Interleukin 15 Protects against Toxicity and Potentiates Antitumor Activity of 5-Fluorouracil Alone and in Combination with Leucovorin in Rats Bearing Colorectal Cancer

Shousong Cao, Anthony B. Troutt, and Youcef M. Rustum

Grace Cancer Drug Center, Roswell Park Cancer Institute, Buffalo, New York 14263 [S.C., Y. M. R.], and Immunex Corporation, Seattle, Washington 98101 [A. B. T.]

ABSTRACT

5-Fluorouracil (FUra) modulated by leucovorin (LV) is active in the treatment of colorectal cancer. Diarrhea and stomatitis are the most common dose-limiting toxicities. We have developed a model system in rats bearing a transplantable colon carcinoma sensitive to FUra therapy with dose-limiting toxicity profiles similar to what is observed in patients treated with either daily or weekly schedules of FUra plus LV. Interleukin 15 (IL-15), a cytokine that shares many biological activities with IL-2, was used at different doses (25, 100, and 400 µg/kg) and schedules (three doses before a single dose of FUra/LV weekly × 4, or three doses before a single dose of FUra or FUra/LV daily × 5, or before each week of FUra/LV weekly × 4) to evaluate its role in the modulation of the therapeutic selectivity of FUra alone and modulated by LV. IL-15 induced a dramatic decrease in chemotherapy-induced gastrointestinal toxicities, significant potentiation of antitumor activity, and an increased therapeutic index of FUra administered on single dose, daily × 5 and weekly × 4 schedules. In contrast, IL-2 (400 µg/kg) significantly potentiated the toxicity of FUra administered as a single LV push, with minimal potentiation of the antitumor activity. Taken together, the results clearly demonstrated the ability of IL-15, but not IL-2, to provide significant improvement of the therapeutic index of FUra alone and in combination with LV. The clinical relevance of the results obtained in this model system needs to be confirmed.

INTRODUCTION

Although recent advances in the treatment of patients with colorectal cancer with FUra/LV have resulted in a significant increase in overall response rate in advanced disease and an increased duration of disease-free survival in the adjuvant setting, severe toxicities represent a major clinical problem (1-5). The profile of FUra/LV-induced toxicity is related to the schedule of FUra/LV treatment (6). With the daily × 5 schedule, the dose-limiting toxicities are stomatitis, diarrhea, and leukopenia; diarrhea is the dose-limiting toxicity with the weekly × 6 schedule (2, 3).

Growth factors such as granulocyte-colony stimulating factor and GMCSF have been used effectively to reduce the incidence of chemotherapy-induced myelosuppression (7, 8), and GMCSF has shown modest effects in the setting of chemotherapy-induced mucosis (9). Although sandostatin has been shown to protect colorectal cancer patients from FUra/LV-induced diarrhea (10), no effective means is available to date for reversal/protection from chemotherapy-induced stomatitis.

IL-15 is a cytokine that shares many of the biological activities of IL-2, including induction of proliferation of phytohemagglutinin-stimulated normal peripheral blood mononuclear cells, NK cells, and B cells and generation of CTL and LAK cells in vitro (11-13). IL-15, like IL-2, also induces production of IFN-γ, tumor necrosis factor α, and GMCSF by activated NK cells from normal individuals and acts synergistically with IL-12 to enhance NK activity (12). IL-15 and IL-2 have been predicted to have similar tertiary structures and share a requirement for components of the IL-2R complex for successful signal transduction (14). IL-15, however, shares no significant sequence homology with IL-2 (15). Unlike IL-2, which is expressed almost exclusively by activated T cells, IL-15 mRNA is expressed in a wide variety of tissues, including placenta, skeletal muscle, kidney, skin, activated macrophages/monocytes, and epithelial and fibroblast cell lines, but not activated T cells (11, 16). There is evidence that suggests IL-15 may have additional biological activities and mechanisms of action not shared by IL-2 (17).

The IEC-18 rat intestinal crypt cell line expresses high-affinity receptors for IL-15. Furthermore, a recent study has shown that IL-15 promotes modest proliferation of the Caco-2 human intestinal epithelial cell line, suggesting that administration of IL-15 might alter the response of the gastrointestinal tract to chemotherapy-induced damage (18). The present report has addressed this possibility.

The clinical toxicity and antitumor activity of FUra/LV has been characterized in rats bearing advanced colorectal cancer (6). Using this in vivo model system, the three clinically used schedules of FUra or FUra/LV, i.e., daily, weekly, and single high-dose i.v. push schedules, were used to evaluate the potential for IL-15 to protect from drug-induced, organ-specific toxicity without reversing the antitumor activity of FUra alone or when modulated by LV.

MATERIALS AND METHODS

Animals and Tumor. Eight- to 12-week-old female Fisher 344/HSD rats (body weight, 150–180 g) were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN) and kept four rats/cage with water and food ad libitum according to an institutionally approved animal protocol. The chemically induced Ward colorectal carcinoma was used in this study (19, 20). Nonneoplastic tumor pieces (0.1 g) were transplanted s.c. via trocar under slight ether anesthesia.

Materials. FUra was purchased from Hoffmann-La Roche Inc. (Nutley, NJ). Stock solution of 50 mg/ml was diluted in sterile 0.9% NaCl solution. LV was obtained from Lederle Laboratories, American Cyanamid Co. (Pearl River, NY). Purified human recombinant IL-15 was provided by Immunex Corp. (Seattle, WA). IL-2 (Proleukin) was obtained from Chiron Co. (Emeryville, CA). IL-15 and IL-2 were diluted to 200 µg/ml in PBS containing 1 mg/ml BSA (Sigma Chemical Co., St. Louis, MO). The stocks were stored at 4°C and used within 2 weeks.

Drug Doses and Schedules. For the schedule of daily × 5 of FUra/LV,FUra was administered by i.v. push daily for 5 days at 25 (MTD) or 35 mg/kg/day; LV was administered at 200 mg/kg/day i.v. push 30 min before each FUra injection. IL-15 (25, 100, or 400 µg/kg/dose) was administered i.p. according to two schedules: (a) at −24, −12, and −2 h relative to the first dose of FUra/LV for a total of three doses; and (b) three doses before initiation of

1 Supported in part by Grant DHP147 from the American Cancer Society and Immunex Corporation.

2 To whom requests for reprints should be addressed, at Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263. Phone: (716) 845-3394; Fax: (716) 845-8857; E-mail: scao@sc3101.med.buffalo.edu.

3 The abbreviations used are: FUra, 5-fluorouracil; LV, leucovorin; IL, interleukin; IL-2R, IL-2 receptor; GMCSF, granulocyte/macrophage colony-stimulating factor; LAK, lymphokine-activated killer; NK, natural killer; MTD, maximum tolerated dose; CR, complete tumor regression; PR, partial tumor regression.

Received 11/7/97; accepted 2/18/98.

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A. Troutt, unpublished data.

[1695]
IL-15 AUGMENTS THE THERAPEUTIC SELECTIVITY OF FUra/LV

FUr/LV treatment and twice daily at 12 and 2 h prior to each subsequent dose of FUra/LV for a total of 11 doses.

For the schedule of weekly × 4 of FUra/LV, FUra was administered by i.v. push once weekly for 4 weeks at 75 (MTD), 100, or 125 mg/kg/week. LV was administered at 200 mg/kg/week by i.v. push 30 min before each FUra administration. IL-15 (400 μg/kg/dose) was injected i.p. at 24, 12, and 2 h before each weekly FUra/LV treatment, for a total of 12 doses.

For the schedule of single i.v. push of FUra, FUra was administered by i.v. push as a single dose of 300 (MTD), 400, or 500 mg/kg. IL-15 (400 μg/kg/dose) was administered i.p. according to three schedules: (a) three doses starting 24 h before FUra (−24, −12, and −2 h) for a total of three doses; (b) twice daily for 4 days immediately after FUra for a total of eight doses; and (c) three doses starting 24 h before FUra (−24, −12, and −2 h) and twice daily for 4 days after FUra, for a total of 11 doses. For comparison, IL-2 (400 μg/kg/dose) was administered i.p. according to two schedules: (a) at 24, 12, and 2 h before FUra for a total of three doses; and (b) twice daily for 4 days immediately after FUra treatment for a total of eight doses.

**MTD and Toxicity Evaluation.** The MTD was defined as the maximum dose that caused no drug-related lethality and which produced animal body weight loss of <20% in normal or tumor-bearing rats. The kinetics of drug-induced toxicities, body weight loss, diarrhea, stomatitis (mouth ulceration), and lethality were determined daily for a minimum of 3 weeks and observed at least twice a week for a period of 3 months after drug treatment.

**Antitumor Activity.** Drug treatments were initiated 12–14 days after s.c. tumor transplantation, when tumor weight was approximately 3.0 g as described previously (6, 19). Each group had four to eight rats per experiment, and each experiment was repeated at least three times. Tumor response was expressed as PR when tumor weight was temporarily reduced by at least 50% and CR when tumor was undetectable by palpation for 90 days after therapy, at which time the rat was sacrificed.

**Statistical Analysis.** The differences between the mean values were analyzed for significance using the unpaired two-tailed Student’s t test for independent samples; P ≤ 0.05 was considered to be statistically significant.

**RESULTS**

**MTD**

The MTD of FUra or FUra/LV administered as a single high-dose i.v. push and on daily × 5 and weekly × 4 schedules was determined in rats bearing Ward colon carcinoma; results are summarized in Table 1. The MTD was schedule dependent: 300 mg/kg/dose, 25 mg/kg/day, and 75 mg/kg/week for the single high-dose i.v. push, daily, and weekly schedules, respectively. The dose of FUra that produced a similar level of lethality was also schedule dependent (Table 1).

**Toxicity Protection**

At the MTD of FUra/LV, the dose-limiting toxicity with the daily × 5 schedule was stomatitis in 38% of treated animals. The data in Table 2 indicate that 35 mg/kg of FUra modulated by LV produced 58% lethality, with 13% of animals developing diarrhea and 60% developing stomatitis. The data also show that administration of IL-15 protected tumor-bearing rats from chemotherapy-induced toxicities; optimal protection from drug-induced toxicities is a function of the dose and schedule of IL-15. With 3-dose and 11-dose schedules, the optimal doses of IL-15 were 400 and 100 μg/kg/dose, respectively. Administration of IL-15 alone produced no observable toxicity.

The data in Table 3 indicate that the dose-limiting toxicity with the weekly schedule of FUra/LV was diarrhea, which occurred in 25% of treated animals at the MTD. At 100 mg/kg/week of FUra, the toxicity observed in animals given chemotherapy alone (33% incidence of diarrhea and 25% incidence of death) were prevented by IL-15 administration. At 125 mg/kg/week of FUra, IL-15 also provided significant protection from drug-induced toxicities.

The data in Table 4 summarize the toxicities and antitumor activity...
of FUra administered as a single high-dose i.v. push alone or with 3, 8, or 11 doses of IL-15 (400 μg/kg/dose). IL-15 offered complete protection from FUra-induced mortality at an FUra dose of 400 mg/kg. Although IL-15 did not offer complete protection from FUra-induced mortality with 500 mg/kg of FUra, the incidences of death, diarrhea, and stomatitis were significantly reduced in all IL-15-treated groups. Furthermore, at each FUra dose, administration of IL-15 was associated with an increase in CR rate.

**Kinetics of Toxicity and Antitumor Activity**

**Daily × 5 of FUra/LV.** The kinetics of treatment-induced toxicity and antitumor activity of FUra/LV ± IL-15 were evaluated, and the results are shown in Fig. 1A. The effects of IL-15 were a function of dose, with highest effect (increase in antitumor activity paralleled decrease in toxicity) observed at 400 μg/kg/dose of IL-15 (three doses).

**Weekly × 4 of FUra/LV.** The data in Fig. 1B demonstrate that the observed decrease in gastrointestinal toxicity and lethality in animals treated with FUra/LV and IL-15 was accompanied by an increase in antitumor activity.

**Single i.v. Push of FUra.** The data in Fig. 1C indicate that with a single high-dose i.v. push of FUra, IL-15 can also provide significant protection from toxicity and in parallel increase antitumor activity of chemotherapy.

**Overall Complete Tumor Regression.** The data in Fig. 2 are a summary of overall CR achieved with FUra ± LV (alone and in combination with IL-15) administered according to the daily × 5, weekly × 4, and single high-dose i.v. push schedules. With all three FUra ± LV treatment schedules, IL-15 significantly potentiated the CR rate in this model system. With the daily × 5 schedule, the CR rate was directly related to the dose of IL-15, with >80% CR at the 400 μg/kg/dose.

**Comparative Effect of IL-15 and IL-2 on Modulation of the Toxicity and Antitumor Activity.** The data in Fig. 3A indicate that, unlike IL-15, IL-2 either provided no protection from FUra-induced toxicities (three doses of pretreatment) or significantly potentiated (eight doses of posttreatment) the toxicities of FUra administered as a single i.v. push of 500 mg/kg. Although IL-2 alone (three or eight doses) had no significant toxicity, increased incidences of diarrhea, stomatitis, and lethality were observed with FUra modulated by eight doses of IL-2. The data in Fig. 3B indicate that both IL-15 and IL-2 (three doses before FUra) produced significant potentiation of the antitumor activity of FUra. With eight doses of IL-2 after FUra, 100% of animals died of chemotherapy-induced toxicity within 8 days after treatment; therefore, antitumor activity could not be observed further.

**DISCUSSION**

Studies were performed to evaluate the ability of IL-15 to modulate the toxicity and antitumor activity of FUra ± LV in vivo using three clinically relevant chemotherapy schedules: daily × 5, weekly × 4, and single high-dose i.v. push administration. The profile of toxicities of FUra/LV in rats bearing colon carcinoma was similar to those documented clinically, i.e., stomatitis, diarrhea, and leukopenia with the daily × 5 schedule and primarily diarrhea with the weekly schedule (2, 3, 6). The results presented here clearly demonstrate that IL-15 is effective in protection of animals from FUra/LV-induced toxicity, paralleled with a significant increase in the proportion of complete tumor regression induced by chemotherapy. Complete protection from FUra/LV-induced lethality was IL-15 dose and schedule...
IL-15 AUGMENTS THE THERAPEUTIC SELECTIVITY OF FUra/LV

In both the MCA-205 and P815 tumor models, although optimal doses of IL-15 or IL-2 induced equivalent antitumor activity, IL-15 had a 3-fold higher therapeutic index (24, 25). Although we do not know the mechanism by which IL-15 improves FUra therapy, it may play an important role in promoting antitumor immunity, including the induction of proliferation of NK and B cells, the generation of CTL and LAK cell activity, activation of NK cells, stimulation of NK cytotoxicity, prevention of apoptosis in NK cells, and induction of production of IFN-γ and tumor necrosis factor α (11—13). IL-15 can drive peripheral blood mononuclear cells to acquire LAK activity and generate an antitumor LAK activity in cancer patient lymphocytes to kill autologous melanoma tumor cells (26). FUra has immunosuppressive effects at both cellular and humoral levels of the immune response (27). Evidence suggests that cytokines could restore or prevent the loss of immunocompetence of the host, and perhaps enhance antitumor responses, in a variety of tumor systems (28, 29). Although IL-15 alone had no significant antitumor activity in rats bearing an advanced colorectal carcinoma, the enhanced antitumor activity of FUra by IL-15 could be the result of the combined cytotoxic effect of FUra on tumor cells together with IL-15.

Fig. 2. CR produced by FUra/LV in daily × 5 (A), weekly × 4 (B) schedules, and by FUra as a single high-dose i.v. push (C) ± IL-15. Each treatment group had 16—48 rats in total, from three to nine experiments. Values shown are the means; bars, SD. The results also indicate that IL-2 does not provide protection from, and may actually exacerbate, FUra-induced toxicities with the dose and schedules of IL-2 tested.

IL-15 and IL-2 stimulate the proliferation of activated CD4+ and CD8+ subsets of T cells and NK cells and facilitate release of IFN-γ and tumor necrosis factor α (11, 12). IL-15 and IL-2 both require the β and γ chain of the IL-2R for signal transduction (14); this may be the basis for their partially overlapping biological activities (11—13). IL-15, however, does not have significant amino acid sequence homology with IL-2 (15). IL-15 mRNA has been detected in a variety of tissues, including activated monocytes/macrophages, placenta, skeletal muscle, kidney, heart, lung, liver, spleen, fibroblasts, epithelial cells, and the dermal layer of skin (11, 16, 21). In contrast, IL-2 is expressed and produced almost exclusively by T cells. IL-15 is an efficient activator of NK cells, and its ability to induce NK cytotoxic activity is not mediated by IL-2 (11, 12, 22). IL-15, but not IL-2, induced expansion of TCRγδ cells (23). In addition, only IL-2 is able to bind to IL-2Ra (12), whereas IL-15 binds to IL-15Ra (17).

Munger et al. (24) reported that high-dose IL-15 or IL-2 treatment resulted in a dramatic decrease in the number of tumor foci in the lung in mice bearing MCA-205. IL-15 therapy also increased the survival of mice bearing P815 tumor (25).
with stimulation of an antigen immune response by IL-15. Histopathological examination of tumor and normal tissues from our laboratory support this hypothesis. The results indicate that there was no specific morphological change in tumor when FUra was administered alone. However, a disintegrated small tumor with dense lymphocyte infiltration was observed in animals treated with IL-15 combined with FUra. Another contributing factor to the increase in CR rate found in rats treated with IL-15 combined with chemotherapy may have been the decrease in FUra-induced lethality in these animals; rats that had been cured of their tumor, but which would have died due to chemotherapy-induced toxicities in the absence of IL-15 therapy, may have survived.

In contrast to IL-2, which provided no protection against FUra-induced toxicities, IL-15 significantly protected tumor-bearing rats against FUra-induced toxicities including lethality, diarrhea, stomatitis, and weight loss. IL-15 also increased the MTD of FUra in three clinically relevant schedules: from 300 to 400 mg/kg; from 25 to 35 mg/kg/day; and from 75 to 100 mg/kg/week, for single high-dose i.v. injection, daily × 5 and weekly × 4 schedules, respectively. These increases in MTDs were associated with an increase in CR rate and an improved therapeutic index of FUra.

IL-2 alone or in combination with LAK has been used clinically for treatment of a variety of cancers, including renal carcinoma, melanoma, and colon and lung carcinoma (30). This treatment, however, provided only limited benefit for the patients with few long-term complete responses and severe side effects (30, 31). IL-2 in combination with FUra did not improve antitumor activity but increased toxicity in patients with advanced colorectal cancer (32, 33). IL-15 exhibited a higher therapeutic index than IL-2 in inhibiting pulmonary metastasis and producing prolongation of the survival of mice bearing MCA-205 and P815 tumors (24, 25) and induced LAK activity in lymphocytes from metastatic melanoma patients to kill autologous tumor cells obtained from fresh tumor biopsies (26). Taken together with our new finding that IL-15, but not IL-2, increased the therapeutic index of FUra, these data suggest that the use of IL-15 may be superior to IL-2 in cancer therapy.

In summary, although the mechanisms of interaction of IL-15 and IL-2 with FUra or FUra/LV treatment need to be investigated further, the finding of effective protection by IL-15, but not by IL-2, from FUra-induced toxicities, along with a parallel increase in antitumor activity and thus a notable increase in the therapeutic index of FUra in rats bearing advanced colorectal tumor, is of significant therapeutic interest. The generality of the observed effects in this model system needs to be confirmed in other tumor models and with other drugs, whereas the potential for clinical verification of this concept is under evaluation.

REFERENCES

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