Exp Oncol 2007
29, 1, 45–48

Experimental ONCOLOGY

SYNERGISTIC THERAPEUTIC EFFECT OF ARSENIC TRIOXIDE AND RADIOTHERAPY IN BALB/C NUDE MICE BEARING NASOPHARYNGEAL CARCINOMA XENOGRAFTS

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It has been shown that arsenic trioxide (ATO) induced apoptosis in human nasopharyngeal carcinoma cells and inhibited the growth of nasopharyngeal carcinoma xenografts (NPCX) in nude mice. *Aim:* The present study was designed to determine whether ATO at the non-toxic dose level could potentiate the therapeutic effectiveness of radiation therapy in nasopharyngeal carcinoma, using a BALB/C nude mouse xenograft model. *Methods:* The mice bearing NPCX were treated with radiation alone (2, 4, and 6 Gy), ATO alone (4 mg/kg/day x 6 days), and ATO plus radiation at the same dosage levels. Time of tumor growth delay (defined as the time necessary for the tumor to grow four-fold of its initial volume after, compared with untreated tumors) and toxic effects were determined. *Results:* The low dose ATO alone has no pronounced effects on tumor growth delay compared to untreated control. However, compared with radiation alone, the combined regimen delayed the tumor growth by 2–10 days and had no significant toxic effects such as the liver function damage. *Conclusions:* Combination of ATO at non-toxic dose level and radiation has synergistic effects on tumor growth inhibition *in vivo* and is well tolerated.

Key Words: **arsenic trioxide, radiation, nasopharyngeal carcinoma xenografts.**

It has been well known that radiation therapy (RT) is one of the major effective treatments for nasopharyngeal carcinoma. However, due to the high dose of radiation, patients frequently suffer from toxic effects, such as xerostomia, hearing loss, hypomnesia, dysphagia [1], neck stiffness [2], and nasal synechia [3]. There is an urgent need for novel approaches to improving response of cancer cells to radiation therapy. Unfortunately, effective non-toxic radiosensitizers for clinical use are hard to find.

Arsenic trioxide (ATO) has been reported as an effective agent in the treatment of patients with leukemia [4–6]. Recently, several studies have been focused on the antitumor effects of ATO on solid tumors [7–11]. It has been reported that ATO enhanced radiation response in glioma [8, 9], cervical cancer [10], head and neck squamous cell carcinomas [11]. In our previous studies, we demonstrated several potential mechanisms by which ATO reduced the invasive and metastatic properties of nasopharyngeal carcinoma cells, including G2/M arrest, up-regulation of p53 and Bax expression, and down-regulation of Bcl-2 expression [12, 13]. In addition, we found that ATO enhanced radiosensitivity of nasopharyngeal carcinoma cells *in vitro* [13]. Thus, it is reasonable to determine whether ATO can increase radiation response of nasopharyngeal carcinoma *in vivo.* The aims of our present study are to determine the therapeutic effects of ATO, RT, and combination of the both treatments on nasopharyngeal carcinoma *in vivo*, to detect the toxic effects of the combined treatment, if any, and to evaluate the potential benefits for future clinical use.

Received: February 7, 2007.
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Abbreviations used: ALT – alanine aminotransferase; AST – aspartate aminotransferase; ATO – arsenic trioxide; RT – radiation therapy.

MATERIALS AND METHODS

Test compound and reagents. ATO was purchased from Harbin-Eda Pharmaceutical Co. Ltd, China. Other reagents are the highest grade available.

Animal model and treatment. BALB/C mice, 7–8 weeks of age and weighing 18–20 g, were obtained from the Experimental Animal Center of Sun Yat-Sen Medical University (Guang Zhou, China). The current study was approved by the Ethic Committee of Cancer Hospital, Shantou University.

The mice were raised under SPF (specific pathogen free) condition. Human nasopharyngeal carcinoma cell line, CSNET-1, was used in the experiment. It is a poorly differentiated squamous cell carcinoma, usually maintained in a frozen state, and has been proven to be transplantable in severe combined immunodeficiency (SCID) mice [14]. Before experiment, 10^6 exponentially growing cancer cells were inoculated subcutaneously into the back of several Balb/c nude mice. When tumors reach approximately 8 mm in length diameter, they were obtained by surgical resection, minced with scissors into fragments of about 1 mm in diameter and then implanted subcutaneously into the armpit of BALB/C mice. When tumors were palpable (~ 4 mm in length diameter), mice were randomly divided into several groups: one untreated control, three groups treated with radiation alone (RT), one ATO alone (ATO), and three groups with ATO plus radiation (ATO + RT). RT and ATO + RT groups were treated with single radiation of 2, 4, and 6 Gy, respectively. There were 8 mice in each group.

ATO (4 mg/kg per day) was administered i. p. for 6 days. In the combined treratment, after the last ATO treatment, local irradiation of 2, 4 or 6 Gy was delivered

with an X-ray machine (Tape $1T_1$-V, Beijing, China), operating at 140 KV, 15 mA, 0.5 mm Cu-equivalent filter, with a dose rate of 107.3cGy/min. During irradiation, unanesthetized mice were placed and fixed in specially designed Perspex boxes, isolated from the air, and the normal tissue around the tumor was shielded as much as possible by placing some lead coverings upon the surface of the boxes.

Measurement of tumor growth. Tumor size was measured with vernier calipers, and tumor volume was calculated by the formula $a \times b^2/2$ (a is the maximal diameter, b is the minimal diameter) [15]. The measurement was performed daily after inoculation of tumor, and weekly after radiation. Relative tumor volume was determined by taking the initial volume before radiation as 1. The time needed for a xenograft to reach 4-fold of its initial volume was defined as T_{GT4}. Time of tumor growth delay (T_{GD}) was the T_{GT4} when compared with the control group, e. g. T_{GD} of RT group compared with untreated group, T_{GD} of RT + ATO group compared with RT alone group.

Detection of toxic effect. In all experiments, mice were monitored daily for signs of toxicity. On the 7th week after radiation, when the relative volume of tumor in each group reached 4 times of the initial volume, the mice were sacrificed. Then the blood samples were collected. Examination of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were performed using an automated biochemistry machine (Olympus, Japan) for evaluation of the toxic effects on liver function. After mice were sacrificed, the toxic effects of therapy on the state of heart, lungs, liver, kidneys were detected histologically.

Additional 15 mice were treated using the same regimen as in the group treated with ATO alone to evaluate the acute toxic effects.

Statistical analysis. The results of T_{GD}, AST, and ALT analyses were expressed as the mean ± standard deviation. The Student's *t*-test was used to determine the significance of the differences among the treated groups compared with controls. A *p*- value of < 0.05 was considered statistically significant.

RESULTS

Tumor response to the combined treatment of ATO and radiation. On the 12th day after the implantation of nasopharyngeal carcinoma xenograft in mice, 90% of tumors were palpable (> 4 mm in length diameter). On the 5th week after radiation therapy, or the 53th day after tumor implantation, tumors in each group grew more than 4-fold compared with that before radiation. There was no obvious change in weight, signs of life, activity among various treatment groups.

The T_{GT4}'s in the ATO alone group and the untreated group were 8.5 ± 0.84 and 8.4 ± 0.58 days, respectively ($p = 0.7$), indicating that ATO treatment alone had no impact on tumor growth. In addition, at the dose used in the present study, ATO had no host toxicity, therefore considering this dose as a non-toxic dose. However, compared with that of RT alone group, the tumor growth delay by radiation was significantly enhanced by ATO in a dose-dependent manner when combined with the non-toxic doses of ATO. As shown in Table 1, when the relative tumor volume reached 4, the tumor growth delay from the combined treatment with ATO and RT was estimated to be about 4 (2 Gy), 13 (4 Gy), and 21 days (6 Gy), respectively, after the effects of ATO were deducted from the results, significantly greater than that from RT alone ($p < 0.05$) (Figure).

Table 1. Tumor growth delay in experimental animals treated by RT alone and combined treatment with ATO and RT

RT dose	Tumor growth delay (days)		
	RT alone	ATO + RT	*p* value
2 Gy	1.9 ± 0.6	3.9 ± 1.3	< 0.05
4 Gy	7.6 ± 1.8	13 ± 2.4	< 0.05
6 Gy	11.9 ± 2.9	21.7 ± 4.4	< 0.05

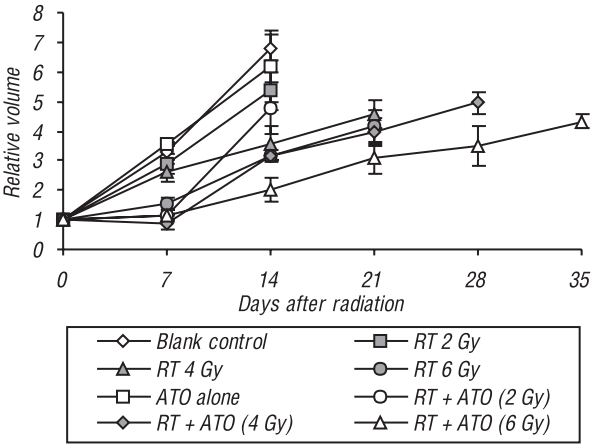

Figure. The effects of combined treatment with ATO and radiation on nasopharyngeal carcinoma xenografts in BALB/C nude mice

Evaluation of AST and ALT. No significant differences in the levels of AST and ALT between control group and group with combined treatment by RT and ATO were observed ($p > 0.05$), suggesting that the combined treatment was well tolerated in the experimental animals (Table 2).

Table 2. AST and ALT levels in untreated group of animals and combined treatment group

Groups	Results of liver function test	
	AST U/L	ALT U/L
Untreated	40.1 ± 24.8	196.3 ± 45.6
RT + ATO (2 Gy)	37.8 ± 32.4	174.7 ± 49.7
RT + ATO (4 Gy)	37.3 ± 34.3	169.5 ± 52.3
RT + ATO (6 Gy)	41.3 ± 35.1	178.2 ± 40.2
Control for toxic effect	47.3 ± 21.4	201.2 ± 25.5

There are no significant difference between the treated group and untreated group ($p > 0.5$).

Moreover, no obvious toxic changes were found histologically in liver, lung, kidney, and heart tissue, and no metastases were not found as well. There were no differences in the toxicity among various treatment groups.

DISCUSSION

Some recent studies have indicated that ATO might enhance radiation response in some solid tumors [7–11, 16], and the combined treatment with ATO and fractionated radiotherapy are effective in the treatment of relapsed acute promyelocytic leukemia [17], but there are no *in vivo* data supporting its effect in nasopharyngeal carcinoma.

High dose of radiation alone or ATO alone was required to treat nasopharyngeal carcinoma effec-

tively, but some pathological changes in liver and cardiac tissues were found [14]. In the current study, T_{GT4} was used to determine the treatment effect, thus, the radiation doses used in the current study should enable all the xenografts to reach 4 times of their initial volumes and should not cause tumor disappearance after irradiation. That is why we chose relatively low radiation doses (2 Gy, 4 Gy, 6 Gy) for the experiment. Before the drug administration, we performed several preliminary experiments to determine the dose of ATO. Three doses (4, 7, and 10 mg/kg) were used, the ATO doses at 7 and 10 mg/kg could lead to severe toxicity including death. In contrast, no mice died at the dose of 4 mg/kg, indicating that low dose of ATO caused less toxic effects. Moreover, ATO alone at the dose of 4 mg/kg had no pronounced antitumor effect compared with the untreated controls. Therefore the combination treatment with low dose of ATO may have clinical implication. Our results showed that the combined treatment with ATO and radiation delayed tumor growth significantly, suggesting the synergistic effects between ATO and radiation.

To determine the time for tumor growth delay when the relative tumor volume reached 4 times of the initial tumor size, the data for ATO alone as control was deducted, thus the inhibitory effect of tumor growth by combined ATO and RT showed the synergistic effects. As shown in Table 1, T_{GD} of ATO combined with RT was higher than that of RT alone, which was dependent on the dose of radiation. These results have demonstrated enhancement of radiation response by ATO in nasopharyngeal carcinoma *in vivo*, and further confirmed our previous results *in vitro* [13].

Considering that liver disfunction is a major adverse event of ATO in clinical treatment of leukemia [18], we investigated the effects of combined treatment with ATO and RT on the level of AST and ALT in mice. To our knowledge, this is the first report on toxic effects of ATO on liver function in solid tumor models. We used two groups of animals for combined treatment, one was sacrificed shortly after the last time of drug administration, another one was sacrificed by the end of the experiments. No significant difference in the levels of AST and ALT was found among the two groups and the untreated group, indicating that no obvious liver disfunction was caused by the combined treatment. Moreover, no significant toxic effects either by observation of activity, weight or pathological morphology, indicating the combination with ATO and RT was a well tolerable treatment.

In conclusion, low dose of ATO enhances the response of nasopharyngeal carcinoma to radiation therapy in nude mouse xenograft models which provides a basis for future studies with this combined regimen in patients with nasopharyngeal carcinoma

ACKNOWLEDGEMENT

This work was supported by the Shantou University Research and Development grant (L03002), the Traditional Chinese Medicine Research grant of Guangdong Province (102053), China.

REFERENCES

1. **Wu Y, Hu WH, Xia YF, Ma J, Liu MZ, Cui NJ.** Quality of life of nasopharyngeal carcinoma survivors in Mainland China. Qual Life Res 2006; **16**: 65–74.

2. **Fang FM, Chiu HC, Kuo WR, Wang CJ, Leung SW, Chen HC, Sun LM, Hsu HC.** Health-related quality of life for nasopharyngeal carcinoma patients with cancer-free survival after treatment. Int J Radiat Oncol Biol Phys 2002; **53**: 959–68.

3. **Chen Y, Huang G, Huang Z, Wen W, Xie C.** Analysis of the nasal complication after radiotherapy in patients with nasopharyngeal carcinoma by logistic regression Lin Chuang. Er Bi Yan Hou Ke Za Zhi 2003; **17**: 461–3.

4. **Soignet SL, Frankel SR, Douer D, Tallman MS, Kantarjian H, Calleja E, Stone RM, Kalaycio M, Scheinberg DA, Steinherz P, Sievers EL, Coutre S, Dahlberg S, Ellison R, Warrell RPJr.** United states multicenter study of arsenic trioxide in relapsed acute promyelocytic leukemia. J Clin Oncol 2001; **19**: 3852–60.

5. **Mathews V, Balasubramanian P, Shaji RV, George B, Chandy M, Srivastava A.** Arsenic trioxide in the treatment of newly diagnosed acute promyelocytic leukemia: a single center experience. Am J Hematol 2002; **70**: 292–9.

6. **Lazo G, Kantarjian H, Estey E, Thomas D, O'Brien S, Cortes J.** Use of arsenic trioxide (As2O3) in the treatment of patients with acute promyelocytic leukemia: the MD Anderson experience. Cancer 2003; **97**: 2218–24.

7. **Monzen H, Griffin RJ, Williams BW, Amano M, Ando S, Hasegawa T.** Study of arsenic trioxide-induced vascular shutdown and enhancement with radiation in solid tumor. Radiat Med 2004; **22**: 205–11.

8. **Kim JH, Lew YS, Kolozsvary A, Ryu S, Brown SL.** Arsenic trioxide enhances radiation response of 9L glioma in the rat brain. Radiat Res 2003; **160**: 662–6.

9. **Ning S, Knox SJ.** Optimization of combination therapy of arsenic trioxide and fractionated radiotherapy for malignant glioma. Int J Radiat Oncol Biol Phys 2006; **65**: 493–8.

10. **Chun YJ, Park IC, Park MJ, Woo SH, Hong SI, Chung HY, Kim TH, Lee YS, Rhee CH, Lee SJ.** Enhancement of radiation response in human cervical cancer cells *in vitro* and *in vivo* by arsenic trioxide (As_2O_3). FEBS Lett 2002; **519**: 195–200.

11. **Huilgol NG.** A phase I study to study arsenic trioxide with radiation and hyperthermia in advanced head and neck cancer. Int J Hyperthermia 2006; **22**: 391–7.

12. **Du CW, Wen BG, Li DR, Peng X, Hong CQ, Chen JY, Lin ZZ, Hong X, Lin YC, Xie LX, Wu MY, Zhang H.** Arsenic trioxide reduces the invasive and metastatic properties of nasopharyngeal carcinoma cells *in vitro*. Braz J Med Biol Res 2006; **39**: 677–85.

13. **Li DR, Xie LX, Lin YC, Du CW, Wu MY.** Arsenic trioxide enhances radiosensitivity of nasopharygeal carcinoma *in vitro*. Exp Oncol 2003; **25**: 248–51.

14. **Li DR, Du CW, Lin YC,Wu MY.** Inhibition of growth of human nasopharyngeal cancer xenografts in SCID mice by arsenic trioxide. Tumori 2002; **88**: 522–6.

15. **Amano M, Monzen H, Suzuki M, Terai K, Andoh S, Tsumuraya A, Hasegawa T.** Increase in tumor oxygenation and potentiation of radiation effects using pentoxifylline, vinpocetine and ticlopidine hydrochloride. Radiat Res 2005; **46**: 373–8.

16. **Lew YS, Kolozsvary A, Brown SL, Kim JH.** Synergistic interaction with arsenic trioxide and fractionated radiation in locally advanced murine tumor. Cancer Res 2002; **62**: 4202–5.

17. **Kai T, Kimura H, Shiga Y, Ogawa K, Sato H, Maruyama Y.** Recurrent extramedullary relapse of acute promyelocytic leukemia after allogeneic stem cell transplantation: successful treatment by arsenic trioxide in combination with local radiotherapy. Int J Hematol 2006; **83**: 337–40.

18. **Shen ZX, Chen GQ, Ni JH, Li XS, Xiong SM, Qiu QY, Zhu J, Tang W, Sun GL, Yang KQ, Chen Y, Zhou L, Fang ZW, Wang YT, Ma J, Zhang P, Zhang TD, Chen SJ, Chen Z, Wang ZY.** Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL): II. Clinical efficacy and pharmacokinetics in relapsed patients. Blood 1997; **89**: 3354–60.

СИНЕРГИЧНЫЙ ТЕРАПЕВТИЧЕСКИЙ ЭФФЕКТ ТРИОКСИДА МЫШЬЯКА И РАДИОТЕРАПИИ У МЫШЕЙ ЛИНИИ BALB/C С КСЕНОГРАФТАМИ КАРЦИНОМЫ НОСОГЛОТКИ

Установлено, что триоксид мышьяка (ТОМ) индуцирует апоптоз в клетках карциномы носоглотки человека и ингибирует рост ксенографта карциномы носоглотки у атимических мышей. *Цель* работы — установить терапевтическую эффективность радиотерапии в комбинации ТОМ в нетоксичной дозе мышам линии BALB/C с ксенографтом карциномы носоглотки. *Методы:* животные с ксенографтом карциномы носоглотки получали либо только радиотерапию (2, 4 и 6 Гр) или ТОМ (4 мг/кг/день в течение 6 дней), или их комбинацию в тех же режимах и дозах. Задержку роста опухоли определяли как различие во времени, необходимом для достижения опухолью 4-кратного объема по сравнению с начальным объемом в опытной группе *versus* такового в контрольной группе. *Результаты:* введение ТОМ в низкой дозе не оказывало выраженного влияния на рост опухоли по сравнению с показателями в контрольной группе, а в комбинации с облучением приводило к задержке роста опухоли на 2–12 сут по сравнению с показателями у животных, получавших только лучевую терапию при отсутствии выраженных побочных эффектов. *Выводы:* комбинация ТОМ в нетоксической дозе и лучевой терапии приводит к ингибированию роста опухоли *in vivo* и не вызывает побочных эффектов.

Ключевые слова: **триоксид мышьяка, радиотерапия, ксенографт карциномы носоглотки.**