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Cytotoxic effect of As(III) in Caco-2 cells and evaluation of its human intestinal permeability

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Abstract

Inorganic arsenic has been classified as a carcinogen for humans (Group I). However, its transit across the human intestinal epithelium has not been characterized. Using Caco-2 cells, the thiol–redox balance and apparent permeability coefficients (P_{app}) for As(III) in the apical to basolateral (AP–BL) and basolateral to apical (BL–AP) direction were evaluated. After As(III) exposure, GSH-induced synthesis was observed, increasing the GSH/GSSG ratio by elevating the As(III) concentration. The AP–BL permeabilities decreased as the As(III) concentrations increased, indicating the existence of a mediated transport mechanism. The (BL–AP)/(AP–BL) permeability ratios were higher than unity, suggesting the existence of a secretion process.

Keywords: Arsenite; Caco-2 cells; Apparent permeability coefficients; Thiol–redox balance; Transport mechanism

1. Introduction

Inorganic arsenic (i-As) comprises two valence states, As(III) and As(V), and has been classified as a carcinogen for humans (Group I) by the International Agency for Research on Cancer (IARC, 1987). In addition, human exposure to i-As has been related to a variety of effects on health, including vascular, genotoxic and neurological alterations (Rossman, 2003). Although most studies on arsenic have evaluated its cytotoxicity in several cell lines (Bode and Dong, 2002), a limitation of such studies is the lack of data on the kinetics of cellular As accumulation (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 bioavailability in foods, and the only existing data in this sense are derived from research relating to i-As solubility in simulated gastrointestinal media (bioaccessibility) carried out by our group (Laparra et al., 2003, 2004). Likewise, no studies have evaluated the human intestinal permeability of As(III) across enterocytes. Consequently, understanding the capability of i-As to cross the intestinal barrier is of prime interest. A first evaluation of this capability and elucidation of the routes involved can be attained by studying the apparent permeability coefficients (P_{app}). Caco-2 cell monolayers, due to their spontaneous enterocytic differentiation at confluence (Pinto et al., 1983), are a well established intestinal epithelial model (Van Campen and Glahn, 1999; Ekmekcioglu, 2002). Based on this cell model, previous reports have characterized the implication of active transport mechanisms for Cd (Blais et al., 1999; Aduayom

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et al., 2003), or diffusion processes for Hg (Aduayom et al., 2003). To our knowledge the only existing data related to inorganic arsenic retention and transport at intestinal level are those reported by our investigation group, evaluating the extent of inorganic arsenic retention and transport by Caco-2 cells (Laparra et al., 2005).

One of the early effects after the intracellular incorporation of i-As is induced oxidative damage—this phenomenon having been previously reported in several cell lines (hepatocytes, lymphocytes, rat liver epithelium etc.) (Bode and Dong, 2002; Qian et al., 2003). Consequently, DNA damage, lipid peroxidation and protein modification, as well as alterations in host antioxidant defenses have been reported (Bode and Dong, 2002; Qian et al., 2003). A primary target of As(III) is GSH, an extremely important cellular antioxidant that appears to be a key in the mediated cytolethality caused (Bode and Dong, 2002)—though no studies have evaluated the effects of As(III) upon the GSH/GSSG ratio in Caco-2 cells. This cell model has been extensively used to explore cellular responses to oxidative stress and nutritional antioxidants (Baker and Baker, 1993; Cepinskas et al., 1994). GSH may be responsible for the early cytotoxicity effects seen in cell cultures, and has been implicated in the control of intercellular junctions; accordingly, it may condition intestinal permeability (Bernkop-Schnürch et al., 2003).

In the present study, Caco-2 cell cultures have been exposed to different concentrations of As(III) to evaluate intestinal permeability to As(III), with quantification of P_{app}, and its effects on intracellular GSH levels.

2. Materials and methods

2.1. Arsenic standard

Stock standard solution of As(III) (1000 mg L^{-1}) was prepared by dissolving 1.320 g of As_2O_3 (Riedel de Haën, Hanover, Germany) in 25 mL 20% (w/v) KOH solution, neutralizing with 20% (v/v) H_2SO_4 and diluting to 1 L with 1% (v/v) H_2SO_4.

2.2. Cell culture

The Caco-2 cell line was obtained from the European Collection of Cell Cultures (ECACC 86010202, Salisbury, UK) and was maintained at pH 7.4 in minimum essential medium, MEM (Gibco BRL Life Technologies, Scotland) redissolved in cellular grade water (B. Braun Medical, S.A., Spain). The MEM was supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Gibco), 1% (v/v) non-essential amino acids (Gibco), 1% (v/v) L-glutamine (BioWhittaker, Belgium, Europe), 1% (v/v) sodium pyruvate (BioWhittaker), 0.22% w/v $NaHCO_3$ (Merck), HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid, BioWhittaker), 1% (v/v) antibiotic solution (penicillin and streptomycin) (BioWhittaker), and 0.1% (v/v) fungizone (Gibco). Incubation conditions were 37 °C, 5% CO_2 and 95% relative humidity atmosphere. The culture medium was changed every two days. Cells were examined periodically for changes in growth by using an inverted-phase contrast microscope (Olympus, CK2 model). The cells at 70% confluence were harvested by using a trypsin (2.5 g L^{-1})–EDTA (0.2 g L^{-1}) solution (Sigma, Spain). After the cells were detached from the flasks, the cells were resuspended in MEM.

2.3. Monolayer integrity

The transepithelial electrical resistance (TEER), was monitored prior to, and during permeability assays. A Millicell electrical resistance system (Millicell-ERS, Millipore Iberia) was employed. Any cultures with TEER $<250\ \Omega\ cm^2$ at the end of the assay were rejected.

2.4. Mitochondrial functionality and thiol–redox balance

For these studies, cells were seeded into independent 6-well plates (Costar Corp., NY, USA) at a density of 5×10^4 cells cm^{-2}. Experiments were performed with completely differentiated cells, 15–18 days after seeding. The culture medium was aspirated, and the cells were washed with phosphate buffered solution (PBS) (all in mM: NaCl, 140; KCl, 2.7; Na_2HPO_4, 6.4; H_2KPO_4, 1.5). Afterwards, cells were incubated for 4 h with As(III) (13, 67, 138, 676, and 1339 μM), using Dulbecco's Modified Eagle Medium (DMEM) without phosphates as treatment medium. Then, DMEM was removed and cells were washed twice with PBS at 37 °C.

The mitochondrial functionality of Caco-2 cell was investigated by using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,3-diphenyl tetrazolium bromide) assay. MTT (0.5 mg mL^{-1} in PBS) was added to the cells, followed by incubation for 2 h at 37 °C/5% CO_2/95% relative humidity atmosphere. Then, the medium was removed and cells were washed twice with PBS. The conversion rate to insoluble formazan was measured using a commercial kit (Sigma, No. 7H258), and absorbance was measured at 570 nm with background subtraction at 690 nm. As control cells in all assays, we used cultures with DMEM lacking As(III).

For evaluating the thiol–redox balance, the cell cultures were treated with 0.5 mL of 20 mM Tris buffer solution containing 0.1% Triton (v/v) to obtain the cell homogenate. The redox balance in cell cultures was determined measuring the formation of a fluorescent complex of *o*-phthalaldehyde (OPT) with reduced glutathione (GSH) and oxidized glutathione (GSSG) (Hissin and Hilf, 1976), as briefly described below.

GSH assay: Cell homogenate (10 μL) was incubated at room temperature with 10 μL of buffered formaldehyde (37–40% formaldehyde: 0.1 M Na_2HPO_4 in proportion 1:4 v/v; pH 8), 170 μL of 0.1 M Na_2HPO_4–5 mM EDTA buffer, and 10 μL of the OPT solution (1 mg mL^{-1} in absolute methanol). After mixing and incubation at room temperature for 45 min, then the fluorescence intensity was measured.