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Pharmacokinetics and tissue distribution of the antileukaemic organoarsenicals arsthinol and melarsoprol in mice

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Abstract

Melarsoprol and arsthinol have been shown to be effective on various leukaemia cell lines. Nevertheless, the tissue distribution of these compounds remains a key point since the bone marrow is considered as the site of action and the central nervous system as the site of the main toxicity. In this study, we have determined the exposure of each organ (blood, liver, bone marrow and brain) to arsenic, irrespectively of the exact nature of arsenic species contained by the organ.

In the bone marrow, arsenic concentrations were very high, especially that of melarsoprol. However, the lower ability of arsthinol to concentrate in the bone marrow could be compensated by its higher antileukaemic activity.

The brain concentrations were high, although lower than in the bone marrow; this fact is in very good accordance with the observation that the brain is mainly involved in the acute toxicity of trivalent organoarsenicals.

Keywords: Arsthinol; Melarsoprol; Arsenic; Pharmacokinetics; Leukaemia

1. Introduction

Beside their well-known toxic effects, organic and inorganic arsenical compounds, that are found in nature [1,2] or synthesized, exhibit important biological activities. In particular, arsenic trioxide is used as an antileukaemic drug [3] and the organic derivative melarsoprol (Fig. 1a) is still used as a typanocide [4,5]. Moreover, melarsoprol has been shown to be very effective on various leukaemia cell lines but its human use has been hampered by severe encephalopathy [4,5]. Recently, we synthesized new derivatives of (2-phenyl-[1,3,2]dithiarsolan-4-yl)-methanol which exhibited an increased cytotoxic activity on U937 and K562 human cell lines, as compared to As_2O_3 and melarsoprol [6]. The tissue distribution of these compounds remains a key point since the bone marrow is considered as the site of action and the central nervous system (CNS) as the site of the main toxicity. Arsthinol (Fig. 1b), also known as Balarsen®, was first time synthesized more than 50 years ago [7], and it has been shown to have amoebicidal [8] and bactericidal [9,10] activities. Moreover, we have previously demonstrated that this drug exhibited also an antileukaemic activity with a best therapeutic index than arsenic trioxide and melarsoprol, as estimated by the ratio LD_{50}/IC_{50} [6]. Thus, this promising compound was chosen for further experiments and this work has compared the blood kinetics and the tissue distribution of melarsoprol and arsthinol in the liver, bone marrow and brain, after IV injection to mouse.

2. Results and discussion

The non-transformed compounds were detected by HPLC only during the first minutes after injection. This proves to be due to a rapid hydrolysis of the dithiarsolanes into the corresponding arsenoxides [11,12]. As it was previously shown for melarsoprol by Keiser et al. [11,13], the half

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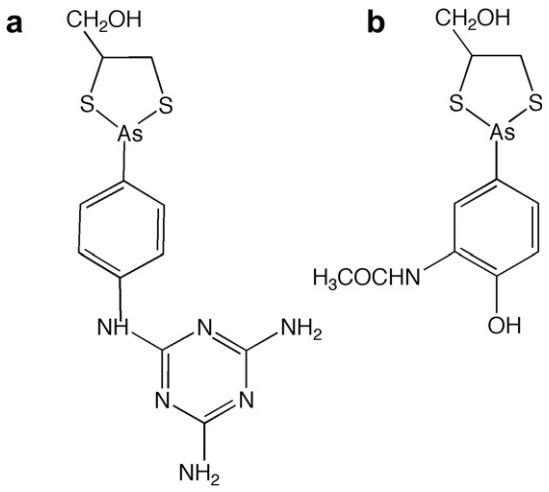

Fig. 1. Chemical structure of melarsoprol (a) and arsthinol (b).

life of the dithiarsolane derivative arsthinol is very short (about 5 min; Fig. 2, inset), while the arsenic concentrations remain high since detectable levels were observed until 20 h after administration (Fig. 2). The total arsenic concentration curve showed a typical bi-exponential shape corresponding to a two-compartment open model described by the equation $C = A \times e^{-k_1 \cdot t} + B \times e^{-k_2 t}$ [14]. Thus, considering the sensitivity of the dithiarsolane ring to hydrolysis leading to the corresponding arsenoxide which avidly bind to sulfhydryl-rich proteins, it was supposed that this second compartment was due to the binding of hydrolyzed arsthinol by plasma proteins. Consequently, the derived pharmacokinetics parameters allow us to estimate this protein binding at 74% using the formula $A/(A + B)$. Finally, the observed binding percentage obtained by *in vitro* experiments was very similar ($77.6 \pm 1.6\%$), confirming the hypothesis.

The total exposure of organs to arsenic following administration of melarsoprol and arsthinol was estimated by the respective area under the concentration–time curves (AUC_{organs}). In order to compare the distribution of both compounds, the total arsenic concentrations (in μmol/g of fresh tissue) were normalized by the amount of drug administered (in μmol/g of body weight). The normalized AUC_{organs} was calculated by the trapezoidal rule and used as exposure index (EI) of each organ to arsenic, irrespectively of the exact nature of arsenic species contained by the organ. Thus, the EI could be considered as representative of the organ/body partition ratio for arsenic [15].

Melarsoprol is known to be rapidly excreted in bile, especially as diglutathione and glucuronide conjugates [16]. As shown in Fig. 3, an initial peak was observed in the liver at 30 min after administration of melarsoprol or arsthinol, However, retarded peaks were also observed at 5 h and 15 h. For melarsoprol, this secondary peak was higher (1.17 ± 0.13 μmol/g per μmol/g of melarsoprol) than the initial peak (0.74 ± 0.13 μmol/g per μmol/g of melarsoprol). Moreover, the curves fit well with the model described by Wajima et al. [17] where the transfer from the bile compartment to the central compartment occurs periodically, and a sine function can be used to represent these periodical changes ($k_b \times \sin(2\pi\,(t + \phi)/\omega)$). Thus, this behavior could be explained by an entero-hepatic recycling of the conjugates. A similar pattern, albeit less pronounced, was observed for arsthinol. The total liver exposure was higher after administra-

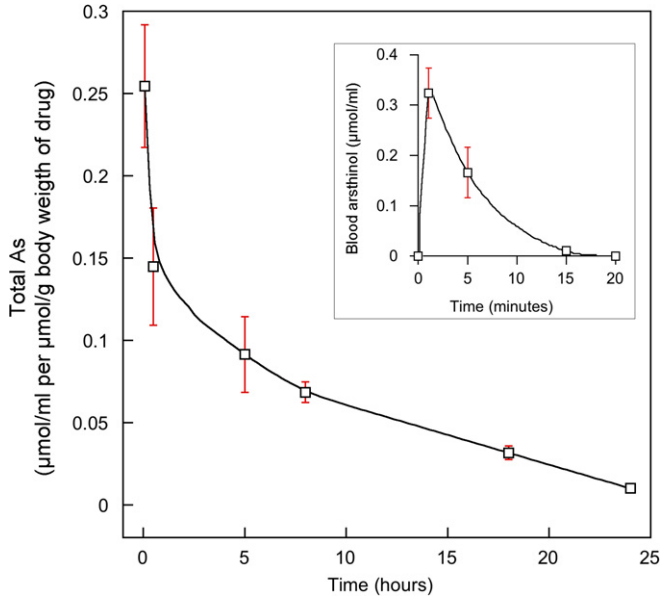

Fig. 2. Blood total arsenic concentrations versus time profiles after injection of arsthinol (0.2 μmol/kg; □). Insert: Concentration of arsthinol after injection (obtained by HPLC). Concentrations were normalized by the injected dose. Symbols represent the means ± SEM of three mice.

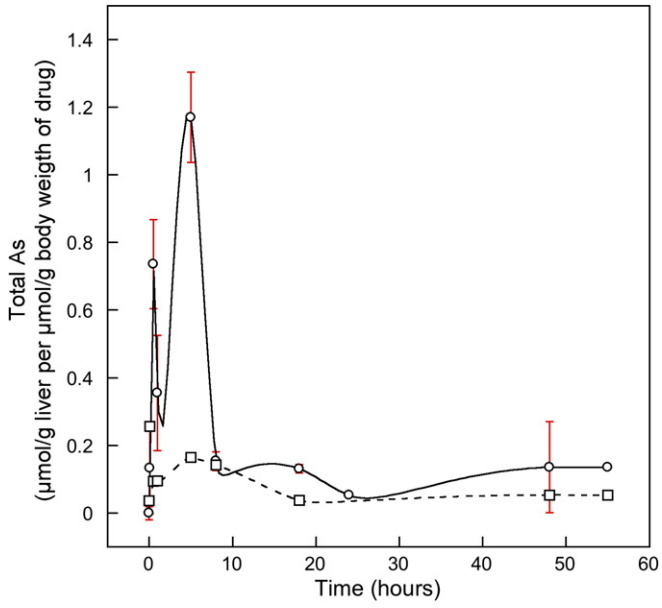

Fig. 3. Liver total arsenic concentrations versus time profiles after injection of melarsoprol (0.056 μmol/g, ○) or arsthinol (0.2 μmol/g; □). Concentrations were normalized by the injected dose. Symbols represent the means ± SEM of three mice.

Table 1
Accumulation of total As in several organs after IV administration of arsthinol and melarsoprol to mice

Organ	Exposure index	
	Melarsoprol	Arsthinol
Blood	ND	1.49 ± 0.26
Liver	10.68 ± 2.90	3.76 ± 0.19
Brain	24.07 ± 6.20	6.79 ± 1.42
Bone marrow	757.44 ± 151.44	51.35 ± 9.17

Isotoxic doses ($LD_{50}/2$) were injected (melarsoprol: 0.056 μmol/g body weight; arsthinol: 0.2 μmol/g body weight). The normalized AUC, expressed in μmol/g organ per μmol/g body weight of administered drug. h, was used as the exposure index to arsenic of each organ. Results are expressed as means ± SE ($n = 3$).

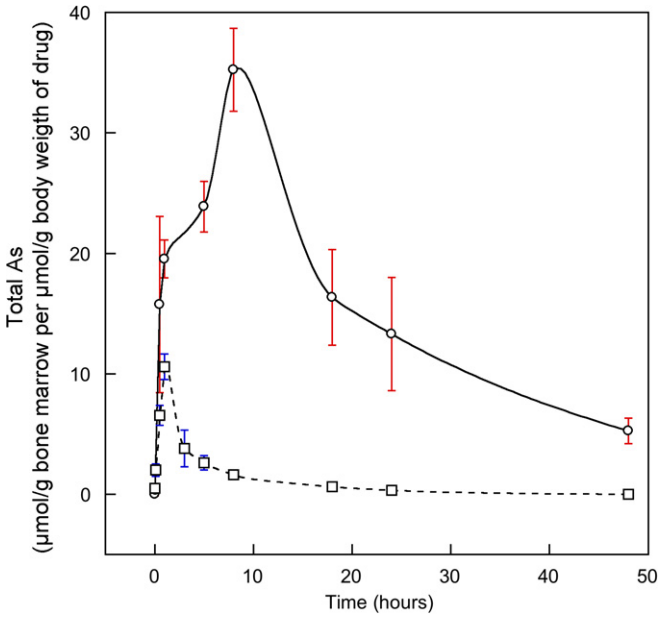

Fig. 4. Bone marrow total arsenic concentrations versus time profiles after injection of melarsoprol (0.056 μmol/g; ○) or arsthinol (0.2 μmol/g; □). Concentrations were normalized by the injected dose. Symbols represent the means ± SEM of three mice.

tion of arsthinol (EI = 10.68 ± 2.90 vs 3.76 ± 0.19, respectively; Table 1). These facts suggest a stronger hepatic first-pass effect for the lipophilic melarsoprol than for the more hydrophilic arsthinol. It could be also postulated that the metabolites or conjugates secreted by the bile were not hydrolyzed and returned to the liver by the portal route without reaching the systemic circulation.

The concentration pattern of arsenic in the lipophilic organs (i.e. bone marrow and brain; Figs. 4 and 5) exhibited a more complex pattern.

In the bone marrow and after injection of arsthinol, the maximal concentration was obtained 1 h after injection followed by a rapid decrease whereas the pharmacokinetics of melarsoprol showed a progressive increase of As concentrations which peak at 8 h ($C_{max} = 35.2 \pm 3.4$ μmol/g of bone marrow per μmol/g of administered melarsoprol)

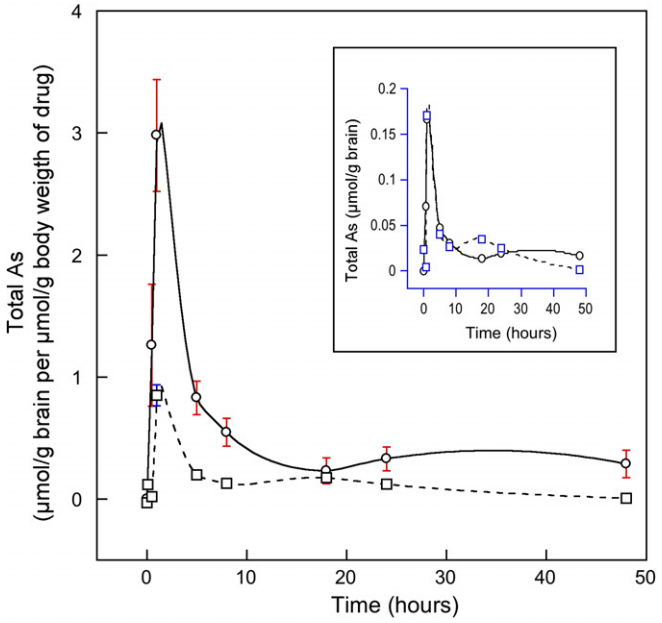

Fig. 5. Brain total arsenic concentrations versus time profiles after injection of melarsoprol (0.056 μmol/g; ○) or arsthinol (0.2 μmol/g; □). Inset: Non-normalized profiles showing identical brain exposure at isotoxic doses of both compounds. Symbols represent the means ± SEM of three mice.

and a more prolonged elimination since the As level at 48 h remained high. The exposure index was very high for both compounds as compared to liver, especially that of melarsoprol (754.44 ± 151.44 vs 51.35 ± 9.17; $p < 0.001$, Fig. 4). This fact is of a crucial importance to explain the excellent activity of As^{III} organic derivatives against leukaemia cells located into the bone marrow. However, the lower ability of arsthinol than melarsoprol to accumulate into bone marrow could be compensated by its higher antileukaemic activity, as we demonstrated on two human cancer cells lines [6]. Actually, the total arsenic level into bone marrow does not entirely reflect the antileukaemic activity of the organoarsenicals.

In the brain, the maximum arsenic peak was obtained after 1 h, both for arsthinol and melarsoprol. As for liver and bone marrow, a higher accumulation was observed for melarsoprol than for arsthinol (EI = 24.07 ± 6.20 vs 6.79 ± 1.42, respectively; $p < 0.001$, Fig. 5). However, when the As levels were expressed in absolute amounts without normalization and considering that isotoxic doses were administered (i.e. half of the respective LD_{50}), the distribution patterns were quite identical (Fig. 5 inset; EI: 1.34 ± 0.60 for melarsoprol vs 1.37 ± 0.49 for arsthinol; *NS*). This fact is in very good accordance with the observation that the brain is mainly involved in the acute toxicity of trivalent organoarsenicals characterized by a major encephalopathy [18,19]. Thus, the modest brain exposure after arsthinol administration could explain its lower acute toxicity as compared to melarsoprol.

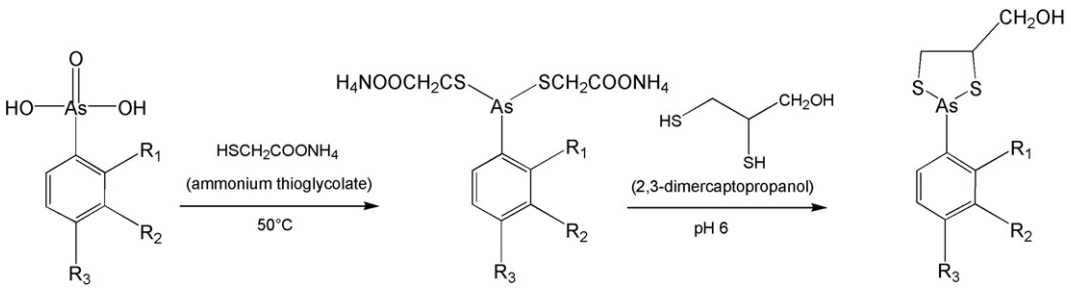

Fig. 6. Typical synthesis pathway for (2-phenyl-[1,3,2]dithiarsolan-4-yl)-methanol derivatives.

Finally, these results demonstrate that bone marrow is more exposed to arsenic than the others organs, especially for melarsoprol.

3. Conclusion

This work is a first step to understand the differences of antileukaemic activity and brain toxicity between several (2-phenyl-[1,2,3]dithiarsolan-4-yl)methanol derivatives. Both melarsoprol and arsthinol lead to very high arsenic levels in the bone marrow, depending of their relative lipophilicity. This strong exposure could be a positive point for a better treatment of refractory leukaemias albeit the antitumor efficacy seems not entirely explained by the bone marrow arsenic content. However, the acute toxicity appears better correlated with the brain arsenic levels. Nevertheless, more complete metabolic studies are required, especially for arsthinol that exhibits one of the most favorable activity/toxicity ratio.

4. Experimental

4.1. Synthesis of (2-phenyl-[1,3,2]dithiarsolan-4-yl)-methanol derivatives

The synthesis of (2-phenyl-[1,3,2]dithiarsolan-4-yl)-methanol derivatives was performed following the method previously described (Fig. 6) [6].

4.2. Animal experimentation

The isotoxic injected dose (i.e. $LD_{50}/2$) was 0.2 mmol/kg for arsthinol and 0.056 mmol/kg for melarsoprol (volume of 0.01 ml/g). These poorly soluble compounds had to be dissolved in a mixture of DMSO/propylene glycol/0.9% NaCl (33/33/33, v/v/v).

Groups of three mice (Female CD1; 20–22 g; Charles Rivers Laboratories, France) were used. At each time point after injection in the caudal vein (5 min, 30 min, 1 h, 5 h, 8 h, 18 h, 24 h and 48 h), blood samples (0.5–1 ml) were collected into heparinized tubes by cardiac puncture under anesthesia (halothane). All sera were frozen at −20 °C until analysis.

Tissue samples (i.e. liver, brain and femurs) were also collected. The bone marrow was extracted from femurs with a syringe (1 ml) of NaCl 0.9%. Then, tissue samples were weighted and stored at −20 °C until analysis.

4.3. Arsenic determination

4.3.1. Quantification of arsenic in the tissues

The tissue concentrations of arsenic were determined using a colorimetric method [20] after digestion with nitric acid (HNO_3; 65%) and hydrogen peroxide (H_2O_2; 3%). In brief, each tissue or blood sample was weighted and placed in a digestion tube with 5 ml of HNO_3(65%) and 5 ml of H_2O_2 (3%). The tubes were heated with a digester apparatus DK-20 (Velp Scientifica), by slowly increasing the temperature from 100 °C to 200 °C. The clear solution was evaporated to dryness, the residue was taken up with 10 ml of HCl (2 N) and introduced into an arsine generator apparatus. The reaction was initiated by zinc powder after reduction to As^{III} with tin chloride ($SnCl_2$; 40%) and potassium iodide (KI; 15%). After 30 min, the pentavalent arsenic (As^V) was completely reduced to arsine (AsH_3) and the gas bubbled through a solution of the silver salt of diethyldithiocarbamate in pyridine. The absorbance of the brown complex was measured at 525 nm. A calibration curve was obtained with increasing amounts of arsenic (As_2O_3, 0–10 μg).

4.3.2. Analysis by HPLC

In the sera, the arsthinol concentrations were also determined by HPLC using a CC250/4.6 Nucleosil 100-5 C18 Column. The mobile phase was a mixture of acetonitrile/acetic acid 0.6% (55/45), the flow rate was 1 ml/min and the wavelength was 254 nm. Proteins were precipitated by 2 vol of cold acetonitrile. After centrifugation (3000 rpm, 5 min), 20 μl of the supernatant was then injected into the chromatographic system.

4.4. Protein binding study

A solution of bovine serum albumin (BSA. MW: 66,000; 60 μM) was prepared, as well as a solution of arsthinol (10 μM) in a mixture of phosphate buffer (0.1 M, pH 7.4) and propylene glycol (80:20). Both solutions were mixed gently at a ratio of 1:1 and incubated at 37 °C. Samples were taken up after 1, 2, 4, 8 and 10 min. Finally, the precipitation of proteins and the quantification of arsthinol

were performed by HPLC at 254 nm as previously described. Protein binding assays were performed in triplicate.

4.5. Statistics

Data were analyzed by t-test with $p < 0.05$ as the level of significance.

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