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Unusual arsenic metabolism in Giant Pandas

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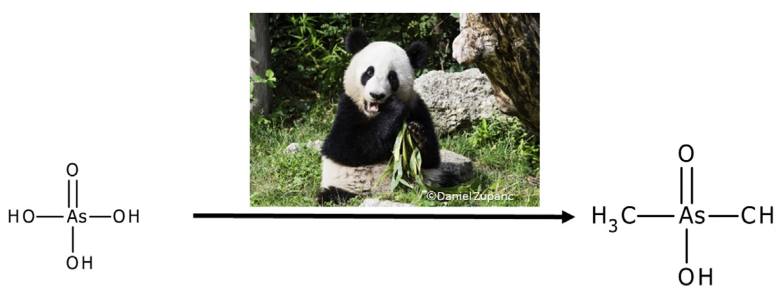
HIGHLIGHTS

- First report on arsenic speciation in Giant Pandas' urine and feces.
- Unusually high arsenic concentrations in the Giant Pandas' urine.
- Only dimethylarsinic acid was found in the urine.
- Bamboo contains mainly inorganic arsenic.
- Giant Pandas are extremely efficient in metabolizing inorganic arsenic.

GRAPHICAL ABSTRACT



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ABSTRACT

The total arsenic concentration and the arsenic speciation in urine and feces samples of the two Giant Pandas living at Vienna zoo and of their feed, bamboo, were determined with ICPMS and HPLC-ICPMS. Urine was the main excretion route and accounted for around 90% of the ingested arsenic. The urinary arsenic concentrations were very high, namely up to 179 μg/L. Dimethylarsinic acid (DMA) was the dominating arsenic compound in the urine samples and ranged from 73 to 92% of the total arsenic, which is unusually high for a terrestrial mammal. The feces samples contained around 70% inorganic arsenic and 30% DMA. The arsenic concentrations in the bamboo samples were between 16 and 920 μg/kg dry mass. The main arsenic species in the bamboo extracts was inorganic arsenic. This indicates that the Giant Panda possesses a unique way of very efficiently methylating and excreting the provided inorganic arsenic. This could be essential for the survival of the animal in its natural habitat, because parts of this area are contaminated with arsenic.

1. Introduction

The Giant Panda (*Ailuropoda melanoleuca*) is one of the best known vulnerable mammals on earth (classification according to the International Union for Concervation of Nature (Saisgood et al., 2016)). Its natural habitat is nowadays restricted to the mountain areas of the provinces Sichuan, Shaanxi and Gansu in China (State Forestry Administration of China, 2015). In these provinces, high arsenic concentrations have been detected in the ground water (He and Charlet, 2013). As a consequence, the free living Giant Pandas are constantly exposed to elevated arsenic concentrations via the water and probably also via their main feed, bamboo. This raises the

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important question whether the animals have somehow adapted to this circumstance or if the chronic exposure to arsenic is negatively influencing the animals' health. However, almost nothing is known about the arsenic metabolism of Giant Pandas. One exception is a recent publication by Chen et al. who investigated the arsenic concentrations in feces samples of wild and captive Giant Pandas (Chen et al., 2016). They found between around 0.05 and 0.5 mg As/kg dry mass (dm) in the fecal droppings, whereupon the samples of the captive animals contained significantly more arsenic than the samples of the wild animals. There is only one other publication reporting arsenic levels in samples of Giant Pandas, namely in hair. According to the authors, 0.04–0.528 mg As/kg was found in the hair of 53 wild Giant Pandas (Zhang and Wei, 2006).

When looking at other terrestrial mammals, a lot of research has been conducted during the last decades. Most of the times, urine is the sample type of choice, because it is easily accessible, non-invasive and usually demands less sample preparation than solid samples. Last but not least, it is generally thought that urine is the main excretion route of arsenic in terrestrial mammals. Arsenic can also be found in bile and feces of mammals, but in much lower concentrations than in urine (Csanaky and Gregus, 2002; Hansen et al., 2003; Hughes et al., 2003).

Urinary arsenic concentrations often serve as a biomarker for arsenic exposure. Urine of humans from uncontaminated areas contains on average less than 10 μg As/L (Caldwell et al., 2009; Lindberg et al., 2006; Navas-Acien et al., 2008; Neamtiu et al., 2015; Schulz et al., 2007). There is far less information on background levels of arsenic in the urine of experimental animals such as mice or rats, but it is considered to be less than 10 μg/L as well (Hughes et al., 2003).

When investigating arsenic, it is crucial to determine not only the concentration, but also the chemical forms (species) in which the element is present. The two inorganic species arsenous acid [As (III)] and arsenic acid [As (V)] are the main arsenic species in natural water (Smedley and Kinniburgh, 2002) and soil (Sun et al., 2015) and are considered to be highly toxic, acutely as well as chronically (Hughes, 2002). In living terrestrial organisms, the small, pentavalent compounds methylarsonic acid (MA) and dimethylarsinic acid (DMA) are found very often. They are far less toxic than the inorganic arsenic species and are the main products of the arsenic metabolism of humans and most of the terrestrial mammals (Vahter, 2002; Watanabe and Hirano, 2013). In the urine of humans, typically 60–80% of the arsenic is present as DMA, 10–20% as MA and also 10–20% as inorganic arsenic (Navas-Acien et al., 2008; Neamtiu et al., 2015; Vahter, 1999). For mice, rats, hamsters, rabbits and rhesus monkeys, the arsenic speciation in urine is similar to that of humans; only the amount of MA can be significantly lower and the percentage of inorganic arsenic slightly higher (Vahter, 2002). Still, the metabolism of arsenic by mammals is not fully understood, and several mechanisms have been proposed (see the short survey by Cullen (2014)). What all of them have in common is that ingested inorganic arsenic is first methylated to MA and then further on to DMA, which is then excreted mainly via the urine. These reactions are catalyzed by arsenic (+3 oxidation state) methyltransferase (As3MT) (Lin et al., 2002). Genetic polymorphisms of this enzyme in humans are associated with differences in the urinary arsenic speciation (Antonelli et al., 2014), especially concerning the percentage of MA.

Some publications report the presence of traces of trivalent arsenic species (methylarsonous acid and dimethylarsinous acid) and even sulfur-containing arsenic compounds in urine samples (Aposhian et al., 2000; Mandal et al., 2001). Depending on the proposed pathway, such trivalent arsenicals are created as intermediates, either in their free forms or bound to biomolecules (Dheeman et al., 2014; Hayakawa et al., 2005). However, these species are usually very labile and easily oxidized, even at low temperatures (Gong et al., 2001). This makes their detection and especially their correct quantification quite challenging.

Interestingly, it has been discovered that guinea pigs, chimpanzees, marmoset monkeys, tamarin monkeys and squirrel monkeys are apparently not metabolizing the element at all and are excreting ingested inorganic arsenic unchanged (Healy et al., 1999; Vahter et al., 1995). Little to nothing is known about the arsenic metabolism of other terrestrial mammals. Exceptions are the investigations of seaweed-eating sheep in Scotland (Feldmann et al., 2000) and of horses which were administered solutions of MA (Assis et al., 2008). In the latter case, MA was the main arsenic species in the horses' urine shortly after the injection, as could be expected. However, after 5 days, DMA accounted for 75% of the excreted arsenic. In case of the seaweed-eating sheep, the main urinary arsenic species was DMA as well, but a couple of other rather unusual arsenic species (some not identified) were also found, for example dimethylarsenoethanol or the tetramethylarsonium ion, which are alleged metabolites of arsenosugars that are present in seaweed (Taylor et al., 2017). This clearly demonstrates that the amount of arsenic and the arsenic speciation that is ingested strongly influence the arsenic concentrations and arsenic speciation in excretes. It is therefore important to also look at the drinking water and feed when investigating the arsenic metabolism of animals.

Concerning arsenic in bamboo, which is the primary feed of Giant Pandas, not much is known until now. Chen et al. found roughly 0.3–0.7 mg As/kg dm in two bamboo species (*Fargesia qinlingensis* and *Bashania fargesii*) that were fed to Giant Pandas in China (Chen et al., 2016). The only other report about arsenic in bamboo was published by Zhao et al., in 2006 (Zhao et al., 2006). They investigated total arsenic concentrations and also the arsenic speciation in moso bamboo shoots (*Phyllostachys pubescens*) and found 27.7–94.0 μg As/kg dm in the samples (Zhao et al., 2006). In water/methanol extracts (extraction efficiency: 66–70%) they were able to detect primarily inorganic arsenic, but also DMA, MA and traces of trimethylarsine oxide. In some samples, the percentage of DMA was even higher than the one of inorganic arsenic. This is a bit surprising, because although organic arsenic compounds have been detected in plant extracts, they usually only account for a small part of the total arsenic, whereas inorganic arsenic is most of the times the main extractable arsenical (Geiszinger et al., 2002; Koch et al., 2000; Ruiz-Chancho et al., 2008).

The aim of our study was to elucidate the pathway of arsenic through the Giant Panda. For this purpose, we collected urine and feces samples of the two adult Giant Pandas living at Vienna zoo at the time of sampling and also of their feed, which consisted of different sorts of bamboo. The samples were investigated for the total arsenic concentrations and for their arsenic speciation with inductively coupled plasma mass spectrometry (ICPMS) and high performance liquid chromatography (HPLC) coupled to ICPMS.

2. Materials and methods

2.1. Chemicals and instruments

All solutions were prepared with ultrapure water (18.2 MΩ*cm, Merck Millipore, Bedford, USA). Nitric acid (≥65% p.a., further purified via sub-boiling), methanol (≥99.5%), triflouroacetic acid (TFA, ≥99.9%), hydrogen peroxide (30% p.a.), ammonia (aqueous solution, ≥25%, p.a) and single element standards of germanium and arsenic were purchased from Carl Roth GmbH + Co.KG (Karlsruhe, Germany). Phosphoric acid was obtained from Sigma-Aldrich (Fluka TraceSELECT™ Ultra, for trace analysis, ≥ 85%). Standard solutions of arsenic species (1000 mg As/L) were prepared as follows. As [V] was prepared from Na_2HAsO_4*$7H_2O$, purchased from Merck (Darmstadt,