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Research paper

Effect of Lead and Arsenic on HEK 293 Kidney Cell Line

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ABSTRACT

Different molecules like drugs, urea, uric acid, other degraded compounds, metal ions, water, and salts present in the blood are encountered by the cells of the kidney. Molecules that could accumulate in the kidney such as heavy metals might lead to renal toxicity. The effect produced by heavy metal toxicity on the protein expression of kidney cell lines has been reported earlier. Here, the alterations in the proteins due to the treatment of the cell line with heavy metals such as lead (Pb) and a metalloid, arsenic (As) were analyzed. HEK 293 kidney cell line was exposed to different concentrations of lead and arsenic. The pattern of cell morphology observed through microscopy was found to be not affected by the 50 ug concentration of lead and the cells survived after 48 hours. On the other hand, protein spots of different molecular weights and pH on the 2D gel were noted to be differentially expressed at different time intervals and treatments of lead. Significant morphological changes in the form of deformity in shape of cells were observed together with cell death in all treatments of arsenic as compared with control. The data suggests exposure to lead and arsenic results in morphological and protein changes.

KEYWORDS HEK 293, Cell line, Lead, Arsenic, Two-dimensional gel electrophoresis

INTRODUCTION

Changing environment due to human activities has resulted in occupational or therapeutic exposure to heavy metals like cadmium (Cd), lead (Pb) etc that have a sound effect on body organs such as the kidney [1]. Various pathways are involved in uptake of heavy metals by the kidneys depending on the form (free or bound) of the metal and the segment of the nephron. The extent of renal damage by heavy metals depends on various factors like the nature, the dose, route and duration of exposure. The structural and functional unit of kidney, the nephron is a tubular structure consisting of mainly four parts, the proximal tubule, loop of Henle, distal tubule and terminal segments [2]. The filtration of blood and the reabsorption process of all essential components take place when the liquid passes through these compartments of the nephron. Nephrotoxicity is a condition that arises from a combination of environmental exposure to agents, therapeutic agents, their alternates, and diagnostic agents together with other risk factors. In such a case all compartments of the kidney are vulnerable to injury [3]. The renal epithelium has a high capacity to transport and accumulate ions, which is why heavy metal ions are of concern. Additionally, these ions can also induce different other human disorders and diseases for example cadmium toxicity can affect the liver and lungs as well. One of the important proteins that can bind metals is metallothionein [4]. It is responsible for cadmium binding and plays an important role in the retention of cadmium in tissues. The unbound and inorganic cadmium in the body is responsible for the toxic effect such as liver disturbances, edema in the lungs, hemorrhage, testicular injury, and even

lethality [5]. Different analytical techniques like 2D gels were utilized to observe these toxic effects on the organs of the body. The serum proteome profile of humans occupationally exposed to arsenic (As) and lead (Pd) was assessed and five un-identified peptides/ proteins were found to be significantly up-or down-regulated in exposed vs. unexposed controls [6]. The change in protein expression of the kidney cell line was identified by using 2D-PAGE followed by MALDI and ESI-MS/MS sequencing [7]. Also, there are different protein and (RNA) molecules that have been identified to be associated with heavy metal toxicity in the case of kidney or parts of nephron such as Sema 3A (semaphoring-3A) and LncRNA (long noncoding RNA) molecules [8, 9]. There are reports that showed that cadmium (Cd) induced toxicity results in the down-regulation of certain protective genes and accumulation of p53 protein and the protective effect of flavones in the case of lead (Pb) toxicity [10, 11]. The current study analyzed the protein differences in cells exposed to lead (Pb) and arsenic (As) at different concentrations. It represents the protein changes that may have occurred due to lead (Pb) toxicity. The differences between the proteins of control/untreated cells and heavy metal-treated cells showed some target proteins which could be of vital importance and could give insights into nephrotoxicity. The study was started to have a baseline understanding of the toxicity, the experience of which could be utilized to conduct human tissue sample-based study on the Pakistani population.

MATERIALS AND METHODS

Cell culture

HEK 293 cells were revived and grown in DMEM containing glucose with 10 % FBS, 1% pen strip (penicillin and streptomycin), and 200 mM L-glutamate. Cells were

treated with cadmium chloride solution 10 µL, 20 µL and 30 µL (1 µg\µL) for 24 hr, 48 hr and 72 hr. Microscopy was performed to determine cell morphology and cell viability (MTT assay). Cells were then trypsinized using 0.25 % trypsin EDTA solution, cells were washed with PBS and 500-750 µL trypsin was added in each flask depending on cell population until the cells detached and were pellet down.

Cell lysis

Supernatant was discarded and pellet was washed with ice cooled PBS twice. Cell pellet was dissolved in 1ml PBS and centrifuged at 3000 g for 1 min at 4 ^{o}C. Cell lysis buffer (Urea 13.5 gm, Triton X-100 0.5 mL, β-merceptoethanol 0.5 mL, DTT 75 mg, PMSF 35 mg, NP-40 1.25 mL, glycerol 1.25, pharmalyte (3-10) 0.5 ml) and 10 µL protease inhibitor cocktail was added to cells for complete lysis, vortex vigorously and centrifuged at 10,000 g for 5 minutes. Cell lysate was stored at -20 ^{o}C until further use.

Protein Estimation

Protein estimation was performed with BCA Protein Assay Kit using a standard protocol with a thermos-fisher scientific kit.

Gel Electrophoresis

The standard Laemmli protocol was used for Gel electrophoresis. SDS PAGE electrophoresis was performed on 12 % separating gel and 4 % stacking gel. The samples were prepared in 20 µL of reducing sample diluting buffer.

Two Dimensional Gel Electrophoresis

For 2D gel electrophoresis, an equal amount of proteins (80 µg) was dissolved in rehydration buffer (8 M urea, 0.2 M EDTA, 0.5 M dithiothreitol [DTT], glycerol, NP-40, ampholyte solution pH 3–10 in 0.5 M Tris–HCl) for passive rehydration of 7 cm IPG strip 3–10 NL overnight at room temperature. Proteins were focused on the IEF Multiphor II system (GE Healthcare) at 20 ^{o}C using a total of 7000 V/h. After IEF, IPG strips were equilibrated using

equilibration buffer I (6 M urea, 30 % glycerol, 2 % sodium dodecyl sulfate [SDS], 50 mM Tris–HCl pH 8.8, and 1 % DTT) and equilibration buffer II (6 M urea, 30 % glycerol, 2 % SDS, 50 mM Tris–HCl pH 8.8, and 2.5 % iodoacetamide). Following equilibration, the focused proteins were separated on 12.5 % SDS-polyacrylamide gel electrophoresis. Gels were visualized by silver staining to detect proteins after electrophoretic separation on 12.0 % polyacrylamide gels.

RESULTS AND DISCUSSION

The HEK 293 cell line was treated with 50, 100, and 150 µg of lead nitrate (Pb $(NO_3)_2$ solution 1ug/uL concentration), and the cells were collected at 24, 48, and 72 hours after treatment. Control cells were treated with PBS only. The concentrations (50, 100, and 150 ug) were selected after the MTT assay performed with 50 to 200 µg. The treatment was carried out in 25 mL cell culture flasks. The 24 h treatment with different concentrations showed minimal effects on the cell line. Although there were patches of cell clustering and breaking the connections cell death was low (Figure 1). Therefore, the normal SDS-PAGE gel was run to see the protein band pattern. The troubleshooting that was encountered during the experiment was the extraction of proteins utilizing the cell lysis buffer. The low amount of proteins extracted from the cell pellets was therefore stained with silver staining in all cases. The protein bands of treated cells at all concentrations showed the same pattern as control cells.

Interesting results were observed with concentrations of Pb $(NO_3)_2$ after 48 h of treatment. Two of the spots were most prominent as differentially expressed towards the basic side of the IPG strip with an approximate apparent weight of ~80 kDa with respect to the molecular weight marker (Figure 2).

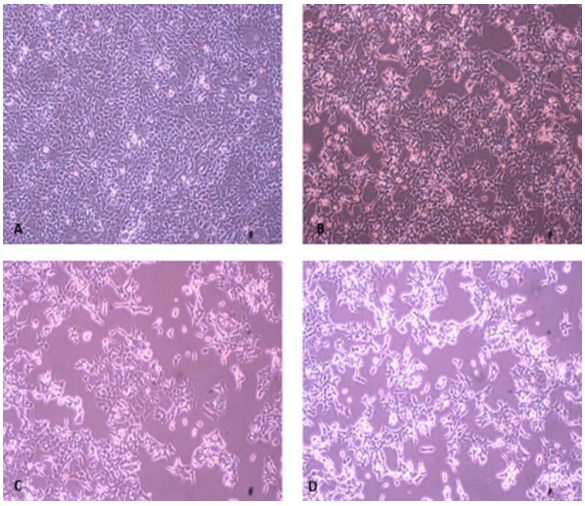

Figure 1: Images of the HEK 293 cell line (10x) after 24 hr of lead nitrate treatment. (A) Normal cell in control conditions, (B) 50 µg of Pb $(NO_3)_2$, (C) 100 µg of Pb $(NO_3)_2$, (D) 150 µg of Pb $(NO_3)_2$.

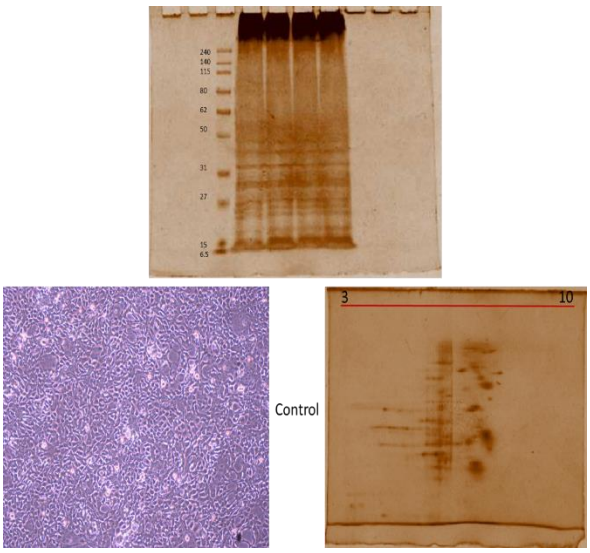

Figure 2: SDS PAGE of the protein extract obtained after 24 hrs of treatment. From left to right, first lane is molecular weight marker (bands mw indicated in kDa), control lane (untreated cells), 50 µg lane, 100 µg lane and 150 µg lane. Lower panel is normal cell in control conditions with respective 2D protein spots pattern after silver staining. The line at the top of the 2D gel represents the IPG strip of 3 to 10 pH gradient.

The spots were expressed after 48 hours with 50 µg treatment (Figure 3). One interesting aspect that was noted during the same treatment was the pattern of cell confluency observed during microscopy where the cells were observed to be not affected by the 50 µg of lead concentration and were surviving even after 48 hours. The same two spots faded with an increase in incubation time and cell death was observed with 100 and 150 µg concentrations. The only reported results of HEK 293 cells treated with Pb $(NO_3)_2$ showed a cell viability assessed by WST protocol, of almost 60% at 10 µg/mL of the salt [11]. However, our results with 50 µg (12.5 µg/mL) showed 35% viability by MTT assay (data not shown) and the viability level remains constant till 48 hours (Figure 1(B), Figure 3).

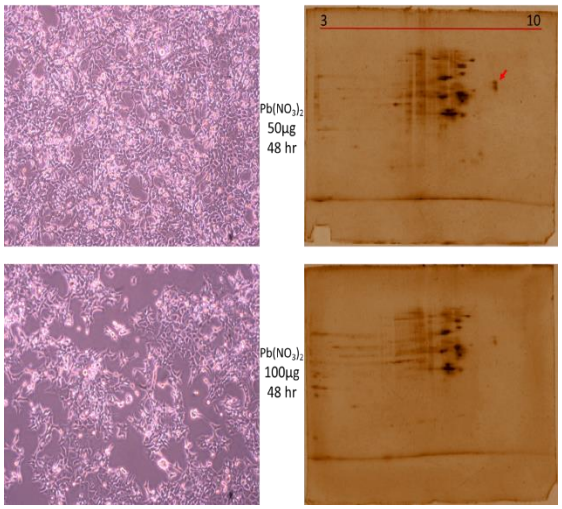

Figure 3: HEK 293 cell treated with lead after 48 h with respective 2D pattern after silver staining. Arrow indicates the differentially expressed protein spots. The line at the top of the 2D gel represents the IPG strip of a 3 to 10 pH gradient.

It dropped significantly only after 72 hours (Figure 4). Whereas, 100 and 150 µg concentrations showed signs of toxicity with an increase in time. Two of the spots encircled in Figure 4 were observed in 150 µg, 48 hours of treatment, and in all concentrations applied for 72 hours of treatment. These were the spots observed in normal cell 2D gel pattern and were less expressed in 48 hours of treatment with 50 and 100 µg of lead nitrate. Lead toxicity has been studied in vivo to observe differentially expressed proteins (12-14).

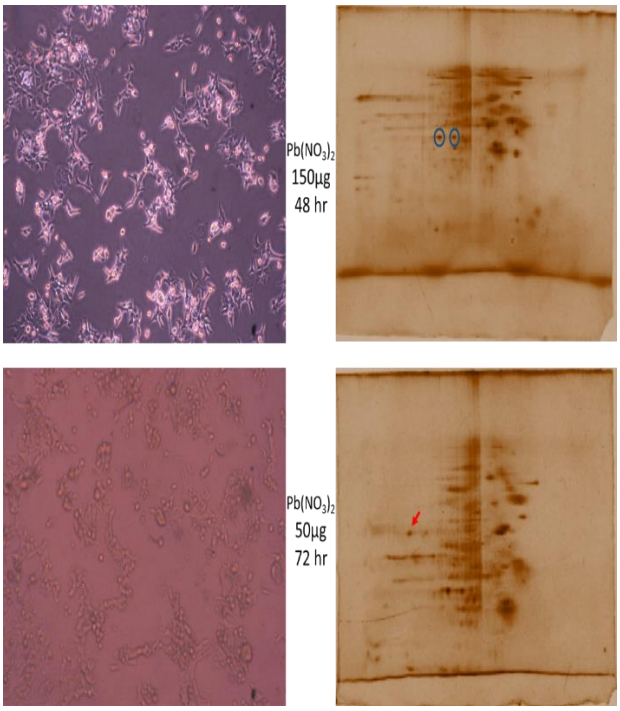

Figure 4: HEK 293 cells treated with lead after 48 and 72 h with respective 2D patterns after silver staining. Arrow indicates the differentially expressed protein spots. The circle indicates spots observed in the control 2D gel pattern.

Similarly, the treatment after 72 hours of time showed increased cell death with a decrease in protein expression as the 150 µg 72 h treatment 2D gel represents in Figure 5. The extracted proteins were difficult to obtain even after the use of a protein clean-up kit. The gel was overstained to visualize the spots making the particle impurities stain resulting in art factual image. The morphological changes observed through the microscopic studies in cell culture represents the particular pattern presented by these cells in response to the lead toxicity.

Arsenic is a metalloid and found in rocks and sediments throughout the earth crust. It can contaminate the ground waters by leaching out from the surrounding. A study published arsenic concentrations in ground water throughout the Indus region of Pakistan showed the concentrations of arsenic in the water from 51-200 µg/L to some areas with 201-500 µg/L [15]. Although arsenic is a metalloid, its different concentrations in the form of sodium arsenate was applied to the HEK 293 cell line to observe the effect. The concentrations used were prepared from a stock solution of 5 µg/mL.

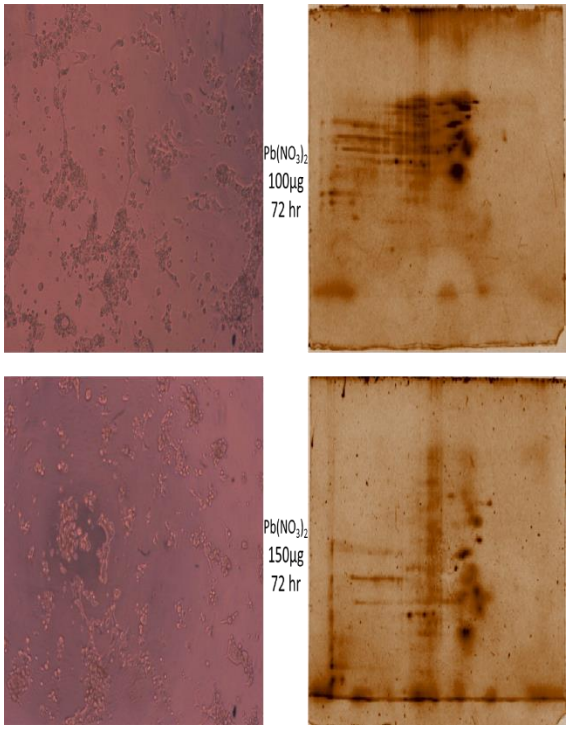

Figure 5: HEK 293 cell treated with lead after 72 hrs with respective 2D pattern after silver staining.

Significant morphological changes were observed together with cell death in all treatments as compared with control. The concentrations of arsenic (As) used in this experiments were higher than the reported over all concentrations of this metalloid in ground water of Pakistan [6] as the effect of the salt was performed for the first time. The toxicity of As on HEK cell linen has been reported to induce changes in stress response genes, signaling molecules, proto-oncogenes, transcription factors proteolytic enzymes and certain receptors [17].

The enhanced expression of heme oxygenase-1 is closely linked to the renal toxicity triggered by arsenic trioxide, and the mechanisms that lead to cell death involve the generation of reactive oxygen species (ROS) and an additional pathway [18, 19]. Our study also demonstrated the clumping/clustering and cell death even at 1 ug concentration of the metalloid (Figure 6).

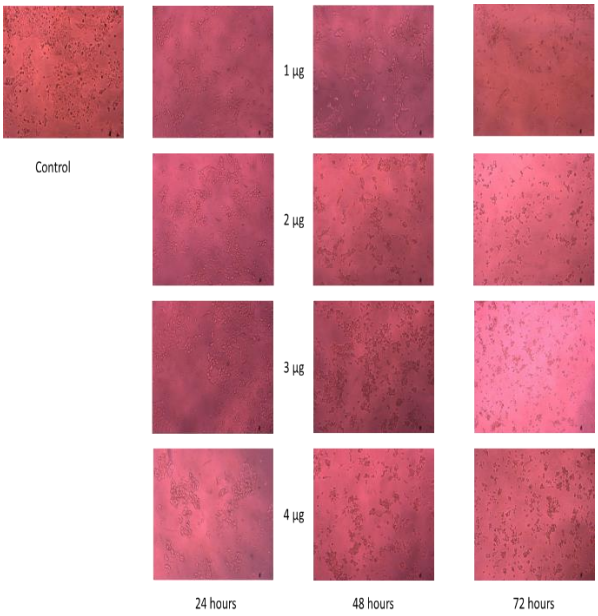

Figure 6: HEK 293 cell treated with Arsenic in 96 well plate with 200 µL total volume per well and concentrations of 1 to 4 ug. Images of the HEK 293 cell line (10x).

CONCLUSION

The lead (Pb) treatment has changed the protein expression level of HEK 293 cells. The 2D gel profiles presented information regarding up and down regulation of protein spots specifically after 48 and 72 hours particularly with 50 µg concentration

of lead. Arsenic (As) exposure, also produced significant cell death on the HEK 293 cell line, suggesting morphological and protein changes due to exposure to arsenic and lead.

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