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Aquaglyceroporins are involved in uptake of arsenite into murine gastrointestinal tissues

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Abstract : Aquaglyceroporins (AQGPs) are members of aquaporin (AQP) family and belong to a subgroup of this water channel family ; they are transmembrane proteins that transport water as well as glycerol and other solutes of small molecules. Recent studies have also identified that AQGPs are important transporters of trivalent metalloid in some mammalian cells. However, the uptake routes of arsenite in mammals are still less defined. In this study, to understand the routes of arsenite intake in mammals, mice were treated with Hg(II), glycerol, and As(III) and uptake of As(III) into the gastrointestinal tissues was measured. The level of inorganic arsenic (iAs) in gastrointestinal tissues after As(III) stimulation was much higher than Hg(II) +As(III) or glycerol+As(III) group. RT-PCR results showed that AQGPs were extensively expressed in gastrointestinal tissues of mice. We also treated Caco-2 cells with Hg(II) and As(III) ; the level of iAs in a group treated with Hg(II)+As(III) decreased compared with As(III)-treated group. Our results suggested that AQGPs could be important transporters in arsenite uptake into gastrointestinal tissues of mice, but more data are need to prove if AQGPs is the only pathway involved in As transport in mammals or just one of them. J. Med. Invest. 56 Suppl. : 343-346, December, 2009

Keywords **:** *aquaglyceroporins (AQGPs), arsenite, gastrointestinal tissues*

INTRODUCTION

Epidemiological studies have shown that chronic arsenic exposure increases the risk of developing vascular diseases and cancers of the skin, bladder, liver, kidney, and lung, as well as skin lesions in human. The International Agency for Research on Cancer (IARC) categorizes arsenic and its compounds as known human carcinogens (1). On the other hand, purified arsenic trioxide (Trisenox) is being successfully used in treatment of acute promyelocytic leukemia (APL), chronic myelogenous leukemia (CML), multiple myeloma (MM), hematological malignancies, and even solid tumors (2). Pathways for arsenite uptake into cells have been discovered only recently. Aquaglyceroporins (AQGPs), a subgroup of the water channel family are transmembrane proteins that transport water as well as glycerol and neutral hydroxides, $Sb(OH)_3$ or $As(OH)_3$, which are structurally similar to glycerol (3-4). G1pF, the glycerol facilitator in *Escherichia coli* was first identified as a trivalent metalloid transporter (5), then Fps1p, an yeast homolog of G1pF in *S. cerevisiae* (6), and AQP7, 9 in mammalian cells

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(7) were all proved to be transporters of arsenite. *Xenopus laevis* oocytes exhibited increased transport of ^{73}As(III) after microinjection with either AQP7 or AQP9 cRNA (7). The results from site-directed mutagenesis showed $As(OH)_3$ and glycerol use the same translocation pathway in AQP9 (8). Pretreatment of cultured primary mouse hepatocytes with an AQP9 inhibitor and siRNA-mediated knockdown of AQP9 decreased uptake of arsenite (9). Up-regulation of AQP9 expression by vitamin D or all-*trans* retinoic acid (ATRA) in HL60, a leukemia cell line, resulted in cell hypersensitivity to Trisenox and enhanced cytotoxicity (10-11). However, until recently, most of these studies were conducted in prokaryotes or primitive eukaryotes, and mostly *in vitro*. The uptake route of arsenite in mammals *in vivo* is still less defined. In this study, we focus on gastrointestinal tissues to study the route of arsenite uptake *in vivo*.

METHODS

Cell culture and treatments Caco-2 cells, a human intestinal epithelial cell line, were maintained in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin. MTT assay was performed to detect the toxicity of different concentrations of As(III) to Caco-2 cells. Cells were cultured on 6 cm-dishes overnight and treated with nontoxic concentration of 32 μM As(III) or 10 μM Hg(II)+32 μM As(III) for 2 h. After having been washed three times with normal saline, the cells in each dish were immersed in 3 ml fuming HNO_3 and incubated overnight at room temperature. The solution was transferred to an acid-washed beaker and hydrolyzed with 3 ml HNO_3 and 3 ml 30% H_2O_2 on a 100°C electric hot plate. The concentrations of inorganic arsenite (iAs) in Caco-2 cells were measured by graphite furnace atomic absorption spectroscopy (GFAAS).

Animals and treatments Thirty-two adult male ICR mice with a mean body weight of 20.0±0.5 g from the animal center of Nantong University were used. The animals were maintained according to the guideline of NIH. The mice were administered by lavage with normal saline (n=8), 10 mg/kg As(III) (n=8), 10 mg/kg As(III) after 10 mg/kg Hg(II) (n=8), and a mixture of equivalent volume of glycerol and As(III) (n=8), respectively. The mice were sacrificed 2 h after administration, and the middle parts of stomach and small intestine were removed for measurement. Concentrations of iAs in gastrointestinal tissues were determined by GFAAS after digestion with fuming HNO_3 and H_2O_2 (12). The levels of iAs in the blood and the small intestine were measured by GFAAS in a semi *in vivo* study after As(III), Hg(II)+As(III), or glycerol+As(III) was injected into the middle-part of ligated small intestine of anesthetized mice.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Total RNA were extracted from both gastrointestinal tissues and Caco-2 cells with Trizol reagent. RT-PCR was performed for mRNA expression of AQP3, 7, 9, and β-actin genes.

RESULTS

Caco-2 cells were treated with 32 μM As(III) for 2 h, with or without pre-treatment for 30 min with 10 μM Hg(II), a specific inhibitor of AQPs. The concentrations of iAs in the cells of Hg(II)+As(III)-treated group significantly decreased to 21.4±3.6 ng/4×10^6 cells compared with As(III) treated group (32.5±7.4 ng/4×10^6 cells). This result indicates that Hg(II) suppressed iAs accumulation in Caco-2 cells

Next, the gastrointestinal tract of mice was lavaged/incubated at high dose of 10 mg/kg arsenic trioxide (1/3 LD_{50}, a tolerated concentration), and/or 10 mg/kg mercuric chloride or glycerol. After 2 h treatment, the accumulation of iAs in gastrointestinal tissues was measured. The result showed that arsenite uptake was suppressed by a specific inhibitor (Hg(II)) and by a competitive inhibitor (glycerol) of AQGPs. Similar results were obtained under semi *in vivo* condition.

Lastly, the expression of AQP3, 7, and 9 in gastrointestinal tissues was detected by RT-PCR analysis. AQP3 was expressed in both stomach and intestines, while AQP9, in stomach and AQP7, intestines only. Furthermore, the expressions of AQP3 and AQP9 were also detected in Caco-2 cells.

DISCUSSION

Arsenite is mainly absorbed by gastrointestinal tissues especially by the small intestine, then distributed to other tissues and organs by way of bloodstream. By control the intestinal arsenite uptake quantitatively, it would become possible to control

the amount of total arsenite redistribution among every tissue throughout the body. In this study, pathways of uptake arsenite into gastrointestinal tissues were explored.

AQPs are a family of hydrophobic, integral membrane proteins. Among 13 identified mammalian AQPs (AQP0-12), AQGPs (AQP3, 7, 9, and 10) have been proven to transport glycerol and other small solutes as well as water (13). AQGPs are widely expressed in mammals. Here we detected expressions of AQP3 and 9 in stomach of mice and in human intestinal epithelial cell line Caco-2, and AQP3 and 7 in small intestine tissues. iAs accumulated in gastrointestinal tissues and Caco-2 cells after administration of As(III). We found the expression levels of AQGPs differed in different parts of the gastrointestinal tissues ; *e.g.*, duodenum and jejunum as the main absorption positions expressed higher level of AQP3 than ileum. The expression level of AQP3 in the stomach was higher than that in small intestines, while the concentration of iAs in stomach (108.4 ± 29.1 μg/g) was much higher than that in intestines (13.5 ± 2.5 μg/g). However, whether AQP3 is the major transporter of arsenite in mammals is still obscure. Liu *et al.* showed that human AQP9 was more effective As(III) transporter than hAQP7, while little or no As(III) was transported by hAQP3 or hAQP10 (8). On the other hand, Lee *et al.*, using RNA interference and ectopic expression, verified that AQP3 participates in As (III) uptake in lung adenocarcinoma cells (1). Our results may give more supportive evidences to the possibility that AQP3 is also an As(III) transporter.

Hg(II) is a specific inhibitor for most of the AQPs, which binds to a unique cysteine residue (C189) locates at the narrowest segment of AQP water channels. Once the Hg(II) ion binds to the sulfhydryl side chain at a channel-lining position, the transportation of water and solutes by AQPs will be blocked (14). In cultured human intestinal epithelial cell line, we found the concentration of iAs was decreased in Caco-2 cells by pre-treatment with Hg(II). Similar results were obtained *in vivo*. Additionally, as a competitive reagent of AQGPs, glycerol also interfered the uptake of As(III) into gastrointestinal tissues. All these results suggested that AQGPs could be important transporters of As(III) not only for a particular mammalian cells but also for whole body in mammals. Certainly, results in this report are preliminary because mammals are complicated organisms requiring more additional evidences to prove if AQGPs is the only pathway in mammals or just one of them.

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