Protection and Potentiation of Nitrogen Mustard Cytotoxicity by WR-2721

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ABSTRACT

The radioprotective agent WR-2721 was examined for its effects on modifying the cytotoxicity of HN2 against normal and tumor cells in the AKR mouse. Quantitation was carried out by the spleen colony assay for both normal hematopoietic stem cells and AKR leukemia cells. Protection from drug toxicity and normal cell cytotoxicity was noted. Potentiation of cytotoxicity to AKR leukemia was found.

INTRODUCTION

WR-2721, a cysteamine analog, is a radioprotective agent which has demonstrated significant protection of a variety of normal tissues from the lethal effects of both radiation and alkylating agents (13). The finding that it has no protective effect against a variety of experimental tumors (10, 11, 13–15) has led to the present interest in its use in clinical radio- and chemotherapy (6), in the hope that higher doses of anticancer agents can be administered, thereby producing more tumor cell killing.

Nearly all studies have used solid experimental tumors; thus no hematological tumor has been examined for the protective effect of WR-2721 with an alkylating agent. We examined this possibility using an AKR leukemia treated with nitrogen mustard and WR-2721.

MATERIALS AND METHODS

Drugs. WR-2721 (Lot AJ-68.1) was obtained from Dr. V. Narayanan, Drug Synthesis and Chemistry Branch, National Cancer Institute Department of Health and Human Services. HN2 (as Mustargen from Merck Sharp & Dohme) was purchased from Narco Drug Co., St. Louis, Mo. Both agents were dissolved in 0.9% NaCl immediately before use.

Mice. Inbred AKR mice, 7 to 9 weeks old, were obtained from our breeding facility with foundation stock supplied by The Jackson Laboratory, Bar Harbor, Maine. Mice weighing 20–24 g were used. Females were used as cell donors and for toxicity studies. Males were used as cell recipients. Mice were housed 5/cage and were provided with free access to food and water.

AKR Leukemia. The tumor cell line was a transplantable, widely disseminating lymphocytic leukemia derived from the thymus of a female AKR mouse. Its method of passage and the preparation of monodispersed leukemia cells have been discussed (9). AKR mice received 5 × 10⁴ leukemia cells 4 days prior to treatment with the drugs.

Assay for LCFU.1 LCFU were enumerated by the spleen colony assay. Twenty-four hr following treatment, groups of 3 leukemic mice were killed, their femurs were removed, and a monodispersed cell suspension of their femoral marrow was prepared (9). Fractions of this suspension were injected in 0.5-ml volumes into the tail veins of 8 recipient AKR mice. They were killed 8 days later, their spleens were removed and placed in Bouin’s fixative, and the macroscopic colonies were counted (2). The number of LCFU in the original donor femur was determined and expressed as fractional survival of LCFU relative to an untreated control group. The average number of LCFU per femoral marrow in the control groups was 2 × 10⁵.

Assay for NCFU. NCFU were assayed in a manner similar to that for LCFU except that the donor mice were nonleukemic, recipient mice received 1000 R whole-body γ-radiation (³²⁰Cs) before i.v. injection of femoral marrow, and spleens were removed 9 days following cell inoculation. The average number of NCFU per femoral marrow in the control groups was 2 × 10⁵.

RESULTS

Since previous investigators had shown a protective effect of WR-2721 when the parameters of host survival or normal hematopoietic stem cell survival were assessed, we carried out similar studies in the AKR mouse. Mice received increasing doses of either nitrogen mustard alone, or nitrogen mustard administered 15 min after the mice had been given WR-2721 (15 mg/mouse). An LD₅₀/₃₀ value of 0.14 ± 0.03 (S.D.) mg/mouse was obtained for the former, and a value of 0.23 ± 0.07 mg/mouse resulted from the latter treatment. This yielded a dose modification factor of 2.0, similar to the value of 2.1 reported by Yuhas (12).

We next examined the response of NCFU. Chart 1 indicates an exponential decrease in survival with increasing dose for HN2 both alone and combined with WR-2721. A D₅₀ of 0.047 mg/mouse for nitrogen mustard alone was calculated, and a value of 0.13 mg/mouse was obtained for WR-2721 plus nitrogen mustard. This dose-modifying factor of 2.8 is comparable although smaller than the value of 4.6 reported by Wasserman et al. (10).

Having shown protective effects in the AKR mouse similar to those reported by others, we next looked for any similar interactions using a transplanted syngeneic, thymus-derived leukemia. Chart 2 shows that, contrary to the results obtained for NCFU, WR-2721 potentiates the cytotoxicity of HN2. The slope of the curve for HN2 alone yielded a D₅₀ of 0.050 mg/mouse, and, in combination with WR-2721, a D₅₀ of 0.025 mg/mouse, i.e., a dose-modifying factor of 2.0.

DISCUSSION

The first study on the ability of aminothiols to modify the toxicity of HN2 was by Pecksenik (5) in 1953. This followed from the work of Brandt and Griffin (1), who showed the ability of the sulfhydryl compound cysteine to reduce the toxicity of nitrogen mustard, which, in turn, developed from the earlier studies on cysteine protection of X-ray toxicity by Patt et al. (4). These early studies also demonstrated the time dependency of administration of the protector. Subsequent studies...
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Chart 1. Survival of spleen colony-forming unit (CFU-S) exposed in vivo either to HN2 alone (●, ▲, ◆) or to WR-2721 administered 15 min before the HN2 (□, △, ○). Different symbols represent different experiments. Bars, S. E.

Chart 2. Survival of LCFU exposed in vivo either to HN2 alone (●, ▲, ◆, ▼) or to WR-2721 administered 15 min before the HN2 (□, △, ○, ▼). Different symbols represent different experiments. Bars, S. E.

provided further evidence and more quantitation of the protective effect of the aminothiols against HN2 toxicity with no effect on tumor growth (3, 7, 8).

The data presented here not only provide confirmation of the phenomenon of protecting normal tissues from cytotoxic effects but also present the first demonstration that WR-2721 exerts a potentiating effect on the cytotoxicity of an anticancer agent and, remarkably, in the same host.

Further studies on the uptake of WR-2721 into AKR leukemia cells are important since the present rationale for the differentiated protective effect of WR-2721 on normal versus tumor cells is that normal cells actively concentrate WR-2721 intracellularly while tumor cells only slowly, passively take up the agent (12).

This study opens up a large area of research to determine whether this action is unique to this specific tumor or to hematological tumors in general, whether similar effects will be noted with other alkylating agents, and a definition of the mechanism for the effect. The therapeutic potential of such a class of agents is obvious.

REFERENCES
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