Synergistic Interaction with Arsenic Trioxide and Fractionated Radiation in Locally Advanced Murine Tumor

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Abstract

We have shown previously that arsenic trioxide (ATO) preferentially shuts down tumor blood flow, leading to pronounced cell death in the central part of the solid tumor with a minimal effect on the surrounding normal tissues. On the basis of the histopathological and tumor perfusion studies, we hypothesized that the tumor control rate of locally advanced solid tumors would increase after the combined treatment of ATO and radiation relative to either radiation or ATO alone. The antitumour action and quantitative tumor perfusion studies were carried out with locally advanced methylcholanthrene-induced fibrosarcoma grown in BALB/c mice. The s.c. methylcholanthrene-induced fibrosarcoma leg tumors were treated with ATO alone (10 mg/kg), radiation alone (30 Gy), or drug plus radiation together. Radiation alone and drug alone delayed the growth of the tumor by a few days compared with untreated tumors. In contrast, when radiation and drug were given together, the tumor growth delay was longer than 1 month, resulting local tumor cure of 55%. The fractionated radiation combined with ATO showed a similar pronounced tumor growth delay relative to the drug alone or radiation alone. Sustained reduction in tumor blood flow after the combined treatment measured using a rubidium uptake method paralleled enhanced tumor response. There was an immediate 10-fold increase in the production of tumor necrosis factor-α in the tumor tissue after the drug treatment, concomitant with the onset of prompt reduction of the tumor blood flow. The present results indicate that tumor response was better with combined treatment than with either treatment alone, suggesting that ATO has potential as an adjuvant to radiotherapy.

Introduction

ATO has a therapeutic benefit in the treatment of some hematologic malignancies. Used as an Asian medicinal for more than 1000 years, ATO recently has been accepted by Western medicine as a part of the arsenal in the treatment of recurrent acute promyelocytic leukemia. A significant percentage of patients with recurrences after conventional chemotherapy subsequently achieve a complete remission with ATO alone (1, 2).

We have demonstrated previously that a single systemic administration of ATO induced a prompt and pronounced vascular shutdown in large murine solid tumors and produced extensive central necrosis in the poorly perfused compartment of the tumor (3). The large tumors were more vulnerable to the action of the antivascular effect of ATO, whereas the peripheral zone of the tumor was minimally affected and became a source of eventual recurrence. Hence, the tumor response to ATO alone produced varying degrees of tumor growth delays in rapidly growing tumors, but the cure was seldom obtained.

The fact that ATO preferentially targets the poorly perfused fraction of the tumor, which may be radiation resistant, whereas radiation therapy is effective against well perfused tissues of the tumor periphery, led us to hypothesize that the combined treatment of drug and radiation would enhance the tumor response relative to that of radiation or drug alone. We report the first in vivo results demonstrating a pronounced enhancement of the radiation tumor response in well established large murine tumors after the combined treatment of drug and radiation.

Materials and Methods

Mice, Tumors, and Compounds. BALB/c male mice, 6–8 weeks of age and weighing 20–25 g, that were obtained from Charles River Laboratories (Portage, MI) were used. ATO was purchased from Sigma-Aldrich (St. Louis, MO). The murine tumor line used was Meth-A grown in mice. For s.c. tumor, which was used for tumor response, approximately 1 × 10^6 viable Meth-A cells in 50 µl of MEM were inoculated into the hind leg s.c. For intradermal tumor, which was used for the study of blood perfusion and TNF-α assay, approximately 1 × 10^6 viable Meth-A cells in 100 µl of MEM were inoculated into the abdominal skin of mice. Maintenance of tumor cells, preparation of cells, and making ATO solution were performed as described previously (3).

Effect of ATO and Radiation on Tumor Growth. s.c. hind leg tumors were used for tumor growth studies. When tumors were palpable, mice were randomly divided into four groups, untreated, ATO alone, radiation alone, and radiation combined with the drug. When mean tumor volume reached 0.3–0.5 cm³, radiation was delivered to the leg with tumors using a cobalt-60 unit (Theratron 780; AECL, Kanata, Ontario, Canada) with a secondary collimator of 2-inch-thick cerrobend. A 2-cm-thick tissue-equivalent bolus was used to bring the maximal radiation dose on the surface of the target tissue. ATO (10 mg/kg) was administered i.p. 1 h after the beginning of each radiation exposure. Tumor growth delay was defined as the additional time necessary for a tumor to grow to 1 cm³ in average volume compared with untreated tumors. Cured tumors were defined as no evidence of palpable tumors 60 days after the treatment.

Blood Perfusion Measurement. Changes in the blood perfusion were assessed by means of ⁸⁶Rb uptake as described elsewhere (4). BALB/c mice with intradermal Meth-A tumors, 1.2 cm in mean diameter, were used. Briefly stated, 5 µCi of ⁸⁶RbCl in a 0.1-ml volume were injected through a tail vein after anesthesia with ketamine (100 mg/kg) and xylazine (10 mg/kg), and mice were sacrificed by cervical dislocation 60 s after the injection. Whole tumors were excised and counted using a well counter (1282 Comugamma Cs; Amersham Biosciences Inc., Piscataway, NJ). The ratio of radioactive counts from the tissue of interest (counts per gram) to the counts in the standard ⁸⁶Rb solution equivalent to the total ⁸⁶Rb activity injected multiplied by 100 gave the percentage of cardiac output to the tissue of interest. Relative blood perfusion values, i.e., a percentage of the untreated tissue, were calculated. ⁸⁶Rb uptake was measured before, at 24 h, and 60 h after treatment. At least five tumor-bearing mice were used at each time point.

Measurement of Tissue TNF-α Levels. The extractable TNF-α levels in tumor, liver, spleen, and serum were measured at 0 min, 30 min, 2 h, and 4 h after i.p. injection of ATO (10 mg/kg). BALB/c mice with intradermal Meth-A tumors, 1.2 cm in mean diameter, were used. Excised tissues were weighed and homogenized in minimal essential medium using a tissue grinder. Homogenates were centrifuged at 2000 × g for 30 min at 4°C, and collected supernatant was kept in a freezer. Tissue TNF-α was assayed in batches on the same day using the same conditions. Samples were thawed and centrifuged a second time before use.
As2O3 IMPROVES TUMOR RADIATION RESPONSE

was given before radiation or 24 h after radiation (data not shown). (b) The next study was to determine whether the initial tumor size would influence the tumor growth delays after the combined treatment, because the initial tumor size limits the number of radiation fractions, and in our previous study, the drug exerted its maximal antivascular effect on a large tumor (3, 5). In contrast to the results with drug alone, both large and small tumors (between 0.3 cm³ and 1.0 cm³ in volume) showed similar enhanced tumor response to the combined treatment. On the basis of these preliminary findings, all subsequent studies with the combined treatment were carried out with Meth-A tumors of moderate size (average of 0.5 cm³), using the following timing and sequence: i.p. administration of As2O3 1 h after the beginning of radiation exposure. Fig. 1A shows the combined effect of radiation and As2O3 after single-dose radiation (30 Gy) or fractionated radiation (12 Gy, four times). The tumor growth delay from the combined treatment was estimated to be about 42 days (29 days if we exclude cured mice), significantly greater than the tumor growth delay after either radiation alone or As2O3 alone, approximately 12 days and 2 days, respectively. Significantly, more than 50% of tumors were cured after the combined treatment. Fig. 1B shows the effect of fractionated radiation and As2O3. As with the foregoing result of single-dose radiation and As2O3, the tumor growth delay was significantly increased (34 days) relative to that of radiation alone (3 days) or As2O3 alone (3 days). Table 1 shows the tumor growth delays obtained with different fractionation schemes.

Relationship Between Tumor Response and Blood Flow Changes in Tumor. Changes in the tumor blood flow were measured using ⁸²Rb uptake to evaluate whether the synergistic effect of the combined treatment was related to blood flow. Fig. 2 illustrates the blood flow changes of whole tumor at 24 h and 60 h after the treatment. The percentage of blood perfusion relative to untreated tumor at 24 h after As2O3 alone, radiation alone, or the combined treatment was 64.3 ± 13.3%, 69.9 ± 13.5%, and 45.9 ± 21.1%, respectively, all showing a significant decrease from pretreatment values. At 60 h, the percentage of blood perfusion after As2O3 alone or radiation alone rebounded to 108.3 ± 22.5% and 121.3 ± 34.2%, respectively. In contrast, the percentage of blood perfusion at 60 h after the combined treatment remained reduced at 61.6 ± 11.9%. The sustained reduction of blood flow in the tumor after the combined treatment paralleled the enhanced tumor response to the treatment.

Intratumoral TNF-α After ATO Treatment. Fig. 3 shows TNF-α levels in the tumor tissue before and after systemic ATO treatment. The TNF-α level in untreated tumor was 0.54 ± 0.12 µg/g. After the drug treatment, there was more than a 10-fold increase in intratumoral TNF-α (6.65 ± 2.98 µg/g) at 30 min. TNF-α gradually decreased by 4 h to 5-fold the initial level. In contrast, the changes of TNF-α in other tissues showed different kinetics, i.e., 2-fold increases time on the day of TNF measurement. TNF levels were measured with the ELISA method using a mouse TNF-α immunoassay kit (Quantikine M, R&D Systems, Minneapolis, MN).

Results

Tumor Response to the Combined Treatment of ATO and Radiation. Before proceeding to a determination of the combined effect of ATO and radiation, we carried out two preliminary studies. (a) We wanted to determine which specific treatment sequence and time of ATO with respect to radiation would produce a maximal local tumor control or tumor growth delay. The maximal synergistic effect was observed only when the drug was administered shortly after irradiation (1-h interval). No synergism was obtained when the drug

<table>
<thead>
<tr>
<th>Dose/fraction × no. of fraction (total dose)</th>
<th>ATO alone (10 mg/kg, i.p.)</th>
<th>RT alone</th>
<th>RT + ATO</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 Gy × 2, once a wk (24 Gy)</td>
<td>2.3 ± 0.9 days</td>
<td>0.8 ± 0.4 days</td>
<td>5.4 ± 2.6 days</td>
</tr>
<tr>
<td>12 Gy × 4, once a wk (48 Gy)</td>
<td>2.4 ± 0.6 days</td>
<td>0.8 ± 0.4 days</td>
<td>27.3 ± 4.8 days</td>
</tr>
<tr>
<td>15 Gy × 4, once a wk (60 Gy)</td>
<td>2.4 ± 0.6 days</td>
<td>1.0 ± 1.4 days</td>
<td>27.0 ± 1.0 days</td>
</tr>
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Table 1 Tumor growth delays by combined treatment with fractionated radiation and ATO

Subcutaneous Meth-A tumors grown in the hind leg of mice were used. The combined fractionated schedule consists of once a week radiation and ATO. ATO (10 mg/kg) was given i.p., 1 h after each radiation exposure in both experiments. △, untreated control; ■, ATO alone; □, radiation alone; ○, combined treatment (×, not cured; +, cured). Arrowheads, treatment time. Data are the means of 9–10 mice per group. Bars, SE.
vasodilation (10). Furthermore, changes in blood flow of the peripheral rim of the tumor, not the central core of the tumor, predicted the recurrence of the tumor after radiation plus ATO.

We sought to determine the kinetic changes of the cytokine, TNF-α, for two reasons, although the mechanism of interaction between ATO and radiation remains unknown. (a) TNF-α increases vascular permeability of the tumor vessels followed by extensive hemorrhagic necrosis of tumor, which was described by Carswell et al. (11) using Meth-A tumors. The gross observation after ATO treatment is similar to that seen with endotoxin-induced necrosis (3). (b) The elevation of intratumoral TNF-α occurs after treatment with other VTAs including flavone acetic acid and 5,6-dimethylxanthenone-4-acetic acid (12, 13). Fig. 3 shows an immediate and pronounced increase in production of TNF-α in the tumor tissue within a half-hour after the drug administration, whereas the TNF-α levels in other organs including the liver showed a delayed elevation at 2 h or later. The implication is that the antivascular effect of ATO is mediated by an increased production of TNF-α. TNF-α has been shown to enhance the antitumor effect of radiation (14, 15). Also, there is evidence that pre-irradiated cell lines including endothelial cells are more sensitive to TNF-α (16, 17). This suggests that the increased production of TNF-α by ATO might be responsible for the synergistic effect of radiation and ATO.

Although VTAs are not curative when delivered alone, they do exhibit major tumor necrosis and, as such, are distinct from antiangiogenic agents that target newly formed tumor vasculature. VTAs have been categorized as one of two types (18). Drugs in one group, flavone acetic acid (19) and related compounds (12), exert their effect through the production of TNF-α. The other group is tubulin-binding agents such as colchicine, the Vinca alkaloids, and the combretastatins, which are toxic to endothelial cells (20). Drugs in both groups enhance the antitumor activity of ionizing radiation. At present, ATO does not clearly belong to one group or the other.

Although the radiation-enhancing effect of ATO is clear, both after single doses and fractionated irradiation, it has been assumed that the drug is augmenting tumor response to irradiation. It is also conceivable that irradiation could affect cellular metabolism in a manner that would sensititize cells to the effects of the drug, because the synergy is obtained with the sequence of radiation followed by the drug administration. Additional studies are needed to examine the interaction and timing of various cytokine production and radiation schedules.

In summary, the results presented demonstrate that ATO retains its synergistic effect with radiation under a fractionated schedule. Furthermore, the data support the use of combining ATO with radiation as a strategy for the treatment of large tumors resistant to conventional therapies.

Acknowledgments

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References


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