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Detection of diphenylarsinic acid and its derivatives in human serum and cerebrospinal fluid

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ABSTRACT

Background: Residents (n = 157) of Kamisu City, Ibaraki, Japan, were orally exposed to diphenylarsinic acid (DPAA) via the ingestion of contaminated underground water. Subsequently, a clinical syndrome associated with a variety of cerebellar and brainstem symptoms, was observed in 20 of the 30 residents who consumed high concentrations of DPAA in the contaminated well water. While the clinical symptoms of DPAA were defined, the toxicokinetics of DPAA remained unclear.
Methods: In order to investigate the underlying toxicokinetics of DPAA, we collected serum and cerebrospinal fluid (CSF) samples from 5 patients with DPAA intoxication, and attempted to estimate the half-life of serum DPAA and the CSF/serum ratio of DPAA.
Results: DPAA, and its derivatives, such as phenylmethylarsinic acid (PMAA) and phenylarsinic acid (PAA), were detected in serum from residents exposed to DPAA. Serum DPAA was observed for >200 days after the last ingestion of contaminated water. The half-life of serum DPAA was 22.5 days in children and 39.4 days in youths and adults, which was nearly double that observed in children. DPAA was found in CSF, and the CSF/serum ratios of DPAA in 2 patients were 3.0% and 3.7%, respectively, suggesting that this toxicant is able to cross the blood–brain barrier.
Conclusion: An established animal model of DPAA intoxication was examined regarding the toxicokinetics, distribution and direct DPAA accumulation in the cerebrum. On the basis of existing animal data, and the present results arising from human subjects, the development of new therapies for DPAA intoxication should be enhanced, such as accelerated DPAA excretion.

1. Introduction

Diphenylarsinic acid (DPAA) is a chemical component of diphenylcyanoarsine (DC) and diphenylchloroarsine (DA) which are used to synthesize chemical weapons. A large amount of this sternutator, referred to by Japanese Agents by its code name “Red No. 1”, was manufactured in Japan by the Imperial Army during World War II [1]. During production of chemical weapons, the chemical component, DPAA, was also synthesized and stored in Japan and neighboring countries, and was believed to have been disposed in an appropriate manner after the war. However, there have been cases where toxic substances from abandoned chemical weapons have leaked to the surrounding environment due to corrosion or damage from construction work, and have caused health hazards by Mustard and the others at Hiratsuka city, Samukawa city and Narashino city in Japan, and at Qiqihar city in Heilongjiang Province, China in recent year [2].

The residents of Kamisu city, Ibaraki, Japan, started to become exposed to DPAA via contaminated well water, prior to the year 2000. The source of contamination was identified as an underground concrete block. Twenty residents suffered from progressing cerebellar and brainstem symptoms including nystagmus, dizziness, ataxic gait, tremors, myoclonus, cerebellar dysarthria, along with temporal and occipital lobe symptoms including memory impairment, sleep disturbance, visual disorders, as well as cerebral atrophy and mental retardation in children. Since DPAA was detected in drinking well water and detected in patient specimens, these neurological symptoms were believed to have been caused by DPAA poisoning [3].

Animal studies revealed that DPAA accumulates more easily than inorganic arsenicals in the brain of mice. This DPAA accumulation in the cerebrum resulted in the spatial learning disabilities demonstrated in the Morris Water Maze tests [4]. DPAA administration on mice induced similar cerebellar dysfunctions to those observed in DPAA-exposed residents. In addition, DPAA was found to produce oxidative stress in Purkinje cells [5]. In a study where DPAA 5 mg/kg/day was administered repeatedly, symptoms including tremors, cerebellar dysfunctions and sensigenous myoclonus were observed, just as in humans [6]. In addition, as observed in repeated administration of DPAA study for 100 days with monkeys, which leads to transient generalized myoclonus [7,8], DPAA administration on animals manifests

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neurologic symptoms similar to those of the residents exposed to DPAA. Therefore, the neurologic symptoms observed in DPAA-exposed residents are caused by DPAA.

An earlier study reported that phenylmethylarsinic acid (PMAA) and phenylarsonic acid (PAA), which are assumed to be metabolites of DPAA, were detected in urine [9] (Fig. 1). However, there has been no investigation of the biological dynamics and metabolism of DPAA, no reported detection of its derivatives in the blood, nor of its potential ability to penetrate to the brain. The present study aimed to assess whether DPAA could cross the blood–brain barrier (BBB) by evaluating the biological half-life of serum DPAA and migration rate to cerebrospinal fluid (CSF) by quantitatively detecting DPAA in the serum and CSF of exposed residents.

2. Subjects, materials and methods

2.1. Patient profiles

Of 157 residents of Kamisu city who had been exposed to DPAA and detected DPAA from biosamples as hair, nails, urine and blood, we studied 30 residents from 10 families who had ingested water from the same contaminated well. The exposed group consisted of 5 residents from 3 families, whose serum and CSF samples were collected during a period in which neurological symptoms were being observed. The remaining 25 exposed subjects included those who had moved away from the places where well water was contaminated with DPAA, those who were still living in contaminated locations but rarely drinking well water, and those who did not cooperate in this study. The concentration of DPAA in water from the drinking well was 4.5 mgAs/l (DPAA 15.5 mg/l), and from 2 to 3 liter per day was ingested in water via cooking and drinking. This would amount to 0.62–0.93 mg/kg/day if body weight was set at 50 kg. Since all 5 residents (patients) were drinking from the same well, we assumed that the level of DPAA exposure was similar within the group [10]. Symptoms of DPAA intoxication such as cerebellar ataxia, tremors and myoclonus appeared in all 5 patients. In addition, Patients #1 and #2 suffered from mental retardation, #2 and #3 from night terrors, and Patients #3, #4 and #5 from memory impairment, sleep disturbance and visual disorders, respectively (Table 1). In addition, since these neurological symptoms had already appeared when the patients had stopped drinking the well water, the level of exposure was assumed to have surpassed the lowest-observed-adverse-effect level (LOAEL).

Table 1
Clinical features of patients with DPAA intoxication.

No.	Age (year)	Sex	Neurological symptoms	Other symptoms
1	2	M	Cerebellar ataxia, tremor, mental retardation,	Bronchitis
2	7	F	Cerebellar ataxia, tremor, myoclonus, night terror, mental retardation	AST elevation
3	15	F	Cerebellar ataxia, tremor, myoclonus, memory impairment, night terror	
4	38	F	Cerebellar ataxia, tremor, myoclonus, memory impairment, sleep disturbance	
5	42	F	Cerebellar ataxia, tremor, myoclonus, memory impairment, sleep disturbance, visual disorder	Edema, dermatitis

AST: aspartate aminotransferase in serum. These cases are referred to Ishii et al. [3].

2.2. Sample collection and storage

Peripheral blood and CSF (via lumbar puncture) were collected from the 5 particular patients with high levels of DPAA intoxication. Two patients of #4 and #5 offered the collection of their CSF, but #1, #2 and #3 did not receive CSF collection. Blood samples were centrifuged at 1500 rpm for 10 min, then the supernatant was separated as serum. The date of sample collection was recorded and used to calculate the number of days after the last ingestion of contaminated water. Patient and sample profiles are shown in Tables 1 and 2. These samples were stored at −40 °C until the measurement of DPAA, PMAA and PAA concentrations. This study was inspected and approved by the Clinical Studies Ethical Review Committee of the University of Tsukuba, and the Ethical Committee of National Institute for Environmental Studies. Each patient gave written informed consent, which was approved by the appropriate Ethical Committees.

2.3. Analysis of phenylarsenic compounds in serum or CSF

Diphenylarsinic acid (DPAA) and phenylarsonic acid (PAA) were supplied by Tri Chemicals Laboratories Inc. $^{13}C_{12}$-DPAA and $^{13}C_6$-PAA were supplied by Hayashi Pure Chemicals Ind. Phenylmethylarsinic acid (PMAA) was synthesized as reported previously [11]. The purity of PMAA (97%) was assessed by HPLC–ICPMS and NMR spectrometry. All other chemicals were of reagent grade or HPLC grade.

An aliquot of either 500 or 200 μl of serum supernate or CSF (depending on the amount of samples available for analysis) was precisely weighed, and treated with 2 ml of 2 mol/l sodium hydroxide solution and 8 mg of bovine serum albumin at 90 °C for 3 h. The solution was

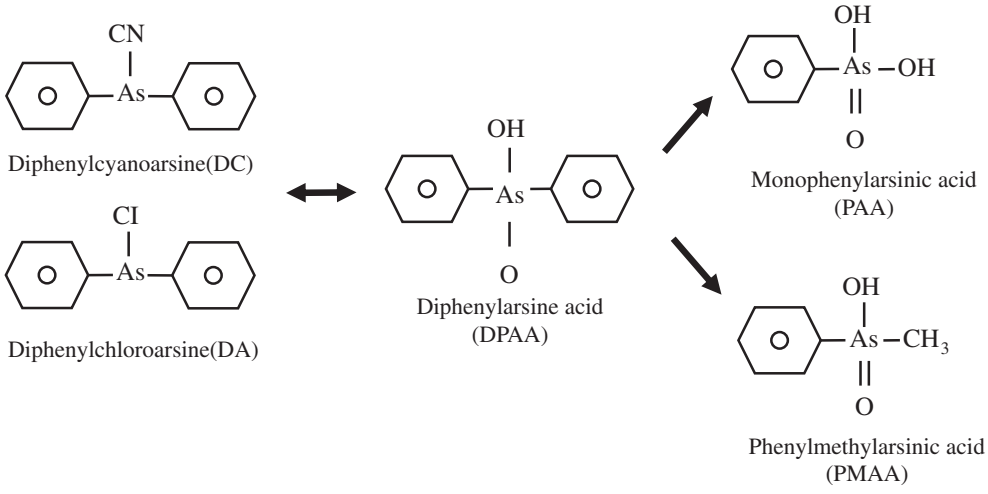

Fig. 1. Decomposition of diphenylarsine compound and its biotransformation. The causal substance of this poisoning is DPAA (diphenylarsine acid), and this substance is produced through the degradation of diphenylcyanoarsine (DC) or diphenylchloroarsine (DA). Also, DPAA is used as a synthetic material for DC and DA. Furthermore, DPAA is thought to transform to PAA or PMAA through bio-metabolism and/or enterobacterial flora.