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Experimental exposure of arsenic in cultured rat intestinal epithelial cells and cell line: Toxicological consequences

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Abstract

Arsenic is a naturally occurring metalloid and the drinking water contamination by inorganic arsenic remains a major public health problem. The trivalent arsenic (arsenite) is more toxic than the pentavalent form (arsenate), and is known to cause gastrointestinal toxicity. Specific immortal cell lines are considered to be suitable for toxicity screening and testing of chemicals as they are easy to handle and possess most of the biochemical pathways present in the corresponding cells present *in vivo*. The present study was designed to evaluate and compare the *in vitro* toxicity of arsenite on rat intestinal epithelial cell line (IEC-6) and primary cultures of rat intestinal epithelial cells (IEC). To evaluate *in vitro* toxicity, cultures of IEC and IEC-6 cells were assessed for viability, morphometric analysis, membrane transport enzymes and structural constituents for membrane damage, dehydrogenase activity test for respiratory and energy producing processes and esterase activity test for intra and extra cellular degradation, following the post exposures to arsenite (0–20 ppm). Significantly similar concentration-dependent changes in these toxicity-screening parameters in IEC and IEC-6 were observed. Highest tested concentration of arsenite (20 ppm) was found to be detrimental in both IEC and IEC-6. Furthermore, to evaluate arsenite toxicity in epithelial cells of rat intestine, intestinal loops were filled with arsenite solutions and incubated for 30 min *in situ. In situ* studies also showed a significant arsenite concentration-dependent decline in epithelial cell membrane transport enzyme activities and total hexose and sialic acid contents. Concomitant release of membrane enzymes, hexose and sialic acid in the intestinal luminal fluid following higher arsenite exposures further indicated partial membrane damage. Similar morphological changes in IEC and IEC-6 were also evident. These findings also suggest that IEC-6 cell lines are suitable for initial screening of gastrointestinal cellular toxicity caused by arsenite.

Keywords: Arsenic; Intestine; Epithelial cells; IEC-6; Gastrointestinal toxicity

1. Introduction

The drinking water contamination by arsenic is a major health problem. Most cases of human toxicity from arsenic have been associated with exposure to inorganic arsenic. Inorganic arsenic (i-As) comprises two valence states, As(III) and As(V) and the trivalent arsenite form is more toxic than the pentavalent arsenate. Acute and chronic arsenic exposure via drinking water has been reported in many countries (Toxicological Profile for Arsenic, 2005). Inorganic arsenic is known to cause irritation of stomach and intestine, with symptoms such as stomachache, nausea, vomiting and diarrhea. Prolonged ingestion can lead to cardiovascular disorders, liver and kidney injuries, neurological and skin disorders (Abernathy et al., 2003; Rossman, 2003; Bashir et al., 2006). Chronic ingestion in drinking water has been associated with increased incidence of human cancer (Jager and Wegman, 1997; Thomas et al., 2001). The main paths for human exposure to As are drinking water and foods; consequently, the intestinal epithelium is the first physiological barrier to As metabolism and distribution towards the tissues through the bloodstream. The literature is lacking in studies on i-As bio-availability in foods, and the only existing data in this sense are derived from research relating to i-As solubility in simulated gastrointestinal media (bio-accessibility) carried out by Laparra et al. (2003, 2004).

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Gastrointestinal tract represents the major site of exposure to chemicals through food and water ingestion and automatically becomes important target organ as well as a site of access of toxicants into the organism. Although intestinal epithelium is equipped to metabolize most of the toxicants and excrete them, but acute and/or chronic exposure of these chemicals may cause cellular damage and lead to a number of biological consequences. Oral uptake is one of the major routes of exposure to toxic heavy metals such as chromium and arsenic, which in turn can cause gastrointestinal cellular toxicity (Shrivastava et al., 2002, 2003; Upreti et al., 2004). Much interest has now been focused on developing *in vitro* toxicity test models evaluating their usefulness in predicting toxicities. Recently, we have shown that resident facultative bacteria isolated from rat gut can be used as an alternate to animals for the preliminary screening of gastrointestinal tract cellular toxicity caused by heavy metals (Upreti et al., 2005a,b).

In last couple of decades, *in vitro* systems using cell lines and primary cultures have been accepted as screening tools to understand the pharmacological and toxicological consequences in a rapid and reliable manner, since most of the biochemical pathways are well expressing in them (Zucco, 1993; Wils et al., 1994; Rossi et al., 1996; Sambruy et al., 2001). Although reports are available on arsenic cytotoxicity in cell lines (Bode and Dong, 2002), however the cellular kinetics comparing primary cultures of intestinal cells and immortal intestinal cell line (IEC-6) is lacking. Thus, in the present investigation, experiments were carried out to study the modulatory effect of trivalent i-As on cellular kinetics and morphometric changes in primary cultures of rat intestinal epithelial cells and immortal rat intestinal cell line (IEC-6) along with a response comparison following *in situ* experimentation in rat intestinal epithelial cells.

2. Materials and methods

2.1. Chemicals and reagents

All the chemicals and reagents were purchased either from Sigma–Aldrich, E. Merck, Gibco, BRL, Hi-Media, India, otherwise stated. These were of analytical grade with highest purity available. Sodium meta-arsenite (Sigma) was used in experiments and arsenite solution containing various concentrations of As(III) was prepared either in distilled water or in incomplete Dulbecco's Modified Eagle's Medium (DMEM).

2.2. Animals

Healthy adult male albino Wistar rats procured from the Animal Breeding Facility of Industrial Toxicology Research Centre, Lucknow, were used for the isolation of intestinal epithelial cells and for *in situ* studies. The animals were housed individually under standard animal house conditions with natural light/dark cycle and a temperature of 25 ± 2 °C. The standard animal food pellets and water were given *ad libitum*. Clearance from the Animal Ethical Committee of the Institute was obtained for the use of animals.

2.3. Cell line (IEC-6)

IEC-6, normal rat small intestine cell line (ATCC CRL 1592), was initially procured from National Centre for Cell Sciences, Pune, India and since then has been maintained at our Institute. Monolayers of cells were grown in DMEM supplemented with 5% fetal bovine serum (FBS), 10 μg/ml insulin, 100 mg/l penicillin, 100 mg/l streptomycin and 2.5 mg/l fungizone (Invitrogen, Groningen, The Netherlands) at 37 °C in a humidified atmosphere of 5%CO_2–95%air. Cells were screened for viability assessment using trypan blue dye exclusion staining method under a phase-contrast microscopy, prior to the start of every experiment. The batches showing more then 95% cell viability were used in the study. All experiments were done on cells between passages 10 and 25.

2.4. Isolation and culture of rat intestinal epithelial cells (IEC)

Intestinal epithelial cells were prepared by the method of Weiser (1973). In brief, the small intestines were flushed gently with normal saline containing 1.0 mM dithiothreitol. The cecal end of the intestine was ligated and solution 'A' containing 1.5 mmol/l KCl, 96 mmol/l NaCl, 27 mmol/l sodium citrate, 8 mmol/l KH_2PO_4, 5.6 mmol/l Na_2HPO_4 (pH 7.3) was filled after clamping the other end with artery forceps. The intestine was immersed in solution 'A' and incubated at 37 °C for 15 min in a constant temperature shaker bath. The intestine was emptied; fluid discarded and was filled with solution 'B' containing 1.5 mmol/l EDTA and 0.5 mmol/l dithiothreitol in PBS (pH 7.2) and immersed in solution 'A' for incubation. After incubation, the contents were emptied into a plastic centrifuge tube to recover the first epithelial cell population. The process of filling with solution 'B' and collecting the washing was repeated at different time periods of incubation (2, 2, 3, and 4 min, respectively) and fractions were pooled. Intestinal epithelial cells were collected by centrifugation (3000 rpm, 15 min at 4 °C). Cells were washed twice with incomplete DMEM and then re-suspended in DMEM supplemented with 5% fetal bovine serum (FBS), 10 μg/l insulin, 100 mg/l penicillin, 100 mg/l streptomycin and 2.5 mg/l fungizone. Cells were seeded in poly-L-lysine pre-coated flasks and grown at 37 °C in a humidified atmosphere of 5%CO_2–95%air. At confluence, cells were expanded by passaging them to subsequent flasks by trypsinization. Cells of 3–6 passages were used in the experiments after ascertaining the cell viability.

2.5. Experimental protocol

Pre-confluent growth were allowed for both rat intestinal epithelial cells and IEC-6 cell line cultures and then the