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Arsenic undergoes significant speciation changes upon incubation of contaminated rice with human colon micro biota

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HIGHLIGHTS

- Postulates pre-systemic arsenic metabolism, even when it is bound in a rice matrix.
- Effect of different matrix of rice on As bio-accessibility at colon level.
- Depending upon content of rice, the most bio-accessible species are differing.
- Depending upon content of rice, pattern of bio-transformations is changing.

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ABSTRACT

Cellular and animal studies involving MMA^{III} (monomethyl arsonous acid) and DMA^{III} (dimethyl arsinous acid) have indicated that their toxicities meet or exceed that of iAs. Thiolated arsenic metabolites were observed in urine after oral exposure of inorganic arsenic in some studies. For these species, the toxicological profile was not yet fully characterized in human cells. Some studies revealed that trivalent organoarsenic species are well absorbed in the intestine compared to iAs. However, other studies also indicated that a significant amount of rice-bound As reaches the colon, which may be attributed to the fibre-rich nature of the rice. Studies have revealed that microorganisms from the gut environment are important contributors to arsenic speciation changes. We aimed to study how the gut microbial metabolism affects As in different rice matrices. This was done *in vitro* using colon suspension from the Simulator of the Human Intestinal Microbial Ecosystem (SHIME system). Significant amounts of MMA^{III}, DMA^{III} and $MMMTA^{V}$ were formed due to microbial metabolic processes like methylation and thiolation. These results suggested that presystemic metabolism by human gut micro biota should not be neglected in risk assessment studies. In this context, also toxicity and absorption of thiolated species by mammalian cells should be further investigated.

1. Introduction

Human exposure to arsenic (As), an important environmental and food contaminant has been associated with several detrimental health effects such as liver, lung, kidney and bladder cancer [1]. A predominant exposure route is the consumption of As contaminated food and water. In a scenario of dietary As exposure, rice has been demonstrated to be one of the major foodstuffs contributing to human As exposure. Rice is widely consumed and it also contains As concentrations that are much higher than in other crops [2]. The latter is due to the use of arsenic-rich groundwater to flood rice fields in several world regions and the conversion of inorganic arsenate (iAs^{V}) to more bioavailable arsenite (iAs^{III}) under reducing conditions in rice fields. In China, daily iAs intake by the human population is around 42 $\mu g\ day^{-1}$, and rice is the largest contributor of total iAs intake accounting for about 60% [3].

While iAs^{V} and dimethylarsinic acid (DMA^{V}) are the predominant As species – or chemical forms of As – in rice, it must be noted that several biotransformation processes can take place in the body upon ingestion. This is important as the As-derived toxicity is largely determined by its speciation. For instance, methylation of iAs^{V} to methylated arsenicals was long considered a detoxification reaction as the toxicity of monomethylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}) is 10-fold lower than that of iAs^{V} [4]. Yet, the finding of trivalent monomethylarsonous acid (MMA^{III}) and dimethylarsinous acid (DMA^{III}) may actually indicate methylation to be a toxification process [5]. Moreover, As thiolation is also possible resulting in monomethyl monothioarsonic acid ($MMMTA^{V}$) and dimethyl monothioarsinic acid ($DMMTA^{V}$) that have been detected

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in the urine of As exposed individuals [6,7]. So far, literature indicates the toxicity of these thiolated arsenicals to be at least equal to trivalent arsenicals [8,9].

Although the liver is thought to be the most important site in the body for biotransformation reactions, it is not well known to what extent As toxification always through a human enzyme mediated process. Several studies indicate an important contribution of the gut microbial community in the conversion of ingested xenobiotics in general [10], and As in particular [11]. The reduction of iAs^{V} to iAs^{III} by rat cecal microbes was already reported in 1981 [12], while As thiolation has been described both for murine [13] and human gut microbes [14]. Hence, arriving to an accurate model of As toxicokinetic behaviour in the body does not only require understanding of the human biotransformation processes but also of the presystemic metabolism, *i.e.* the metabolism by gut micro biota prior to intestinal absorption.

In the scenario of As exposure through consumption of contaminated rice, this presystemic metabolism may even become a more dominant process. Given the fibre rich nature of rice, it is plausible that significant amounts of fibre-bound As reach the colon environment. Due to extensive breakdown of fibre by colon microbial metabolism, As is released in to the colon environment and becomes available to microbial metabolism. Such contaminant release upon colonic fibre breakdown was earlier demonstrated for Hg, Pb and Cd [15].

We previously developed an analytical protocol for successfully detecting and quantifying trivalent and pentavalent inorganic and methylated arsenicals as well as pentavalent thiolated methylarsenicals in complex gastrointestinal suspensions [16]. Moreover, we recently developed an extraction method for analysing As-contaminated rice that completely preserves the As speciation [17]. Given the importance of contaminated rice in contributing to dietary As exposure and the putative role of fibre content to the As release and subsequent microbial metabolism, we investigated whether rice fibre content is a determining factor towards the metabolic potency of human gut microorganisms towards arsenic. To this end, we tested three rice types that differ in fibre content. Polished (fibre poor (2%)), basmati (medium fibre (12%)) and parboiled (fibre rich (22%)) rice were incubated with *in vitro* cultured human colon micro biota and both the As bioaccessibility as the microbial As speciation changes were evaluated.

2. Experimental

2.1. Preparation of mobile phase

Ammonium carbonate ($NH_4(CO_3)_2$, Merck, Darmstadt, Germany), trimethylammoniumhydroxide (TMAH, Merck, Darmstadt, Germany), double distilled deionized water (prepared using MilliQR (MQ), Millipore, Brussels, Belgium) and sodium hydroxide (NaOH,) were used to prepare mobile phase. Nitric acid (HNO_3, Merck, Darmstadt, Germany) was used to adjust the pH of the mobile phase.

2.2. Arsenic standards

The following products were used to prepare the standards: $NaAsO_2$ solution (VWR, Belgium) for As^{III}, $Na_2HAsO_4{\cdot}7H_2O$ (Fluka, Switzerland) for As^{V}, $(CH_3)_2AsO_2Na{\cdot}3H_2O$ (Fluka, Switzerland) for DMA^{V}, and $(CH_3)AsNa_2O_3{\cdot}6H_2O$ (Chemservice, Belgium) for MMA^{V}. Monomethyl monothioarsonic acid ($MMMTA^{V}$) was prepared in the lab. MMA^{III} and DMA^{III} were purchased from Argus Chemicals (Italy).

2.2.1. $MMMTA^{V}$ preparation procedure

A 40 μg As mL^{-1} monomethylarsonic acid (MMA^{V}) solution was prepared by mixing 60 μL of 1850 μg As mL^{-1} MMA^{V} solution with 2.94 mL of 10% v/v formic acid solution. The pH of this solution was 2.4. The conversion yield of MMA^{V} to MMMTA is highest at a pH near 3.0 [18]. Saturated H_2S solution was prepared by adding 2 mL of HCl (Fisher Scientific, United Kingdom) along with 4 mL of double-deionized water to 1.0 g of iron (II) sulphide (Harshaw Scientific, Cleveland, OH). The released H_2S gas was captured into 15 mL of double-deionized water until the effervescence in the round bottom flask subsided. An amount of 100 μL of saturated H_2S solution was added to 900 μL of the 40 μg As mL^{-1} MMA^{v} solution in a 1 mL glass vial. The mixture was shaken overnight on a mechanical shaker. Progress of the reaction was verified using HPLC–ICP-MS (Perkin Elmer Series 200 HPLC and Elan DRC-e ICP-MS) [14]. The retention times of $MMMTA^{V}$ in the standard and $MMMTA^{V}$ in the sample were slightly offset because the matrix caused decreased retention of $MMMTA^{V}$ on the C_{18} column. The structure of the product was checked by LC–ESI-MS and MS/MS. Tandem MS (MS/MS) of m/z 155 yielded a product ion of m/z 137 (loss of H_2O) and, to a lesser extent, a product ion of m/z 121 (due to $CH_2AsO_2^-$) and m/z 140 (loss of CH_3). The molecular mass of 155 and corresponding fragments were consistent with other reports for $MMMTA^{V}$ [19] (Fig. 1). MMA^{III} and DMA^{III} were purchased from Argus Chemicals (Italy).

2.3. Certified reference material (CRM) and rice samples

NIST 1568a Rice Flour (National Institute of Standards and Technology, NIST, USA) was used to check the recovery of total arsenic. The certified value of total As in NIST SRM 1568a is 290 ± 0.03 μg kg^{-1}, while the As speciation in this reference material is not defined.

Three different rice samples of Indian origin were purchased in a local supermarket: polished (fibre poor (2%)), basmati (medium fibre (12%)) and parboiled (fibre rich (22%)) rice.

2.4. Analysis of rice samples for As content

All rice samples were microwave digested at 80 °C for 30 min using water as extraction solvent. This method has previously been proven to be successful for extracting total As from rice [17]. NIST 1568a Rice Flour (National Institute of Standards and Technology, NIST, USA) was used to check the recovery of total arsenic. The certified value of total As in NIST SRM 1568a is 290 ± 0.03 μg kg^{-1}. Digested samples were filtered using a 0.45 μm syringe-type PVDF membrane filter and the filtrate was diluted to 25 mL using double distilled deionized water. This filtrate was analysed for total arsenic content using ICP-MS. PerkinElmer Elam DRC-e ICP-MS (Sunnyvale, CA, USA) is used to perform total As analysis. For total analysis following are the optimized conditions for ICP-MS: (1) oxygen is used as reaction gas to detect As as arsenic oxide (As0) at 91 amu. (2) Nebulized gas flow is maintained in between 0.7 and 1.1(basing on daily optimization). (3) Plasma RF power was 1250 W and Rpq of cell was 0.6. (4) Lens voltage and autolens are optimized prior to analysis.

The same filtrate was used for speciation analysis using HPLC–ICP-MS [16].

2.5. SHIME reactor to create colon matrix

As a representative matrix for the human gastrointestinal environment, *in vitro* cultured colon suspension was sampled from the colon compartments of the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) [20]. This is a dynamic model