can even cross placenta and cause harm to developing fetus. In the present study we investigated the anti-proliferative effects of arsenic, cadmium and lead on human placental cells (PCs). PCs were isolated from placental tissue and cell line was developed. Anti-proliferative effects of arsenic, cadmium and lead were tested by neutral red uptake assay. Both arsenic and cadmium proved to be very toxic for PCs. There was marked decrease in cells proliferation when cells were exposed to metal concentrations for a period of 24 hrs. Reduction in proliferation was recorded on exposure to lead but the effect of lead was not as severe as arsenic and cadmium. Arsenic, cadmium and lead are very toxic for PCs. Proper measures should be taken for the disposal of heavy metals to protect the environment and humans from exposure.

Keywords: Placental cells, Heavy metals, Proliferation, Cytotoxicity, Morphology.

INTRODUCTION

During pregnancy placenta, amniotic fluids and fetal membranes help in growth and development of fetus by facilitating exchange of gases, nutrients, and waste products between mother's circulation and fetus (Gude *et al.*, 2004; Kippler *et al.*, 2010). Placenta also prevents pollutants and harmful substances from entering into fetus with the help of macromolecular protein complexes present on its cell membrane (Osman *et al.*, 2000; Kuhnert *et al.*, 1987). Placenta is also known as dual marker in case of certain heavy metals that can effect the fetus (Iyengar and Rapp, 2001).

Cadmium exposure to human occurs by cigarette smoking, consumption of polluted food including cereals, vegetables, nuts, pulses, starchy roots or potatoes, meat, meat products and contaminated

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water (Zenzes *et al.*, 199; Jarup *et al.*, 1998) and retains in placenta with the help of metallothionin and does not enter in fetus (Brambila *et al.*, 2002). But cadmium alters placental transport of calcium and zinc, causes early decidualization of human endometrial stroma cells, inhibits trophoblast cell migration by affecting actin cytoskeletal organization, reduces leptin synthesis, enhances corticosterone concentrations and interferes with placental progesterone production (Ronco *et al.*, 2009; Lin *et al.*, 1997; Tsutsumi *et al.*, 2009; Alvarez and Chakraborty, 2011; Stasenکو *et al.*, 2010; Kawai *et al.*, 2002). Endoplasmic reticulum (ER) is essential for survival and normal functioning of cells and cadmium also has an effect on ER (Ferri and Kroemer, 2001; Rao *et al.*, 2004). ER chaperons causes the folding of nascent proteins but if these client proteins exceed the chaperons, ER undergoes stress which in turn reduces the placental development and fetal growth (Iwawaki *et al.*, 2009; Lian *et al.*,

2011; Yung *et al.*, 2008). If the fetus is exposed to cadmium then it can impair child's IQ, birth weight, birth length and head circumference (Tian *et al.*, 2009; Gonzalez-Cossio *et al.*, 1997; Salpietro *et al.*, 2002; Osman, 2000; Ronco *et al.*, 2009; Gundacker *et al.*, 2010; Zhu *et al.*, 2010; Lin *et al.*, 2011). Cadmium toxicity is found to be responsible for this stress related damage to placenta (Wang *et al.*, 2012). Since Cadmium has a long half-life in the human body; the placental burden is affected by the length of gestation (Kantola *et al.*, 2000).

Arsenic is a metalloid that occurs in environment both naturally and due to human activities. It is present in glasses, pesticides, food preservatives and pigments etc. Human are exposed to arsenic via contaminated drinking water, rice, other grains and fish etc. (ACSH, 2002). The two oxidation states of inorganic arsenic, arsenite (AsIII) and arsenate (AsV) can cross and accumulate in placenta (DeSesso *et al.*, 1998; Lindgren *et al.*, 1984) upto a level five folds more than control (Concha *et al.*, 1998a). Its accumulation can have deleterious vascular effects leading to placental abnormalities and less flow of blood which eventually lead to retarded fetal growth (Hopenhayn *et al.*, 2003). Higher exposure to arsenic causes increased miscarriage rates, premature deliveries and lower birth rates (Aschengrau *et al.*, 1989). If the pregnant lady is exposed to lead contaminated air or food then lead may cross the placenta to enter into fetus by passive diffusion (Goyer, 1990) and accumulates in syncytiotrophoblast cells of placenta and minimise cytochrome oxidase activity (Reichrtova *et al.*, 1998), thus may cause neurodevelopmental disorders and subclinical brain dysfunction in neonates.

Transportation of lead and cadmium through placenta occurs by binding to glutathione (GSH) forming a complex which is then transferred with the help of ABC family such as MRP1, MRP2 and P-glycoprotein (Gundacker *et al.*, 2010; Thevenod, 2010, UNECB).

In the present study placental cells were used to

investigate the effect of heavy metals such as arsenic, cadmium and lead on proliferation and morphology of cells.

MATERIALS AND METHODS

Heavy Metals: Three heavy metals were used in this study including lead, cadmium and arsenic. Stock solutions were prepared and filter sterilized with 0.2 µm filter (Orange Scientific). Heavy metals solutions were stored at room temperature.

Isolation of placental cells (PCs): The placental tissue was collected after normal delivery. The tissue was cut into small pieces and added in sterile phosphate buffer saline (PBS) containing penicillin and streptomycin (20 ml PBS with 1 ml penicillin/streptomycin). The samples were immediately transferred to cell culture lab. The tissue pieces were washed multiple times with PBS and chorion layer of placenta was separated by surgical knife. The placental tissue was cut into small pieces and added the piece on tissue culture dish. Let the tissues to adhere for short time (5min) and completed medium (DMEM containing glutamine, 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin) was added. The dish was incubated in CO₂ incubator at 37°C with 5% CO₂ in humidified environment. Observe the dish on daily basis to see migrating cells from tissue.

Cell Culture and CV Staining: When enough cells migrated out from tissue explants, the explants were removed and cells were treated with Trypsin-EDTA (GIBCO, USA). The numbers of cells were counted and cultured them into new culture flasks under standard culturing conditions. When cells became confluent, the cells were stained with crystal violet (CV) (Sigma). For staining, cells were washed twice by PBS, stained with 0.5% CV solution for 5-10 min. The stain was removed from plate with dH₂O, until no stain come out. Images were taken by inverted microscope.

Cytotoxicity assay: PCs were cultured in 75 cm²

tissue culture flask. The cells were incubated for 24 hrs at 37°C in a humidified environment with 5% CO₂ to grow the cells in monolayer. When cells grew to 90% confluency, they were washed with PBS, trypsinized with 1X Trypsin-EDTA. The cells were counted with hemocytometer and 5×10^3 cells were added in each well of 96 wells plate with a total volume of 200 μ l of complete DMEM medium. Cells were incubated for 24 hrs at 37 °C in a 5% CO₂ incubator with humidified environment. The old medium was replaced by 200 μ l of fresh medium containing 0-10 μ g/ml cadmium, 0-10 μ g/ml arsenic and 0-100 μ g/ml lead respectively and incubated the plates under the same culture conditions for 24 hrs. Cytotoxic effects were tested by neutral red uptake method. Aspirate treatment medium and incubated cells with Neutral red medium for 3 h at 37°C. Cells were washed twice with PBS and 150 μ l of Neutral red stain solution was added in each well and put the plate on shaker at 100 rpm for 10 min. The supernatant was taken and measured the differential absorbance at 492nm and 630 nm using ELISA reader (Humareader plus, HUMAN). All assays were done in triplicate.

RESULTS

Isolation and Crystal Violet Staining of PCs: When explant were cultured under standard culture condition, cells started moving out from explant after 3 days and in 5 days they covered the surface of whole plate. Cells were trypsinized and cultured again. Initially the cells were showing spindle shaped morphology and also these were very rapidly dividing cells.

When cells were stained with CV to observe the morphology of cells after 2nd passage, the cells were appearing in between the spindle and flattened shape (Fig. 2).

Cytotoxic effect of arsenic, cadmium and lead on PCs: PCs were exposed to different concentration of arsenic, cadmium and lead for 24 hrs but all the metals have different effect on proliferation of PCs.

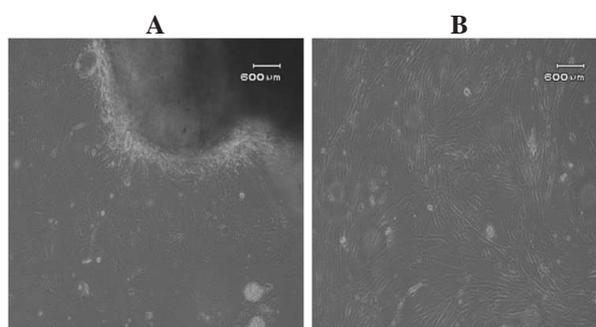


Figure 1. Growth of PCs. (A) The cells are migrating out from placental explant tissue. (B) Morphology of PCs after confluent growth. The cells are appearing spindle shaped and showing similar morphology to that of mesenchymal stem cells (MSCs).

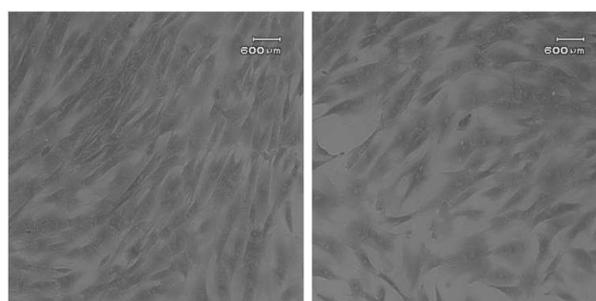


Figure 2. Morphology of PCs. The morphology of cells is in between spindle and flattened shape as clearly visible in both the images after CV staining.

The maximum effect was observed by arsenic followed by cadmium and least was observed in case of lead. When LC₅₀ was calculated for arsenic and cadmium, it was 2.52 and 6.2 μ g/ml respectively. The percentage reduction in growth in case of lead on 24 hrs of exposure was 46% only and LC₅₀ could not be calculated as lead did not have drastic effects on proliferation of PCs compared to arsenic and lead.

DISCUSSION

In the present study we investigated in vitro effect of three heavy metals on human placental cells. A significant difference in arsenic content in the human placenta under exposed (5.9 ng/g) and non-exposed (2.6ng/g) environmental conditions have been demonstrated (Iyengar and Rapp, 2001). It was observed in this study that when PCs were exposed to arsenic for 24 hrs, its effect was most severe compared to other heavy metals used in this study

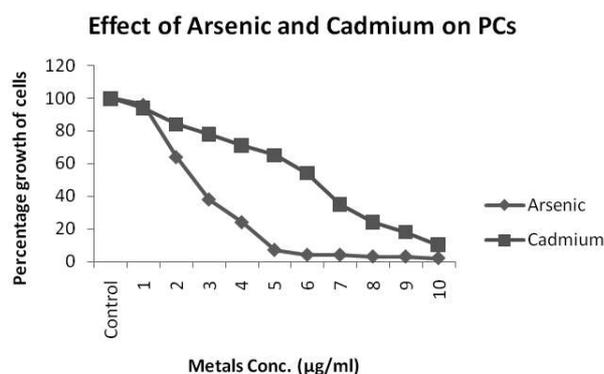


Figure 3. Graphical representation of effect of arsenic and cadmium on PCs. There is gradual reduction in proliferation of cells with increase in concentration of arsenic and lead but arsenic has more severe effect even at lower concentration on proliferation of cells as is clearly shown in figure. There was gradual decrease in proliferation of cells on increase in concentration of cadmium.

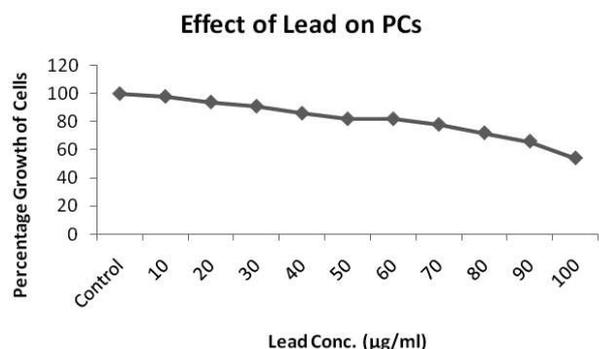


Figure 4. Graphical representation of effect of lead on PCs. Lead did not have severe effect on proliferation of PCs and even at 100 µg/ml concentration there is just 46% reduction in proliferation of cells.

of LC50 which came out to be 2.52µg/ml. When inside the cells arsenic mainly effects mitochondria, this result in changes in the trans-membrane potential and ultimately leads to death of cells (Haga *et al.*, 2005).

Cadmium also proved to be fatal for PCs but to a lesser extent than arsenic. On exposing PCs to cadmium for a period of 24 hrs the LC50 was calculated to be 6.2µg/ml. According to a study the average concentration of cadmium under non-exposed environmental conditions was 4 ng/g, with a range of 1–6 ng/g based on the wet weight of placenta (Iyengar and Rapp, 2001). The placental cells causes interruption in cadmium passage either

completely or partially (Baranowska, 1995; Kuhnert *et al.*, 1982; Roels *et al.*, 1978; Schramel *et al.*, 1988; Korpela *et al.*, 1986; Needham *et al.*, 2011) so there are chances that fetus get exposed to cadmium when higher level of cadmium is present inside the maternal body. According to different studies increase in double-stranded RNA-activated kinase (PKR)-like ER kinase (PERK)-peIF2a signaling is associated with decreased cellular proliferation in placenta (Lian *et al.*, 2011) which is enhanced due to cadmium exposure (Wang *et al.*, 2012).

Effect of lead was found to be much different than that of arsenic and cadmium and that could be due to permeability of lead to PCs. On exposure to lead for 24 hrs, the percentage reduction in proliferation of cells was calculated to be 46%. The average concentration of lead in placental tissue is approximately 34 ng/g, (normal range 5–60 ng/g) (Iyengar and Rapp, 2001). There does not exist maternal-fetal barrier for lead and thus level of lead in fetus blood is nearly equal to maternal blood lead level (Goyer, 1990), so lead exposure to mother during fetal development could be very deleterious for the health of developing fetus especially on nervous system.

Heavy metals are present in environment and it is very hard to avoid exposure of heavy metals. The exposure of heavy metals during pregnancy is great concern because it is not only dangerous for the life of mother but it could be lethal for developing fetus. The present study was conducted to show the anti-proliferative activity of arsenic, cadmium and lead on human PCs. It was concluded that among these heavy metals arsenic is the most toxic heavy metal for PCs as it had maximum anti-proliferation effect, followed by cadmium and least anti-proliferative effect was observed in the case of lead. There is an alarming increase of heavy metals in environment in developing countries and it can result in many abnormalities and malignancies in human and this is also a serious danger to our future generations.

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