行政院國家科學委員會專題研究計畫成果報告

氧化砷治癌及抗藥性機轉之研究

Mechanism of cytotoxicity and resistance of arsenic trioxide in cancer cells

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一、中文摘要

砷元素廣泛的散佈在自然界中。長期的暴露在過量的砷會引起各種疾病，包括皮膚病變、神經病變及臺南沿海常見的烏腳病等等，也會增加得到皮膚癌、肺癌、膀胱癌等癌症的機會。最近中國大陸的哈爾濱醫科大學發現，氧化砷是治療急性前骨髓型白血病非常有效的藥物，使得這個古老的藥物又重新被科學界認識。在哈爾濱、大連及上海的血液科醫師分別發現，氧化砷能治療對化學治療、全反式維生素甲酸完全沒有效用的急性前骨髓型白血病病人，並無交互抗藥性。大陸學者後來也發現，有一部分一開始使用氧化砷治療有效的病人，繼續治療仍產生抗藥性，使白血病無法控制。

在先前計畫中，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。

本計畫的目的在探討癌細胞對氧化砷抗藥性的可能機轉。

在本計畫中，我們測試氧化砷在對化學治療敏感的細胞及多重抗藥癌細胞株的細胞毒性及這些細胞中 Glutathione-s-transferase pi(GST-pi)，GSH 及 multidrug resistance-associated protein (MRP1)蛋白質的表現，並和細胞毒性比對，此外也用 GSH 的抗藥逆轉劑 buthionine sulfoximine (BSO)，嘗試增強氧化砷對癌細胞的毒殺能力。結果發現細胞中 GSH 的升高或 MRP1 的高度表現都能使多重抗藥癌細胞產生抗藥性。使用 BSO 使 GSH 下降可使這兩株抗藥細胞的氧化砷敏感度提高。氧化砷應可應用在固態腫瘤治療之臨床試驗。由於高 GSH 往往引起細胞的抗藥性，使用 BSO 可能可以加強治療的效果。本研究支持氧化砷必須和 GSH 結合才能由 MRP1 排到細胞外的假說。

關鍵字：氧化砷，癌症治療，抗藥性

Abstract

Arsenic trioxide is a novel anticancer agent, which has been found to induce remission in acute promyelocytic leukemic patients following daily intravenous administration. The therapeutic value of arsenic trioxide in other cancers is still largely unknown. Our previous study showed that bladder cancer, acute promyelocytic leukemic and gastrointestinal cancer cells were the most sensitive to arsenic trioxide. Cellular glutathione system plays an important role in arsenic detoxification in mammalian cells. Cancer cells that were intrinsically sensitive to arsenic trioxide contained lower levels of glutathione whereas resistant cancer cells contained higher levels of glutathione. On the other hand, there was no association of glutathione-s-transferase-pi or multidrug resistance-associated protein 1 levels with arsenic sensitivity in these cancer cells. In the present study we have shown that multidrug resistant cancer cells that were cross-resistant to arsenic contained higher levels of glutathione or multidrug-resistance

associated protein 1 than their drug-sensitive parental cells. Cancer cells become more sensitive to arsenic after depletion of cellular glutathione with L-buthionine sulfoximine. We concluded that cellular glutathione level is the most important determinant of arsenic sensitivity in cancer cells. Cellular glutathione level and its modulation by buthionine sulfoximine should be considered in designing clinical trials using arsenic in solid tumors.

二、緣由與目的

Arsenic trioxide (As_2O_3) is a novel anticancer agent. Daily intravenous infusion of 10mg As_2O_3 induced complete remission in acute promyelocytic leukemia patients who were refractory to conventional chemotherapy and/or all-trans retinoic acid[1,2]. The role of arsenic in the treatment of this unique form of leukemia is still under investigation. Arsenic compounds have been tested in other hematological malignancies in vitro[3]. It is not known at present whether these compounds will prove useful in the treatment of solid tumors. We have screened a panel of cancer cell lines with As_2O_3. In the previous study we have shown that bladder cancer cells NTU-B1 and BFTC905 were most susceptible to As_2O_3. Apoptosis can be induced in NTU-B1 cells at 1μM As_2O_3 . IC_{50}s of the bladder cancer cell lines was substantially lower than reported As_2O_3 peak plasma levels (4-6μM) in patients[1]. Thus, it is conceivable that As_2O_3 may be effective in the treatment of bladder cancer and other solid tumors that show similar sensitivity to arsenic. It will be important to further study the acquired arsenic resistance mechanisms in cancer cells.

Multiple mechanisms of arsenic resistance in either bacteria or mammalian cells have been described in the literature. One of the most important arsenic detoxification mechanisms is the glutathione (GSH) system. Trivalent arsenic was shown to bind to GSH in a cell free system[4]. Overexpression of glutathione-s-transferase-pi (GST-pi)[5], GSH[6] or multidrug resistance-associated protein (MRP1)[7] has been shown to confer arsenic resistance. To investigate the correlation of the GSH detoxification system with arsenic resistance, we examined GSH content, MRP1 and GST-pi expression in a panel of cancer cells and in multidrug resistant cancer cells that were cross-resistant to arsenic. L-buthionine sulfoximine (BSO) was used to modulate cellular GSH content and to enhance arsenic sensitivity of cancer cells.

Specific aim

1. To study whether GSH, GST-pi and MRP1 is involved in As_2O_3 resistance in cancer cells.
2. To study if BSO is useful to reverse resistance in As_2O_3 resistant cancer cells.

Importance of the result achieved:

The results will be important to determine which biologic marker should be tested in As_2O_3 clinical trials. It is also important to know whether BSO can be useful to reverse arsenic resistance.

三、結果與討論

Cytotoxicity of As_2O_3 and GSH content in drug sensitive and resistant cancer cells.

Concentrations of As_2O_3 that inhibit 50% of cell growth (IC_{50}s) of cancer cells and their GSH contents are shown in Table 1.

GST-pi protein expression in cancer cells.

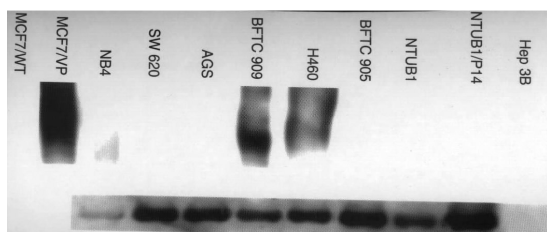
Overexpression of GST-pi may facilitate conjugation of trivalent arsenic to GSH. GST-π protein expression in the cancer cells was measured by western blot as shown in Figure 1. Arsenic sensitive NB4 cells contained very low level of GST-pi protein. However, several arsenic sensitive cells such as BFTC905 and SW620 cells expressed high level of GST-pi protein whereas arsenic resistant H460, Hep3B and BFTC909 cells expressed low level of GST-pi protein. There was no correlation of GST-pi levels to As_2O_3 IC_{50}s in cancer cells. Multidrug resistant NTU-B1/P14 cells overexpressed GST-pi compared to their drug sensitive parental NTU-B1 cells.

MRP1 expression in cancer cells.

MRP1 may facilitate export of conjugated

GSH out of the cells[8] and thus, may affect arsenic resistance in cancer cells. MRP1 expression in the membrane protein of cancer cells was measured by western blot as shown in Figure 1. H460, BFTC909 and one multidrug resistant MCF7/VP cells contained measurable levels of MRP1 whereas MRP1 expression was very low in other cancer cells. MRP1 seemed to confer resistance to arsenic, however, not all arsenic resistant cancer cells expressed high level of MRP1.

Figure 1. Western blot of MRP (upper lane) and GST-pi (lower lane) in cancer cells. MRP (190KD) was expressed in measurable amount only in MCF7/VP, BFTC909 and H460 cells. GST-pi (26KD) was expressed in almost all cancer cells except for Hep3B cells.



Cross-resistance of arsenic in multidrug resistant cancer cells.

IC50s of two multidrug resistant cancer cells are listed in Table 1. Cisplatin resistant NTU-B1/P14 was 5.5-fold resistant to arsenic. Etoposide resistant MCF7/VP cells was 4.8-fold resistant to arsenic. Glutathione content of NTU-B1/P14 was 6.7-fold higher than that of NTU-B1 cells. On the other hand, there was no difference of glutathione content between MCF7/WT and MCF7/VP cells. MCF7/VP expressed high level of MRP1 that may account for its arsenic resistance whereas NTU-B1/P14 expressed no measurable level of MRP1 (Figure 1).

Modulation of GSH content in cancer cells by BSO.

BSO is known to deplete cellular GSH via inhibition of gamma-glutamylcysteine synthetase which is required for GSH biosynthesis. NTU-B1, NTU-B1/P14, MCF7/WT and MCF7/VP cells were incubated with various concentrations of As_2O_3 and 10 μM of BSO for 4 days. Ten μM of BSO was not toxic to these cancer cells (IC_{10}s of BSO in NTU-B1, NTU-B1/P14, MCF7/WT and MCF7/VP cells were 37μM, >50μM, 27μM and 24μM respectively). The representative cytotoxicity curves of NTU-B1 and NTU-B1/P14 cells in As_2O_3 with or without co-incubation with 10μM of BSO are shown in Figure 2. IC_{50}s of As_2O_3 and GSH contents in BSO-treated GSH depleted cells (drug-sensitive and –resistant NTU-B1 and MCF7/WT cells) are shown in Table 1. All 4 cancer cells became very sensitive to arsenic (IC_{50}s 0.1μM to 0.4μM) when glutathione was depleted by BSO.

Figure 2. Representative cytotoxicity curves of As_2O_3 in NTU-B1 cells (A) and NTU-B1/P14 cells (B). Cells were incubated in the presence (- -) or absence (- ~ -) of 10 μM BSO.

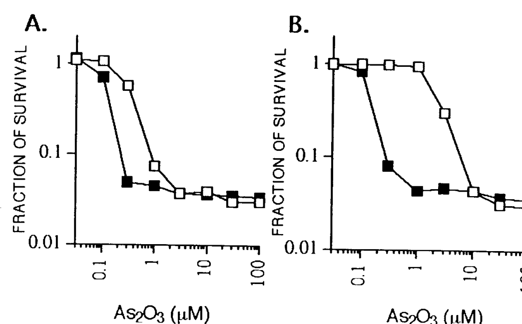


Table 1. Fifty percent growth inhibitory concentrations (IC_{50}s) of arsenic trioxide on drug sensitive and resistant human cancer cells after 96-hour of treatment. Shown in the table are the means and standard errors of at least 3 independent experiments. * μM, # μg GSH/mg protein.

Cell lines	As_2O_3*	GSH content#
NTU-B1	0.47!0.08	7.59!1.16
NTU-B1/P14	2.59!0.41	50.9!10.9
MCF-7	2.08!0.40	28.6!2.3
MCF7/VP	9.89!1.74	20.1!0.8
Coincubation 10uM BSO		
NTU-B1	0.19!0.04	2.28!0.64

NTU-B1/P14	0.14!0.01	14.20!1.59
MCF-7	0.40!0.12	3.65!0.23
MCF7/VP	0.20!0.02	2.10!0.12

四、計劃成果自評

Cisplatin resistant NTU-B1/P14 cells were cross-resistant to arsenic, the GSH content of these cells was higher than in steady state parental cells. When GSH was depleted by BSO, resistant cells became sensitive to arsenic treatment. BSO may also enhance arsenic toxicity in wild type MCF7 cells and multidrug resistant and MCF7/VP cells. When cellular GSH were depleted, all drug sensitive and multidrug resistant cancer cells became very sensitive to arsenic.

We demonstrated complete reversal of arsenic resistance in MRP1-overexpressing MCF7/VP cells when glutathione was depleted by BSO. The result suggests that MRP1 overexpression may not protect cancer cells from arsenic toxicity when glutathione was depleted. Overexpression of MRP1 may contribute to arsenic resistance, but MRP1 expression is not the main determinant of arsenic sensitivity in cancer cells.

Use of As2O3 in the solid tumor clinical trials is clearly warranted. Our study suggests that GSH content in tumor cells may be the main determinant of arsenic sensitivity. Attempts should be made to measure tumor GSH content and correlate to arsenic response in clinical trials. Therefore, adding BSO to arsenic treatment may potentially be useful to reverse acquired arsenic resistance in acute promyelocytic leukemic patients or to treat tumors that are intrinsically resistant to arsenic.

In conclusion, GSH content correlates well with arsenic resistance in cancer cells. Depletion of cellular GSH by BSO enhanced arsenic toxicity in both arsenic sensitive and resistant cancer cells. Further animal studies and human trials evaluating arsenic as an anticancer drug are warranted. Our study suggests that As_2O_3 should be tested in solid cancers, especially patients with bladder and gastrointestinal cancer. This study suggests that measurement and modulation of cellular GSH content in cancer cells should be deployed in designing future clinical trials.

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