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Short communication

Unexpected radiation protection with 13-cis-retinoic acid plus interferon α-2a

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Abstract. A recent clinical protocol combining retinoic acid, interferon, and radiotherapy for advanced cervical cancer produced proctitis severe enough to necessitate dose reductions. In an attempt to model this biological response to multimodality therapy, we used a murine model to study the response of the colonic epithelium. Mice were treated with retinoic acid (100 μg/day) and interferon (3×10^4 units/day) for 5 days before undergoing whole-body irradiation. The functional response of the bowel was assessed by the ability to maintain body weight, and reproductive cell survival was quantified by the colon crypt assay. Both assays indicated a moderate protective effect of treatment with retinoic acid and interferon prior to irradiation. Protection factors in the range of 1.1–1.4 were observed.

Introduction

Recently, Lippman et al. reported on the use of 13-cis-retinoic acid (RA) combined with interferon-α (IFN) as a systemic therapy for previously untreated advanced squamous-cell carcinoma of the cervix [4, 6] and advanced inoperable squamous-cell carcinoma of the skin [5]. In each trial, 50% or more of the treated patients had major clinical responses. These reports are noteworthy because the positive response to therapy occurred in epithelial cancers, the most common histopathologic malignancies in humans [2], with about 3,000 deaths per year being attributed to squamous-cell carcinoma of the skin [5] and 4,500, to cervical cancer [4, 6] in the United States.

This combination therapy was relatively well tolerated, although dose reductions were necessitated by severe fatigue in patients treated for squamous-cell carcinoma of the skin [5]. However, when RA and IFN were combined with radiation for treatment of cervical carcinoma, severe proctitis occurred (J.J. Kavanagh, personal communication). In an attempt to explain the clinically observed proctitis, experiments were designed to assess whether systemic treatment with RA plus IFN prior to radiotherapy alters the in vivo dose-response characteristics of normal colonic epithelial cells in mice.

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Materials and methods

Mice. Female C3Hf/Kam mice were used at an age of 16 weeks. Radiation dose groups contained six mice each. The experiments were repeated three times and data were combined for presentation in figures. The responses of 220 mice were evaluated.

Drug treatment. Drug treatment schemes were designed to simulate those used clinically [4–6]. RA was dissolved in dimethylsulfoxide (DMSO) and 0.1 ml was injected i.p. at a dose of 100 μg/mouse daily for 5 days. IFN was prepared in saline and 0.1 ml was injected s.c. at a dose of 30,000 units/mouse daily for 5 days. Mice were irradiated 4 h after the fifth daily dose of RA-IFN.

Radiation. Mice were whole-body-irradiated in groups of six using 250 kvp X-rays at a dose rate of 1.56 Gy/min. A single dose in the range of 9.5–19.5 Gy was given 4 h after the last drug injection.

Assay. Mice were observed daily for signs of drug-related toxicity. Mice irradiated with doses of 9.5–12.5 Gy were weighed daily for 10 days following treatment. Radiation dose-response curves for clonogenic cells of the colonic epithelium were obtained by the microcolony assay of Withers and Mason [9]. Briefly, mice were killed 5 days, 10 h after irradiation and a 2-cm length of colorectal tissue was excised. Formalin-fixed tissue was histologically prepared and four transverse sections per mouse were cut at 4-μm thickness and stained with hematoxylin and eosin. The surviving crypts per colon cross section were counted microscopically at 100x magnification. After application of a Poisson correction to the raw crypt counts to account for the multiplicity of surviving clonogenic cells per crypt [3, 9], data were plotted on semilogarithmic coordinates as surviving cells per circumference of colon as a function of radiation dose.

Statistical analysis. Error bars were drawn to represent the standard error of the mean unless the error was smaller than the symbol. Differences between groups were tested for significance using Student's-

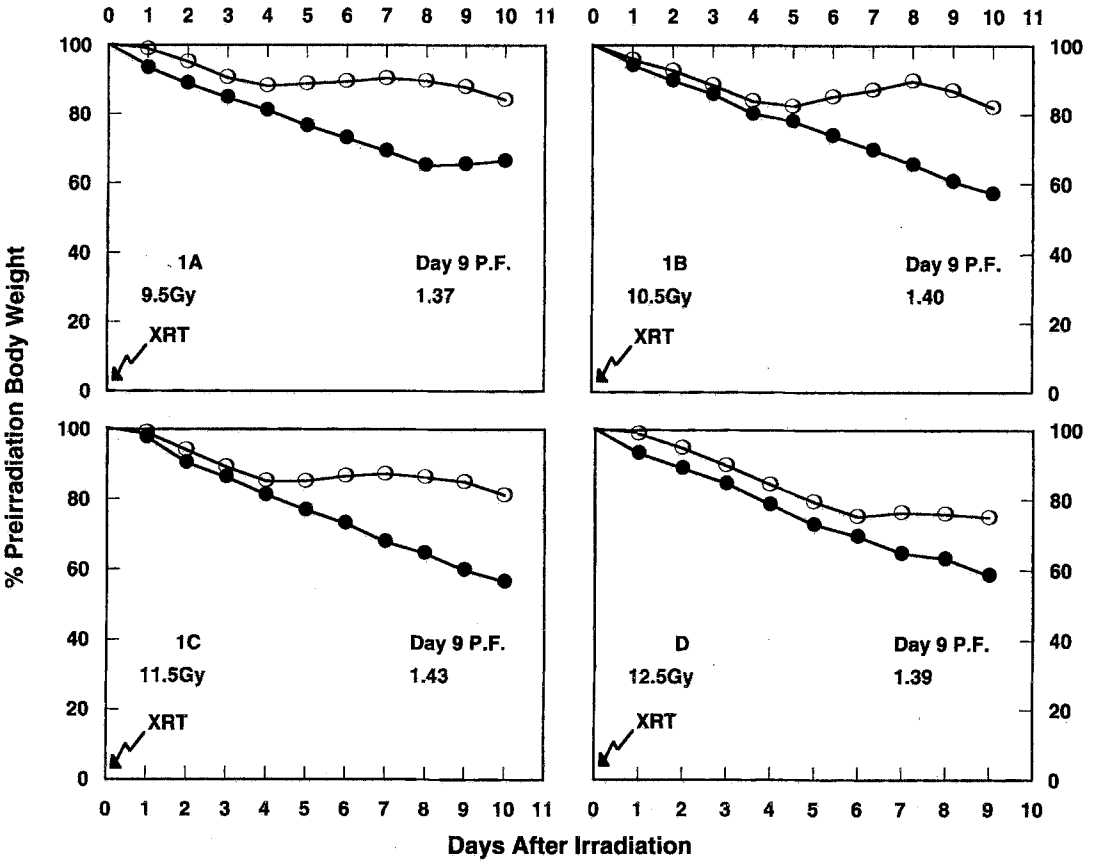

Fig. 1 A–D. Weight loss expressed as a percentage of the preirradiation body weight as a function of time in days after irradiation with doses of 9.5 (**A**), 10.5 (**B**), 11.5 (**C**) and 12.5 Gy (**D**). *Filled circles,* Control mice (6/dose group); *Open circles,* mice treated with RA-IFN for 5 days before irradiation (6/dose group). Protection factors (wt. loss of RA-IFN treated mice/wt. loss of control mice) are shown on each panel. From day 7 onward, differences in weight between control and RA-IFN-treated mice are highly significant for all radiation doses tested (P <0.001)

test. Survival curves were fit to the data using a weighted least-squares regression analysis.

Results

Mouse weight loss

Figure 1 shows the weight loss observed in mice following radiation treatment with doses of 9.5, 10.5, 11.5 and 12.5 Gy. Mice lost weight in a linear manner from 1 to 5 days after radiation exposure. In no case did mice treated with RA-IFN prior to irradiation lose more weight than their normal irradiated counterparts. Normal mice continued their weight loss for the entire 10-day period of observation, losing a maximum of 40% of their body weight in 10 days. Mice were killed at 10 days because death from hematologic insufficiency begins to occur at this time in whole-body-irradiated mice.

Mice pretreated with RA-IFN showed weight loss similar to that observed in controls for the first 5 days and then maintained their weight at about 80%–90% of their preirradiation level. By 7 to 9 days after irradiation, RA-IFN-treated mice lost significantly less weight than did controls (P<0.001 for all doses). Protection factors (P.F.) shown in Fig. 1 ranged from 1.37 to 1.43 at 9 days after irradiation. Mice pretreated with RA-IFN lost about half as much weight in 10 days as did normal mice irradiated with the same dose. Thus preirradiation treatment with RA-IFN improved the functional status of the gastrointestinal tract as evidenced by an enhanced ability to maintain body weight.

Clonogenic cell survival

The reproductive survival of cryptogenic cells of the colonic epithelium is shown in Fig. 2. The number of crypts per colon cross section at the time of irradiation was 156 ± 2 in control mice and 162 ± 4 in those treated with RA-IFN. Following irradiation, the number of surviving cells was higher for a given radiation dose in the mice pretreated with RA-IFN. An isoeffect of 20 surviving cells was achieved with a dose of 14.96 Gy in control mice and a dose of 16.95 Gy in those treated with RA-IFN. Therefore, RA-IFN protected the reproductive survival of colonic epithelial stem cells by a factor of about 1.13. In addition, RA-IFN decreased the terminal slope of the cell-survival curve from a D_0 value of 169 to that of 213, a protection factor of 1.26 (Fig. 2).

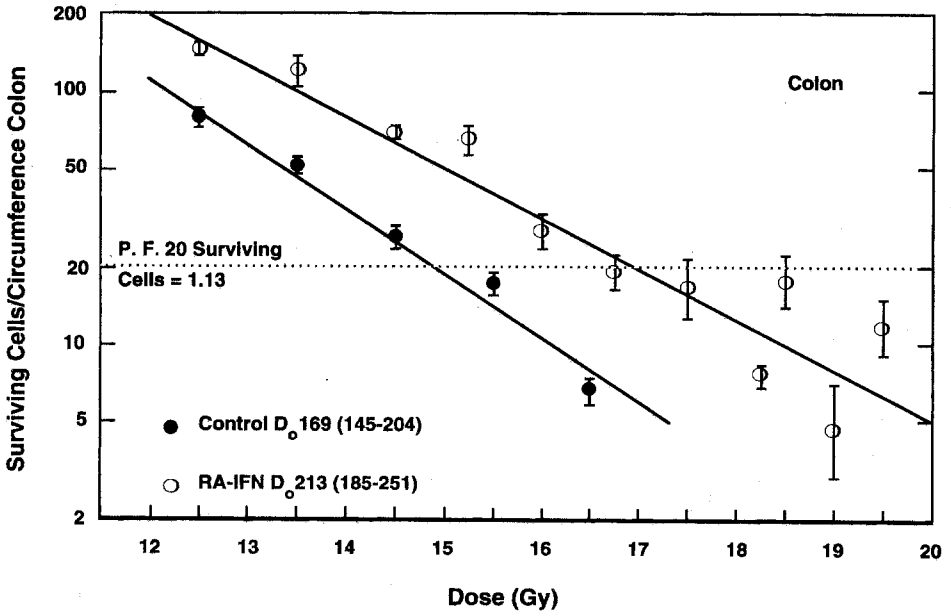

Fig. 2. Reproductive cell survival of colon cryptogenic cells at 5 days, 10 h after irradiation. RA-IFN treatment for 5 days prior to irradiation protected the bowel by a factor of 1.13 at an isoeffect of 20 surviving cells/circumference and resulted in a more shallow slope of the dose-response curve (D_0 *169* versus D_0 *213*)

Discussion

A variety of functions related to cellular differentiation and inhibition of proliferation of malignant cells and of hematopoiesis have been identified for RA and IFN [2]. To our knowledge, no study has investigated the effects of multimodality therapy with RA-IFN and radiation on the digestive tract, although single-agent studies of human tumor cell lines in vitro have shown radiosensitization [1, 8]. For cervical cancer patients treated with RA-IFN, the response of the normal bowel to this drug combination may be particularly important if multimodality therapy is to include radiotherapy. Most clinical complications of treatment to the pelvis are due to injury to the intestinal mucosa [7]. The present data suggest a beneficial effect of RA-IFN on both the functional (weight loss) and reproductive integrity (crypt cell survival) of the irradiated bowel, the mechanism of which is currently unknown. However, they do not verify or explain the clinically observed proctitis in cervical patients treated with this regimen. The difference in response of these mice, which were moderately protected by RA-IFN prior to radiation, and patients treated with combined radiotherapy and RA-IFN could be related to species differences, the mode of drug delivery (oral versus i.p. administration of RA), the duration of drug treatment (≥2 months vs 5 days), the timing of drug administration relative to radiotherapy, or preexisting disease and inadequate nutrition in the patient population under study [4, 6].

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