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

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Abstract.- Heavy metals present in environment can effect human health in different ways. These heavy metals can even cross placenta and cause harm to the developing fetus. In the present study the anti-proliferative effects of arsenic, cadmium and lead were studied on human placental chorion cells (PCCs). The cells were isolated by explant method from placental tissue. 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 PCCs. There was marked decrease in cell proliferation when cells were exposed to different metal concentrations for 24 h. There was reduction in proliferation of cells on exposure to lead but the effect of lead on PCCs was not as severe as that of arsenic and cadmium. It is concluded that arsenic, cadmium and lead are toxic to PCCs and hence there is a need to adopt proper measures to reduce the exposure of animals and human.

Keywords: Placental chorion cells, heavy metals, proliferation, cytotoxicity.

INTRODUCTION

Human placenta is a specialised organ through which nutritious substances, gases and electrolytes are transported from the mother to fetus and waste products from fetus to mother during gestation. Certain metals have also been reported to cross the placental barrier if the mother becomes exposed directly or idirectly to these toxic metals (Magdalini, 2010). Lead, cadmium and arsenic are some of the common industrial environmental pollutants in Pakistan.

Lead enters placenta during pregnancy via dust, polluted food and water (Hubermont *et al*. 1978; Carrington *et al*., 1993), by simple diffusion, (Georgieff *et al.*, 2000) facilitated by divalent metal transporter 1 (DMT1) which has high affinity for iron and lead (Gunshin *et al*., 1997) and is present in cytoplasm and membrane of syncytiotrophoblast since 8th gestation week (Georgieff *et al*., 2000). Another mechanism of lead transportaion is through specialised binding proteins (CaBPs) which are present in trophoblastic cells of placenta (Hosking, 1996) and exihibt strong affintity for lead. Lead toxicity can cause low birth weight (McClain and Becker, 1972) and dcreased total uptake of amino acids by fetus (Gerber *et al*., 1978).

Cadmium enters the maternal circulation by tobacco smoke (Zenzes *et al*, 1995), polluted food and water (Jarup *et al*., 1998). Cadmium does not pass on to fetus and is retained in placenta (Loiacino *et al*., 1992; Berlin *et al*., 1992). During gestation, increased need of iron induces DMT1 pathway which enhances cadmium uptake that competes with iron for DMT1 thus leading to iron deficiency and elevated toxicity of cadmium in both maternal organs and placenta. Cadmium toxicity causes decreased blood flow to placenta, hemorrhage, necrotic foci, which harm the endocrine function of placenta (Magdalini, 2010) and decreased zinc availability to fetus (Suzuki *et al*., 1990).

Humans get exposed to arsenic through various sources such as contaminated drinking water, certain eatables rice, grains, fish etc. (ACSH, 2002). Arsenic has the ability to cross the placenta with a transfer rate of 80% and even pass on to fetus (Rudge *et al*., 2009). Arsenic exposure can lead to abortion due to aberrant placental vasculogenesis and placental insufficiency (Wenjie *et al*., 2007).

In the present study PCCs were first isolated and later effect of arsenic, cadmium and lead was observed on proliferation and morphology of cells, with an objective to assess the damage done to these

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cells during gestation, which may end up with some teratological abnormalities.

MATERIALS AND METHODS

Heavy metals

Three heavy metals were used in this study including Lead (Lead nitrate), Cadmium (Cadmium chloride) and Arsenic (Sodium arsenite). Stock solutions were prepared and filter sterilized with 0.2 µm filter (Orange Scientific) and stored at room temperature for future use.

Isolation of placental chorion cells (PCCs)

The placental tissue was collected after normal delivery of a baby boy in Lady wellington Hospital, Lahore after obtaining the consent of mother. The tissue was cut and added in sterile PBS containing penicillin streptomycin (20 ml PBS with 1 ml penicillin/streptomycin). The sample was immediately transferred to cell culture lab. The tissue pieces were washed multiple times with PBS and upper layer of placenta was separated by surgical knife. The chorion layer was cut into small pieces with a sharp razor, which were then added to the tissue culture plates. After five min, when the tissue adhered to the surface of the culture plates, complete medium was added and incubated at 37°C with 5% CO_2 in humidified environment in a CO_2 incubator. The culture plates were observed on daily basis for migration of the cells away from the tissue.

Cell culture and crystal violet staining

When enough cells migrated out from tissue explants, the explants were removed by tip of micropipette and cells were treated with Trypsin-EDTA (GIBCO, USA). The number of cells were counted and cultured them into new culture flasks under standard culturing conditions. When the cells became confluent, they were stained with 0.5% crystal violet (Sigma) for 5-10 min. After washing the cells twice with phosphate buffered saline (PBS). The stain was removed from plate and cells washed with distilled water, until no stain came out. Images were taken by inverted microscope.

Cytotoxicity

PCCs were cultured in 75 cm^2 tissue culture flask. The cells were incubated for 24 h at 37°C in a humidified environment with 5% CO_2 to grow the cells in monolayer. When cells grew to 90% confluency, they were washed with PBS, trypsinized with 1 ml of 1X Trypsin-EDTA. The cells were counted with hemocytometer and 5 x 10^3 cells were added in each well of 96 wells plate with a total volume of 200 µl of complete DMEM medium. Cells were incubated for 24 h at 37°C in a 5% CO_2 incubator. The old medium was replaced by 200 µl of fresh medium containing 0-10 µg/ml cadmium, 0-10 µg/ml arsenic and 0-100 µg/ml lead, respectively and incubated the plates under the same culture conditions for 24 h.

Cytotoxic effects were tested by neutral red uptake method. The medium was aspirated treatment medium, while the cells were incubated with neutral red medium for 3 h at 37°C. Cells were washed with PBS and images were taken. Neutral red destainer solution (150 µl) was added in each well and the plates were shaken on a shaker at 120 rpm for 10 min. The differential absorbance of supernatant was recorded at 492nm and 630 nm using ELISA reader (Humareader plus, HUMAN). All assays were done in triplicate.

RESULTS

Characteristics and viability of PCCs

When explant were cultured under standard culture condition, cells started moving out from explant in couple of days and after five days they covered the surface of whole plate. The cells were trypsinized and sub-cultured. Initially the cells divided very fast (Fig. 1) and showed spindle to flattened shape (Fig. 2) after crystal violet staining.

Effect of arsenic, cadmium and lead on PCCs

When PCCs were treated with arsenic, cadmium and lead, there was great reduction in proliferation of cells on exposure to arsenic and cadmium (Fig. 3). There was also change in morphology of cells on exposure to arsenic and cadmium and at 10 µg/ml concentration, no cell survived (Fig. 5).

The effect of lead on PCCs were not as severe and (Fig. 4) there was not much change in morphology of cells (Fig. 5). LC_{50} values were

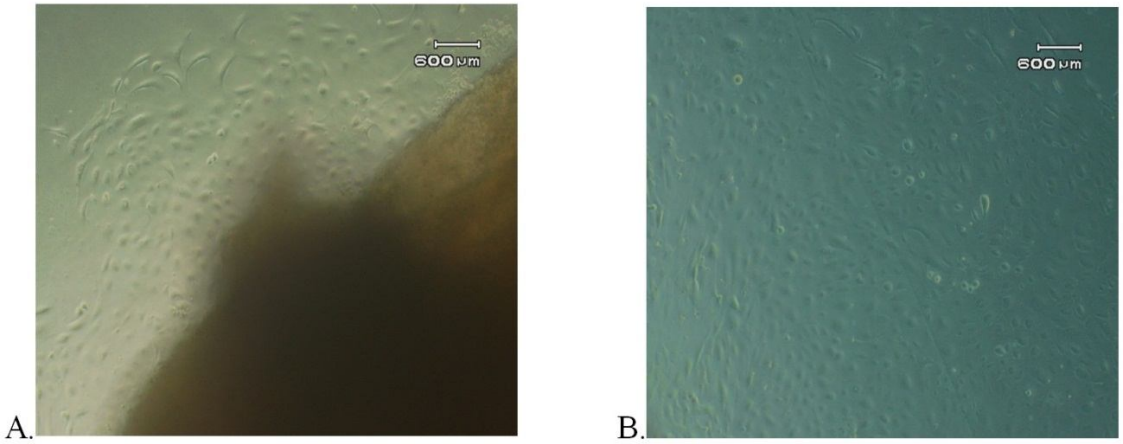

Fig. 1. Growth of PCCs. A. The cells are migrating out from chorion layer of explant. B. Morphology of PCCs after confluent growth. Dividing cells are appearing round in shape. The dark area in figure A is explant piece of chorion membrane.

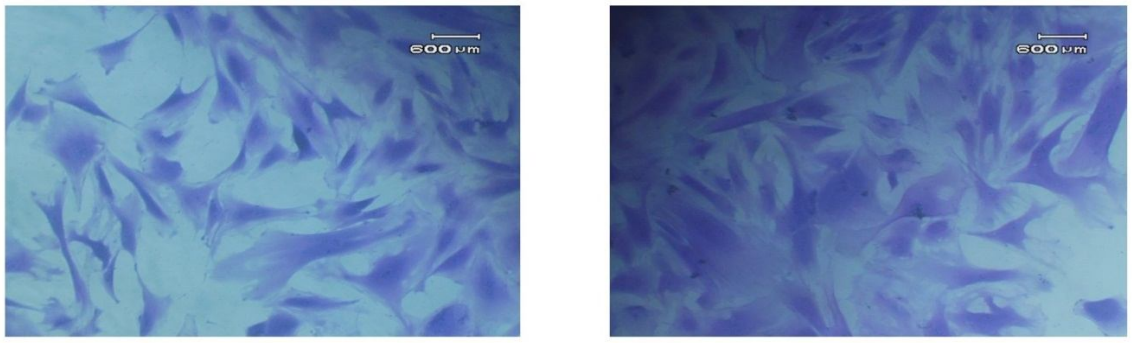

Fig. 2. Morphology of PCCs. The cells appear as all gradations between spindle and flattened shape. All the cell are of same shape so there is no mixed growth of cells.

calculated in arsenic and cadmium treated cells and these were 1.98 and 9.9 µg/ml, respectively which clearly indicated that arsenic has more severe inhibitory effect on proliferation of PCCs, than cadmium. Lead was not very toxic for cells and there was 40% reduction in growth of PCCs at 100 µg/ml concentration of lead.

DISCUSSION

The present study was conducted to investigate *in vitro* effect of arsenic, cadmium and lead on placental chorion cells (PCCs) of human.

When PCCs were exposed to arsenic, there was reduction in proliferation and also change in morphology of cells. LC_{50} on exposure of arsenic for 24 h was 1.98 µg/ml. Placenta partially blocks arsenic penetration, so exposure of arsenic may result in exposure of fetus, due to transport of arsenic across placental membrane (Iyengar and Rapp, 2001). Arsenic exposure alters mitochondrial trans-membrane potential (Haga *et al*., 2005) and due to production of reactive oxygen species (ROS), it results in apoptosis of cells. In the present study these factors could be the cause of arsenic induced chorionic cell death (Kim *et al*., 2006; Miller *et al*., 2002).

Cadmium exposure also proved fatal to PCCs but to a lesser extent then arsenic. It also altered cell proliferation and morphology. When PCCs were

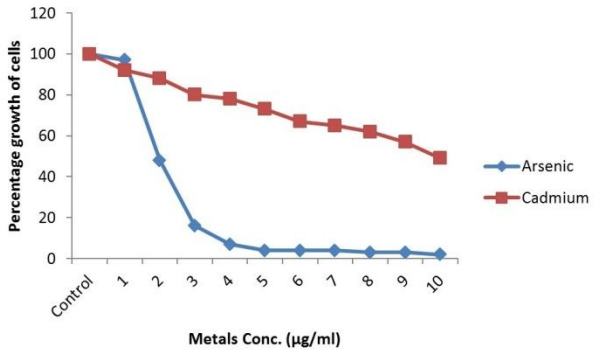

Fig. 3. Effect of arsenic and cadmium on PCCs. As compared to cadmium, arsenic had severe effect on proliferation of PCCs and after 1 µg/ml concentration there was rapid decrease in growth of cells but similar trend was not observed in case of cadmium treated cells.

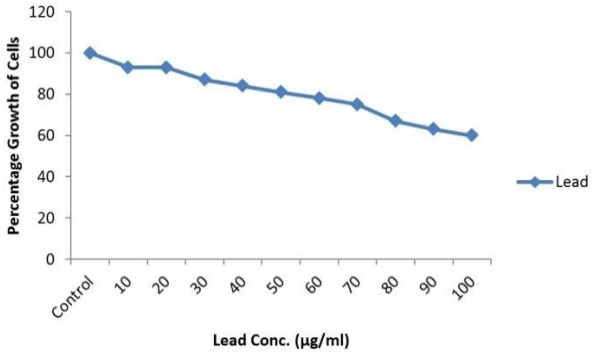

Fig. 4. Effect of lead on PCCs. There is gradual decrease in proliferation of PCCs on increase in concentration of lead. The cells were exposed for a total period of 24 hrs.

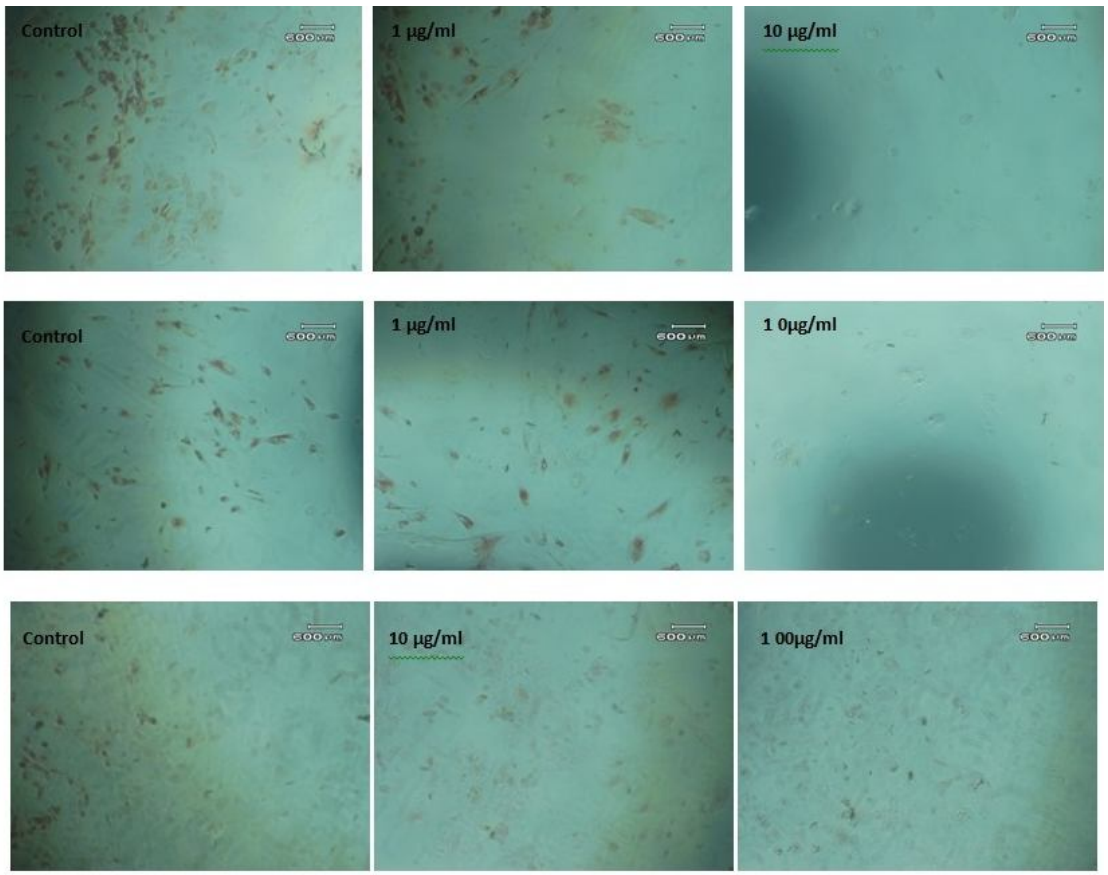

Fig. 5. Effect of arsenic (top), cadmium (middle) and lead (bottom) on PCCs. The treated concentration of arsenic, cadmium and lead are given on each image. The living cells appear red in color due to uptake of neutral red dye, while dead cells did not uptake neutral red and did not stain. At 10 µg/ml concentration in both arsenic and cadmium treated cells, no living cells can be seen due to the toxic effect of both these metals.

exposed to cadmium for 24 h, LC_{50} was observed to be 9.9 µg/ml which was much higher concentration compared to arsenic. Cadmium does not enter into fetus from placenta and retain inside placental cells with the help of metallothionein (Brambila *et al.*, 2002). Inside the placental cells cadmium impairs the transport of important elements including calcium and zinc and effect number of important processes including alteration of hormonal balance of placental tissue (Ronco *et al*, 2009; Tsutsumi *et al.*, 2009; Alvarez and Chakraborty, 2011; Stasenko *et al.*, 2010).

The effect of lead on PCCs was much different than arsenic and cadmium as it proved to be much less toxic for cells and LC_{50} could not be obtained even when cells were exposed to 100 µg/ml lead concentration, only 40% reduction in growth was observed. When inside the cells, lead usually substitutes zinc in zinc finger DNA binding protein and results in impairment in protein function (Zawia *et al*., 1998). In addition lead is also reported to impair the function of regulatory protein genes (Hanas *et al*., 1999). So this could be probable cause of reduction of proliferation of cells on exposure to lead.

The present study showed that arsenic, cadmium and lead are very fatal for PCCs. Arsenic proved to be the most toxic for chorionic cells followed by cadmium and lead. On exposure to these metals there was reduction in cell proliferation to a great extent and morphology of cells was also altered. These heavy metals not only cause reduction in growth of cells but also cause change in shape and finally lead to cell death due to their cytotoxic and genotoxic effects. There is great need to take proper measures to reduce the exposure of these metals to human and especially to females during pregnancy so to avoid lethal effect of heavy metals to mother and the fetus.

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