Pharmacodynamics of S-dimethylarsino-glutathione, a putative metabolic intermediate of inorganic arsenic, in mice

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A R T I C L E  I N F O

Article history:
Received 25 August 2016
Accepted 18 November 2016
Available online 23 November 2016

Keywords:
S-dimethylarsino-glutathione
Arsenite
Tissue distribution
Albumin
HPLC-ICP-MS

A B S T R A C T

Inorganic arsenicals are well-known carcinogens, whereas arsenite (iAsIII) compounds are now recognized as potent therapeutic agents for several leukemias, and arsenic trioxide has been used for the treatment of recurrent acute promyelocytic leukemia (APL). However, recent clinical trials revealed that arsenite is not always effective for non-APL malignancies. Another arsenical, S-dimethylarsino-glutathione ([DMAIII(GS)]), which is a putative metabolic intermediate in the hepatic metabolism of iAsIII, shows promise for treating several types of lymphoma. However, the metabolism of [DMAIII(GS)] has not been well investigated, probably because [DMAIII(GS)] is not stable in biological fluids where the concentration of glutathione is low. In the present study, we injected [DMAIII(GS)] intravenously into mice and compared the tissue distribution and metabolic dynamics of [DMAIII(GS)] with those of sodium arsenite (NaAsO2). We found a unique organ preference for the distribution of [DMAIII(GS)] to the lung and brain in comparison to NaAsO2. Furthermore, [DMAIII(GS)] appeared to bind to serum albumin by exchanging its glutathione moiety quickly after administration, providing novel insights into the longer retention of [DMAIII(GS)] in plasma.

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1. Introduction

Inorganic trivalent and pentavalent arsenicals (iAsIII and iAsV) are present in food and drinking water [23] and are metabolized to DMAV as an end product in mammals. Thus, methylated metabolites of inorganic arsenic (iAs) such as methylarsonate (MMAV) and dimethylarsinic acid (DMAV) were first recognized as detoxified metabolites in 1973 [2]. Two metabolic pathways have been proposed. One is a classical “oxidative methylation” mediated through tandem methylations on arsenic (first from iAsIII to MMAV, and then from MMAIII to DMAV) [4]. The other one is “glutathione-mediated methylation”, where iAs is first conjugated with reduced glutathione to generate arsenic triglutathione, then sequentially methylated by the S-adenosyl-L-methionine (SAM)-dependent arsenic 3 methyltransferase AS3MT/Cyt19 [9]. Thus, S-dimethylarsino-glutathione ([DMAIII(GS)]) is presumed to be a metabolic intermediate.

The methylation of arsenic by AS3MT is now considered to be an activation rather than a detoxification process because both [DMAIII(GS)] and dimethylarsinous acid (DMAV) are more toxic in vitro than iAsIII [19]. It was reported that [DMAIII(GS)] is more efficiently taken up by cells and more potently inhibits growth and induces apoptosis in various malignant hematologic cell lines compared to iAsIII [6]. Despite its potentially higher toxicity in vitro, the LD50 of [DMAIII(GS)] was about 50-fold higher than that of iAsIII in mice [8].

Those remarkable competencies have pushed [DMAIII(GS)] into clinical trials in patients with refractory solid tumors, multiple myeloma, and other types of non-Hodgkin’s lymphoma such as diffuse large B-cell lymphoma as well as peripheral T-cell lymphomas (PTCL) [1,22,24,11]. In the present study, we compared the metabolic dynamics of [DMAIII(GS)] and iAsIII in blood. We report that [DMAIII(GS)] binds to plasma components, resulting in a unique tissue distribution of arsenic in mice following intravenous injection.

2. Materials and methods

2.1. Chemicals

Trizma base, bovine serum albumin (BSA), sodium arsenite (NaAsO2) and dimethylarsinic acid (DMAV) were purchased from...
Heparinized blood was collected and centrifuged at 1600 g. Control mice were injected with an equivalent volume of saline. Sacrificed 5 min after injection under pentobarbital anesthesia.

2.2. Preparation of [DMA\text{III}(GS)]

[DMA\text{III}(GS)] was synthesized according to previously published procedures with minor modifications [5,12,10]. Briefly, DMA\text{V} (2 mmol) and GSH (6 mmol) were dissolved in 10 mL deionized water and the solution was stirred overnight under a nitrogen atmosphere. Then, the water was evaporated under reduced pressure without heating using a centrifugal concentrator (5305, Eppendorf, Hamburg, Germany). [DMA\text{III}(GS)] was extracted from the viscous residue using ice-cold methanol. A white powder was obtained after evaporating the methanol, and the powder was further dried and stored in a desiccator. [DMA\text{III}(GS)] solution was freshly prepared before each experiment.

2.3. Animals

The protocols and procedures were in accordance with the institutional guidelines for the care and use of laboratory animals and were approved by the Animal Care and Use Committee of National Institute for Environmental Studies. Specific-pathogen-free 7 week-old male C57BL/6 mice were purchased from Clea Co. (Tokyo, Japan). The animals were kept under a 12-h light/dark cycle, allowed free access to commercial chow and water, and were acclimated for one week before the experiment. Groups of five mice, weighing from 19.5 to 26.1 g, were injected with NaAsO\text{2} or [DMA\text{III}(GS)] via a tail vein under anesthesia with isoflurane at a dose of 0.1 or 0.5 mg As/kg body weight as 0.05 mg As/mL or 0.25 mg As/mL solutions, respectively. In the clinical trial of [DMA\text{III}(GS)] as an anti-leukemic drug (darinaparsin), the dose range from 200 to 300 mg/m² which corresponds to ca. 1.1–1.6 mg As/kg body weight [11]. Organs and blood were collected 1 h after injection under anesthesia with pentobarbital. In another experiment, mice were treated similarly with NaAsO\text{2} or [DMA\text{III}(GS)] at a dose of 0.5 mg As/kg body weight and were sacrificed 5 min after injection under pentobarbital anesthesia. Control mice were injected with an equivalent volume of saline. Heparinized blood was collected and centrifuged at 1600 g for 10 min to separate plasma from red blood cells (RBCs).

2.4. Measurement of arsenic concentration by inductively coupled plasma mass spectrometry (ICP-MS)

Concentrations of arsenic in tissues (liver, kidney, lung, spleen, testis, heart, cerebrum, cerebellum, skin, bone, and muscle) and blood were determined by ICP-MS (Agilent 7500 cx) or ICP-MS/MS (Agilent 8800, Tokyo, Japan) after wet-ashing. Briefly, 0.1 g of tissues or 0.03 to 0.05 mL of liquid samples were digested with 600 μL of HNO\text{3} and 200 μL of 30% H\text{2}O\text{2} at 135 °C for 2 days. Then, each sample was diluted with deionized water to a final volume of 3.0 mL or 5.0 mL. Total arsenic concentrations were measured in the normal mode (7500 cx; m/z 75: 75As\text{+}). The analytical method was validated by measuring the arsenic concentration in the CRM.

2.5. Chemical species analysis for arsenicals in blood plasma by HPLC-ICP-MS

Each plasma sample was diluted two folds with elution buffer (50 mM Tris-HNO\text{3} buffer pH 7.4), filtered through a 0.44 μm pore membrane filter (Millipore, Bedford, MA, USA), and then a 20-μL sample was applied to the HPLC column. The measurement conditions for HPLC were: LC-20AB, SPD-M20A, and CTO-20AC HPLCs (SHIMADZU, Kyoto, Japan); column: Shodex Asahipak GS-220HQ or GS-520HQ (300 mm × 7.5 mm) (Showa Denko, Tokyo, Japan); flow rate: 0.5 mL/min or 1.0 mL/min. The column was eluted with 50 mM Tris-HNO\text{3} buffer (pH 7.4, 25 °C, filtered through a 0.22 μm membrane) and the concentrations of arsenic and sulfur were continuously measured by ICP-MS. The arsenic standard solution contained iAs\text{V}, iAs\text{III}, and [DMA\text{III}(GS)] at a concentration of 100 ppb As and arsenobetaine and DMA\text{V} at 50 ppb As.

2.6. In vitro reaction of [DMA\text{III}(GS)] with fractionated plasma

Untreated plasma was collected from control male C57BL/6 J mice. Twenty-μL samples were applied to the GS-520HQ column and the eluate was collected as 3 fractions (8–14 min, 14–15 min, 15–17 min) under a N\text{2} atmosphere. The protein concentration of each fraction was measured using the BCA method and was adjusted to 0.01 mg/mL to normalize the samples. [DMA\text{III}(GS)] was added to a final arsenic concentration of 50 ppb As. Each sample was incubated for 1 h at 37 °C with mixing using a thermomixer (Eppendorf). Chemical speciation analysis for arsenic was conducted by HPLC-ICP-MS using a GS-520HQ column as described above.

2.7. In vitro reaction of [DMA\text{III}(GS)] with albumin-depleted plasma

Plasma was depleted of albumin using a BioMag\textsuperscript{®} ProMax Albumin Removal Kit. Albumin removal from the plasma was confirmed by SDS-PAGE (NuPAGE\textsuperscript{®} Bis-Tris 4–12% gel, Life Technologies, Carlsbad, CA) followed by Coomassie Brilliant Blue (CBB) attaining and also by HPLC. [DMA\text{III}(GS)] was added to a final arsenic concentration of 50 ppb. After incubation for 1 h at 37 °C, speciation analysis was conducted by HPLC-ICP-MS using a GS-520HQ column as described above.

2.8. Statistical analysis

Data are presented as mean ± SD. Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test. Statistical significance was considered when p < 0.05.

<table>
<thead>
<tr>
<th>Recovery of As (% of dose)</th>
<th>Lung</th>
<th>Liver</th>
<th>Urine</th>
<th>Feces</th>
<th>Others</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mg As/kg DMA\text{III}(GS)</td>
<td>0.045 ± 0.019</td>
<td>1.11 ± 0.76</td>
<td>76.6 ± 11.8</td>
<td>8.02 ± 3.45</td>
<td>6.56 ± 1.31</td>
<td>93.0 ± 11.7</td>
</tr>
<tr>
<td>0.5 mg As/kg NaAsO\text{2}</td>
<td>0.046 ± 0.103</td>
<td>0.604 ± 0.50</td>
<td>68.5 ± 18.7</td>
<td>8.19 ± 3.76</td>
<td>2.06 ± 0.74</td>
<td>80.3 ± 17.0</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. Others: cerebellum + cerebrum + heart + spleen + kidney + testis + bone + skin + muscle + whole blood + plasma.
### Table 2
Concentrations of arsenic in the organs and blood at 1 h after intravenous injection of S-dimethylarsino-glutathione ([DMAIII(GS)]) or sodium arsenite (NaAsO2) at doses of 0.1 and 0.5 mg As/kg body weight.

<table>
<thead>
<tr>
<th>Concentrations of arsenic (ng/g)</th>
<th>Cerebrum</th>
<th>Cerebellum</th>
<th>Lung</th>
<th>Heart</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
<th>Testis</th>
<th>Bone</th>
<th>Skin</th>
<th>Muscle</th>
<th>Whole blood</th>
<th>Plasma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.7 ± 5.2</td>
<td>18.2 ± 3.3</td>
<td>46.6 ± 4.7</td>
<td>34.0 ± 5.1</td>
<td>42.6 ± 10.4</td>
<td>24.0 ± 4.4</td>
<td>312.6 ± 285</td>
<td>33.4 ± 17.0</td>
<td>63.2 ± 77.9</td>
<td>38.2 ± 29.0</td>
<td>53.5 ± 58.6</td>
<td>31.2 ± 4.1</td>
<td>32.7 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>0.1 mg As/kg DMAIII(GS)</td>
<td>30.9 ± 5.9</td>
<td>39.3 ± 5.0</td>
<td>387.4 ± 80.5</td>
<td>91.1 ± 2.8</td>
<td>220.8 ± 7.5</td>
<td>82.1 ± 6.8</td>
<td>317.4 ± 54.0</td>
<td>36.6 ± 14.6</td>
<td>84.5 ± 20.1</td>
<td>41.8 ± 12.3</td>
<td>47.1 ± 5.5</td>
<td>85.3 ± 24.4</td>
<td>106.0 ± 15.7</td>
<td></td>
</tr>
<tr>
<td>0.1 mg As/kg NaAsO2</td>
<td>33.5 ± 12.2</td>
<td>36.5 ± 3.3</td>
<td>191.7 ± 17.5</td>
<td>97.4 ± 6.2</td>
<td>217.1 ± 12.3</td>
<td>120.0 ± 17.1</td>
<td>486.5 ± 66.2</td>
<td>38.6 ± 5.5</td>
<td>59.6 ± 29.9</td>
<td>80.2 ± 39.6</td>
<td>50.6 ± 7.0</td>
<td>160.0 ± 24.4</td>
<td>209.4 ± 19.6</td>
<td></td>
</tr>
<tr>
<td>0.5 mg As/kg DMAIII(GS)</td>
<td>176.2 ± 68.8</td>
<td>171.2 ± 34.7</td>
<td>1989 ± 423</td>
<td>402.6 ± 77.6</td>
<td>869.3 ± 158</td>
<td>346.0 ± 61.2</td>
<td>1302 ± 319</td>
<td>94.6 ± 15.6</td>
<td>315.4 ± 103</td>
<td>176.4 ± 47.7</td>
<td>221.3 ± 25.8</td>
<td>337.9 ± 100</td>
<td>345.9 ± 64.5</td>
<td></td>
</tr>
<tr>
<td>0.5 mg As/kg NaAsO2</td>
<td>73.4 ± 61.4</td>
<td>54.9 ± 10.0</td>
<td>803.5 ± 63.7</td>
<td>433.3 ± 46.2</td>
<td>1600 ± 427.2</td>
<td>422.9 ± 41.1</td>
<td>1417 ± 189</td>
<td>71.0 ± 12.3</td>
<td>196.6 ± 56.4</td>
<td>207.8 ± 53.7</td>
<td>168.1 ± 21.8</td>
<td>233.5 ± 17.9</td>
<td>277.9 ± 118.4</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. *, significantly different from the corresponding NaAsO2-treated group (p < 0.05).

### Table 3
Recovery of arsenic in the organs and blood at 1 h after intravenous injection of S-dimethylarsino-glutathione ([DMAIII(GS)]) or sodium arsenite (NaAsO2).

<table>
<thead>
<tr>
<th>Recovery of As (% of the dose)</th>
<th>Cerebrum</th>
<th>Cerebellum</th>
<th>Lung</th>
<th>Heart</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
<th>Testis</th>
<th>Bone</th>
<th>Skin</th>
<th>Muscle</th>
<th>Whole blood</th>
<th>Plasma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mg As/kg DMAIII(GS)</td>
<td>0.29 ± 0.1</td>
<td>0.073 ± 0.02</td>
<td>20.2 ± 0.2</td>
<td>1.77 ± 0.24</td>
<td>2.2 ± 0.16</td>
<td>0.26 ± 0.03</td>
<td>8.5 ± 0.02</td>
<td>0.28 ± 0.01</td>
<td>0.99 ± 0.02</td>
<td>0.28 ± 0.01</td>
<td>0.28 ± 0.01</td>
<td>2.3 ± 0.02</td>
<td>3.68 ± 0.02</td>
<td>2.69 ± 0.02</td>
</tr>
<tr>
<td>0.1 mg As/kg NaAsO2</td>
<td>0.29 ± 0.1</td>
<td>0.064 ± 0.02</td>
<td>2.69 ± 0.19</td>
<td>0.30 ± 0.09</td>
<td>7.39 ± 0.87</td>
<td>0.28 ± 0.12</td>
<td>2.32 ± 0.04</td>
<td>0.28 ± 0.04</td>
<td>0.28 ± 0.04</td>
<td>0.28 ± 0.04</td>
<td>7.39 ± 0.87</td>
<td>2.7 ± 0.04</td>
<td>10.4 ± 0.04</td>
<td>7.39 ± 0.87</td>
</tr>
<tr>
<td>0.5 mg As/kg DMAIII(GS)</td>
<td>0.46 ± 0.21</td>
<td>0.082 ± 0.03</td>
<td>2.08 ± 0.48</td>
<td>0.33 ± 0.07</td>
<td>7.49 ± 1.52</td>
<td>0.17 ± 0.04</td>
<td>2.37 ± 0.80</td>
<td>0.10 ± 0.06</td>
<td>0.12 ± 0.04</td>
<td>0.08 ± 0.02</td>
<td>0.08 ± 0.02</td>
<td>2.08 ± 0.48</td>
<td>10.4 ± 0.04</td>
<td>2.7 ± 0.04</td>
</tr>
<tr>
<td>0.5 mg As/kg NaAsO2</td>
<td>0.18 ± 0.18</td>
<td>0.020 ± 0.01</td>
<td>0.79 ± 0.08</td>
<td>0.37 ± 0.03</td>
<td>14.3 ± 3.45</td>
<td>0.20 ± 0.03</td>
<td>2.67 ± 0.23</td>
<td>0.072 ± 0.02</td>
<td>0.054 ± 0.02</td>
<td>0.18 ± 0.08</td>
<td>5.88 ± 1.63</td>
<td>2.74 ± 0.21</td>
<td>1.81 ± 0.91</td>
<td>29.3 ± 4.02</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. *, significantly different from the corresponding NaAsO2-treated group (p < 0.05).
3. Results

3.1. Tissue distribution and excretion of [DMAIII(GS)] and NaAsO2

First, we examined how quickly [DMAIII(GS)] was excreted from the body by quantitative measurement of arsenic in the urine and feces by ICP-MS (Table 1). This preliminary examination revealed that most of the injected [DMAIII(GS)] was excreted from the body into feces or urine within 24 h after injection. The total recovery of arsenic was 93.0% for the [DMAIII(GS)]-treated group and 80.3% for the NaAsO2-treated group, suggesting that most injected arsenicals were excreted from the body within 24 h. Then, we intravenously administered [DMAIII(GS)] or NaAsO2 at two doses, and collected the organs and blood 1 h after injection. As shown in Table 2, several notable differences were observed between [DMAIII(GS)] and iAsIII at the higher dose. Concentrations of arsenic in the cerebellum and lung of the [DMAIII(GS)]-treated group were significantly higher than those of the NaAsO2-treated group, and similar tendencies were observed in the cerebrum, testis, bone, and whole blood. In contrast, the arsenic concentration in the liver was higher in the NaAsO2-treated group, suggesting that there are...
To elucidate the early metabolic dynamics of [DMAIII(GS)] in blood plasma, we further examined the tissue distribution of arsenic (Tables 4 and 5) and the elution profiles of arsenic in plasma (Fig. 1) 5 min after intravenous injection of [DMAIII(GS)] or NaAsO2 at a dose of 0.5 mg As/kg. Concentrations of arsenic were higher in the brain (cerebrum and cerebellum) and lung in the [DMAIII(GS)]-treated group than in the NaAsO2-treated group, whereas the arsenic concentration of the liver was higher in the NaAsO2-treated group, which is consistent with the data obtained 1 h after injection. The elution profiles of arsenic in HPLC-ICP-MS analysis revealed that the unidentified peak was remarkably higher in the [DMAIII(GS)]-treated group (Fig. 1), suggesting that a large part of the unidentified peak was incorporated into organs in 1 h after injection.

3.3. Partial characterization of plasma components reacted with [DMAIII(GS)]

We attempted to identify the arsenic peak in plasma of the [DMAIII(GS)]-treated mice that eluted at 10 min in HPLC-ICP-MS measurements. We presumed that [DMAIII(GS)] was easily hydrolyzed in plasma and that DMAIIIIII, a putative hydrolysis product of [DMAIII(GS)], would bind to thiol-containing peptides/proteins. Consequently, we analyzed the plasma collected from [DMAIII(GS)]-treated mice again monitoring UV absorbance (280 nm) of the eluate from HPLC operated in the same condition as the HPLC-ICP-MS measurement. The unidentified arsenic peak was observed on the UV absorbance at 280 nm and also co-eluted with sulfur, suggesting that the unidentified arsenic-binding compound was a protein (Fig. 2). Then, we fractionated the control plasma (Fraction #1, 8–14 min; Fraction #2, 14–15 min; and Fraction #3, 15–17 min) reacted each fraction with [DMAIII(GS)] for 1 h, and analyzed the reaction products by HPLC-ICP-MS. Fig. 3 shows that only Fraction #3 contained arsenic.

3.4. [DMAIII(GS)] binds to albumin forming a complex in blood

To estimate the molecular weight of the candidate substance, we measured the retention time of BSA by monitoring sulfur by HPLC-ICP-MS (Data not shown). The retention time of BSA was very similar to that of the unidentified peak, suggesting that the molecular weight of the arsenic-binding protein was about 60 kDa. Finally, we removed albumin from plasma using the albumin removal kit and confirmed that a large portion of albumin was removed from the blood plasma by SDS-PAGE (Fig. 4(a)). Moreover, HPLC-ICP-MS analysis for sulfur confirmed that albumin was removed from the plasma (Fig. 4(b)). The albumin-depleted plasma was incubated with [DMAIII(GS)] for 1 h and the reaction product was analyzed by HPLC-ICP-MS. No sign of the unidentified arsenic peak was found in albumin-depleted plasma (Fig. 5).

4. Discussion

4.1. Difference in the tissue distribution of arsenic between [DMAIII(GS)] and NaAsO2

In the present study, we showed that more arsenic was accumulated in the lung and brain in the [DMAIII(GS)]-treated mice than those in the NaAsO2-treated mice (Tables 2–5), suggesting that [DMAIII(GS)] was preferentially taken up by the lung and brain compared to NaAsO2. It was previously shown that [DMAIII(GS)] was unstable in culture medium and that a gaseous form of arsenic was likely generated from [DMAIII(GS)] [10]. It is plausible that a small amount of [DMAIII(GS)] was converted to more volatile arsenic substances and that those arsenicals were absorbed by the lungs and brain more efficiently than NaAsO2. However, this hypothesis requires further investigation. Alternatively, it is
possible that [DMAIII(GS)] deposited preferentially in brain tissue compared to iAsIII because the two methyl groups endow [DMAIII(GS)] more hydrophobic or amphiphilic characteristics, aiding absorption through the blood–brain barrier in a manner similar to methyl mercury [14]. In the clinical investigation one report (Phase I) showed that the treatment with [DMAIII(GS)] achieved stable disease in 7 of 40 patients with colorectal or renal tumors or chordoma. Interestingly, 6 out of those 7 patients had metastatic sites in the lung [22]. However, [DMAIII(GS)] was not effective for advanced hepatocellular carcinoma in another clinical study (Phase II) [24], suggesting a potential tissue preference of [DMAIII(GS)]. Our current observation that [DMAIII(GS)] was more preferentially accumulated in the lung and brain than NaAsO2 may lead to another clinical trial of [DMAIII(GS)] for solid tumors.

4.2. Behavior of [DMAIII(GS)] in blood

We demonstrated that the metabolic behavior of [DMAIII(GS)] was different from that of iAsIII in plasma and that albumin reacted immediately with [DMAIII(GS)]. Because pentavalent arsenicals are

Fig. 3. HPLC-ICP-MS elution profiles of arsenic aligned with each UV absorbance (280 nm) in the fractionated plasma for the detection of [DMAIII(GS)]-binding plasma components. The fractionated plasma samples (Fraction 1 – 3) were mixed with [DMAIII(GS)] in vitro with [DMAIII(GS)] and incubated for 1 h at 37 °C. Column, GS-520HQ; flow rate, 0.5 mL/min; eluent, 50 mM Tris-HNO3 (pH 7.4); temperature, 25 °C; injection volume, 20 μL; ICP-MS, Agilent 8800. (a) UV at 280 nm for Fraction 1, (b) 75As16O+ for Fraction 1, (c) UV at 280 nm for Fraction 2, (d) 75As16O+ for Fraction 2, (e) UV at 280 nm for Fraction 3, (f) 75As16O+ for Fraction 3.

Fig. 4. Confirmation of albumin removal by SDS-PAGE (a) and HPLC-ICP-MS (b). (a) The gel (4–12%) was stained with Coomassie Brilliant Blue (CBB) after electrophoresis. (1) Ten-fold diluted untreated control plasma, (2) Supernatant after reaction with separation beads, (3) Proteins remained on the separation beads after washing; The beads were directly boiled with SDS sample buffer for 5 min. (4) Supernatant in the elution buffer (50 μL), (5) Supernatant in the elution buffer (100 μL), (6) Proteins remained after elution; The beads were directly boiled with SDS sample buffer for 5 min. (b) HPLC-ICP-MS elution profiles of sulfur (32S16O+) aligned with the UV absorbance at 280 nm. The peak (15–17 min) disappeared in the albumin-depleted plasma. Column, GS-520HQ; flow rate, 0.5 mL/min; eluent, 50 mM Tris-HNO3 (pH 7.4); temperature, 25 °C; injection volume, 20 μL. (1) UV at 280 nm of the untreated plasma, (2) 32S16O+ of the untreated plasma (3) UV at 280 nm of the albumin-depleted plasma, (4) 32S16O+ of the albumin-depleted plasma.
HPLC-ICP-MS. As we found DMAV alone as a major arsenic metabo-
not seem to bind to plasma proteins as measured by the current Fig. 1(c), was detected only after 1 h. Inorganic arsenic itself did
one reason that the small arsenic peak, labelled as unknown in
[DMAIII(GS)] was originally selected as a promising and heart, limits its versatile application to non-APL malignancies.
dimethylarsinothioyl glutathione [DMMTAIII] was reported to
lite in the urine of [DMAIII(GS)]-injected mice (data not shown), it
is reasonable to suppose that DMAV was eventually released from
the DMAIII-albumin complex.

The transition from [DMAIII(GS)] to DMAIII is proposed to be
involved in the formation of thiolated dimethylmonothioarsinous acid ([DMMTAIII]) [20]. Recently, a novel thiolated pentavalent, dimethylarsinothiol glutathione [DMMTAIII(GS)] was reported to be produced possibly through an as-yet-unknown enzymatic reac-
tion in human multiple myeloma cell lines upon [DMAIII(GS)] exposure [25]. Together, the metabolism of the DMAIII-albumin complex should be studied more precisely including thiolated intermediates.

4.3. [DMAIII(GS)] as an anti-cancer drug

iAsIII has been used as a therapeutic drug for acute promyelo-
cytic leukemia (APL). Although up to 87% of APL patients respond well to iAsIII [18], a degree of systemic toxicity, especially in liver and heart, limits its versatile application to non-APL malignancies. [DMAIII(GS)] was originally selected as a promising anti-cancer compound from more than 100 organic synthetic arsenicals in the screen utilizing the NCI-60, an authentic cancer cell collections provided by NIH [8].

[DMAIII(GS)], which is also called Darinaparsin in clinical studies, is one of the novel organic arsenical compounds for the clinical trial treating patients with relapsed or refractory T-cell lymphomas (TCLs), uncommon and aggressive non-Hodgkin lymphoma that are difficult to treat, and refractory and relapsed Hodgkin lymphoma. Particularly, PTCL is categorized as unfavorable TCLs and accounts for 7–10% of the non-Hodgkin lymphoma. Accordingly, [DMAIII(GS)] is now recognized as a potent orphan drug for PTCL in Europe and the USA following the particular success in PTCL in the phase II trial [11]. Our present study indicates that [DMAIII(GS)] has two advantages as a cancer therapeutic compared to iAsIII: glutathione masks the toxicity of DMAIII, and [DMAIII(GS)] exhibits a longer residence time in blood. Moreover, most [DMAIII(GS)] is excreted from the body in urine or feces within 24 h. The mecha-
nism for the cellular clearance and uptake of iAsIII has been exam-
ined extensively. Multi-drug resistant proteins (MRPs) or aquaglyceroporins are suggested to be involved because MRP-1- [15], MDR1a/1b- [13], and AQP9-null [3] mice were vulnerable to iAsIII and more arsenic accumulated in their organs compared to wild-type mice. Several reports shed some light on the clearance and uptake of [DMAIII(GS)]. The excretion of [DMAIII(GS)] was much less affected by the expression level of MRP-1 than was iAsIII [6]. Furthermore, it has been shown that [DMAIII(GS)] is degraded by γ-GT (gamma-glutamyltransferase) and subsequently by di-
peptidase dissociating S-dimethylarsino-cysteine (DMAC) [7]. In this context, DMAC has a favorable conformation for transport through cystine/cysteine transporters such as xCT and xAG [7]. These findings suggest that [DMAIII(GS)] has unique chemical char-
acteristics that are different from iAsIII.

It has been reported that [DMAIII(GS)] activated several tumor-
related signaling pathways such as the Jun N-terminal kinase (JNK) pathway, promoting apoptosis [6], and the SHP1-dependent extracellular signal-regulated kinase (ERK) pathway [17]. Also, [DMAIII(GS)] appears to effectively radio-sensitize solid tumors in a reactive oxygen (ROS)-independent manner [21]. Further examination of the metabolic dynamics of [DMAIII(GS)] in the plasma from PTCL patients and/or using malignant cells may help elucidate the clinical effectiveness of [DMAIII(GS)] and understand the efficacy of [DMAIII(GS)] for treating leukemia [11]. Although more mechanistic studies are required to fully understand the effectiveness of [DMAIII(GS)] as an anti-cancer drug, the present study revealed the basic metabolic behavior of intravenously injected [DMAIII(GS)] and shed light on the therapeutic use of [DMAIII(GS)].

Conflicts of interest

There are no conflicts of interest to declare.
Acknowledgements

The authors would like to thank staffs of the Animal Care, Ms. Mihoko Tatano, and Ms. Masako Hirano of NIES for their technical assistance with the animal experiments and ICP-MS measurements. This work was partially supported by the Sasakawa Scientific Research Grant from The Japan Science Society (No. 28-601) and JSPS (#16K15386).

References

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