Protection by S-2-(3-Aminopropylamino)ethylphosphorothioic Acid against Radiation-induced Leg Contractures in Mice

Nancy Hunter and Luka Milas

Department of Experimental Radiotherapy, The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, Houston, Texas 77030

ABSTRACT

S-2-(3-Aminopropylamino)ethylphosphorothioic acid (WR-2721) was shown to provide marked protection against development of radiation-induced leg contractures in C3Hf/Kam mice whose legs were exposed to single doses of γ-radiation. The radiation doses ranged from 3300 to 6200 rads delivered to the right hind thighs from two parallelly opposed 137Cs sources. WR-2721 was given i.p. 30 min before irradiation. The severity of radiation-induced leg contractures in untreated and WR-2721-treated mice was followed for 342 days after irradiation. The degree of leg contractures in both control and WR-2721-treated mice increased up to 100 days after radiation, when the change stabilized, remaining more or less at the same level to the end of the observation period. During this entire period, the severity of contractures was less in WR-2721-treated mice. The dose-modifying factor for the level of 5 mm reduction in leg extension was 1.5 at 182 days after irradiation. Since WR-2721 did not prevent the radiocurability of 8-mm fibrosarcomas growing in the same legs, these data imply that WR-2721 has a high potential for increasing therapeutic gain when combined with irradiation in the treatment of tumors of an appreciable size.

INTRODUCTION

With the advent of megavoltage radiotherapy, acute response of the epithelial tissue to irradiation, especially that of the skin, has become rarely a dose-limiting factor in tumor radiotherapy. Instead, the late radiation damage commonly expressed as tissue fibrosis and ulceration that develops long after completion of therapy has emerged as a serious limitation to the effective treatment of cancer with ionizing radiation. Consequently, attention in radiobiology studies has recently turned more to tissues with relatively slowly progressing expression of radiation damage, such as lungs, kidneys, spinal cord, and s.c. tissue (1-3, 4, 8, 14, 15).

Thus, the need for avoiding or minimizing development of late radiation sequelae is obvious. It has been observed recently that the ratio β/α of the radiation survival curve parameters is larger for late than for acutely responding tissues, which implies that the use of low fractional doses of radiation should lead to less expression of the late damage (11). In fact, this observation is a major reason for a recent trend for using hyperfractionation in clinical radiotherapy (10). An alternative approach in minimizing late radiation damage is the application of agents capable of radioprotecting normal tissues but not tumors. Recently, there has been a considerable body of evidence that a thiol compound, WR-2721, protects normal tissues better than it protects tumors against the damage inflicted by either radiation (5, 7, 19) or alkylating cytotoxic agents (16, 18). However, most studies on radioprotection of normal tissues with WR-2721 have dealt with protection against early rather than late radiation damage (reviewed in Ref. 7, 19). In addition to being a potent radioprotector against early radiation damage, WR-2721 was found to effectively protect against late damage of kidney (8), lung (12), muscle (13), and parotid gland (9).

We recently reported that WR-2721 reduced the severity of radiation-induced leg contractures of mice, which was assessed up to 100 days after radiation exposure (6). During that observation period, the extent of developing contractures was still in progress both in mice exposed to radiation alone and in mice that received WR-2721 and radiation. We extended the period of observation on the protective effect of WR-2721 against radiation-induced contractures up to 342 days after irradiation and report here our findings.

MATERIALS AND METHODS

Mice used in this study were 12-week-old C3Hf/Kam males bred and maintained in our own specific-pathogen-free mouse colony. They were given injections of syngeneic fibrosarcoma cells into the hind right thighs, and when the tumors grew to 8 mm in diameter the tumor-bearing legs were locally irradiated with single doses of 3300 to 5200 rads γ-radiation (6). Individual doses of irradiation are listed in the legend of Chart 1. Radiation was performed with a small-animal irradiator with 2 parallelly opposed 137Cs sources at a dose rate of 917 rads/min, as measured by lithium fluoride dosimetry. The overall time required to deliver irradiation ranged between 3.6 and 6.76 min. The mice were immobilized without anesthesia in a jig. During radiation, the right hind thigh containing the tumor was centered in the circular radiation field 3 cm in diameter. The heel was 1 to 2 mm outside the 3 cm opening of the jig. Approximately one-half of the mice to be irradiated were given i.p. injections of WR-2721 in the dose of 400 mg/kg/mouse 30 min before irradiation. [WR-2721 (Batch AJ-68.2) was kindly supplied by Dr. David A. Pistonmaa, National Cancer Institute, Bethesda, Md.] Immediately before the injection into the mouse, the drug was dissolved in 0.9% sodium chloride solution and injected in a volume equal to 0.01 ml/g body weight. Results of the radiation-induced tumor control and the effect of WR-2721 on that control were reported previously (6). WR-2721 had no protective effect against the radiation damage of the tumor as evidenced by no change in the dose of radiation yielding local tumor control in 50% of the animals.

Mice that had no recurrent tumors present were used for determination of the radiation-induced leg contracture (reduction in the leg extension).

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2To whom requests for reprints should be addressed, at Department of Experimental Radiotherapy, The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, Houston, Texas 77030.

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Radioprotection with WR-2721

The dynamics of the development of the radiation-induced leg contracture in normal mice and in mice treated with WR-2721 are presented in Chart 2. The contracture was evident already at 36 days after irradiation with most radiation doses used. The degree of leg contracture in untreated and WR-2721-treated mice increased up to about 100 days after irradiation when the change stabilized, remaining more or less at the same level up to the end of the observation period, which was 342 days. In general, higher doses of radiation caused more profound changes in the leg extension. However, during the whole observation period, the contracture in mice treated with WR-2721 was less severe than that in irradiated-only mice. The same mice were used for determination of skin epilation at 36 days after irradiation. The reaction was more prominent in mice receiving higher doses of irradiation, and WR-2721 was quite preventive (DMF, 1.24) (6).

To determine the DMF, the data were analyzed by the linear regression analysis of the degree of contracture at 182 days after irradiation, at the time when the development of the leg contracture had already stabilized in both WR-2721-treated and untreated mice (Chart 1). The DMF for the damage resulting in a 5-mm reduction of leg extension was 1.5. Although WR-2721 reduced the severity of damage from all doses of radiation, the data suggest that the protection against lower doses of radiation might be greater.

DISCUSSION

The results presented here clearly indicate that WR-2721 is a potent protector against the development of radiation-induced leg contractures. Since our earlier report (6) showed that radio-curability of an 8-mm fibrosarcoma growing in the same legs was not influenced by WR-2721 treatment, it follows that WR-2721 was, under experimental conditions used in the present study, capable of achieving an appreciable therapeutic gain when combined with tumor radiotherapy. Our results suggest that WR-2721 was more effective against lower doses of irradiation within the range of doses used. The reason for this effect is not known, but it is likely that the injury of oxic cells, which dominates in the pathogenesis of tissue damage after lower doses of radiation, is more amenable to radioprotection than the injury of hypoxic cells (5). The protective effect of WR-2721 against several other late damages of ionizing radiation was studied by other investigators, and a brief account of these findings is as follows. WR-2721 protected against radiation-induced atrophy of rat skeletal muscles by a DMF of 1.5 to 2.0, as measured by changes in the cross-sectional diameters of muscle fibers (13). Phillips et al. (8) reported that WR-2721 protected murine kidneys from radiation damage by a DMF of 1.5, as measured by assay of the dose of radiation that caused death of 50% of mice in 365 days. They also reported protection of late lung damage by a DMF of 1.2, as measured by the dose of radiation that caused death of 50% of mice in 160 days. Using breathing rate and mouse lethality to assess lung damage from 6 to 12 months after irradiation, Travis et al. (12) observed that WR-2721 was protective by a DMF of 1.5 to 1.6. Furthermore, Sudolfoff et al. (9) showed that WR-2721 was effective by a DMF of 2.3 in minimizing the radiation-induced loss of weight of rat parotid glands that occurs from 60 to 90 days after irradiation. As already mentioned in the "Introduction," WR-2721 has been studied more often for its ability to protect acute rather than late tissue responses to radiation, and DMFs there ranged from 1.4 to 3.4, depending mainly on the tissue studied (7-9). Therefore, this compound appears to be quite effective in protection of both late and early radiation damage.

The underlying mechanisms in the development of leg con-
tracts are not well understood. A recent study on this aspect of radiation damage by Stone has indicated that the leg contraction was due to fibrosis and atrophy of the skin, s.c. tissues, muscles, and possibly joints. Shortening of the bone was not a contributing factor since it does not occur in mice when irradiated as adults. Stone investigated the correlation between skin contraction (shrinkage) and leg contractures and found that skin contraction was a significant factor in leg contractures, since the extent of the latter was reduced in skinned legs. A recent study from this laboratory (4) suggested that skin shrinkage in the early weeks after radiation reflects depletion of basal epithelial cells but that skin contraction evident at more than 100 days after radiation exposure resulted to a large extent from damage in the s.c. tissues. Pathogenesis of the development of late radiation damage, in general, including skin and leg contractures, is still a matter of controversy with proponents advocating the cellular depletion of slowly proliferating tissues as a major pathogenetic event (4, 10, 11) and those regarding vascular damage as a primary underlying cause (3). Which of the above damages that participated in the radiation-induced leg contractures were protected by WR-2721 is not known, but the findings of the following studies imply that it is likely that most of them were protected. WR-2721 is capable of protecting skin against acute responses (7, 13, 19) and late contraction (13). Also, Utley et al. (13) recently reported that radiation-induced vascular damage and radiation-induced skeletal muscle atrophy were greatly prevented by WR-2721.

The presence of the tumor within the irradiated area and a mere disuse of irradiated legs might have influenced the extent of the development of leg contractures, as well as the protective effect of WR-2721, but this has not been investigated yet. We reported earlier (6) that WR-2721 prevented radiation-induced hair loss from the skin overlying the 8-mm tumor by a DMF of 1.24, which is a lower protection value than most investigators observed for the hair loss (7, 19). We hypothesized that a tumor of this size might have compromised the blood circulation in tissues that surrounded the tumor, including skin, which resulted in deficient delivery of WR-2721 to the skin and, consequently, in a relatively low value of DMF (6). By analogy, one would expect higher protection against leg contractures of irradiated non-tumor-bearing legs, but this must be determined. Stone observed that muscle atrophy is one of the features in pathogenesis of radiation-induced leg contractures, but the extent of contribution to it of a mere disuse of muscles of irradiated legs is uncertain. It should also be noted here that repeated measurements (repeated stretching of damaged extremity) do not significantly influence the degree of the leg contraction. Since 8-mm leg fibrosarcoma was not radioprotected by WR-2721 (6) while normal tissues were protected highly (the present study), one might conclude that WR-2721 is a selective protector of normal tissues. Although the majority of tumors studied thus far were not protected by WR-2721 (reviewed in Ref. 19), one should be cautious when applying the term of selective radioprotector of normal tissues to WR-2721 because a variety of factors, tumor size in particular, determine whether tumors will be protected or not (5). It should be noted, however, that even in situations in which tumor radioprotection was achieved it was smaller than radioprotection of critical normal tissues, implying that WR-2721 protects normal tissues preferentially rather than selectively.

Therefore, our present observation and studies reported by others (8, 9, 12, 13) show clearly that WR-2721 is capable of a significant protection against radiation-induced fibrosis development, which is the underlying pathogenesis in the development of late radiation damage, and thus imply that this compound may be of potential clinical use in tumor radiotherapy. It should be stressed, however, that these reports on the late damage have almost exclusively dealt with large single doses of radiation. Therefore, studies with fractionated doses of radiation, especially with clinical-range doses, are necessary for better assessment of the therapeutically relevant radioprotective potential of this drug.

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