Effect of Tumor Size on S-2-(3-Aminopropylamino)ethylphosphorothioic Acid and Misonidazole Alteration of Tumor Response to Cyclophosphamide

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ABSTRACT

The influence of tumor size on the ability of S-2-(3-aminopropylamino)ethylphosphorothioic acid (WR-2721) or misonidazole (MISO) to alter cyclophosphamide (CY) antitumor activity was investigated, using a chemically induced fibrosarcoma (FSA) and a spontaneous fibrosarcoma (NFSA) in C3Hf/Kam mice. Tumors were of two sizes at the time of treatment, 8-mm leg tumors and 4-day-old micrometastases in the lung. The antitumor activity of CY and its modification were assessed by growth delay of leg tumors and the reduction in the number of lung metastases. Both measures of tumor response were more pronounced as the dose of CY increased, and FSA was more sensitive to CY than was NFSA. WR-2721 (400 mg/kg), given 30 min before treatment with CY, reduced the effectiveness of CY on both FSA and NFSA. This reduction in effectiveness of CY was only minimal for leg tumors (dose-modifying factors were 1.1 for FSA and 1.03 for NFSA) but remarkable for lung micrometastases (dose-modifying factors were 1.81 for FSA and 1.55 for NFSA). Protection increased with the increase in the dose of WR-2721 and was also dependent on the time of injection relative to CY. The greatest protection occurred when WR-2721 was given within 30 min before to 15 min after CY. Tumor size had the opposite effect on MISO from that on WR-2721. MISO (1 mg/g) enhanced the effect of CY more effectively for leg tumors than for lung micrometastases: dose-modifying factors were 1.74 for FSA and 2.21 for NFSA growing in the leg and 1.27 for FSA and 1.11 for NFSA lung micrometastases. Therefore, tumor size appears to be a very important factor in determining the extent of WR-2721- and MISO-induced modification of CY antitumor effect.

INTRODUCTION

Recently, there has been significant interest in studies of the radioprotective and chemoprotective effects of WR-2721. Most studies have shown that WR-2721 protects normal tissues more effectively than tumors against irradiation or alkylating agents (reviewed in Refs. 16 and 27), suggesting that WR-2721 might provide a therapeutic benefit in radiotherapy and chemotherapy. We reported recently that WR-2721 was highly protective against radiation in several normal tissues in mice but had no effect on the cure rate of 8-mm fibrosarcomas growing in the leg exposed to irradiation (13). However, WR-2721 protected lung micrometastases of this tumor from radiation damage, although still to a lesser extent than it protected normal tissues (13). These observations show that tumor size may be an important factor in determining whether tumors will be radioprotected or not.

In reported studies on the effect of WR-2721 on the response of murine tumors to alkylating agents, large solitary tumors were used, and the chemoprotection was minimal if any (22, 24, 25). The purpose of the present studies was to determine whether, as with the radioprotective effect of WR-2721, the size of tumors influences WR-2721 protection against tumor damage caused by CY.

Furthermore, we studied the effect of MISO, a potent hypoxic cell radiosensitizer and chemosensitizer, on the response of lung metastases and solitary leg tumors to CY. Since MISO selectively sensitzes hypoxic cells, it was assumed that it would affect the response of leg tumors to CY more than lung micrometastases.

MATERIALS AND METHODS

Mice

Inbred C3Hf/Kam mice of both sexes bred and maintained in our own specific-pathogen-free mouse colony were used. Mice were 11 to 13 weeks old at the beginning of the experiments. Within each experiment, the mice were of the same sex, and they were housed 4 to 6 per cage.

Tumor

Leg Tumor. A methylcholanthrene-induced FSA and a NFSA syngeneic to C3Hf/Kam mice were used. Single-cell suspensions from both tumors were prepared by trypsin digestion of nonnecrotic tumor tissue (11). Viability of cells was more than 95% as assessed by phase-contrast microscopy. Mice received injections of 5 × 10⁶ viable tumor cells in the right hind thighs. When tumors were 8 mm in diameter, mice were treated with CY. To obtain tumor growth curves, 3 mutually orthogonal diameters of tumors were measured 3 times a week with a vernier caliper, and the mean values were calculated. Tumor growth was followed until the animals died or when the mean tumor diameter was approximately 19 mm. At that time, lungs of mice were removed, and the number of spontaneous lung metastases was determined (9). Each group contained 10 mice.

Lung Micrometastases. To produce tumor micrometastases in the lung, 1.42 × 10⁷ or 2 × 10⁷ viable FSA or 2.5 × 10⁷ NFSA cells were suspended in 0.25 ml Hau’s medium (Grand Island Biological Co., Grand Island, N. Y.) and injected i.v. into groups of 7 mice. Four days later, mice were given i.p. injections of CY ranging from 20 to 100 mg/kg. Twelve days after CY treatment, mice were killed, and the number of lung nodules was determined. The number of lung nodules in mice not exposed to CY was determined 14 days after tumor cell injection.

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4 The abbreviations used are: WR-2721, S-2-(3-aminopropylino)ethylphosphorothioic acid; CY, cyclophosphamide; MISO, misonidazole; FSA, chemically induced fibrosarcoma; NFSA, spontaneously developed fibrosarcoma; DMF, dose-modifying factor.

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CY, WR-2721, and MISO

CY (Mead Johnson, Evansville, Ind.) was dissolved in distilled water at concentrations ranging from 2 to 20 mg/ml and was administered i.p. at doses of 20 to 200 mg/kg body weight. WR-2721 was dissolved in 0.9% sodium chloride solution and injected i.p. in doses ranging from 50 to 500 mg/kg. The pH of sodium chloride was 6.0, but immediately upon addition of WR-2721, it became 7.0 and remained at that value for 4 hr. WR-2721 dissolved in this way was incapable of protecting in vitro culture cells against γ-irradiation. MISO was dissolved in Ringer’s solution and injected i.p. into mice at a dose of 1 or 0.5 mg/g. MISO was given 30 min before CY, as was WR-2721, with the exception of one experiment. In the majority of experiments, a single dose of 400 mg WR-2721 per kg was used. Both WR-2721 and MISO were obtained from the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. (kindly supplied by Dr. David H. Pistenmaa). All 3 drugs used here were injected into animals within 1 hr after having been dissolved.

RESULTS

Leg Tumors. Tumors in the legs were generated by injecting $5 \times 10^5$ FSA or NFSA cells into the right thighs of mice. When tumors reached 8 mm in diameter (12 days after tumor cell injection), mice with FSA tumors were treated with 100 or 200 mg CY per kg, and those with NFSA tumors were treated with 80, 120, or 160 mg CY per kg. Groups of mice were given i.p. injections of 400 mg WR-2721 per kg or 1 mg MISO per g 30 min before treatment with CY. Controls were tumor-bearing mice that received either WR-2721 or MISO alone or remained untreated.

At 2- to 3-day intervals after drug treatment, mice were checked for tumor regression and regrowth. The results with FSA are presented in Chart 1, and those with NFSA are in Chart 2. Treatment of FSA-bearing mice with 100 mg CY per kg delayed tumor growth, whereas treatment with 200 mg CY per kg temporarily reduced tumor size, followed by resumption of tumor growth. WR-2721 given before CY only slightly protected tumors from the CY effect. Conversely, MISO markedly enhanced the effect of CY. It should be mentioned, however, that 200 mg CY per kg were toxic when combined with MISO, resulting in 80% mortality of mice by 1 month after treatment. NFSA tumor transplants were less sensitive to CY than was FSA. However, similar to the situation with FSA, treatment with WR-2721 only slightly protected NFSA from CY, whereas MISO was quite effective in increasing NFSA damage caused by CY.

To determine the DMFs produced by treatment with WR-2721 or MISO, we calculated the time in days for tumors to reach 12 mm after treatment with CY plus WR-2721 (Chart 3A) or 9 mm after treatment with CY plus MISO (Chart 3B). The latter tumor size was chosen because only 2 of 10 mice treated with MISO plus CY survived long enough for their tumors to reach 12 mm in diameter. Parallel lines were obtained, and the DMFs were determined at the tumor growth delay level of 16 days. WR-2721 protected FSA by a DMF of 1.1, and MISO sensitized it by a DMF of 1.7. DMFs were determined in a similar way for NFSA, WR-2721 protection being by a DMF of 1.03, and MISO sensitization by a DMF of 2.21.

An additional observation was made in the above experiment with NFSA. When tumors reached approximately 19 mm in diameter, mice were killed, and their lungs were examined for the number of metastases (Chart 4). Mice bearing leg tumors that received no treatment or that were treated with WR-2721 alone were killed 15 to 18 days after the tumors reached an 8-mm diameter; essentially, they were without metastases (Chart 4A). Mice treated with CY or CY plus WR-2721 were killed 20 to 25 days after treatment. Mice that received CY alone had more metastases per lung than did mice treated with both WR-2721 and CY.

Lung Micrometastases. To investigate the effect of WR-2721 or MISO on the effect of CY on tumor micrometastases in the lung, mice were given i.v. injections of $2 \times 10^5$ FSA or $2.5 \times 10^5$ NFSA cells, and 4 days later, they were treated with CY, CY plus WR-2721, or CY plus MISO. Other groups of mice, in addition to receiving i.v. injections of tumor cells, received either WR-2721 or MISO alone, or they received no further treatment.
The dose of WR-2721 was 400 mg/kg, and that of MISO was 1 mg/g 30 min prior to chemotherapy. Doses of CY ranged from 10 to 60 mg/kg in the experiment with FSA and from 20 to 100 mg/kg in the experiment with NFSA.

The number of both FSA and NFSA metastases was determined 12 days after CY treatment (Chart 5). CY effectively reduced the number of lung colonies of both tumors, with the reduction being more pronounced as the dose of CY was increased. As with the leg tumors, FSA metastases responded better to CY than did NFSA metastases. Treatment of mice with WR-2721 before CY protected tumor colonies from the CY damage. DMFs were calculated at the level of 50% reduction in the number of tumor colonies (93 colonies for FSA and 33 colonies for NFSA). FSA metastases were protected better than

**Chart 3.** Growth delay in days of FSA as a function of the dose of CY. These data are derived from results presented in Chart 1. The drugs were given i.p. when leg FSAs had grown to 8 mm in diameter. Treatments: CY alone (O); 400 mg WR-2721 per kg and CY (•); or 1 mg MISO per g and CY (A). WR-2721 and MISO were given 30 min before CY. DMFs were determined at the tumor growth delay level at 16 days, and it was 1.1 for WR-2721 and 1.74 for MISO. FSa, FSA.

**Chart 4.** Number of spontaneous metastases in mice bearing NFSA in the leg. Mice were treated with CY or CY and WR-2721 when tumors had grown to 8 mm in diameter (see Chart 2). In A, controls were untreated mice (O) and mice treated with 400 mg WR-2721 per kg (•). In B, C, and D, mice were treated with CY alone (C) or with WR-2721 and CY (•). Mice were killed, and the number of metastases was determined when primary tumors had grown to 19 mm in diameter.

**Chart 5.** Effect of WR-2721 or MISO on the CY-induced reduction in the number of FSA (A) or NFSA (B) metastases in the lung. Mice were given injections i.v. of 2 × 10⁶ FSA or 2.5 × 10⁶ NFSA cells, and 4 days later, when tumor nodules in the lung were microscopic, they were given i.p. graded doses of CY alone (C); 400 mg WR-2721 per kg and CY (•); or 1 mg MISO per g and CY (A). WR-2721 and MISO were given 30 min before CY. Bars, S.E; FSA, FSA; NFSA, NFSA.
were NFSA metastases; the DMF was 1.81 for FSA and 1.55 for NFSA. Thus, WR-2721 protection of pulmonary metastases was more pronounced than that of leg tumors. In contrast to WR-2721, MISO chemosensitized leg tumors more than pulmonary micrometastases, increasing the antimetastatic effect of CY by a DMF of 1.27 in FSA and 1.11 in NFSA. DMFs were determined at the 50% colony reduction level. However, neither MISO nor WR-2721 alone affected the number of colonies in the lung.

Reduction in the number of lung nodules prolonged, while increase in their number reduced, the survival of mice. We reported previously that the higher numbers of lung nodules in mice treated with WR-2721 and local thoracic irradiation than in mice that received local thoracic radiation only were associated with earlier death (12). In that study, WR-2721 and thoracic irradiation were given 4 days after i.v. injection of FSA cells into mice. Results (Chart 6) show that MISO significantly increased the beneficial effect of CY on the survival of mice with FSA metastases in the lung. Mice were given i.v. injections of $1.42 \times 10^6$ FSA cells, and 4 days later, they were exposed to 0.5 mg MISO per g, 200 mg CY per kg, or both. Fifty % of the mice were cured when MISO was given 30 min before CY, whereas there was only a significant prolongation in the survival of mice given CY alone. The mean survival time of mice treated with CY was $64.7 \pm 10.2$ days, compared to $>121$ days for mice treated with MISO and CY ($p = 0.012$; Mann-Whitney $U$ test).

Additional experiments on the dose and timing of WR-2721 were performed. In one experiment, doses of WR-2721 ranging from 50 to 500 mg/kg were given 30 min before 20 mg CY per kg at Day 4 after i.v. injection of $2 \times 10^6$ FSA cells into mice (Chart 7). All doses of WR-2721 used were chemoprotective, and, in general, higher doses protected tumor micrometastases better. In another experiment, 400 mg WR-2721 per kg were given at different times from 4 hr before to 2 hr after CY, 4 days after i.v. injection of $2 \times 10^6$ FSA cells. Results (Chart 8) show chemoprotection was achieved if WR-2721 was given 2 hr before to 1 hr after treatment with CY. However, the highest protection was observed when WR-2721 was given within 30 min before and 15 min after CY.

DISCUSSION

Our results show that the magnitude of the tumor-chemoprotecting effect of WR-2721 and of the tumor-chemosensitizing effect of MISO in the CY therapy of murine tumors greatly depended on the size of tumors. WR-2721 was only marginally protective for 8-mm leg tumors, but it protected lung micrometastases of both FSA and NFSA tumors to a considerable degree. The opposite effect was produced by MISO, which enhanced the therapeutic effect of CY on lung micrometastases to a lesser extent than on 8-mm leg tumors. The reason for this influence of tumor size on the effect of WR-2721 and MISO is not clear. In order to modify cell response to ionizing radiation or to alkylating agents, WR-2721 and MISO have to be present within cells that interact with these agents, or, to a lesser extent, they can interfere with pharmacokinetics of alkylating drugs (3, 4). Therefore, a probable explanation for the tumor size effect could be that, because of the development of hypoxic regions within the tumor during the tumor growth, there exists interference with
either distribution or metabolism of these agents within the tumor. An 8-mm FSA contains nearly 30% hypoxic cells (18), whereas 4-day-old micrometastases in the lung would not be expected to contain a high percentage of hypoxic cells, if any at all. It follows then that MISO is expected to be more effective against tumors with large amounts of hypoxic cells, since this compound selectively binds to hypoxic cells and sensitizes them to ionizing radiation and alkylating agents (3, 4). Consistent with this is our observation that MISO induced a much more effective response of 8-mm leg tumors than of lung micrometastases to CY. In the case of WR-2721, one can assume that more drug would be available to tissues that are well supplied with blood, which include oxic areas of tumors, thus providing better protection for those tissues. Based on this assumption, it was expected that better chemoprotection should occur in lung micrometastases than in large solitary tumors. Our results are consistent with the reports in the literature that large solid tumors are not protected, or are protected only minimally, against radiation by WR-2721 treatment (reviewed in Refs. 16 and 27), whereas 1-day i.v. transplants of a murine leukemia were protected equally as well as were normal bone marrow cells (16). A recent report by Denekamp et al. (6) clearly shows that oxygen concentration in tissues is a critical factor in the extent of WR-2721 radioprotection. Radioprotection of skin was maximal in air-breathing conditions and was nonexistent in 100% nitrogen. However, it still remains to be determined whether WR-2721 reaches hypoxic regions of the tumor or remains in oxic cells only. Yuhas (24) reported that, in an in vitro situation, WR-2721 diffuses into both viable and necrotic pieces of the tumor, which suggests that in vivo WR-2721 could be expected to reach hypoxic areas of the tumor as well. Yuhas (24), however, found that tumor cells absorb WR-2721 only passively, whereas normal tissue cells absorb it actively, which results in higher concentrations of WR-2721 in normal cells and, consequently, in better radio- and chemoprotection of normal cells. We reported recently that tumor cells from mice treated with WR-2721 were protected only minimally by radiation given when all tumor cells were hypoxic (10).

Therefore, the extent of hypoxia (or compromised blood circulation) within tumors is likely to be a major reason for the observed effect of tumor size on WR-2721 protection of tumors against CY damage, either by influencing delivery of WR-2721 to tumor cells or by affecting the ability of WR-2721 to express chemoprotection, or by both mechanisms. However, other factors might have also been involved. For example, WR-2721 might have affected distribution of CY within organs and tissues of animals so that there was a low concentration of CY in the lung and thus less damage of micrometastases. This possibility is indirectly supported by evidence that a significant splenomegaly due to vasodilatation develops within 1 hr after injection of WR-2721 into mice (26). This splenomegaly was associated with a reduced peripheral oxygen tension, a change that could alter distribution of drugs throughout tissues and organs. However, if the redistribution of CY did take place, one would expect that large solid tumors would also be protected more profoundly, but this did not occur. Whatever the reasons were for the observed effect of tumor size, the finding has important therapeutic implications in that micrometastatic foci are expected to be protected to some extent, whereas large solitary tumors will most likely escape chemoprotection.

WR-2721 protected 4-day-old FSA micrometastases against radiation damage by a DMF of 1.28 (13) and 4-day-old NFSA micrometastases by a DMF of 1.22, which is less protection than we observed against CY (Chart 5). The reason for this difference is not clear. Both radiation and alkylating agents kill tumor cells by damaging DNA. While radiation damages DNA by either direct hit or through formation of highly reactive free radicals, DNA damage by alkylating agents is through formation of alkyl groups. Recent investigations show that intracellular thiols are a significant determinant of the extent of cell and tissue damage by irradiation or alkylating agents (3, 4). While for radiation damage, thiols scavenge free radicals and thus reduce mainly indirect injury, thiols influence various aspects of interaction between cells and alkylating agents, which could lead to proportionally higher protection of tissues against injury caused by these agents when compared to radiation. Therefore, this might be a possible reason for greater WR-2721 protection against elimination of micrometastases by CY than by radiation. The possible alternative explanation, that WR-2721 caused redistribution of CY among tissues so that less of it was available to micrometastases, is a situation not applicable in the case of radiation delivery.

WR-2721 has been used to protect against tumor injury caused by ionizing radiation for more than a decade (reviewed in Refs. 16 and 27). Most studies have indicated that this compound protects normal tissues more than tumors, implying that WR-2721 could increase the therapeutic benefit of tumor radiotherapy. We reported recently that large leg tumors, but not lung micrometastases, are spared from the radioprotective effect of WR-2721 (13). It was also reported recently that WR-2721 can effectively protect normal tissues against the damage inflicted by several alkylating agents without concomitantly interfering with tumor damage (22, 24, 25). Those studies, however, used solitary tumors of an appreciable size. The data we reported here show clearly that solid tumors of an 8-mm diameter were only marginally protected by WR-2721 and that tumor protection was a problem in the treatment of microscopic tumor foci. Whether a therapeutic gain can be achieved by introducing WR-2721 prior to chemotherapy for microscopic foci cannot be concluded from our results, since parallel studies on chemoprotection of normal tissues were not performed. However, previous studies using normal hematopoietic tissue show that WR-2721 protected mice from CY-induced hematopoietic death by a factor of 1.5 (24) and from CY-induced destruction of colony-forming units by a factor of 2.4 (22). WR-2721 was also effective in protecting hematopoietic tissue against damage inflicted by other alkylating agents by factors of 1.5 to 4.4 (22) and in protecting kidney from cis-platinum-induced damage by a factor of 1.7 (25). These observations, together with our present data, show that WR-2721 should provide remarkable therapeutic benefit when combined with chemotherapy in the treatment of large solitary tumors.

The extent of micrometastasis chemoprotection by WR-2721 depended on the dose and the time it was given in relation to CY application. A certain degree of protection of lung micrometastases was achieved with 100 mg of WR-2721 per kg, and protection increased as the dose was increased. In many cases of WR-2721-induced radioprotection (13), the same dose of WR-2721 protected normal tissues better than tumors. Recently, Twentyman (21) reported that WR-2721 was capable of pro-

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tecting both tumors and normal hematopoietic tissues against CY damage but that therapeutic gain was achieved with a dose of 200, and not with 400, mg WR-2721 per kg. These results suggest that different tissues might require different amounts of WR-2721 for their differential protection. Regarding the time of WR-2721 injection, protection was very effective when WR-2721 was given within 30 min before to 15 min after CY treatment. The ability of WR-2721 to protect tissues when given after chemotherapy differs from its effect on radiation treatment, where it protects tissues only when given before. This can be explained by the difference in duration of damage infliction by the 2 treatments.

Mice bearing NFSA leg tumors developed fewer spontaneous metastases in the lung when treated with WR-2721 plus CY than when they were treated with CY only (Chart 4). The reason for this unexpected observation is unclear. Since the number of observed metastases did not decrease with the increase in CY dose, as one would expect, it can be assumed that the majority of metastatic foci in the lungs developed from cells released from the primary tumor after CY treatment. Therefore, WR-2721 might have been influencing the metastatic process rather than the established metastases. CY is known to increase markedly the formation of metastases from i.v.-injected cells by damaging lung tissue (15) and suppressing antitumor resistance (7, 14). CY enhancement of spontaneous metastasis formation is still uncertain, but it might occur when the primary tumor is insensitive to CY or develops resistance to CY. As shown in Chart 2, NFSA was only slightly sensitive to CY and therefore most likely retained its ability to release tumor cells into the circulation following treatment with CY. If this was the case, then it is likely that WR-2721 protected either the lung or immune system from CY damage and thus reduced the hypothetical CY-induced enhancement of metastases formation. As discussed above, WR-2721 could have protected the lung tissue from CY, either by being absorbed by lung tissue or by causing a change in the pharmacokinetics of CY so that a smaller amount of it was present in the lung to cause damage. WR-2721 can also protect against radiation-induced suppression of immune reactions mediated by B-lymphocytes (23), T-lymphocytes (8), and natural killer lymphocytes. At least some of these immune cells may be involved in restricting tumor spread. For example, it has been reported recently that CY increases formation of murine lung metastases from i.v.-injected tumor cells by suppressing natural killer cell activity (7). Therefore, it is possible that WR-2721 protection of these cells could have led to a smaller number of spontaneous metastases in mice treated with both WR-2721 and CY than in mice that received CY only. A remote possibility for WR-2721 prevention of spontaneous metastasis is a direct antitumor metastatic effect. This hypothesis is based on the observation by Apffel et al. (2) that multiple treatments of tumor-bearing mice with WR-2721 can cause tumor regression. However, our experiments, reported here and before (12, 13), in which treatment of lung micrometastases with WR-2721 alone did not affect the number of micrometastases, argue against this possibility.

In general, there has been more interest in using MISO treatment to increase therapeutic gain of radiotherapy and chemotherapy than in WR-2721. MISO increases radiocurability of a variety of experimental animal tumors, and more recently, it has been shown to increase the therapeutic effect of chemotherapy for animal tumors as well (reviewed in Ref. 1). The compound increases the radioreponse of some normal tissues but less so overall than that of tumors (5). Like those of WR-2721, most studies of MISO used solitary tumor transplants of appreciable size, which contain significant fractions of hypoxic cells that can be sensitized by MISO.

Our data show that the response to CY of both FSAs, growing as leg tumors, can be markedly increased by treatment with MISO. This enhancement of chemotherapeutic effect was still present, though greatly reduced, in the treatment of lung micrometastases. Recently, Siemann (17) reported that MISO given prior to 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea reduced the volume of tumor nodules and increased the survival of mice, compared with the effect of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea alone. If one assumes that MISO selectively sensitizes hypoxic cells, then the effect of MISO on 4-day-old lung micrometastases is surprising, since they are unlikely to contain hypoxic cells. On the average, the 4-day-old micrometastases were less than 10 cells in diameter, whereas it is generally considered that cells within tumors become hypoxic when they are more than 150 μm from the blood vessels (20). However, we observed recently that 4-day-old micrometastases in the lung responded better to ionizing radiation if micrometastases were exposed to hyperbaric oxygen during radiation. This implies that there is some hypoxia in micrometastases, which can explain the effect of MISO on the CY effect. It should be emphasized that it is difficult to understand why such tiny metastatic foci contain hypoxic cells, unless the mere presence of tumor cells changed the microenvironment, reducing the ventilation and perfusion of microanatomic sites in the lung occupied by tumor cells. It is also possible that the very process of initiation of micrometastases could produce local hypoxia if it occurred within the capillary strictures and associated with formation of tiny dots. Another possibility for the chemosensitizing effect of MISO on lung micrometastases is a change in the pharmacokinetics of CY in MISO-treated animals. Recently, Tannock (19) reported that MISO increased the half-life of active metabolites of CY in the blood of mice, a phenomenon which could easily account for the enhanced reduction of lung micrometastases in mice treated with both MISO and CY.

In conclusion, the effect of both WR-2721 and MISO on the response of FSAs and NFSA to CY treatment greatly depended on the tumor size at the time of CY treatment. WR-2721 marginally protected 8-mm leg tumors but appreciably protected lung micrometastases. On the other hand, the enhancement of CY damage by MISO was less pronounced for lung micrometastases than it was for 8-mm leg tumors. Although the reasons for this effect of tumor size are not fully understood, this observation might have a significant bearing on the tumor therapy strategies in which WR-2721 or MISO are combined with chemotherapeutic agents.

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