5-Iododeoxyuridine Increases the Efficacy of the Radioimmunotherapy of Human Tumors Growing in Nude Mice

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Recently, there has been much interest in the use of radionuclide conjugated monoclonal antibodies for the treatment of human malignancies. One way to potentially maximize the therapeutic effectiveness of radioimmunotherapy would be to sensitize tumor cells to the radiation dose delivered by the antibody. Since radioimmunotherapy can potentially treat disseminated disease, including micrometastasis, we chose to study a halogenated pyrimidine radiosensitizer, a class of compounds that affect nonhypoxic cells. 5-Iododeoxyuridine, administered with pyrimidine metabolism modulators, increased the therapeutic effectiveness of radioimmunotherapy, resulting in individual cures of human tumors growing in BALB/c nu/nu (nude) mice. 5-Iododeoxyuridine was administered with N-(phosphonacetyl)-L-aspartic acid and 5-fluorodeoxycytidine plus tetrahydrouridine. This drug treatment was combined with radioimmunotherapy using ^{131}I conjugated to a monoclonal antibody, Mc5. Mc5 binds to a mucin component of the human milk fat globule. This antigen is expressed on the surface of MX-1 cells, the transplantable human tumor used in this study. Tumor-bearing mice treated with both the drug protocol and ^{131}I-Mc5 (540 μCi, 10 μCi/μg) showed a regression in average tumor volume. The average tumor volume was reduced below the initial size at treatment for 50 days; two of five cures were obtained. Neither cures nor regressions were observed with either the drug or antibody treatments alone. Our results indicate the potential for increasing the therapeutic effectiveness of radioimmunotherapy of human solid tumors with halogenated pyrimidines.

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Various problems continue to limit the clinical usefulness of radioimmunotherapy (RIT). These problems include the limited access of the antibodies to the tumor, low antigenic expression of tumor cells, the lack of truly tumor specific antigens, immune complex formation due to the presence of circulating antigen and conjugate breakdown with the release of free radionuclide. All of these problems result in only a small percentage of the administered radioactivity accumulating at the tumor site (*1*). In addition, the formation of anti-mouse antibodies (HAMA) in human patients limits repeated treatment (*2*). Nevertheless, various murine monoclonal antibodies (Mabs) have been used clinically for RIT of human tumors with moderate success (*3–9*).

One way to increase the therapeutic effectiveness of RIT would be to make the tumor cells more sensitive to the radiation dose delivered by the antibody. In a previous study, 5-bromodeoxyuridine, a halogenated pyrimidine analog, was shown to enhance the cell kill obtained in vitro by an ^{131}I conjugated polyclonal antibody (*10*). In this study, we utilized 5-iododeoxyuridine (IdU), another less toxic halogenated pyrimidine analog, in an attempt to radiosensitize tumors during RIT. This compound incorporates into DNA in place of thymidine, where it acts as a "target" for radiation damage (*11*). Previous studies have shown this compound to be an effective radiosensitizer of murine tumors to external beam X-irradiation (*12,13*). Long-term infusions of this drug have resulted in modest clinical radiosensitization (*14,15*).

The major obstacles associated with the use of IdU as a tumor radiosensitizer are: the rapid breakdown of IdU by serum and liver phosphorylases, necessitating the use of long-term infusions of the drug, and its toxicity to rapidly proliferating normal tissues, primarily to bone marrow and gut epithelium. One way to increase the effectiveness of IdU would be to selectively lower the cellular levels of thymidylate (TMP), which competes with 5-iododeoxyuridylate (IdUMP) for incorporation into DNA, in tumor cells. In a previous study, it was shown that pretreatments with N-(phosphonacetyl)-L-aspartic acid (PALA), and 5-fluorodeoxycytidine (FdC) coadministered with tetrahydrouridine (H_4U), preferentially increased the level of incorporation of a halogenated pyrimidine analog into tumor DNA in vivo (*16*). These pretreatments were also shown to dramatically increase the tumor radiosensitization obtained with bolus doses of a halogenated pyrimidine analog in vivo (*16,17* and Santos O, Gottlieb C, Schwade J, Greer S, *written communication*). PALA is an inhibitor of aspartate transcarbamoylase, blocking de novo pyrimidine synthesis at an early step (*18*). FdC, when coadministered with H_4U, has been shown to be metabolized

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preferentially in tumor cells to 5-fluorodeoxyuridylate (FdUMP) (*19*). FdUMP is an inhibitor of thymidylate synthetase, blocking the conversion of deoxyuridylate (dUMP) to thymidylate (TMP).

In this study, we combined the use of IdU as a tumor radiosensitizer with RIT. We utilized the modulators of pyrimidine metabolism discussed above to achieve substantial radiosensitization with relatively low bolus doses of IdU.

MATERIALS AND METHODS

Immunodeficient BALB/c nu/nu (nude) mice bearing a transplantable human mammary tumor (MX-1) were treated with ^{131}I conjugated to Mc5, a murine Mab directed against a high molecular weight (400 kDa) mucin, a glycoprotein component of the human milk fat globule (HMFG). This monoclonal antibody and its radioiodination procedure have been described previously (*20, 21*). The purified antibody was prepared by Coulter Immunology, Hialeah, FL. Radioimmunotherapy was combined with a radiosensitizing drug treatment. For each experiment, 40 mice were surgically implanted in the left side (slightly above the hip) with an approximately 3 mm^3 tumor fragment. After about 16 days, the animals' tumors were measured in three dimensions using calipers. Tumor volumes were calculated as one-half the product of the three measurements. Animals were selected and grouped in a manner that produced experimental groups with similar initial average tumor volumes and standard deviations. The experiments consisted of four groups of five animals: Group 1 the control; Group 2 received only the drug treatment; Group 3 received only RIT; and Group 4 was given the combination drug treatment and RIT.

All groups received the same total number of injections. Sham injections of sterile phosphate-buffered saline replaced the drug and/or the radioimmunoconjugate injections in the control, drug treatment alone or RIT alone groups.

The drug treatment protocol was as follows:

Day −1	PALA (100 mg/kg)
Day 0	FdC (12 mg/kg) + H_4U (25 mg/kg)
	IdU (300 mg/kg)
Day 1	FdC (12 mg/kg) + H_4U (25 mg/kg)
	IdU (300 mg/kg)
Day 2	IdU (300 mg/kg)
Day 3	IdU (300 mg/kg).

We also examined several variations of this protocol omitting the pyrimidine metabolism modulators or IdU and varying the total IdU dose with constant doses of the modulators. Each injection was administered intraperitoneally (i.p.). Animals undergoing RIT were given a single i.p. dose of ^{131}I conjugated Mc5 on Day 0, roughly 1 hr following the IdU injection. Initial ^{131}I-Mc5 dose and whole-body clearance of radioactivity was monitored with a dose calibrator in order to ensure that the correct dose had been delivered; the reported doses are the average dose administered to all animals undergoing RIT.

Average tumor volumes (as described above) were calculated and plotted versus time. Animals were killed before their tumor volumes reached 1000 mm^3. Body weights were measured over time, and the maximum percent weight loss was calculated as the difference between the initial body weight and the lowest weight attained by the animal, divided by the initial body weight, multiplied by 100. Average maximum percent weight loss, a measure of generalized toxicity, and standard deviations are reported.

Statistical analyses were made using the SAS/STAT® 6.03 edition software (SAS Institute Inc., Cary, NC). Weight loss data were compared by an analysis of variance followed by a comparison of the means using the following tests: t-tests (LSD), Waller-Duncan K-ratio t-test, Duncan's multiple range test and Tukey's studentized range (HSD) test. Significant differences reported were significant in all tests. Tumor volume data from the groups undergoing RIT and the combined therapies were compared using a t-test.

RESULTS

Figure 1 displays the effect of combining RIT with IdU radiosensitization. The animals that received 540 μCi (10 μCi/μg) of ^{131}I-Mc5 alone experienced a cessation in average tumor growth (no increase in tumor volume) for 17 days. The animals receiving the combined drug treatment and RIT underwent a regression resulting in an average tumor volume that was below the initial size at treatment for 50 days; 2/5 cures were obtained. Their average tumor volume was 90% smaller than the initial size by Day 29. Neither cures nor a regression in average tumor volume was observed with the RIT alone. Drug treatment alone had only a minimal effect, producing only a 5-day lag in tumor growth. Drug treated animals experienced a transient weight loss averaging 15% (s.d. = 4.3) of their initial body weight, the same weight loss as animals receiving the combination therapies (s.d. = 6.4). Animals administered only the ^{131}I-Mc5 temporarily lost an average of 4% (s.d. = 4.1) of their body weight. Control animals did not lose weight.

The experiment was repeated, but this time utilizing a

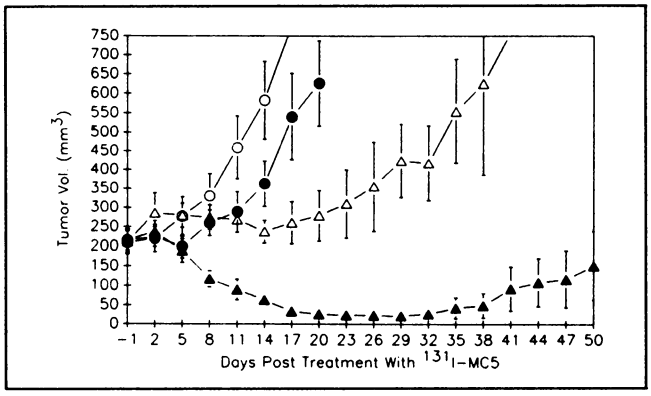

FIGURE 1. Radioimmunotherapy combined with IdU radiosensitization. Drug treatment included IdU and the pyrimidine metabolism modulators, PALA and FdC + H_4U. An average of 540 μCi (standard deviation = 64) of ^{131}I-Mc5 was administered to the animals undergoing radioimmunotherapy. Shown are average tumor volumes versus time. A significant difference ($p < 0.05$) between animals undergoing radioimmunotherapy alone and those given the combined treatments was found on Days 8–32 and Days 41–47. ○: control, no treatment; ●: the drug treatment protocol; △: radioimmunotherapy; and ▲: combined drug treatment and radioimmunotherapy.

higher dose of ^{131}I-Mc5, 625 μCi (8.3 μCi/μg), in an attempt to maximize the therapeutic effect, and in order to make comparisons with the lower dose experiment. Figure 2 shows the results obtained. The combination therapies again produced a regression in average tumor volume, this time lasting 38 days (time below initial size at treatment); one of five cures was obtained. Their average tumor volume was 79% smaller than the initial size by Day 20. Radioimmunotherapy without drug treatment also produced a regression, resulting in the average tumor volume being below the initial size at treatment for 35 days (average tumor volume was 40% smaller than the initial size by Day 26); no animals were cured and all the tumors resumed growth. Again, drug treatment alone had only a minimal effect on tumor growth, resulting in only a 5-day cessation of tumor growth. Drug treated animals experienced a transient weight loss averaging 6% (s.d. = 8.2) of their initial body weight. Animals administered only the radioimmunoconjugate also lost an average of 6% (s.d. = 5.1) of their initial body weight. Animals given the combined therapies lost an average of 22% (s.d. = 2.6) of their initial body weight. All animals regained the weight lost. Control animals did not lose weight.

We wished to determine if the total dose of IdU could be reduced without loss of efficacy in an attempt to lower chemotoxicity. Figure 3 shows the results of an experiment lowering the total dose of IdU by omitting the fourth IdU injection from the drug treatment protocol described in the Materials and Methods section. Combined RIT (500 μCi ^{131}I-Mc5, 10 μCi/μg) and drug treatment resulted in a cessation of average tumor growth between Days 1 and 5, average tumor volume then decreased and was below the value on Day 1 from Day 7 through Day 19. The average tumor volume was smaller than the initial size by Day 16. Due to the availability of ^{131}I, this experiment was begun

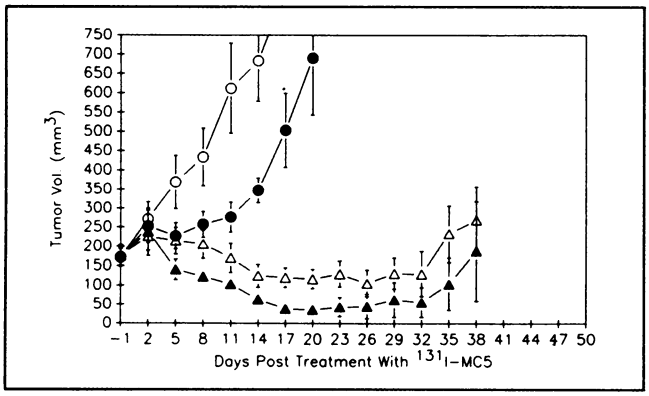

FIGURE 2. High-dose radioimmunotherapy combined with IdU radiosensitization. An average of 625 μCi (standard deviation = 16) of ^{131}I-Mc5 was administered to the animals undergoing radioimmunotherapy. Shown are average tumor volumes versus time. A significant difference ($p < 0.05$) between animals undergoing radioimmunotherapy alone and those given the combined treatments was found only on Days 17 and 20. O: control, no treatment; ●: the drug treatment protocol; △: radioimmunotherapy; and ▲: combined drug treatment and radioimmunotherapy.

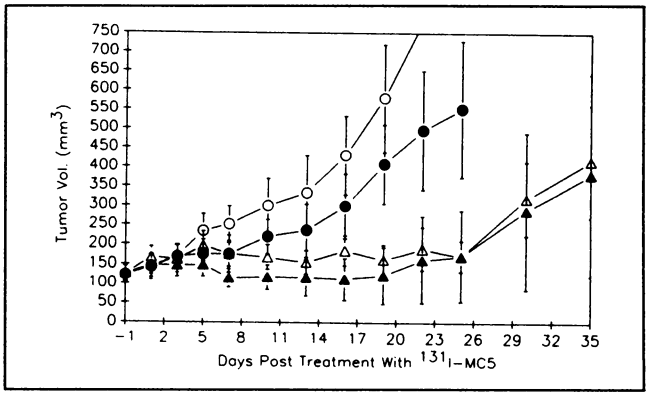

FIGURE 3. Radioimmunotherapy combined with low-dose IdU. The dose of IdU used previously was lowered by omitting the final IdU injection from the drug treatment protocol. The PALA and FdC + H_4U doses were unmodified. Animals undergoing radioimmunotherapy were administered approximately 500 μCi of ^{131}I-Mc5 (whole-body radioactivity was not monitored in this experiment). Shown are average tumor volumes versus time. The average tumor volumes of animals undergoing radioimmunotherapy alone were not significantly different at the 0.05 level than those of the animals receiving the combined treatments. O: control, untreated; ●: the low dose IdU protocol; △: radioimmunotherapy; and ▲: low dose IdU drug protocol combined with radioimmunotherapy.

using animals with somewhat smaller tumors than in the previous or subsequent experiments. This may have resulted in a lower level of IdU DNA incorporation and may have contributed to the lower level of radiosensitization obained in this experiment. Radioimmunotherapy without drug treatment resulted in a cessation of tumor growth for about 25 days. Drug treatment alone slightly reduced the rate of average tumor growth compared to the untreated controls. The animals administered the drug treatment alone lost an average of 7% (s.d. = 4.2) of their initial body weight, 1% (s.d. = 1.8) with the RIT alone, and 10% (s.d. = 4.4) with the combined treatments. All animals recuperated from this weight loss. The untreated controls did not lose weight.

In the next two experiments, we examined the effects of PALA and FdC + H_4U treatment. In Figure 4, pyrimidine metabolism modulators were omitted from the standard drug protocol. The drug treatment consisted of four IdU injections (300 mg/kg) spaced 24 hr apart. A single injection of ^{131}I-Mc5 (516 μCi, 4.9 μCi/μg) was administered about 1 hr following the first IdU injection (Day 0). The animals undergoing this combined treatment experienced a regression, resulting in the average tumor volume being below the initial size on Day 0, for 29 days, from Day 7 through Day 36. Average tumor volume was 50% smaller than the initial size by Day 36. No cures were obtained. Animals undergoing RIT without drug treatment experienced a reduction in tumor volume between Days 7 and 20, however, average tumor volume never regressed below the initial size at treatment. Animals treated with only the four injections of IdU displayed only a slight reduction in

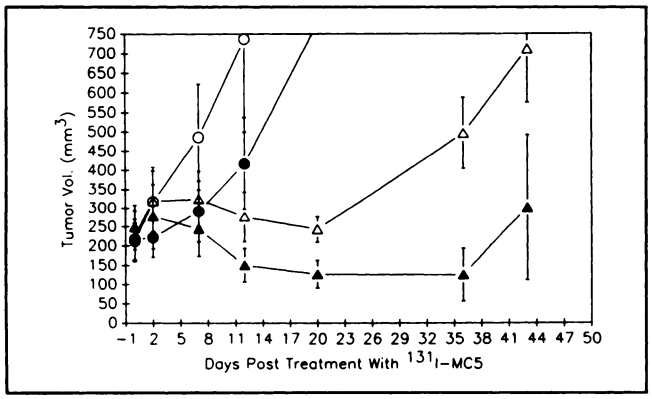

FIGURE 4. Omission of the pyrimidine metabolism modulators. The PALA and FdC + H_4U injections were omitted from the drug treatment protocol. Animals undergoing radioimmunotherapy received an average of 516 μCi (standard deviation = 64) of ^{131}I-Mc5. Shown are average tumor volumes versus time. A significant difference ($p < 0.05$) between animals undergoing radioimmunotherapy alone and those given the combined treatment was found only on Day 36. O: control, untreated; ●: IdU treatment; △: radioimmunotherapy; and ▲: combined IdU treatment with radioimmunotherapy.

the rate of tumor growth compared to the controls. Animals given the combined IdU treatments and RIT lost an average of 6% (s.d. = 2.6) of their initial body weight. Drug treatment alone resulted in a 3% (s.d. = 3.7) weight loss. The untreated controls and those undergoing RIT without IdU treatments did not lose weight.

The next experiment (data not shown) examined the effect of combining the PALA and FdC + H_4U pretreatments, without IdU, with RIT. The treatment protocol was identical to that described in the Materials and Methods section, minus the IdU injections. A dose of 527 μCi (9.6 μCi/μg) of ^{131}I-Mc5 was used. No difference was seen in the tumor response to RIT with or without treatment with pyrimidine metabolism modulators. Drug treatment alone produced no effect in comparison to the untreated controls.

DISCUSSION

Treatment with IdU and pyrimidine metabolism modulators enhanced the therapeutic effectiveness of RIT. The combined treatments resulted in cures of a transplantable human breast carcinoma with acceptable toxicity. An analysis of variance was performed on the weight loss data indicating a significant ($p < 0.0001$) added variance component among groups. A comparison of the means demonstrates that toxicity was due to the drug treatment. There was not a significant difference at the 0.05 level between animals receiving drug treatment alone or the combined drug treatment and radioimmunotherapy. Both of these groups were significantly different at the 0.05 level from the untreated controls and from the group undergoing radioimmunotherapy without drug treatment. Weight loss in the animals treated with just the radioimmunoconjugate was not significantly different at the 0.05 level than the untreated controls. Similar results were obtained with the lower dose of IdU, and when IdU was used without the modulators of pyrimidine metabolism.

In the second experiment, where a higher dose of the radioimmunoconjugate was used, it can be seen that the higher dose of radioactivity alone was less effective than the low dose combined with drug treatment used in the first experiment. This indicates that the apparent dose enhancement ratio (based on therapeutic effect) obtained by drug treatment was greater than 1.2 (625 μCi/540 μCi). Unfortunately, it is not possible to further increase the dose of ^{131}I-Mc5 in order to achieve cures with RIT alone without killing the animals. Due to variability in the radioiodination and purification procedures a lower specific activity was obtained in this experiment compared to the first experiment. Nevertheless, as evidenced by the biological response, a greater radiation dose was delivered to the tumor in the second experiment. However, the dose enhancement ratio calculated above must be taken as an estimate, lacking direct dosimetry. The higher radioactivity dose used in the second experiment did not increase the efficacy of the combined treatments over that obtained with the lower dose in the first experiment. Perhaps a greater effect can be obtained by increasing the IdU dose. An analysis of variance was again performed on the weight loss data. There was a significant ($p < 0.0001$) added variance component among groups. A comparison of the means showed that only the combined treatment group was significantly different at the 0.05 level from the other groups in this experiment. Neither drug treatment alone nor radioimmunotherapy alone produced significant weight loss compared to the controls.

In agreement with previous results, the use of the inhibitors of thymidylate biosynthesis (PALA, FdC + H_4U) increased the radiosensitization obtained with halogenated pyrimidine analogues (*16*). Inclusion of the modulators resulted in a greater reduction in average tumor volume than when IdU was used alone in combination with RIT. Cures were only obtained with the complete protocol. The pyrimidine metabolism modulators slightly increased the toxicity (weight loss) of the drug treatment, although not significantly (a t-test comparing drug treated animals from Figs. 1 and 2 to those receiving only IdU from Fig. 4 shows $p = 0.1626$). The modulators enhanced IdU radiosensitization but did not radiosensitize the tumors when administered without IdU.

By lowering the total dose of IdU in the treatment protocol, a slight reduction in toxicity was obtained, but this also reduced the anti-tumor effect. Recently, Pedley et al. reported that a very low dose of IdU (200 mg/kg total) resulted in radioresistance of tumors treated with ^{131}I conjugated anti-CEA antibodies (*22*).

CONCLUSION

Our results demonstrate the potential for increasing the effectiveness of the radioimmunotherapy of human tu-

mors using halogenated pyrimidine analogs. Substantial radiosensitization was obtained with bolus doses of IdU when coadministered with the modulators of its metabolism. The use of this radiosensitizing drug treatment should allow the clinician to achieve a therapeutic effect while minimizing the radiation dose given to the patient. The combined drug treatment, including pyrimidine metabolism modulators, with RIT provided the greatest antitumor effect in this study. We hope to include halopyrimidine radiosensitization in our ongoing clinical trials of breast cancer radioimmunotherapy in the near future.

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