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Molecular mechanism of the increased tissue uptake of trivalent inorganic arsenic in mice with type 1 diabetes mellitus

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ABSTRACT

Arsenic is associated with several adverse health outcomes, and people with diabetes may be more susceptible to arsenic. In this study, we found that arsenic levels in some tissues such as liver, kidney, and heart but not lung of type 1 diabetes mellitus (T1DM) mice were higher than in those of normal mice after a single oral dose of arsenic trioxide for 2 h. However, little is known about the molecular mechanism of the increased tissue uptake of trivalent inorganic arsenic in mice with T1DM. This study aimed to investigate the expression of the mammalian arsenic transporters aquaglyceroporins (AQPs) and glucose transporter 1 (GLUT1) in T1DM mice and compare them with those in normal mice. Results showed that the levels of AQP9 and GLUT1 mRNA and protein were higher in T1DM mouse liver than in the normal one. The levels of AQP7 mRNA and protein were higher in T1DM mouse kidney. In the heart, we observed that the levels of AQP7 and GLUT1 mRNA and protein were higher in T1DM mice, but the levels of AQP9 mRNA and protein in the lung had no significant difference between both mice. These results suggested that T1DM may increase the expression of transporters of trivalent inorganic arsenic and thus increase the arsenic uptake in specific tissues.

1. Introduction

Inorganic arsenic is widely distributed in water and food [1]. Generally, trivalent inorganic arsenic is considered to be more hazardous than its pentavalent counterpart. Several studies have shown that trivalent inorganic arsenic can be transported into mammalian cells by aquaglyceroporins (AQPs) [2,3]. The important AQP3, AQP7, and AQP9, which exist in the cell membrane, efficiently transport water and glycerol and participate in the uptake of trivalent inorganic arsenic [4]. AQP9 is reportedly expressed in the liver [5]; AQP7 is abundantly expressed in the kidney, adipose, and testis [6–8]; and AQP3 is mainly expressed in the skin, esophagus, and kidney [7,9]. Meanwhile, glucose transporter 1 (GLUT1), which widely exists in various cell membranes, is known to transport glucose into the cell and is important in the uptake of trivalent inorganic arsenic [10,11].

Diabetes mellitus is a chronic metabolic disorder that has become a major health threats. Type 1 diabetes mellitus (T1DM) is caused by the destruction of pancreatic β-cells, resulting in an absolute lack of insulin. T1DM has widespread adverse effects on many tissues, including the kidney, heart, retina, liver, lung, vasculature, and skeletal muscle. Several studies have shown that gene-expression profile is altered in T1DM rat liver and lung [12–14]. Due to such alterations, diabetes can be suspected to increase susceptibility to toxic hazards [15,16]; for example, diabetic rats are more susceptible to cadmium nephrotoxicity than normal rats [16]. Streptozotocin (STZ) is widely used as an agent to induce T1DM in mice [17–19]. As far as we know, no study has investigated the tissue uptake of trivalent inorganic arsenic in T1DM mice.

The present study aimed to determine whether T1DM can increase the tissue uptake of trivalent inorganic arsenic and to elucidate the molecular mechanism of increased tissue uptake of arsenic under diabetic condition.

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2. Materials and methods

2.1. Arsenic trioxide treatment in control mice

Male ICR mice (7–8 weeks old, weighing 20–24 g) were purchased from the Experimental Animal Center of Nantong University, China. To determine the peak concentration of arsenic uptake in various tissues at different time points, 45 male ICR mice were treated with 10 mg/kg arsenic trioxide by intragastric administration after fasting for 5 h (with free access to water). Every five mice were euthanized at 0, 0.5, 1, 2, 3, 6, 12, 24, and 48 h after treatment, and tissue samples were removed and stored in a −80 °C freezer for measurement of arsenic concentration.

To clarify the mechanism of trivalent inorganic arsenic uptake in mice, the expression levels of AQPs and GLUT1 associated with trivalent inorganic arsenic uptake were studied. Normal mice were divided into two groups: the control group (n = 4) was treated with saline, and the experimental group (n = 4) was treated with 10 mg/kg arsenic trioxide by intragastric administration after fasting for 5 h. Mice were euthanized 2 h after treatment, and tissues were removed and stored in a −80 °C freezer for semi-quantitative RT-PCR analysis. All animal procedures were carried out according to the requirements of the Provisions and General Recommendation of Chinese Experimental Animals Administration Legislation. This study, including the use of animal subjects, was approved by the Lab Animal Ethical Committee of Nantong University (approval number: 20170303–001).

2.2. Induction of T1DM in mice by STZ

Male ICR mice were housed in our animal facility under standard conditions at least 3 days before being subjected to experiments. Mice were randomly divided into two groups: control group (n = 18) and T1DM group (n = 18). Mice in the T1DM group were intraperitoneally injected with STZ dissolved in citric acid–sodium citrate buffer (pH 4.4) at a dose of 60 mg/(kg.bw), whereas the control group was intraperitoneally injected with the same amount of the vehicle. STZ and vehicle were injected once a day for 5 days. The mice were then raised 12 days following the last injection. Body weight and amount of food and water intakes were recorded daily, and the level of fasting blood glucose was measured from blood taken from the tail vein on the last day after fasting for 12 h with free access to water. The level of fasting blood glucose was measured using an Accu-Chek Advantage glucometer (Roche Diagnostics GmbH, Mannheim, Germany). The mRNA and protein expression levels of AQPs and GLUT1 in the tissues of control (n = 6) and T1DM (n = 6) mice were analyzed by semi-quantitative RT-PCR, real-time quantitative RT-PCR, and Western blotting.

2.3. Arsenic trioxide treatment in control and T1DM mice

After fasting for 5 h (with free access to water), control (n = 12) and T1DM (n = 12) mice were treated with 10 mg/kg arsenic trioxide by intragastric administration and then euthanized 2 h after treatment. Tissues were washed with saline three times to remove blood and stored in a −80 °C freezer for arsenic measurement.

2.4. Measurement of arsenic in mice tissues

The concentration of arsenic in tissues was determined by atomic fluorescence spectrometry. Tissue samples weighing about 200 mg were placed in a beaker, mixed with 5 mL of ultrapure concentrated HNO_3, and stored at room temperature overnight. The beakers were placed on a heat plate, which controlled the temperature to between 90 and 100 °C. After hydrolysis by concentrated HNO_3, about 6 mL of a freshly prepared mixture of concentrated HNO_3 and H_2O_2 (1:1, *v*/*v*) was added to each beaker, which was heated at 100 °C. When the solution was about to dry, 3 mL of 30% H_2O_2 was added and heated was continued until the solution became colorless. Following hydrolysis, all materials in the beaker were dissolved in distilled water and transferred to a 15 mL glass test tube. The residue in beakers was then thoroughly washed with distilled water. The final volume of the mixture was exactly made to reach 9 mL. Lastly, 0.5 mL of 150 g/L thiourea solution and 0.5 mL of 36% concentrated hydrochloric acid were added to each tube. Arsenic concentration was measured by atomic fluorescence spectrometry (AFS-9700, Kchaiguang Instrument Co., Ltd., Beijing, China) with high purity argon as carrier gas, 5% hydrochloric acid as carrier liquid, and potassium borohydride as reducing agent.

2.5. Semi-quantitative RT-PCR and real-time quantitative RT-PCR

Total RNA was extracted using RNAiso™ Plus (TaKaRa Biotechnology Co., Ltd., Dalian, China) following the manufacturer's instructions. Single-stranded cDNA was synthesized from 3.5 μg of total RNA with M-MLV reverse transcriptase kit (TaKaRa Biotechnology Co., Ltd., Dalian, China). For a visual evaluation of the mRNA expression of genes, routine RT-PCR was also performed. In a typical procedure, specific hypoxanthine phosphoribosyltransferase (HPRT) primers were used as the internal control. PCR was performed in a thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) for cycles starting at 94 °C for 4 min, denaturation at 94 °C for 30 s, annealing at specific temperature (Table 1) for 30 s, and extension at 72 °C for 1 min, followed by a final 10 min extension at 72 °C. A 10 μL aliquot of PCR product was electrophoresed on 1.5% agarose gel containing 0.5 μg/mL ethidium bromide. The gel was exposed to UV light, and pictures were taken with a Gel Doc™ XR Gel Documentation System.

The synthesized cDNA was also subjected to real-time PCR reaction (10 μL) in LightCycler® 480 II (Roche Diagnostics GmbH, Mannheim, Germany) using SYBR enzyme (TaKaRa Biotechnology Co., Ltd., Dalian, China). The reaction mixture was heated at 95 °C for 15 s, followed by 40 cycles of PCR consisting of 60 °C for 15 s and 72 °C for 20 s. The melting curve was determined to confirm the purity of the PCR product. As an internal control, HPRT mRNA was amplified and mRNA expression was analyzed through the $2^{-\Delta\Delta Ct}$ method.

2.6. Western blotting

Mice tissues were homogenized in RIPA buffer supplemented with a protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany) to obtain total protein extracts. Protein concentration was quantified with a Bio-Rad protein assay kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using bovine serum albumin as a standard. For Western blotting, the proteins were mixed with 2×SDS sample buffer and denatured at 95 °C for 5 min. Protein aliquots were separated by 10% SDS-PAGE. After electrophoresis, separated proteins were electrophoretically transferred onto polyvinylidene fluoride membranes (Roche Diagnostics GmbH, Mannheim, Germany). The blotted membrane was blocked with Tris-buffered saline and Tween 20 (TBST) containing 5% nonfat dry milk for 2 h at room temperature, followed by incubation with rabbit anti-GLUT1 antibody (1:500; sc-7903, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), goat anti-AQP3 antibody (1:500; sc-9885, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), rabbit anti-AQP7 antibody (1:200; sc-

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