

Potential of Radioimmunotherapy by Inhibition of Topoisomerase I¹

Steve R. Roffler,² James Chan, and Ming-Yang Yeh

Institute of Biomedical Sciences, Academia Sinica [S. R. R.], and Department of Microbiology and Immunology, National Defense Medical Center [J. C., M-Y. Y.], Taipei, 11529 Taiwan, Republic of China

ABSTRACT

Cancer therapy with radiolabeled antibodies is limited by the low uptake of radioimmunoconjugates into tumor masses. In this study, camptothecin, a topoisomerase I poison, was examined *in vitro* and *in vivo* for potentiation of radioimmunoconjugate therapy. γ -Ray irradiation of AS-30D rat hepatoma cells followed by a 2-h exposure to camptothecin was found to act additively at low radiation doses (<200 rad) and synergistically at higher radiation doses as shown by isobologram analysis with 20% survival used as the end point. A monoclonal antibody, RH1, was developed against AS-30D cells and shown to localize in hepatoma ascites in SD rats. Therapy of established ascites tumors with four weekly rounds of either camptothecin administered i.m. in a slow release form or ¹³¹I-labeled monoclonal antibody RH1 administered by i.p. injections prolonged rat survival but was ineffective at curing animals of tumors. In contrast, four weekly rounds of combined therapy consisting of i.m. injections of 5 mg/kg camptothecin suspended in lipiodol followed 24 h later by i.p. injection of 200 μ Ci ¹³¹I-labeled monoclonal antibody RH1 cured 86% of animals. Treatment with camptothecin and a ¹³¹I-labeled control antibody was no more effective than treatment with drug alone. These results show that camptothecin can potentiate the effects of radiation both *in vitro* and *in vivo* and suggest that topoisomerase I inhibitors may be useful for increasing the efficacy of radioimmunoconjugates for the treatment of cancer.

INTRODUCTION

Much attention has been focused during the past decade on developing imaging and therapeutic agents by radiolabeling monoclonal antibodies that can recognize and bind to tumor-associated antigens (1-4). Successful radioimmunotherapy of cancer, however, has been difficult due to the small amounts of antibody that can be targeted to tumors and the dose-limiting toxicity associated with systemic administration of large quantities of radiolabeled antibody (5, 6). A variety of methods have been investigated to improve the tumor targeting of radioimmunoconjugates including the use of antibody fragments (7, 8), targeting with recombinant single-chain antibodies (9, 10), administration of agents to increase tumor blood flow (11) or antigen expression (12, 13), and the use of two-step delivery methods (14, 15). An alternative approach is to potentiate the therapeutic action of radioimmunoconjugates at the tumor site and thus improve the efficacy of radioimmunotherapy by decreasing the effective dose required for antitumor activity.

Several anticancer drugs have been examined for synergistic effects with radiation including cyclophosphamide, cisplatin, mitomycin C, 5-fluorouracil, doxorubicin, and taxol (16-19). Recent work has suggested that topo I³ inhibitors may potentiate the lethal effects of ionizing radiation (20-24). Topo I is a nuclear enzyme that plays an important role in DNA replication and RNA transcription (25, 26). Camptothecin, a natural plant alkaloid isolated from *Camptotheca acuminata* (27), has been shown to be a potent topo I poison (28, 29).

Camptothecin acts to stabilize a normally transient complex formed between topo I and DNA during the relaxation of torsional stress in DNA (28). The stabilized complex inhibits the religation of the single-strand DNA break formed during the action of topo I and is believed to cause double-strand DNA breaks at the replication fork with concomitant cell death (30). In the current study, interactions between camptothecin and γ -ray irradiation on cultured rat hepatoma cells were investigated. Camptothecin was also examined for potentiation of the efficacy of radioimmunoconjugate therapy of hepatoma tumors *in vivo*.

MATERIALS AND METHODS

Reagents and Cells. Camptothecin and common biological reagents were purchased from Sigma Chemical Company (St. Louis, MO). [^{6-³H}]Thymidine was from Amersham (Buckinghamshire, England). ¹³¹I was purchased from Syncor Taiwan, Inc. (Taipei, Taiwan). Immunochemicals were purchased from Organon (Durham, NC). Sepharose CL-4B protein A was from Pharmacia LKB Biotechnology (Uppsala, Sweden). Lipiodol ultra-fluide was from Laboratoire Guerbet (Bois Cedex, France). Hematoxylin was purchased from Surgipath Medical Industries (Grayslake, IL) while OCT compound was from Miles Diagnostic Division (Elkhart, IN). Camptothecin stock solutions were made in DMSO at 2.5 mg/ml, sterilized by filtration, and stored at -70°C. AS-30D rat hepatoma cell line was provided by Dr. J. P. Chang, Institute of Zoology, Academia Sinica, Taipei. Cells were maintained in Dulbecco's modified Eagle's medium (Gibco BRL, Grand Island, NY) supplemented with 5% heat-inactivated bovine serum, 100 units/ml penicillin, and 100 μ g/ml streptomycin. HepG2 human hepatoma cells, available from American Type Culture Collection (Rockville, MD), were maintained in RPMI 1640 supplemented with 5% heat-inactivated fetal calf serum, 100 units/ml penicillin, and 100 μ g/ml streptomycin.

Animals. Female BALB/c mice and SD rats were obtained from the animal room of the Institute of Biomedical Sciences, Academia Sinica.

Antibodies. A hybridoma secreting Mab RH1, specific for AS-30D rat hepatoma cells, was generated by immunization of female BALB/c mice with 4 injections of 5×10^6 AS-30D cells on days 1, 21, 45, and 60. Three days after a final i.p. injection of 1×10^7 cells on day 75, splenocytes were fused with NS-1 myeloma cells as described previously (31). Hybridoma supernatants were screened by ELISA, and selected hybridomas were cloned three times and cultured in RPMI 1640 (Gibco BRL, Grand Island, NY) supplemented with 15% heat-inactivated fetal calf serum, 100 units/ml penicillin, and 100 μ g/ml streptomycin. Control hybridoma H16-L10-4R5, which secretes an IgG_{2a} antibody (Mab HB65) against nucleoprotein of influenza type A viruses, was obtained from the American Type Culture Collection. Mabs were purified from ascites produced in BALB/c mice by protein-A affinity chromatography (32).

Characterization of Mab RH1. The antigen-binding activity of Mab RH1 was determined by ELISA using whole AS-30D cells coated on 96-well plates as antigen (33). Monoclonal antibody isotype was determined using a kit (Mono-Ab-ID-EIA kit; Zymed Labs) according to the manufacturer's instructions. Antibody binding to solid AS-30D tumors was examined by immunohistochemistry. Solid tumors were generated by injecting 10^7 AS-30D cells under the liver capsule of anesthetized SD rats. Tumors were allowed to grow to about 1 cm in diameter, at which time animals were killed, and the tumor as well as surrounding normal liver tissue was removed and fixed in OCT compound. Cryostat sections (5 μ m) of tissue were pretreated with acetone at -20°C, incubated in 3% H₂O₂ in methanol to deactivate endogenous peroxidase activity, and blocked with normal human serum (1:5 in PBS) to decrease nonspecific background. Antibody binding was assayed by the peroxidase antiperoxidase technique (34). Briefly, Mab RH1 was diluted to 50 μ g/ml and incubated with tissues. Sections were then incubated with rabbit anti-mouse IgG and mouse peroxidase anti-peroxidase conjugate. Binding was visualized

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² To whom requests for reprints should be addressed.

³ The abbreviations used are: topo I, topoisomerase I; Mab, monoclonal antibody; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; DMSO, dimethyl sulfoxide.

with 3,3'-diaminobenzidine. Tissues were counterstained with hematoxylin for 1 min, washed with water, and dehydrated in increasing concentrations of ethanol followed by xylene.

The size and cellular location of the antigen recognized by Mab RH1 were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblotting of AS-30D cellular fractions (35, 36). AS-30D cells (10^8) were washed with PBS and resuspended in 1 mM Na_2CO_3 containing 1 mM phenylmethylsulfonyl fluoride. Cells were disrupted by 50 strokes in a dounce homogenizer at 4°C. Cell nuclei were recovered by centrifugation at $3000 \times g$ for 5 min. The resulting supernatant was centrifuged at $100,000 \times g$ for 45 min to recover membrane (pellet) and cytosol (supernatant) fractions. Protein concentrations of cellular fractions were measured by the bicinchoninic acid assay (37) and frozen at -70°C until used for electrophoresis and immunoblotting.

In Vitro Cytotoxicity of Camptothecin. The effect of camptothecin on cells was evaluated *in vitro* by measuring cellular DNA synthesis. Cells were plated at 3×10^4 cells/well in 96-well microtiter plates and incubated overnight at 37°C in a 5% CO_2 -95% air atmosphere. After spinning the plates for 5 min at $1000 \times g$, medium was removed, and 200 μl of fresh medium spiked with camptothecin from DMSO stock solutions were added at time zero. Control cells were treated with fresh medium containing an equal concentration of DMSO. After the indicated treatment periods, drug-containing medium was removed, cells were washed with PBS, and 200 μl of fresh medium were added to each well until 46 h after time zero. One μCi [$6\text{-}^3\text{H}$]thymidine was then added to wells, and cells were incubated for 2 h. Incorporation of [$6\text{-}^3\text{H}$]thymidine into cellular DNA was measured in a Beckman LS 5801 scintillation counter after washing cells three times with 6% trichloroacetic acid. Results are expressed as relative DNA synthesis rate of treated cells compared with control cells as

$$\text{Relative DNA synthesis rate} = \frac{\text{Sample cpm} - \text{background cpm}}{\text{Control cpm} - \text{background cpm}}$$

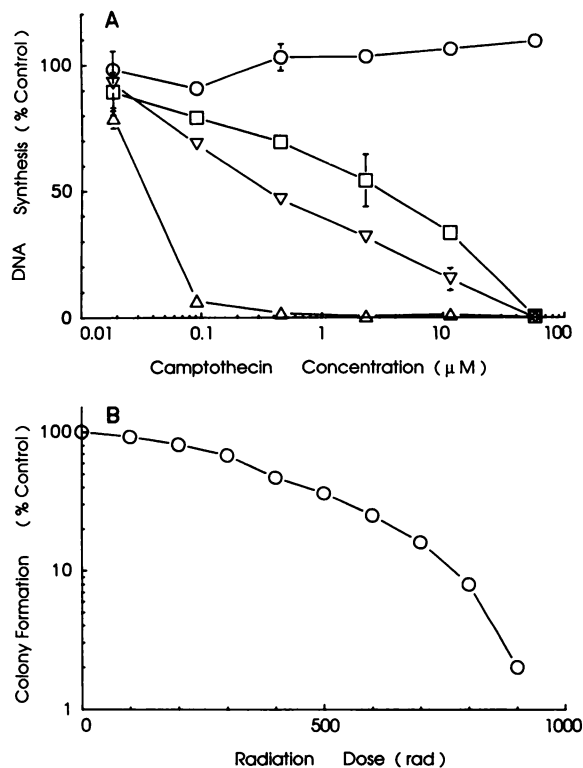


Fig. 1. *In vitro* cytotoxicity of camptothecin or radiation to AS-30D cells. A, inhibition of DNA synthesis by camptothecin. AS-30D rat hepatoma cells were exposed to DMSO for 24 h (○) or camptothecin in DMSO for 1 h (□), 2 h (◇), or 24 h (△). Incorporation of [$6\text{-}^3\text{H}$]thymidine into cellular DNA was measured at 48 h and is compared with untreated cells. Bars, SE of two experiments, each consisting of triplicate determinations. B, cell survival after radiation treatment. AS-30D cells were exposed to γ -ray irradiation produced by a ^{60}Co source and replated for colony formation. The number of colonies surviving treatment was compared to control colonies after 7 days of culture.

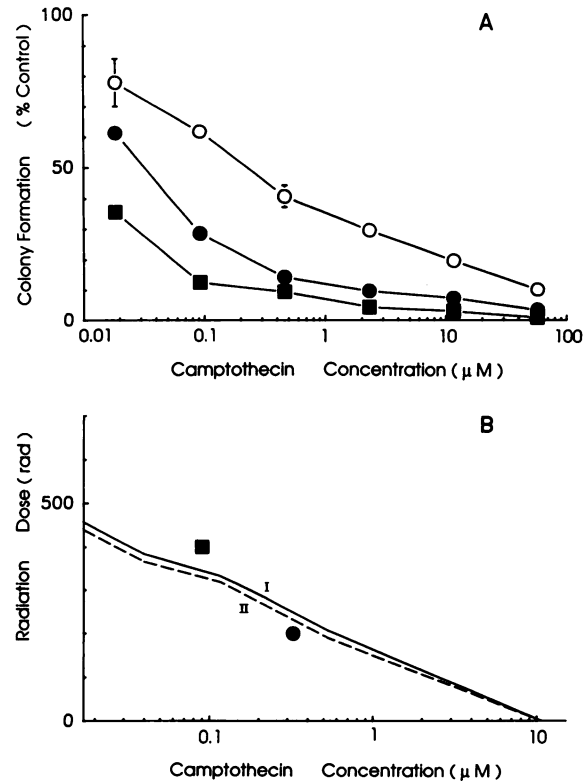


Fig. 2. Combination treatment of AS-30D cells with camptothecin prior to irradiation. A, AS-30D cells were exposed to camptothecin for 8 h, washed, and incubated in fresh medium for 16 h before exposure to 0 (○), 200 (●), or 400 (■) rad. Cell survival was determined by clonogenic assay and is shown as percentage survival relative to untreated AS-30D cells. Bars, SE of triplicate determinations. B, isobologram for treatment of cells with camptothecin prior to irradiation. Isoeffect mode I and mode II curves were calculated from data displayed in Figs. 1B and 2A (0 rad) as described in (38) taking 20% survival an end point. The combined effects of camptothecin and 200 rad (●) were slightly supraadditive, whereas pretreatment of cells with camptothecin followed by 400 rad (■) resulted in subadditive toxicity.

In Vitro Cytotoxicity of Camptothecin and Radiation. The combined effects of camptothecin and radiation on cultured cells were evaluated by clonogenic assay. AS-30D cells (5×10^6) were plated overnight in 100-mm dishes. After exposure to camptothecin, DMSO, or medium for 8 h, cells were washed with PBS and incubated in fresh medium for 16 h before γ -ray irradiation with a ^{60}Co source. Cells were then replated in 6-well plates at 500 cells/well and incubated at 37°C in 5% CO_2 for 7 days. Postexposure studies were carried out by first irradiating cells and then immediately adding camptothecin for 2 h. After washing, cells were replated in fresh medium and processed as above. Colony-forming ability was evaluated by counting colonies under a microscope.

Construction of Isoeffect Curves. Analysis of the effects of combination treatment was performed by constructing isoeffect curves (isobolograms) as described by Steel and Peckham (38). Mode I isoeffect curves were constructed by reading the doses of radiation or camptothecin from the respective single-agent dose-response curves, starting at zero dose of each agent, that added up to 20% cell survival. Mode II curves were formed in the same way except that the steepest portion of the radiation dose-response curve was used in the construction. Combination treatment results were plotted on the isobologram by interpolation of the combination dose-response curves at 20% cell survival. Combinations resulting in points falling in the region to the left of the isoeffect curves correspond to positive or supraadditive interactions between the combined agents, points to the right of the curves represent subadditive effects, and combination doses falling in the region between the mode I and mode II curves are attributed to additive interactions of the single agents (38).

Radiolabeling of Mabs and Nuclear Imaging. Mabs RH1 and HB65 were radiolabeled with ^{125}I using the Chloramine T-procedure (39) to a specific activity of about 4.5 $\mu\text{Ci}/\mu\text{g}$. Iodinated Mabs were tested by direct binding to AS-30D and HepG2 cells to ensure maintenance of specificity and immunoreactivity. For nuclear imaging studies, rats bearing AS-30D ascites were given

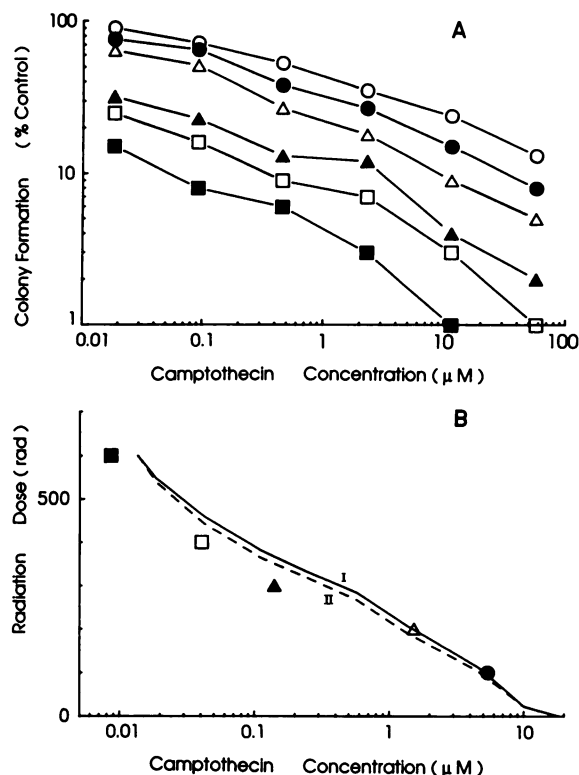


Fig. 3. Combination treatment of AS-30D cells with radiation followed immediately by camptothecin. A, AS-30D cells received 0 (○), 100 (●), 200 (◐), 300 (◑), 400 (◒), or 600 (◓) rad before exposure to camptothecin for 2 h. Cell survival was determined in triplicate by clonogenic assay and is depicted as percentage cell survival relative to untreated cells. B, isobologram for cells exposed to camptothecin immediately after irradiation. Isoeffect mode I and mode II curves were calculated as described (38). Treatment with camptothecin after exposure to 100 (●) or 200 (◐) rad resulted in additive cell killing, whereas higher radiation doses of 300 (◑), 400 (◒), and 600 (◓) rad produced supraadditive cytotoxicity.

Lugol's solution (1% KI) in their drinking water 2 days before injection of radiolabeled antibodies to prevent thyroid uptake of dehalogenated radioiodine. Two hundred µCi of ¹³¹I-RH1 or ¹³¹I-HB65 were injected i.p., and each rat was then imaged from the dorsal surface with a gamma camera (Elsint Model Apex 400) at 24, 48, 72, and 96 h.

Biodistribution of Radiolabeled Mabs. Eight groups of three rats were killed and dissected at 24, 48, 72, and 96 h after i.p. injection of 200 µCi ¹³¹I-RH1 or ¹³¹I-HB65. Blood, organs, and ascites cells were weighed on an analytical balance and assayed for radioactivity using a multichannel gamma counter (4). Results are expressed as specific uptake of antibody in tumor or

tissue (cpm/mg) and localization index, defined as the ratio of specific (Mab RH1) to control (Mab HB65) antibody uptake in tissue or tumor, normalized to the radioactivity in the muscle tissue of different rats. Localization index is calculated as

$$\text{Localization index} = \frac{\left[\frac{\text{Cpm/mg tissue}}{\text{Cpm/mg muscle}} \right]_{\text{Mab RH1}}}{\left[\frac{\text{Cpm/mg tissue}}{\text{Cpm/mg muscle}} \right]_{\text{Mab HB65}}}$$

A localization index of 1 indicates that Mab RH1 and HB65 binding to a particular tissue were equivalent, whereas a higher index value indicates specific binding of Mab RH1.

In Vivo Therapy with Camptothecin. Groups of three rats were injected i.p. with 5×10^6 AS-30D cells on day zero. Treatment was initiated 5 days after tumor implantation to allow development of ascites. Camptothecin was formulated in a slow release form by sonication at 10 mg/ml in lipiodol ultra-fluid. Camptothecin in lipiodol was administered i.m. at the indicated doses by deep muscle injection through a 27-gauge needle into the posterior leg. Treatments were repeated on days 12, 19, and 26. Animal survival was monitored until day 40, at which time surviving animals were killed and examined for residual tumors.

In Vivo Therapy with Radiolabeled Antibody. Nine SD rats received i.p. injections of 5×10^6 exponentially growing AS-30D cells. These rats received Lugol's solution in their drinking water on days 3 and 4. On day 5, groups of 3 rats were treated by i.p. injection of 200 µCi ¹³¹I-RH1, 200 µCi ¹³¹I-HB65, or PBS. Treatments were repeated on days 12, 19, and 26.

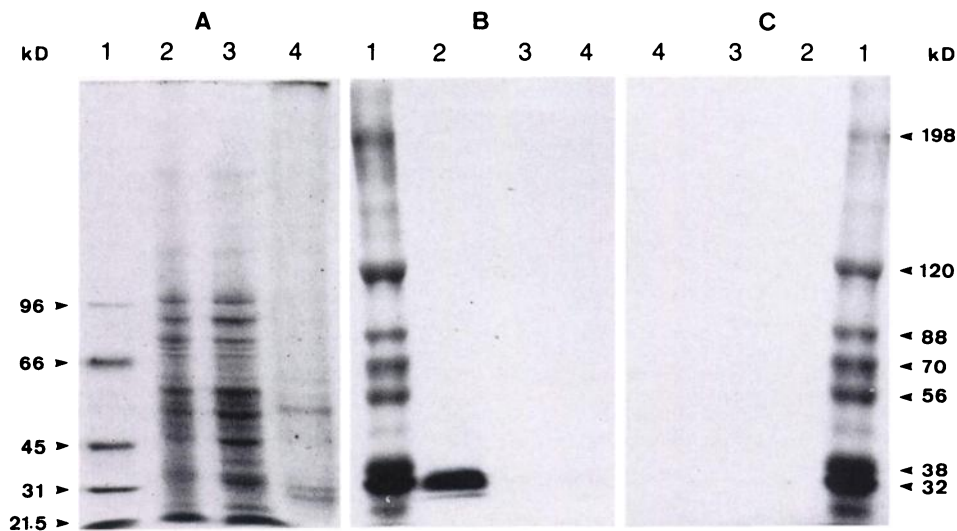
Combination Treatment with Camptothecin and Radiolabeled Antibody. Fifty-six SD rats received i.p. injections of 5×10^6 exponentially growing AS-30D cells and were randomly divided into 8 treatment groups. On day 5, groups of seven rats received a slow release formulation of camptothecin in lipiodol by i.m. injection at doses of 0, 2, or 5 mg/kg. Twenty-four h later, rats received 200 µCi ¹³¹I-RH1, 200 µCi ¹³¹I-HB65, or PBS. Treatment was repeated on days 12, 19, and 26. Animal survival was monitored until day 60.

Statistical Analysis. The significance of differences between survival curves was estimated by the Mantel-Haenszel χ^2 test of association (40). Mean survival times were estimated by dividing the sum of individual survival times by the number of animals in that group. For experiments with long-term survivors, the survival time for the mean survival time calculation was taken as the last day of observation.

RESULTS

In Vitro Cytotoxicity of Camptothecin or Radiation Alone. The cytotoxicity of camptothecin to cultured AS-30D rat hepatoma cells was assessed by measuring inhibition of cellular DNA synthesis.

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblots of the antigen recognized by Mab RH1. Samples of cellular fractions isolated from AS-30D cells were electrophoresed on a nonreduced polyacrylamide gel and stained with Coomassie Blue R-250 (A) or transferred to nitrocellulose paper and immunoblotted with Mab RH1 (B) or Mab HB65 (C). Lane 1, molecular weight standard (prestained standard in immunoblots); Lane 2, AS-30D membrane fraction; Lane 3, AS-30D cytosol fraction; Lane 4, AS-30D nuclear fraction. kD, molecular weight in thousands.



Camptothecin inhibition of DNA synthesis was both dose and time dependent with the concentration of drug causing 50% inhibition of DNA synthesis values at 3.1, 0.35, and 0.035 μM for 1-, 2-, or 24-h exposures to camptothecin, respectively (Fig. 1A). DMSO was also tested for its effect on cellular DNA synthesis since this solvent was used to prepare camptothecin stock solutions. DMSO did not affect DNA synthesis at the concentrations used in these experiments (Fig. 1A). Exposure of AS-30D cells to γ -ray irradiation resulted in dose-dependent survival as determined by clonogenic assay (Fig. 1B) with 50% of cells being killed at 384 rad.

In Vitro Cytotoxicity of Camptothecin and Radiation. The ability of camptothecin to radiosensitize AS-30D cells was first examined *in vitro*. In the first experiment, cells were exposed to camptothecin for 8 h, washed, and incubated in fresh medium for 16 h before being irradiated. This strategy was used to examine whether camptothecin-induced accumulation of cells in G_2 (41, 42) could increase the radiosensitivity of these cells (19). Fig. 2A shows the dose-response curves of AS-30D cells pretreated with camptothecin followed

by irradiation with 0, 200, or 400 rad. Combination treatment with camptothecin and radiation resulted in increased cell death compared to cells treated with drug alone. Isoeffect curves for 20% cell survival were constructed as described in "Materials and Methods" to quantify the interactions between camptothecin and radiation treatment. Pretreatment of cells with camptothecin followed by exposure to 200 rad resulted in slightly supraadditive cell killing, whereas the combination of camptothecin with 400-rad radiation gave a slightly subadditive response (Fig. 2B). Isobologram analysis of treating cells with camptothecin before γ -ray irradiation with inhibition of DNA synthesis as opposed to clonogenic assay used to assay cell survival revealed subadditive interactions at both 200 and 400 rad (data not shown).

The effect of treating AS-30D cells with camptothecin immediately after radiation exposure was also examined. Fig. 3A shows survival curves for cells exposed to 0, 100, 200, 300, 400, or 600 rad followed by treatment with camptothecin for 2 h. Irradiation with 100 or 200 rad in combination with subsequent drug treatment resulted in additive

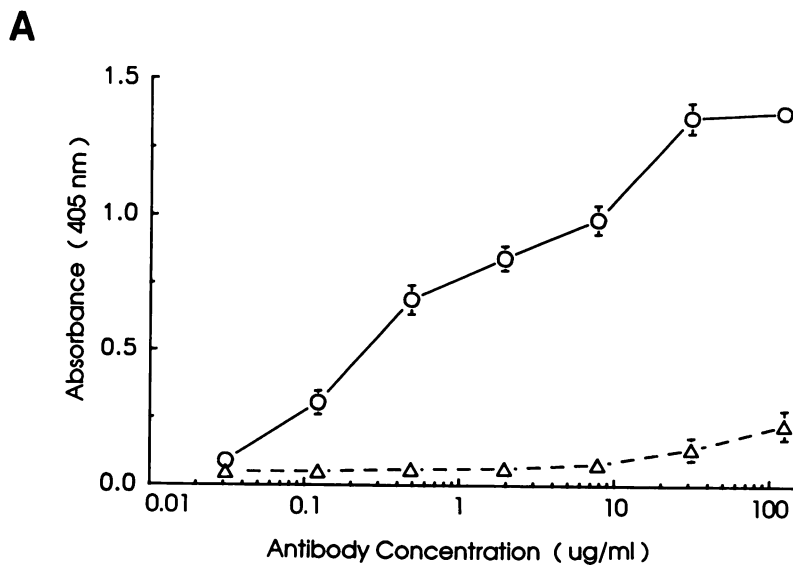


Fig. 5. Binding of Mab RH1 to AS-30D cells and tumors. *A*, ELISA measurement of antigen-binding activities of Mab RH1 (O) or control Mab HB65 (Δ) to AS-30D cells. Bars, SE of triplicate determinations. *B*, immunohistochemical demonstration of Mab RH1 binding to AS-30D tumors. AS-30D solid tumors propagated in the liver of Sprague-Dawley rats were excised and fixed. Cryostat sections of tumor and adjacent normal liver tissue were sequentially incubated with Mab RH1, rabbit anti-mouse IgG, and mouse peroxidase anti-peroxidase conjugate. After Mab RH1 binding was visualized by reaction with 3,3'-diaminobenzidine, tissues were counterstained with hematoxylin. *T*, AS-30D tumor tissue; *N*, normal liver.



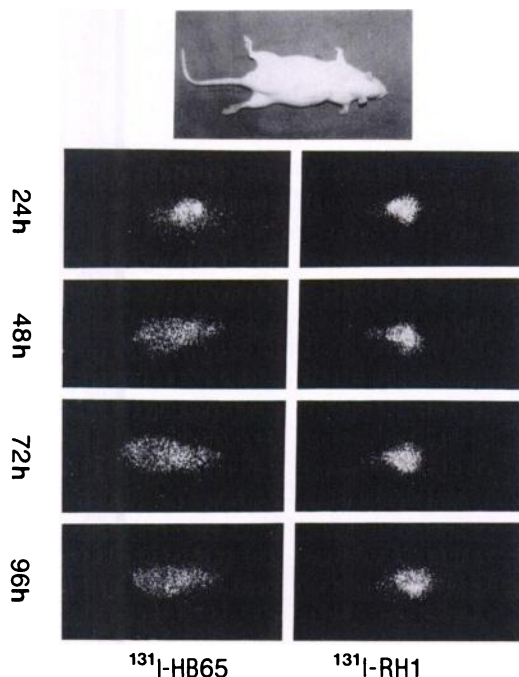


Fig. 6. *In vivo* localization of ^{131}I -RH1. Sprague-Dawley rats bearing established AS-30D ascites tumors received i.p. injections of 200 μCi ^{131}I -RH1 (right) or ^{131}I -HB65 (left) and were imaged at 24, 48, 72, and 96 h with a gamma camera.

cell killing (Fig. 3B), whereas higher doses of radiation acted synergistically with camptothecin (Fig. 3B, points left of the envelope of additivity).

Characterization of Mab RH1. A monoclonal antibody against AS-30D cells was generated to examine the ability of camptothecin to potentiate radioimmunotherapy of AS-30D tumors *in vivo*. Mab RH1 (IgG_{2a}) was produced by immunization of BALB/c mice with live AS-30D cells and fusion of splenocytes with NS-1 myeloma cells. Analysis of Mab RH1 binding to AS-30D cells by immunofluorescence indicated that Mab RH1 binds to an antigen expressed on the membrane of these cells (data not shown). Surface expression of the antigen was further confirmed by Western blot analysis of cellular fractions isolated from AS-30D cells. Mab RH1 was found to bind to an antigen present in the membrane fraction of AS-30D cells. The antigen has an estimated molecular weight of 32,000 after sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting (Fig. 4B, Lane 2). No such antigen could be detected in the cytosol or nuclear fractions from AS-30D cells (Fig. 4B, Lanes 3 and 4). Mab HB65, a control IgG_{2a} antibody, did not bind to any fraction of AS-30D cells on immunoblots (Fig. 4C). Mab RH1 binding to immobilized and fixed AS-30D cells was also evaluated by ELISA (Fig. 5A). Mab RH1 but not control Mab HB65 bound to AS-30D cells coated on microtiter plates. Half-maximal binding was exhibited by Mab RH1 at a concentration of 4.5 nM. The ability of Mab RH1 to bind to AS-30D tumors that had been propagated *in vivo* was assessed by immunohistochemistry. Solid tumors were grown in the liver of Sprague-Dawley rats by injection of AS-30D cells under the liver capsule. Fig. 5B shows that Mab RH1 clearly bound to tumor tissue but did not bind to adjacent normal rat liver. Control IgG_{2a} antibody (Mab HB65) bound to neither AS-30D tumor nor normal liver tissue (data not shown).

***In Vivo* Localization of Mab RH1.** Radioimmunotherapy requires localization of radiolabeled antibody at tumors *in vivo*. Mab RH1 localization was examined in Sprague-Dawley rats bearing established AS-30D ascites tumors. External scintigraphic images were obtained with a gamma camera at 24, 48, 72, and 96 h after injection of 200 μCi

of ^{131}I -RH1 or ^{131}I -HB65. Fig. 6 shows that Mab RH1 remained localized in the peritoneal cavity of tumor-bearing rats throughout the observation period. Control antibody, in contrast, did not localize at ascites tumor cells but rather was distributed throughout the bodies of rats.

The biodistribution of radiolabeled antibodies was also determined. Eight groups of three rats bearing AS-30D ascites tumors received i.p. injections of ^{131}I -RH1 or ^{131}I -HB65. Ascites tumor cells, blood, and organs were removed from separate groups of rats after 24, 48, 72, and 96 h and counted for radioactivity. Fig. 7A shows that Mab RH1 localized in AS-30D ascites tumors, reaching a maximum specific activity of 414 cpm/mg after 24 h. After 96 h, the specific activity of ^{131}I -RH1 was 6 times higher in tumor than in lung or spleen and 12 times higher than in blood. Mab RH1 elimination followed first-order kinetics with a half-life of 108 h from ascites, much slower than elimination from the blood pool with a half-life of 27 h, indicating that Mab RH1 was specifically retained at AS-30D cells in the peritoneal cavity. Mab HB65, in contrast, entered the blood pool much more rapidly with a half-life in ascites of only 42 h (data not shown). Elimination of Mab HB65 from the blood pool was similar to Mab RH1 with a half-life of 28 h. The specificity of Mab RH1 retention at AS-30D ascites cells is shown in Fig. 7B, which depicts the localization index of Mab RH1 relative to Mab HB65, normalized to the uptake of radioimmunoconjugate in muscle tissue. After 96 h, over seven times more Mab RH1 than Mab HB65 had localized at tumor cells. Mab RH1 also localized to a small extent in kidney, stomach,

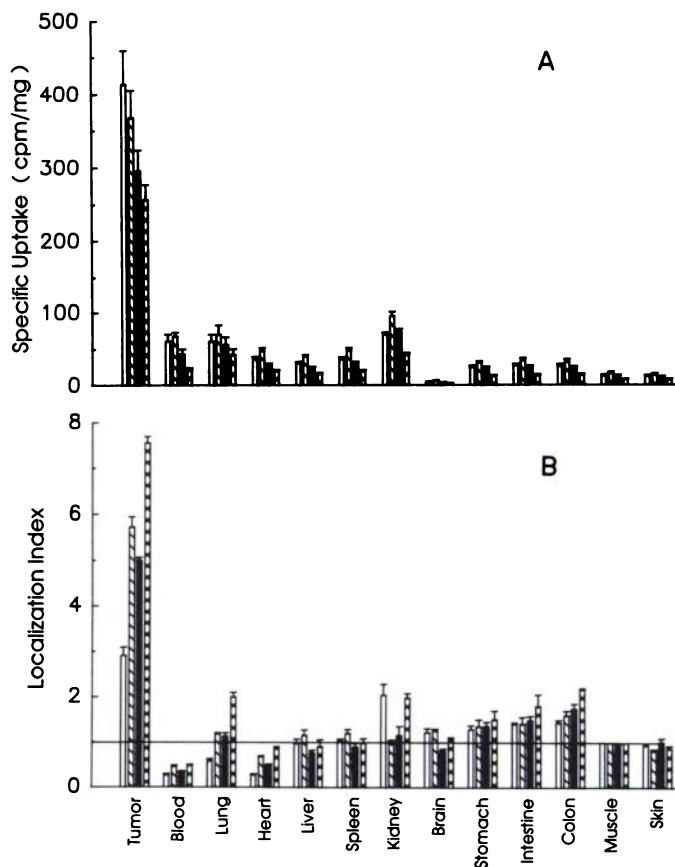


Fig. 7. Biodistribution of ^{131}I -labeled antibodies in tumor-bearing rats. Sprague-Dawley rats bearing established AS-30D ascites tumors were administered 200 μCi ^{131}I -RH1 or ^{131}I -HB65. A, distribution of ^{131}I -RH1 in rat tissues. Specific activity of ^{131}I -RH1 was determined after 24 (\square), 48 (\triangle), 72 (\blacksquare), and 96 (\square) h. Results represent the mean of three rats. Bars, SE of the mean. B, specificity of Mab RH1 tumor localization. The localization index, the ratio of ^{131}I -RH1 to ^{131}I -HB65 uptake in tissues, normalized to uptake of radioactivity in muscle tissue is shown. Indices greater than one indicate specific uptake of Mab RH1.

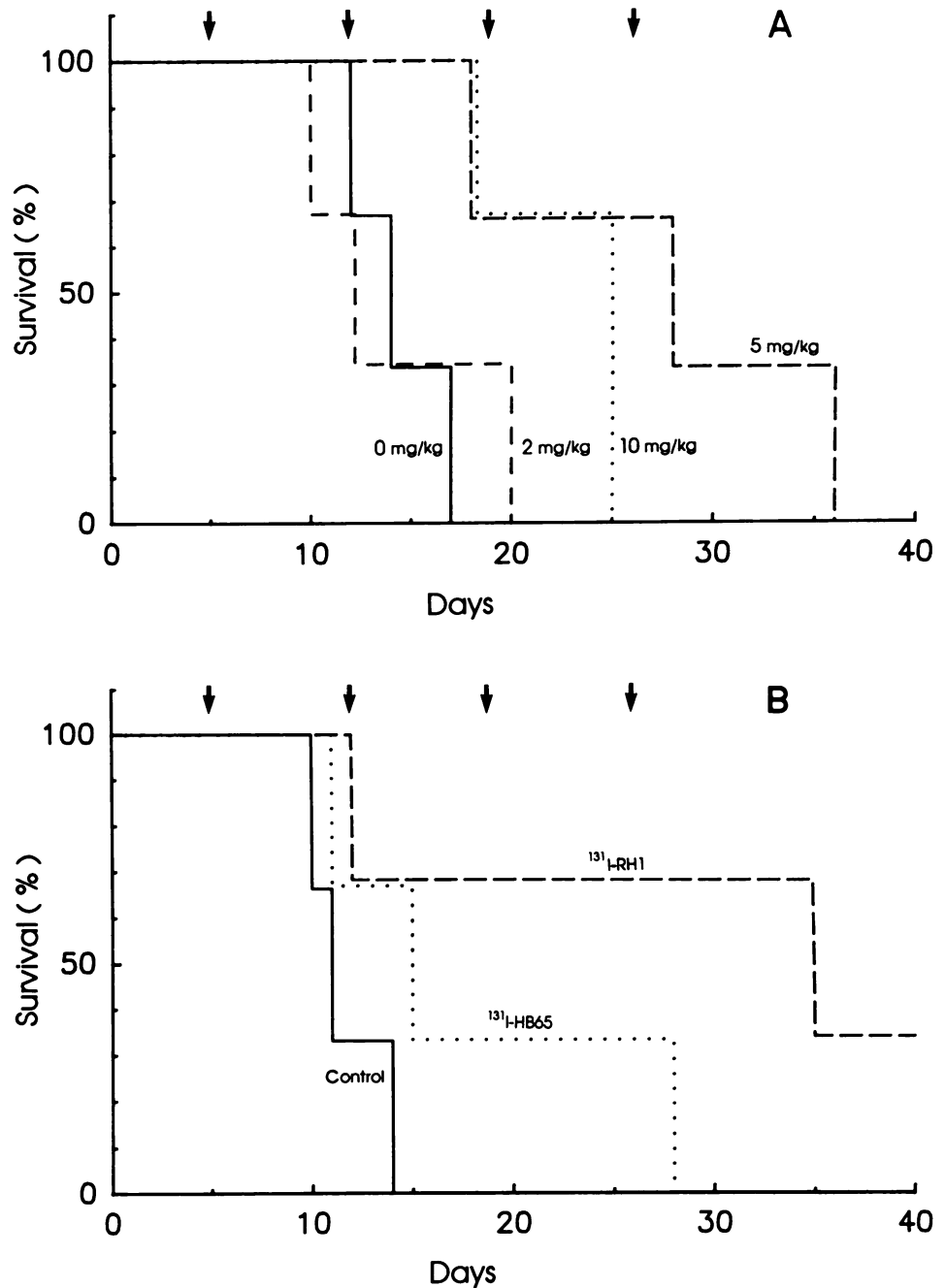


Fig. 8. Survival of tumor-bearing rats treated with single agents. Sprague-Dawley rats received i.p. injections of 5×10^6 AS-30D cells on day 0. A, groups of three rats received i.m. injections in the rear leg muscles of 0 mg/kg (—), 2 mg/kg (---), 5 mg/kg (— · —), or 10 mg/kg (···) camptothecin in lipiodol on days 5, 12, 19, and 26. B, groups of three rats received i.p. injections of PBS (—), 200 μ Ci 131 I-RH1 (---), or 200 μ Ci 131 I-HB65 (···) on days 5, 12, 19, and 26. Arrows, treatments.

colon, and intestine tissue but had a similar distribution pattern to Mab HB65 in the liver, brain, spleen, and skin. The low localization index for blood reflects the retarded partition of Mab RH1 from ascites cells to the blood pool.

In Vivo Therapy with Single Agents. To establish a baseline against which to compare the ability of camptothecin to potentiate the radioimmunotherapy of hepatoma ascites tumors, we examined the ability of each single agent to prolong the survival of Sprague-Dawley rats bearing AS-30D tumors. Treatment was initiated 5 days after rats received i.p. injections of 5×10^6 AS-30D cells to allow establishment of ascites. Fig. 8A shows the survival curves of rats that received four weekly i.m. injections of camptothecin suspended in lipiodol. Control tumor-bearing rats treated with lipiodol died within 16 days due to accumulation of ascites tumors. Treatment with four rounds of camptothecin at 2 mg/kg did not significantly prolong rat survival ($P > 0.25$). Mean survival times of rats given four 5-mg/kg doses of

camptothecin were significantly increased ($P < 0.05$) from 14.3 to 27.3 days, but all rats died by day 36. Mild toxicity was evidenced by transient diarrhea in some rats. Increasing the dose of camptothecin to 10 mg/kg resulted in severe toxicity; rats died by day 25 even though ascites tumors were minimal.

Rats bearing established ascites tumors were also treated with radiolabeled antibodies (Fig. 8B). Rats treated with four weekly i.p. injections of 131 I-RH1 had increased survival time (35.7 days and 1 cure compared to 11.7 days for controls), although the difference was not significant ($P > 0.10$). Treatment with radiolabeled control antibody HB65 also did not result in a significant increase in survival ($P > 0.25$). These results show that treatment of tumor-bearing rats with either camptothecin or radioimmunoconjugates alone was ineffective at curing animals.

Combination Drug and Radioimmunotherapy. The ability of camptothecin to potentiate the therapeutic efficacy of radioimmuno-

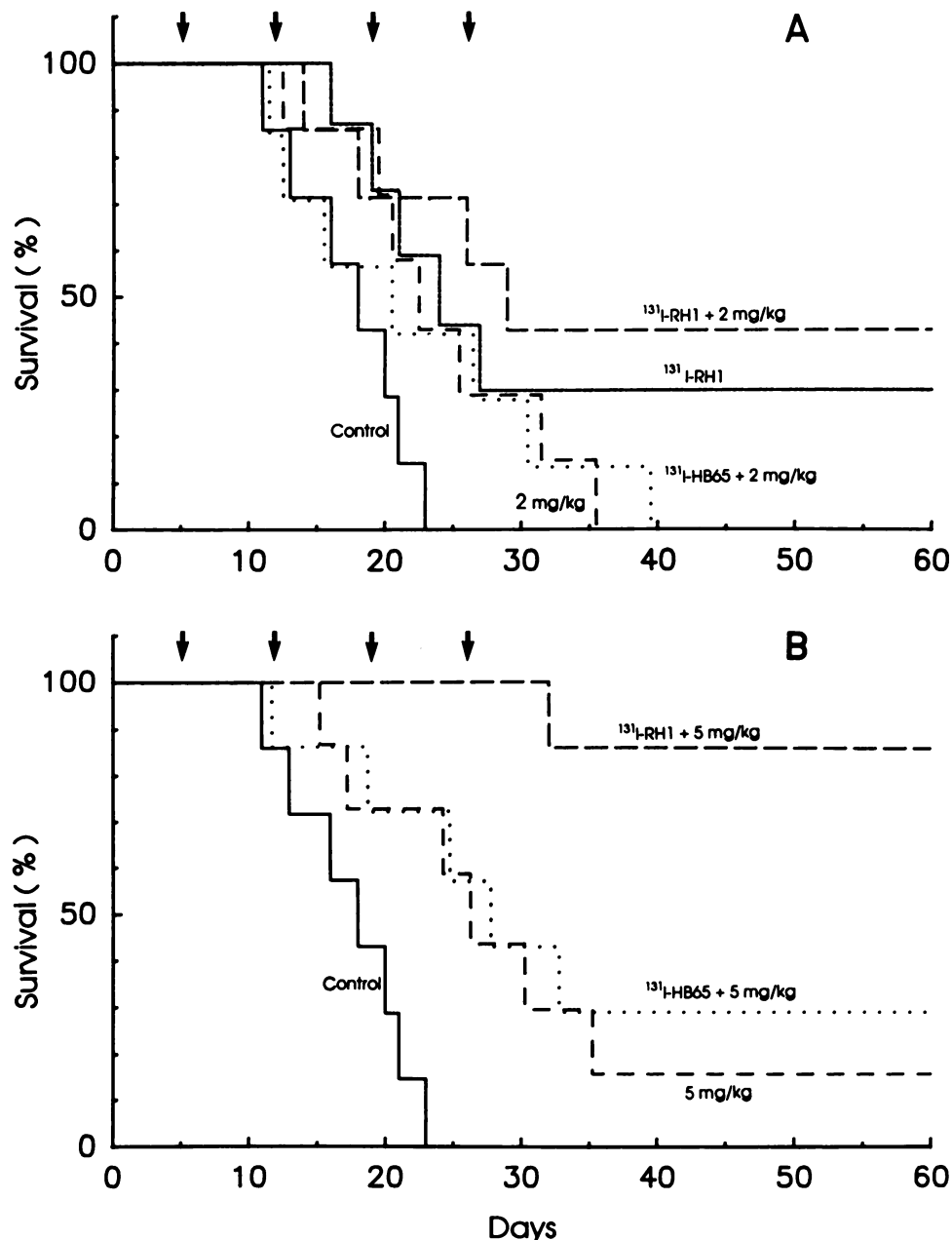


Fig. 9. Potentiation of radioimmunotherapy by combination treatment with camptothecin. Sprague-Dawley rats received i.p. injections of 5×10^6 AS-30D cells on day 0. On day 5, groups of seven rats received i.m. injections of camptothecin in lipiodol followed by an i.p. injection of PBS or radioimmunoconjugates 24 h later. Treatments were repeated on days 12, 19, and 26. Rat survival is displayed on two graphs for clarity. A, survival of rats receiving lipiodol only (—), lipiodol plus 200 μCi ^{131}I -RH1 (- · - ·), 2 mg/kg camptothecin (- - -), 2 mg/kg camptothecin plus 200 μCi ^{131}I -HB65 (···), or 2 mg/kg camptothecin plus 200 μCi ^{131}I -RH1 (— · —). B, survival of tumor-bearing rats treated with lipiodol only (—), 5 mg/kg camptothecin (- - -), 5 mg/kg camptothecin plus ^{131}I -HB65 (···), or 5 mg/kg camptothecin plus ^{131}I -RH1 (— · —). Arrows, treatments.

therapy was examined in groups of seven Sprague-Dawley rats bearing established hepatoma ascites. Treatment, initiated 5 days after tumor implantation, consisted of weekly i.m. injections of camptothecin suspended in lipiodol, a mixture of the ethyl esters of the iodized fatty acids of poppy-seed oil, followed 24 h later by i.p. administration of radiolabeled antibody. Camptothecin was administered as an oily suspension to provide sustained release of the drug during localization of radiolabeled antibody at tumor cells (43). Fig. 9 shows the survival curves of different treatment groups. Summaries of survival times and statistical analysis of differences in survival times are shown in Table 1. Untreated rats had a mean survival time of 17.4 days, and all animals died by day 23. Treatment with 2 mg/kg camptothecin did not significantly prolong survival ($P > 0.05$). Rats receiving 5 mg/kg camptothecin had significantly increased survival times ($P < 0.025$), but six of seven rats eventually succumbed to tumors. Radioimmunotherapy with ^{131}I -RH1 resulted in two long-term survivors of seven rats. Combination therapy with camptothecin and ^{131}I -RH1, however, was much more effective. Eighty-six % (6 of 7) of animals receiving

5 mg/kg camptothecin plus ^{131}I -RH1 were cured of cancer. This treatment was significantly more effective than 5 mg/kg camptothecin alone ($P < 0.005$) or ^{131}I -RH1 alone ($P < 0.025$). Enhancement of RH1 radioimmunotherapy by camptothecin was specific, as shown by the finding that combination of camptothecin with control radioimmunoconjugate ^{131}I -HB65 was not better than treatment with camptothecin alone ($P > 0.25$).

DISCUSSION

Increasing evidence is accumulating that topo I inhibitors can potentiate the lethal effects of ionizing radiation on tumor cells. Type I topoisomerases generate single-strand DNA breaks and relieve torsional stress by unwinding duplex DNA (44). During the relaxation reaction, a covalent bond is formed between a tyrosine group of topo I and the 3' phosphoryl end of the broken DNA strand, creating a transient protein-DNA crosslink (45). Camptothecin acts to stabilize this complex, preventing the religation of the DNA strand (28). In-

Table 1 Survival times of hepatoma-bearing rats after single agent or combination treatment with camptothecin and radiolabeled antibodies

Groups of 7 rats bearing established AS-30D hepatoma ascites received four rounds of treatment as described in the legend to Fig. 9. Statistically significant differences in the survival outcomes between treatment and comparison groups were estimated by the Mantel-Haenszel χ^2 test of association (40). *P* values less than 0.05 are considered significant.

Treatment group	Mean survival time (days)	Increase in life span (%)	Cures	Statistical analysis	
				Comparison group	<i>P</i>
Control	17.4	0	0/7		
Camptothecin (2 mg/kg)	23.4	34	0/7	Control	>0.05
Camptothecin (5 mg/kg)	29.6	70	1/7	Control	<0.025
¹³¹ I-RH1	32.5	87	2/7	Control	<0.025
Camptothecin (2 mg/kg) + ¹³¹ I-HB65	22.9	32	0/7	Control	>0.10
Camptothecin (2 mg/kg) + ¹³¹ I-RH1	38.2	120	3/7	Control	<0.025
Camptothecin (5 mg/kg) + ¹³¹ I-HB65	33.9	95	2/7	Control	<0.025
				Camptothecin (5 mg/kg)	>0.25
Camptothecin (5 mg/kg) + ¹³¹ I-RH1	>60	>245	6/7	Control	<0.001
				Camptothecin (5 mg/kg)	<0.005
				¹³¹ I-RH1	<0.025
				Camptothecin (5 mg/kg) + ¹³¹ I-HB65	<0.05

teraction between the stabilized tertiary complex and the replication fork is thought to convert single-strand breaks into double-strand breaks and cause cell death (30).

Camptothecin has been reported to potentiate the lethal effects of radiation on radioresistant human malignant melanoma cells (23), a bladder carcinoma cell line (20), and a human squamous carcinoma cell line (24). Topotecan, a water-soluble analogue of camptothecin which also acts as a topo I poison, has also been reported to act synergistically with radiation in several cell models (21, 22). Documentation of synergistic effects, however, is complicated by the non-linearity of the survival curves resulting from camptothecin or radiation treatment. Claims of supraadditive interactions must take into account the nonadditive responses of each agent.

In the present study, isobologram analysis was used to rigorously determine the nature of the effects of interactions between camptothecin and radiation on cells. Isobologram analysis involves generating curves of equal cytotoxic effect from data on each individual agent and allows valid comparisons to be made between agents with different modes of action and different response curves (38). Using this method, we found that addition of camptothecin immediately following γ -ray irradiation resulted in synergistic lethality to cultured cells at radiation doses greater than 200 rad. Synergy between camptothecin and radiation could result from stimulation of processes involved in the repair of damage caused by ionizing radiation. The increased interaction of the camptothecin stabilized topo I-DNA complex with the replication machinery involved in the repair process could lead to increased numbers of double-strand breaks (46). Detailed molecular analysis of the interaction between camptothecin and radiation is needed to determine the actual mechanism of synergy. Pretreatment of cells with camptothecin, in contrast, was ineffective at potentiating the effect of radiation and, in fact, resulted in subadditive cell killing in most cases. The extended period between camptothecin treatment and radiation exposure may have allowed repair of drug-induced lesions. Subadditive interaction between cells preexposed to camptothecin and later treated with radiation could result from a number of mechanisms, such as perturbation of the cell cycle to less radiation-sensitive phases (47, 48) or stimulation of processes involved in repairing DNA damage.

The primary purpose of this work was to determine whether camptothecin can act to potentiate the efficacy of radioimmunotherapy.

Mab RH1, generated against AS-30D hepatoma cells, was found to bind a M_r 32,000 antigen expressed on the cell membrane of these cells. Animal studies demonstrated that ¹³¹I-RH1 could specifically localize in hepatoma ascites tumors *in vivo*. Mab RH1 remained associated with ascites tumor cells for an extended period with a half-life exceeding 100 h. Control antibody, in contrast, did not localize in AS-30D ascites tumors, demonstrating the specificity of Mab RH1 localization.

In vitro results indicated that camptothecin should be present immediately after radiation exposure to potentiate radiation lethality to tumor cells. During radioimmunotherapy, however, tumor cells are exposed to radiation over an extended period as the radioimmunoconjugate accumulates in the tumor. Camptothecin was, therefore, injected into the leg muscles as an oily suspension 24 h prior to administration of radiolabeled antibodies to allow slow release of drug during radioimmunotherapy (43). Therapy was initiated 5 days after implantation of a large number of AS-30D cells to allow development of established ascites. AS-30D ascites grew rapidly *in vivo*; control animals consistently succumbed to tumors within 2–3 weeks. Four rounds of treatment with camptothecin and ¹³¹I-RH1, in contrast, resulted in the cure of 86% of animals without any apparent increase in toxicity compared to treatment with camptothecin alone. This treatment was superior to therapy with either single agent. In fact, camptothecin was ineffective at eliciting cures in tumor-bearing animals. These results show that camptothecin can potentiate the efficacy of radiation delivered to tumors by radiolabeled antibodies.

A fundamental limitation of radioimmunotherapy for human cancer treatment is the low amounts of antibody that can localize in tumors. Monoclonal antibody uptake into solid tumors normally accounts for only 0.0006–0.02% of the total injected dose per gram of tumor (49–51). Dose-limiting toxicity associated with large amounts of radiolabeled antibodies circulating throughout the body is often reached before a therapeutic radiation dose can be delivered to the tumor site (5, 6). The results of the current study suggest that camptothecin treatment combined with radioimmunoconjugates could increase the therapeutic outcome of radioimmunotherapy. Although ascites tumors were examined in the current study, camptothecin may also be effective against solid tumors in combination with radioimmunotherapy. Early clinical studies of camptothecin documented unpredictable toxicity in human patients (52–54). These studies, however, used the

sodium salt of camptothecin, which has subsequently been shown to possess much lower antitumor activity than the lactone form of camptothecin (46). More recent studies have found that the lactone form of camptothecin displays impressive activity against a wide range of solid human tumors (55) and shows activity against multiple-drug resistant tumor cells (56, 57). Several analogues of camptothecin with improved formulation characteristics have also been shown to display activity against solid tumors (58, 59). Since these analogues also act by inhibiting topo I activity, they are also expected to potentiate the effects of radiolabeled antibodies.

In summary, this report provides *in vitro* and *in vivo* evidence that camptothecin can increase the therapeutic efficacy of radioimmunotherapy. It is suggested that the combination of topo I inhibitors with radioimmunoconjugates may help overcome the limitations of low antibody uptake in tumors and increase the usefulness of radioimmunotherapy as a treatment modality for cancer.

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REFERENCES

- Larson, S. M. Radioimmunology. Imaging and therapy. *Cancer* (Phila.), 67 (Suppl.): 1253s-1260s, 1991.
- Britton, K. E. Overview of radioimmunotherapy: a European perspective. *Antib. Immunoconjug. Radiopharm.*, 4: 133-150, 1991.
- Order, S. E., Sleeper, A. M., Stillwagon, G. B., Klein, J. L., and Lechner, P. K. Radiolabeled antibodies: results and potential in cancer therapy. *Cancer Res.*, 50 (Suppl.): 1011s-1013s, 1990.
- Yu, M-H., Roffler, S. R., Hwang, W-S., Tung, E., Chen, W-L., and Yeh, M-Y. Localization and therapy of human cervical tumor xenografts with radiolabeled monoclonal antibody 1H10. *Gynecol. Oncol.*, 47: 93-101, 1992.
- Sands, H., and Jones, P. L. Physiology of monoclonal antibody accretion by tumors. In: D. M. Goldenberg (ed.), *Cancer Imaging with Radiolabeled Antibodies*, pp. 97-122. Norwell, MA: Kluwer Academic Publishers, 1990.
- Larson, S. M. Improved tumor targeting with radiolabeled, recombinant, single-chain, antigen-binding protein. *J. Natl. Cancer Inst.*, 82: 1173-1174, 1990.
- Brown, B. A., Comeau, R. D., Jones, P. L., Liberatore, F. A., Neacy, W. P., Sands, H., and Gallagher, B. M. Pharmacokinetics of the monoclonal antibody B72.3 and its fragments labeled with either ¹²⁵I or ¹¹¹In. *Cancer Res.*, 47: 1149-1154, 1987.
- Munz, D. L., Alavi, A., Koprowski, H., and Herlyn, D. Improved radioimmunotherapy of human tumor xenografts by a mixture of monoclonal antibody F(ab)₂ fragments. *J. Nucl. Med.*, 27: 1739-1745, 1986.
- Colcher, D., Bird, R., Roselli, M., Hardman, K. D., Johnson, S., Pope, S., Dodd, S. W., Pantoliano, M. W., Milenic, D. E., and Schlom, J. *In vivo* tumor targeting of a recombinant single-chain antigen-binding protein. *J. Natl. Cancer Inst.*, 82: 1191-1197, 1990.
- Schlom, J. New approaches to improved antibody targeting. *Antib. Immunoconjug. Radiopharm.*, 4: 819-828, 1991.
- Smyth, M. J., Pietersz, G. A., and McKenzie, I. F. Use of vasoactive agents to increase tumor perfusion and the antitumor efficacy of drug-monoconal antibody conjugates. *J. Natl. Cancer Inst.*, 79: 1367-1373, 1987.
- Greiner, J. W., Guadagni, F., Goldstein, D., Smalley, R. V., Borden, E. C., Simpson, J. F., Molinolo, A., and Schlom, J. Intraperitoneal administration of interferon- γ to carcinoma patients enhances expression of tumor-associated glycoprotein-72 and carcinoembryonic antigen on malignant ascites cells. *J. Clin. Oncol.*, 10: 735-746, 1992.
- Greiner, J. W., Guadagni, F., Noguchi, P., Pestka, S., Colcher, D., Fisher, P. B., and Schlom, J. Recombinant interferon enhances monoclonal antibody-targeting of carcinoma lesions *in vivo*. *Science* (Washington DC), 235: 895-898, 1987.
- Hawkins, G. A., McCabe, R. P., Kim, C-H., Subramanian, R., Bredehorst, R., McCullers, G. A., Vogel, C-W., Hanna, M. G., Jr., and Pomato, N. Delivery of radionuclides to pretargeted monoclonal antibodies using dihydrofolate reductase and methotrexate in an affinity system. *Cancer Res.*, 53: 2368-2373, 1993.
- Rowlinson-Busza, G., Hnatowich, D. J., Ruszkowski, M., Snook, D., and Epenetos, A. A. Xenograft localization using pretargeted streptavidin-conjugated monoclonal antibody and ¹¹¹In-labeled biotin. *Antib. Immunoconjug. Radiopharm.*, 6: 97-109, 1993.
- Belli, J. A., and Piro, A. J. The interaction between radiation and Adriamycin damage in mammalian cells. *Cancer Res.*, 37: 1624-1630, 1977.
- Phillips, T. L., and Fu, K. K. The interaction of drug and radiation effects on normal tissues. *Int. J. Radiat. Oncol. Biol. Phys.*, 4: 59-64, 1978.
- Vokes, E. E., and Weichselbaum, R. R. Concomitant chemoradiotherapy: rationale and clinical experience in patients with solid tumors. *J. Clin. Oncol.*, 8: 911-934, 1990.
- Choy, H., Rodriguez, F. F., Koester, S., Hilsenbeck, S., and Von Hoff, D. D. Investigation of taxol as a potential radiation sensitizer. *Cancer* (Phila.), 71: 3774-3778, 1993.
- Musk, S. R., and Steel, G. G. The inhibition of cellular recovery in human tumour cells by inhibitors of topoisomerase. *Br. J. Cancer*, 62: 364-367, 1990.
- Mattern, M. R., Hofmann, G. A., McCabe, F. L., and Johnson, R. K. Synergistic cell killing by ionizing radiation and topoisomerase I inhibitor topotecan (SK&F 104864). *Cancer Res.*, 51: 5813-5816, 1991.
- Kim, J. H., Kim, S. H., Kolozsvary, A., and Khil, M. S. Potentiation of radiation response in human carcinoma cells *in vitro* and murine fibrosarcoma *in vivo* by topotecan, an inhibitor of DNA topoisomerase I. *Int. J. Radiat. Oncol. Biol. Phys.*, 22: 515-518, 1992.
- Boothman, D. A., Wang, M., Schea, R. A., Burrows, H. L., Strickfaden, S., and Owens, J. K. Posttreatment exposure to camptothecin enhances the lethal effects of X-rays on radioresistant human malignant melanoma cells. *Int. J. Radiat. Oncol. Biol. Phys.*, 24: 939-948, 1992.
- Boscia, R. E., Korbut, T., Holden, S. A., Ara, G., and Teicher, B. A. Interaction of topoisomerase I inhibitors with radiation in *cis*-diamminedichloroplatinum(II)-sensitive and -resistant cells *in vitro* and in the FSAIIC fibrosarcoma *in vivo*. *Int. J. Cancer*, 53: 118-123, 1993.
- Fleischmann, G., Pflugfelder, G., Steiner, E. K., Javaherian, K., Howard, G. C., Wang, J. C., and Elgin, S. C. R. *Drosophila* DNA topoisomerase I is associated with transcriptionally active regions of the genome. *Proc. Natl. Acad. Sci. USA*, 81: 6958-6962, 1984.
- Brill, S. J., DiNardo, S., Voelkel-Meiman, K., and Sternglanz, R. Need for DNA topoisomerase activity as a swivel for DNA replication for transcription of ribosomal RNA. *Nature* (Lond.), 326: 414-416, 1987.
- Wall, M. E., Wani, M. C., Cook, C. E., Palmer, K. H., McPhail, A. T., and Sim, G. A. Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. *J. Am. Chem. Soc.*, 88: 3888-3890, 1966.
- Hsiang, Y-H., and Liu, L. F. Identification of mammalian DNA topoisomerase I as an intracellular target of the anticancer drug camptothecin. *Cancer Res.*, 48: 1722-1726, 1988.
- Eng, W-K., Faucette, L., Johnson, R. K., and Sternglanz, R. Evidence that DNA topoisomerase I is necessary for the cytotoxic effects of camptothecin. *Mol. Pharmacol.*, 34: 755-760, 1988.
- Hsiang, Y-H., Lihou, M. G., and Liu, L. F. Arrest of replication forks by drug-stabilized topoisomerase I-DNA cleavable complexes as a mechanism of cell killing by camptothecin. *Cancer Res.*, 49: 5077-5082, 1989.
- Yeh, M-Y., Hellstrom, I., Brown, J. P., Warner, G. A., Hansen, J. A., and Hellstrom, K. E. Cell surface antigens of human melanoma identified by monoclonal antibody. *Proc. Natl. Acad. Sci. USA*, 76: 2927-2931, 1979.
- Ey, P. L., Prowse, S. J., and Jenkin, C. R. Isolation of pure IgG₁, IgG_{2a} and IgG_{2b} immunoglobulins from mouse serum using protein A sepharose. *Immunochemistry*, 15: 429-436, 1978.
- Yeh, M-Y., Roffler, S. R., and Yu, M-H. Doxorubicin-monoconal antibody conjugate for therapy of human cervical carcinoma. *Int. J. Cancer*, 51: 274-282, 1992.
- Johnstone, A., and Thorpe, R. *Immunochemistry in Practice*. Ed. 2, pp. 280-282. Oxford, England: Blackwell Scientific Publications, 1987.
- Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* (Lond.), 227: 680-685, 1970.
- Roffler, S. R., Yu, M-H., Chen, B. M., Tung, E., and Yeh, M-Y. Therapy of human cervical carcinoma with monoclonal antibody-*Pseudomonas* exotoxin conjugates. *Cancer Res.*, 51: 4001-4008, 1991.
- Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Klenk, D. C. Measurement of protein using bicinchoninic acid. *Anal. Biochem.*, 150: 76-85, 1985.
- Steel, G. G., and Peckham, M. J. Exploitable mechanisms in combined radiotherapy-chemotherapy: the concept of additivity. *Int. J. Radiat. Oncol. Biol. Phys.*, 5: 85-91, 1979.
- Moshakis, V., Bailey, M. J., Ormerod, M. G., Westwood, J. H., and Neville, A. M. Localization of human breast-carcinoma xenografts using antibodies to carcinoembryonic antigen. *Br. J. Cancer*, 43: 575-581, 1981.
- Selvin, S. *Statistical Analysis of Epidemiologic Data*, pp. 302-305. New York: Oxford University Press, 1991.
- Tobey, R. A. Effects of cytosine arabinoside, daunomycin, mithramycin, azacytidine, Adriamycin, and camptothecin on mammalian cell cycle traverse. *Cancer Res.*, 32: 2720-2725, 1972.
- Tsao, Y-P., D'Arpa, P., and Liu, L. F. The involvement of active DNA synthesis in camptothecin-induced G₂ arrest: altered regulation of p34^{cdc2}/cyclin B. *Cancer Res.*, 52: 1823-1829, 1992.
- Konno, T. Targeting cancer chemotherapeutic agents by use of lipidol contrast medium. *Cancer*, 66: 1897-1903, 1990.
- Zhang, H., D'Arpa, P., and Liu, L. F. A model for tumor cell killing by topoisomerase poisons. *Cancer Cells* (Cold Spring Harbor), 2: 23-27, 1990.
- Liu, L. F. DNA topoisomerase poisons as antitumor drugs. *Annu. Rev. Biochem.*, 58: 351-375, 1989.
- Slichenmyer, W. J., Rowinsky, E. K., Donehower, R. C., and Kaufmann, S. H. The current status of camptothecin analogues as antitumor agents. *J. Natl. Cancer Inst.*, 85: 271-291, 1993.
- Terasima, R., and Tolmarch, L. J. X-ray sensitivity and DNA synthesis in synchronous

- populations of HeLa cells. *Science* (Washington DC), *140*: 490–492, 1963.
48. Sinclair, W. K., and Morton, R. A. X-ray sensitivity during the cell generation cycle of cultured Chinese hamster cells. *Radiat. Res.*, *29*: 450–474, 1966.
 49. Douillard, J. Y., Lehur, P. A., Aillet, G., Kremer, M., Bianco-Arco, A., Peltier, P. and Chatal, J. F. Immunohistochemical antigenic expression and *in vivo* tumor uptake of monoclonal antibodies with specificity for tumors of the gastrointestinal tract. *Cancer Res.*, *46*: 4221–4224, 1986.
 50. Greager, J. A., Brown, J. M., Pavel, D. G., Garcia, J. L., Blend, M., and Das Gupta, T. K. Localization of human sarcoma with radiolabeled monoclonal antibody. A preliminary study. *Cancer Immunol. Immunother.*, *23*: 148–154, 1986.
 51. Rosenblum, M. G., Kavanagh, J. J., Burke, T. W., et al. Clinical pharmacology, metabolism, and tissue distribution of ⁹⁰Y-labeled monoclonal antibody B72.3 after intraperitoneal administration. *J. Natl. Cancer Inst.*, *83*: 1629–1636, 1991.
 52. Gottlieb, J. A., Guarino, A. M., Call, J. B., Oliverio, V. T., and Block, J. B. Preliminary pharmacologic and clinical evaluation of camptothecin sodium (NSC-100880). *Cancer Chemother. Rep.*, *54*: 461–470, 1970.
 53. Muggia, F. M., Creaven, P. J., Hansen, H. H., Cohen, M. H., and Selawry, O. S. Phase I clinical trials of weekly and daily treatment with camptothecin (NSC 100880). Correlation with preclinical studies. *Cancer Chemother. Rep.*, *56*: 515–521, 1972.
 54. Gottlieb, J. A., and Luce, J. K. Treatment of malignant melanoma with camptothecin (NSC 100880). *Cancer Chemother. Rep.*, *56*: 103–105, 1972.
 55. Giovanella, B. C., Hinz, H. R., Kozielski, A. J., Stehlin, J. S., Jr., Silber, R., and Potmesil, M. Complete growth inhibition of human cancer xenografts in nude mice by treatment with 20-(S)-camptothecin. *Cancer Res.*, *51*: 3052–3055, 1991.
 56. Naito, M., Hamada, H., and Tsuruo, T. ATP/Mg²⁺-dependent binding of vincristine to the plasma membrane of multidrug-resistant K562 cells. *J. Biol. Chem.*, *263*: 11887–11891, 1988.
 57. Chen, A. Y., Yu, C., Potmesil, M., Wall, M. E., Wani, M. C., and Liu, L. F. Camptothecin overcomes MDR1-mediated resistance in human KB carcinoma cells. *Cancer Res.*, *51*: 6039–6044, 1991.
 58. Giovanella, B. C., Stehlin, J. S., Wall, M. E., Wani, M. C., Nicholas, A. W., Liu, L. F., Silber, R., and Potmesil, M. DNA topoisomerase I-targeted chemotherapy of human colon cancer in xenografts. *Science* (Washington DC), *246*: 1046–1048, 1989.
 59. Burris, H. A., Rothenberg, M. L., Kuhn, J. G., and Von Hoff, D. D. Clinical trials with the topoisomerase I inhibitors. *Semin. Oncol.*, *19*: 663–669, 1992.

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