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※ 台灣烏腳病流行區泌尿系統移形上皮癌生物分子學之探討研究—無機砷暴露後泌尿上皮細胞之分子變化及砷在泌尿上皮癌之抗藥性機制(三年計畫) ※

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Report 第一篇(已刊出)

Arsenic trioxide as a novel anticancer agent against human transitional carcinoma—characterizing its apoptotic pathway

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Arsenic trioxide (As_2O_3) has been shown to be an active agent against acute promyelocytic leukemia. Little is known about its therapeutic efficacy in human transitional carcinomas. In this study, the arsenic-mediated apoptotic pathway in transitional carcinoma cells was investigated. Three bladder transitional carcinoma cell lines were used, including a parental sensitive line and two resistant daughter lines (cisplatin and As_2O_3 resistant). The As_2O_3-mediated cytotoxicity to the three cell lines was studied *in vitro* in the presence or absence of buthionine sulfoximine (BSO), a chemotherapy modulator. In results, although a lesser extent of apoptosis was seen in cells treated with As_2O_3 alone, more significant apoptotic events were observed in the combined treatment of As_2O_3 and non-toxic concentrations of BSO (up to 10 μM). These included the accumulation of sub-G_1 fractions and internucleosomal DNA breakdown, which were preceded by production of reactive oxygen species, loss of mitochondrial membrane potential and activation of caspase-3. In conclusion, As_2O_3 in the presence of BSO may be an active agent against both chemonaive and cisplatin-resistant transitional carcinomas. The As_2O_3-mediated cytotoxicity appeared to go through the conventional apoptotic pathway. Our results have clinical implications and warrant further investigation. [© 2002 Lippincott Williams & Wilkins.]

Key words: Bladder neoplasms, buthionine sulfoximine, caspases, glutathione, NTUB1 cells, reactive oxygen species.

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Introduction

Although arsenic compounds are known as poisons, they have been used in traditional Chinese medicine for centuries. Interestingly, arsenic compounds, such as arsenic trioxide (As_2O_3) and arsenic disulfide, were recently shown to be effective in the treatment of acute promyelocytic leukemia (APL).[1] The mechanisms of action were shown to be associated with the induction of apoptosis and differentiation.[2] Moreover, *in vitro* studies revealed that clinically achievable concentrations of As_2O_3 could trigger apoptosis of leukemia[3] and lymphoma[4] cells as well as some solid tumor cells, including esophageal cancer,[5] neuroblastoma,[6] prostate cancer,[7] ovarian cancer,[7] etc. This suggests that As_2O_3-induced apoptosis may also be seen in a variety of tumor models. Although As_2O_3-mediated apoptosis has been explored in many tissue systems, little is known about the cytotoxic effects of As_2O_3 on human transitional carcinoma cells.

About 30–50% of advanced transitional cell carcinomas do not respond to cisplatin-based chemotherapy. Treatment failure is not uncommon and an effective salvage therapy for patients who failed cisplatin-based regimens is urgently needed. If As_2O_3 is to be used as a second-line agent against transitional carcinoma, apoptosis should be seen in arsenic-treated cisplatin-resistant cells. Data of this kind are also lacking.

We have previously shown that intracellular glutathione (GSH) content has a decisive effect on As_2O_3-induced apoptosis.[8] Cells that have a low GSH

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第二篇(已付印中)

CYTOTOXICITY OF ARSENIC TRIOXIDE TO TRANSITIONAL CELL CARCINOMA CELLS

YEONG-SHIAU PU, TZYH-CHYUAN HOUR, JUN CHEN, CHAO-YUAN HUANG, JING-YI GUAN, AND SHIU-HUI LU

ABSTRACT

Objectives. Arsenic is a natural substance that has been used medicinally for centuries. Recently, arsenic trioxide (As_2O_3) was shown to be an active agent against acute promyelocytic leukemia. However, little is
AQ: 1 known about its therapeutic efficacy in human transitional cell carcinomas. We investigated its potential use
AQ: 2 and cross-resistance with cisplatin in transitional cell carcinoma.
Methods. Three bladder transitional cell carcinoma cell lines, NTUB1, NTUB1/P (cisplatin-resistant), and
AQ: 3 NTUB1/As (As_2O_3 resistant), were used. The chemosensitivity of the three cell lines to cisplatin and As_2O_3 was determined by the microculture tetrazolium assay. The modulatory effect of buthionine sulfoximine (BSO) on As_2O_3 cytotoxicity was studied by combining the two agents simultaneously or sequentially and evaluated using the median-effect analysis. Cellular glutathione contents were determined using a biochemical method.
Results. There was evident cross-resistance between cisplatin and As_2O_3 in the cell model used. BSO significantly enhanced As_2O_3 cytotoxicity in the three cell lines, indicating synergism in combination. In the presence of 3 μM BSO, the sensitivity of NTUB1, NTUB1/P, and NTUB1/As to As_2O_3 was increased 3, 7.4, and 8.4-fold, respectively. Among the three different combination schedules, greater cytotoxic effects were obtained by concurrent exposure to both agents. A significant dose-response relationship was found between the BSO concentrations and glutathione contents in NTUB1 ($P = 0.007$) and NTUB1/As ($P = 0.05$) but not NTUB1/P ($P = 0.1$) cells.
Conclusions. As_2O_3 in the presence of BSO may be an active agent against transitional cell carcinoma. Our results have clinical implications and warrant further investigation. UROLOGY **60:** 000–000, 2002. © 2002, Elsevier Science Inc.

Arsenic is a natural substance that has been used medicinally for centuries. In the 1970s, Thomas Fowler developed "Fowler's solution" (potassium arsenite) for the treatment of a variety of diseases, including asthma, pernicious anemia, and Hodgkin's disease.[1] In 1910, Paul Ehrlich, the founder of chemotherapy, introduced salvarsan, an organic arsenical that could cure syphilis.[1] Recently, arsenic compounds, such as arsenic trioxide (As_2O_3) were used to treat acute promyelocytic leukemia.[2] The mechanism appeared to be associated with the induction of apoptosis and differentiation.[3] Clinically achievable concentrations of As_2O_3 could trigger apoptosis of leukemia[4] and lymphoma[5] cells, as well as some solid tumor cells in vitro, including esophageal cancer,[6] prostate cancer,[7] ovarian cancer.[7] This suggests that As_2O_3-induced apoptosis may be seen in a variety of tumors. However, little is known about the cytotoxic effects of As_2O_3 in human transitional cell carcinoma cells.

About 30% to 50% of advanced transitional cell carcinomas do not respond to cisplatin-based chemotherapy. Effective salvage regimens for cisplatin-refractory tumors are urgently needed. The interaction or cross-resistance between As_2O_3 and cisplatin has never been reported in transitional cell carcinoma. If As_2O_3 is to be used as a second-

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第三篇(已投稿, 91年4月). Journal of Urology

CHARACTERIZATION OF MOLECULAR EVENTS IN A SERIES OF TRANSITIONAL CARCINOMA CELLS WITH PROGRESSIVE RESISTANCE TO ARSENIC TRIOXIDE

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Running title: molecular changes in arsenic-resistant bladder cancer cells

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ABSTRACT

Purpose: Arsenic trioxide (As_2O_3) is a novel anti-cancer agent. Our previous studies have shown that it may be active against transitional carcinomas. A series of bladder transitional carcinoma cells with progressive As_2O_3 resistance were established to reveal the molecular events that may be related to the mechanisms of resistance to As_2O_3.

Materials and methods: A sensitive parental line and three As_2O_3-resistant sublines were used with their IC50s being 0.9, 1.2, 2.5, and 4.9 μM., respectively. Western blotting was used to study the levels of the three cell proliferation markers (p53, $p21^{Waf1/Cip1}$, and c-Myc), the apoptosis-related factor (Bcl-2), the reactive oxygen species scavenger (superoxide dismutase (Cu/Zn)), and DNA mismatch repair enzymes (hMSH2 and hMLH1). A colorimetric biochemical assay was used to study the glutathione (GSH) content. The activity of two transcription factors, NF-κB and AP-1 were determined by using the electrophoretic mobility shift assay.

Results: Cellular resistance to As_2O_3 was associated with a lowered proliferation profile (increased p53 and $p21^{Waf1/Cip1}$ and decreased c-Myc levels) and a greater resistance to apoptosis (elevated Bcl-2 levels). Cells with a stronger resistance had higher expressions of superoxide dismutase (Cu/Zn)

and hMSH2 (but not hMLH1). GSH contents were up-regulated in resistant cells in a dose-dependent manner. The DNA binding activities of NF-κB and AP-1 were down-regulated in resistant cells also in a dose-dependent manner.

Conclusions: Profound molecular alterations occur during the acquisition of secondary As_2O_3 resistance. Our cell model can help to reveal the resistance mechanisms to As_2O_3 in transitional carcinoma cells.