# 行政院國家科學委員會專題研究計畫成果報告

## 氧化砷治療癌症可行性之探討

**Preclinical Study of Arsenic Trioxide in the Treatment of Solid Tumor**

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### 一、中文摘要

砷元素廣泛的散佈在自然界中。長期的暴露會引起各種疾病，也會增加得到皮膚癌、肺癌、膀胱癌等癌症的機會。但自古以來，砷就被用來治療多種疾病，包括關節炎、氣喘及白血病等癌症。

最近在哈爾濱及上海的血液科醫師分別發現，注射氧化砷能治療對化學治療、全反式維生素甲酸失效的急性前骨髓型白血病病人。效果很好，毒性也可接受。

氧化砷可抑制細胞生長，它可干擾蛋白質硫氫基(-SH)及磷酸化的功能，從而抑制細胞代謝、結構。砷也可以破壞 DNA、改變細胞的染色體。我們發現，氧化砷在病人身上可達到的濃度下，即可抑制癌細胞的生長，可能有臨床應用價值。

本計畫的目的在探討氧化砷用在各種癌症治療的可行性，以及初步瞭解氧化砷毒殺癌細胞及癌細胞抗藥性的可能機轉。

在本計畫中，我們發現氧化砷對膀胱癌及白血病細胞有極高毒性，對卵巢癌及腸胃癌症的細胞毒性為中等，對其他癌症之效果則不佳。氧化砷對癌細胞之毒性似乎是經由引起細胞凋亡之機轉。氧化砷應可應用在固態腫瘤治療之臨床試驗。由於高的 GSH 往往引起細胞的抗藥性，使用 BSO 可能可以將加強治療的效果。

**關鍵字: 氧化砷，癌症治療，抗藥性**

**Abstract**

Arsenic is a ubiquitous element in environment. Chronic exposure of arsenic compound may cause various diseases and is also associated with increased risk of skin cancer, lung cancer, bladder cancer. Despite these chronic toxicity, arsenic is an ancient medication and was used widely in both western and Chinese medicine. It has been used to treat asthma, arthritis and several types of cancer, including leukemias.

Arsenic trioxide has revived after the revelation that it is very effective in the treatment of acute promyelocytic leukemia patients who were refractory to both chemotherapy and all-trans retinoic acid.

Our preliminary cytotoxicity results indicated that at concentrations attainable in patient plasma, $As_2O_3$ may inhibit the growth of some solid tumor cell lines.

The objective of this project is to test feasibility of using $As_2O_3$ in the treatment of solid tumors. The secondary aim is to find out mechanisms of toxicity and resistance of tumor cells to arsenic compounds.

In this study, we found that $As_2O_3$ is very cytotoxic to bladder cancer and APL cells. It is moderately active in ovarian and gastrointestinal cancer cells. Other types of cancer are resistant to $As_2O_3$. $As_2O_3$ seems to kill tumor cells through induction of apoptosis. Our results indicate that it is warranted to use $As_2O_3$ in clinical trials to treat solid cancer patients, especially for bladder cancer. High GSH content was associated with intrinsic arsenic resistance in cancer cells. BSO may be considered to enhance cytotoxicity of arsenic treatment.

### 二、緣由與目的

**Arsenic in environment**

Arsenic is widely distributed in water,

mineral, soils, air, plants and animals. Human exposed to arsenic from inhaled air, drinking water and seafood. Artesian wells in Tainan county contain up to 1.8mg/L of arsenic in contrast to less than 0.01mg/L in most of water supply[1].

**Arsenic in traditional medicine**

Arsenic compounds have been used for a long time in both western and Chinese medicine. Fowler's Solution contains $As_2O_3$ and was administered for the treatment of leukemia, psoriasis and asthma. Pearson's solution (0.5% arsenate) can be used up to 20mg (4.8mg of As) per day. Salvarsan and neoarphenamine are arsenic compounds used for the treatment of syphilis.[1] $As_2O_3$ is present in **砒霜**. **雄黃** contains $As_2S_3$. Daily intake of arsenic may approach 10mg in some formulas.

**Arsenic in the treatment of cancer**

Local application of arsenic was used in the past in the treatment of cancer of skin, oral cavity, cervix etc. Doctors have been using arsenic in the treatment of chronic myelogenous leukemia, lymphoma, stomach cancer and esophageal cancer in Mainland China. However, there were no reports on its usefulness and efficacy in the treatment of cancer in the era of contemporary medicine.

**$As_2O_3$ in the treatment of acute promyelocytic leukemia (APL)**

$As_2O_3$ has revived recently with the revelation that arsenic may be the most effective treatment APL.

Chemotherapy and all-trans retinoic acid are two effective treatments for APL patients. However, more than 40% patients still died of their disease because of the high recurrence rate. In 1971, a group at Harbin began to treat leukemic patients with a preparation called "Ailing-1" (**癌靈一號**). In a recent follow-up study, several APL patients are still alive without disease[2]. The longest surviving patient lived more than 23 years. They found that $As_2O_3$ is the active component in Ailing-1. Ten mg of $As_2O_3$ were given intravenously daily for 28 days. Patients then may rest for 7-14 days. Two to 3 cycles were given. Up to 52% to 100% complete remission were observed in APL patients.[3]

Toxicity of $As_2O_3$ treatment include infection 11.97%, nausea 22.41%, vomiting 8%, diarrhea 4.27%, numbness 9.4%, increase liver enzyme 4.27%, edema 1.71%. Chronic toxicities are few.

**Mechanism of action of $As_2O_3$ in the treatment of APL**

$As_2O_3$ at concentrations achievable in patients' plasma (1-2μM) were found to kill NB4 cells, an APL cell line. There were no cytotoxic effect at the same $As_2O_3$ concentrations in other non-APL cell lines (HL60, U937). $As_2O_3$ induce apoptosis in NB4 cells. Inhibition of oncoprotein PML-RARα was suggested. In addition, partial induction of differentiation was noted in NB4 cell treated with 0.25μM of $As_2O_3$ with long period of incubation[4].

**Other mechanism of cytotoxicity of $As_2O_3$**

Other mechanisms have been proposed to contribute to arsenic toxicity. Arsenic was known to induce DNA strand breaks associated with DNA-protein cross-links[5], induce sister-chromatid exchanges[6]. Arsenic was also shown to inhibit DNA repair.[7] It may interfere protein phosphorylation and inhibit the function of proteins carrying sulfhydryl group (-SH).

**$As_2O_3$ plasma levels in human**

Pharmacokinetics study was done in APL patients treated with daily infusion of 10mg $As_2O_3$. Peak levels were 4.2 to 6.7μM and were reached at 3-4 hours after infusion. Drug concentrations in the blood were between 0.5 and 3μM most of the time.[8].

**$As_2O_3$ cytotoxic test in cancer cell**

The first step of exploring effectiveness of chemicals in the treatment of cancer is cytotoxicity test.

**Arsenic detoxification system in cells**

Cells contain several detoxification systems in response to environmental hazardous toxins. These include phase I and phase II enzymes that metabolize toxins, heat shock proteins, ATP-cassette binding proteins (mdr-1) and a variety of other stress-responsive proteins.[9] Glutathione and/or

glutathione-s-transferase pi (GST-π) over-expression were linked to arsenic resistance[10]. A mdr-1 homologue multidrug resistant-associated protein (MRP) transfectants of HeLa cells were shown to be resistant to arsenic[11]. A combination of overexpression of GST-π and MRP, therefore, may theoretically confer high resistance to arsenic in cancer cells[12].

**Specific aim**

1. To study the feasibility of using $As_2O_3$ as anticancer drug in solid tumors by cytotoxicity test.
2. To study the intrinsic resistance mechanism of $As_2O_3$ in cancer cells.
3. To study the mechanism of $As_2O_3$ toxicity to cancer cells.

**Importance of the result achieved:**

$As_2O_3$ may be effective in the treatment of solid tumor.

## 三、結果與討論

**1. cytotoxicity of $As_2O_3$ in cancer cells.**

Cancer cells were added to each well in 96-well plates for 24 hours. $As_2O_3$ was diluted in water and added to each wells resulted in final concentrations from 30nM to 100μM. Cells were grown for 96 hours. MTT or SRB assays were performed to viable cells in each well after drug treatment. Cytotoxicity curves were shown below:

**2. $IC_{50}$s of $As_2O_3$ in these cells:**

| | Origin | IC50s# |
|---|---|---|
| BFTCC905 | Bladder | 0.34!0.03* |
| NTU-B1 | Bladder | 0.49!0.16* |
| NB4 | APL | 0.64!0.11* |
| T24 | Bladder | 0.93!0.20* |
| A2780 | Ovary | 1.12!0.33* |
| SW620 | Colon | 1.16!0.15* |
| AGS | Stomach | 1.16!0.20* |
| TSCH8302 | Cervix | 2.50!0.69* |
| MCF7 | Breast | 2.67!0.66* |
| BFTCC909 | Bladder | 2.84!0.79* |
| H460 | Lung | 3.27!0.49* |
| A172 | Glioblastoma | 3.40!0.40* |
| CL-1 | Lung | 4.17!0.50* |
| Hep3B | Liver | 5.17!1.02* |
| HepG2 | Liver | 7.17!1.20* |

# in μM *average and standard error of at least 3 independent experiments

**Three groups of cancer can be identified.**

**Group1: bladder cancer, APL are very sensitive to $As_2O_3$.**

**Group2: gastrointestinal cancer are moderately sensitive to $As_2O_3$.**

**Group3: lung cancer, liver cancer, cervical cancer, breast cancer and glioblastoma. Relatively resistant to $As_2O_3$.**

**2. $As_2O_3$ induce apoptosis in NTU-B1 cells.**

Cells were treated with 0, 0.3, 1 and 3μM of $As_2O_3$ for 48 and 72 hours. DNA were isolated and subjected to gel electrophoresis. DNA ladder signifying apoptosis of the cells were observed in cells treated with 3μM for 2 days or 1μM for 3 days.

**3. Glutathione content in cancer cells.**
Glutathione content was measured by colorimetric assay.

| | **GSH content#** |
|---|---|
| NTUB1 | 7.59!1.17* |
| H460 | 21.8!2.9* |
| NB4 | 6.12!0.96* |
| SW620 | 4.91!0.17* |
| AGS | 7.30!0.84* |
| BFTC905 | 6.03!1.35* |
| BFTC909 | 17.1!2.9* |
| Hep3B | 17.4!1.7* |

#(μg/mg protein) *average and standard error of at least 3 independent experiments
There is good correlation between GSH content and cytotoxicity of As2O3 in cancer cells. Lower GSH content is associated with higher toxicity.

**4. BSO deplete glutathione in cancer cells**

| | **GSH content#** |
|---|---|
| NTUB1 | 7.59!1.17* |
| NTUB1+10μM BSO | 2.28!0.64* |
| NTUB1+50μM BSO | 1.00!0.44* |
| H460 | 21.8!2.9* |
| H460+10μM BSO | 4.95!0.17* |
| H460+50μM BSO | 3.17!0.27* |

#(μg/mg protein) *average and standard error of at least 3 independent experiments

**5. BSO sensitize cancer cells to arsenic trioxide.** IC50 of $As_2O_3$ in NTU-B1 cells was 0.6 μM. IC50 was 0.2 μM when cells are co-incubated with 50 μM of BSO.

## 四、計劃成果自評

Our study suggest that $As_2O_3$ may be useful in the treatment of bladder cancer, ovarian cancer and gastrointestinal cancer. Together with the demonstration that arsenic probably kill tumor cells through induction of apoptosis, it is warranted to use $As_2O_3$ in solid cancer clinical trials. Since the toxicity of $As_2O_3$ is not well described in trials from China. A phase I protocol to find out the optimal dose and toxicity is preferred.

We also found that GSH content correlate well with arsenic toxicity. On the other hand, there were no correlation between glutathione transferase or MRP content and cytotoxicity. This finding lead to the strategy to use BSO to enhance arsenic toxicity to cancer cells or to reverse arsenic resistance.

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