行政院國家科學委員會專題研究計畫　成果報告

三氧化砷治療之慢性心臟毒性：著重於病理變化、組織砷化合物之分佈及停藥後組織沉積與心臟毒性之可逆性研究

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一、中文摘要

三氧化砷靜脈注射是最新且有效的治療方法，但其可能之毒性資料仍有限。本研究探討其藥理動態指標及組織各代謝物之濃度變化。主要之發現如下：

一、實驗兔接受靜脈三氧化砷單一劑量（0.2、0.6 或 1.5mg/kg）之表現：

血液中主要為 As（III），且在 1.5mg/kg 劑量呈現代謝之限值。而組織中，DMA 為主要的成分。而 As（III），DMA 與 MMA 濃度與劑量之曲線呈 線性變化。

二、實驗兔接受靜脈三氧化砷 30 天每天注射後之表現：

長期給藥後可能誘發部分組織對砷化合物之代謝能力，例如在 kidney 其 As（III）下降且 DMA 增加。此外，在 liver DMA 原本濃度最高，但在 30 天給藥後，其 normalized 的組織濃度變成 non-linear 表示 liver 在長期高劑量給藥後，對 DMA 代謝能力的增加。

三、長期給藥 30 天後再停藥 30 天後之實驗兔表現：

As（III），DMA 與 MMA 在組織之堆積大都可消除，但 hair 中的 As（III）與 DMA，heart，lung 與 kidney 劑量 DMA 仍存在。

四、結論：

三氧化砷靜脈給藥單一或長期給藥，其藥理動力學指標大致相同，1.5mg/kg/dose 為其限值。組織主要之代謝物為 DMA，而停藥後組織內 As（III），DMA 與 MMA 大多可清除，但仍有組織之選擇性。

關鍵詞：三氧化砷、砷代謝物、藥理動力學

Abstract

Parenteral administration of As_2O_3 has recently been recognized as an effective antineoplastic therapy, especially for the treatment of acute promyelocytic leukemia. Its efficacy and toxicity are concentration-dependent and are related to the fractions of different arsenic species and the degree of methylation. In this study, arsenic trioxide was given parenterally to rabbits as a single dose or as a daily dose (0.2, 0.6 and 1.5 mg/kg) for 30 days. The blood and organ concentrations of the arsenic species, including As(III), DMA and MMA, were studied on day-1 (single-dose study), day-30 (multiple dosing study) and day-60 (reversibility study). The results showed that As(III) was the major detectable arsenic species in the blood. The pharmacokinetic parameters (total clearance, area under the curve, etc) for As(III) indicated a limit for the capacity to eliminate As(III) at the dose of 1.5 mg/kg, and were quite the same after a single or chronic multiple dosing. In tissues, DMA was found to be the major metabolite and the concentrations of DMA, As(III), and MMA in general increased with the dose, with the increase most significant at a dose of 1.5 mg/kg. However, normalized tissue distribution of As(III) in the kidney on day-1, but not on day-30, was nonlinear. Along with decreased levels of As(III) and increased

levels of DMA, an inducible capacity of methylating As(III) to DMA after chronic dosing in kidney was suggested. The tissue concentration of DMA was highest in lung and liver, and the normalized tissue distributions in liver on day-30 were nonlinear, suggesting a limit in eliminating DMA after a chronic high load of As(III). Tissue concentrations of As(III), DMA and MMA in bladder increased dramatically after chronic dosing. However, after wash-out for 30 days, As(III), DMA and MMA were all undetectable in bladder and liver. But, As(III) in hair and low levels of DMA in lung, kidney, heart and hair were still detected. In conclusion, in rabbits we found a similar pharmacological profile after a single or chronic multiple dosing of parenteral arsenic trioxide, with a limiting metabolizing capacity at the dose of 1.5 mg/kg. Tissue accumulation of arsenic species, mainly DMA, and its reversibility after washout were tissue-selective. The potential for late toxicities of arsenic trioxide in organs with significant tendency for arsenic accumulation and low reversibility should be closely monitored.

Keywords: arsenic trioxide, heart, MMA, DMA, electrophysiology

二、緣由與目的

In contrast to their notorious carcinogenic properties in environment pollutants, arsenic compounds have been used for medical purposes for a long time [1]. Recent studies have shown the high effectiveness of parenteral administration (10 mg/day) of arsenic trioxide (As_2O_3) for the treatment of acute promyelocytic leukemia (APL) [2]. The antineoplastic effects of As_2O_3 are related to partial cytodifferentiation and activation of cysteine proteases instrumental in apoptosis. Study using APL cells and NB4 cell line indicated that these antineoplastic effects of As_2O_3 appear to be dose-dependent [3-4]. As_2O_3 triggered apoptosis in the range of 0.5 to 2.0 μM (i.e., 98.9-395.7 ng/ml) and induced partial differentiation at concentrations of 0.1 to 0.5 μM. However, major adverse effects of As_2O_3, including lethal cardiac dysfunction and liver injury, have been reported [5-9]. The toxicity of arsenic compounds depends on the fractions of different chemical species of arsenics and on the various degrees of methylation. In humans, inorganic arsenic is methylated to monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and, to lesser extent, trimethylarsine oxide (TMAO). Methylated arsenic species are more rapidly excreted in urine than inorganic arsenics and are usually believed to be less toxic [10-11]. However, recent evidence indicates that the trivalent intermediates of MMA and DMA may have a role in arsenic toxicity or carcinogenicity [12-17]. Therefore, data of plasma and tissue concentrations of arsenic compounds after As_2O_3 therapy are mandatory in order to define the therapeutic strategy using As_2O_3. While the body burden of trivalent inorganic arsenite (As III) as a result of environmental pollution or intoxication has been reported, the pharmacokinetic data of inorganic and organic arsenics at therapeutic levels after parenteral administration of As_2O_3 are still limited. Shen et al. [2] had reported data for some pharmacokinetic parameters and the arsenic levels in the nail and hair after intravenous infusion of 0.16 mg/kg As_2O_3 in patients with relapse of APL. However, the dose-concentration relationship of As_2O_3 and its metabolites in blood and tissues are still unknown. The New Zealand White rabbit has been shown to have an arsenic methylation process similar to that in humans [18-21]. This study used this rabbit as a model to determine blood and tissue concentrations of arsenite and the metabolites (DMA and MMA) after parenteral administration of As_2O_3 starting from a therapeutic dose of 0.2 mg/kg to 1.5 mg/kg. The effects of single and multiple dosing of As_2O_3 were also compared.

三、結果與討論

RESULTS

Arsenic species in the blood

After single dose of As_2O_3 of 0.2 mg/kg, 0.6 mg/kg or 1.5 mg/kg was administered to

rabbits, only As(III) and trace amounts of DMA were detected in the blood (Figure 1). On day-1, total body clearance significantly decreased as the dose increased(Table1). Accordingly, the AUC increased significantly as the dose increased. When AUC values were normalized by the given doses, it was noted that the increase in AUC was disproportional to the dose. The AUC/D ratio slightly increased from doses of 0.2 mg/kg to 0.6 mg/kg, while the ratio increased nearly 2 folds at doses of 1.5 mg/kg compared to that of 0.2 mg/kg (1.88±0.13 vs. 1.07 ±0.18). The decrease in clearance and the subsequent increase in AUC indicate that the capacity for the elimination of As(III) would reach a limit with the increasing doses, and that this limit had already been reached at a dose of 1.5 mg/kg. Subsequently, the mean residence time of As(III) increased as the dose increased, reaching the highest value at the dose of 1.5 mg/kg. Although not statistically significant, the half-life of As(III) tended to increase as the dose increased. On the other hand, the volume of distribution (Vss) appeared to be unaffected by the dose. Since Vss reflects the relationship between dose and blood levels, the lack of change in Vss suggests that the tissues provide a reservoir for the distribution of As(III) to compensate for changes in the dose/concentration ratio. This phenomenon was corroborated by the data collected on day-30.

After repetitive dosing for 30 days, blood levels and pharmacokinetic parameters of As(III) remained unchanged compared to those on day-1 (Figure 1) which suggests that either the accumulation of As(III) in tissues was negligible or tissues provided an adequate buffering effect for As(III) distribution. The latter postulation was supported by the following tissue data.

Arsenic species in the organs

In contrast to blood data in which As(III) was the only detectable chemical species, As(III), MMA and DMA were all detected in organ tissues and DMA levels were several folds higher than the As(III) and MMA levels. In samples from both day-1 and day-30, tissue distributions of the three arsenic species in general increased with the dose, with the increase most significant at a dose of 1.5 mg/kg. When tissue contents of arsenic species were normalized by the doses (Figure 2), the dose-distribution relationships for most arsenic species appeared to be linear, suggesting that sufficient tissue capacity could be provided under the current dose range. However, nonlinear dose effects for the tissue distribution were observed for As(III) in kidney on day-1 and for DMA in liver on day-30 after multiple dosing.

As shown in Table 2, on day-1 after the single dose, spleen contained the highest concentration of As(III) followed by hair, liver, lung, kidney, heart, bladder and bone. However, at highest test dose (1.5 mg/kg), the tissue concentration of As (III) was highest in the kidney and that in bladder was only lower than in kidney, hair and liver. The dose-distribution relationships obtained after normalizing the tissue contents by the doses were non-linear in the kidney at the day-1 after a single dose of As_2O_3 (Figure 2). After multiple dosing for 30 days, the accumulation of As(III) was evident in bladder (>3 fold). The tissue accumulation of As(III) in hair and heart also showed a trend of tissue accumulation after chronic multiple dosing, but to a less extent than bladder. On the contrary, As(III) concentrations in kidney and to a less extent in liver were even lower after chronic multiple dosing compared to those after a first single dose.

The distribution of MMA was summarized in Table 3. On day-1, after a single dose, MMA could only be detected in lung at the dose of 0.2 mg/kg, but MMA could be detected in the lung as well as kidney, liver, heart and bladder at higher (0.6 or 1.5 mg/kg) dose of As_2O_3. On day-30 after multiple dosing, organs including liver, kidney, bladder and heart that initially did not have detectable MMA on day-1 after a single dose (0.2 mg/kg and 0.6 mg/kg) were found to have detectable levels of MMA. Significant accumulation of MMA caused by multiple dosing was observed in bladder and liver. The tissues with high tissue contents of As(III), including spleen and hair, showed no or very low levels of MMA or DMA.

On day-1 after single dose of As_2O_3, the tissue concentrations of DMA were much higher than those of As(III), and MMA, being highest in lung, followed by liver, kidney, heart and spleen (Table 4). In general, the tissue concentrations of DMA of most organs were higher after multiple dosing than after a single dose. However, similar to As(III) and MMA, it was noted that the accumulation of DMA in bladder after multiple dosing was more prominent compared to other organs. In hair and bone, only after chronic multiple dosing, DMA could be found. The dose-distribution relationship of DMA was found to be non-linear in liver on day-30 after chronic multiple dosing.

DISCUSSION

Parenteral As_2O_3 therapy is highly effective for the induction of remission in adults or children with promyelocytic leukemia. However, the pharmacokinetic characteristics of such therapy, especially after multiple dosing, are far from clear. The main results of this study using a rabbit model were as follows: 1) A decreased clearance of As(III) and nonlinear blood levels of As(III) after a higher single dose (1.5 mg/kg) of As_2O_3 suggest the existence of saturable enzyme systems which catalyze As(III); 2) after chronic dosing of As_2O_3, the pharmacokinetic parameters and blood levels of As(III) remained unchanged; 3) DMA, rather than As(III) and MMA, was the major arsenic compound in tissues; and 4) DMA, MMA as well as As(III) accumulated with tissue selectivity after multiple chronic parenteral As_2O_3 and could be washout completely (e.g., in bladder), partially (e.g., in liver, heart, lung and kidney) or minimally (e.g., in hair). Although great care must be taken in the direct extrapolation of results from experimental study involving animal models to clinical therapy, our data are important references for the therapy using the As_2O_3.

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附件：封面格式

行政院國家科學委員會補助專題研究計畫成果報告

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