Experimental Photoimmunotherapy of Hepatic Metastases of Colorectal Cancer with a 17.1A Chlorin_{e6} Imunoconjugate

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ABSTRACT

Photoimmunotherapy (using a monoclonal antibody-targeted photosensitizer and red light) may be a strategy to overcome the limitations inherent in photodynamic therapy of liver tumors. The aims of this study were (a) to test the efficacy of selective treatment of hepatic metastases of colorectal cancer in an orthotopic murine xenograft using the murine monoclonal antibody 17.1A conjugated to the photosensitizer chlorin_{e6}, and (b) to compare the tumor response after the same light dose was delivered at two different fluence rates. Based on previous biodistribution studies that had shown that the photoimmunoconjugate with a polyanionic charge had both a higher absolute tumor chlorin_{e6} content and a greater tumor:normal liver ratio than those obtained with a photoimmunoconjugate bearing a polycationic charge, mice were treated 3 h after i.v. injection of the polyanionic 17.1A chlorin_{e6} conjugate or unconjugated photosensitizer. Red light was delivered into the liver tumor by an interstitial fiber, and tumor response end points were total tumor weight in the short term and survival in the long term. There was a highly significant reduction (<20% of controls; \( P = 0.0035 \)) in the weight of the tumors in the mice treated with photoimmunotherapy, and the median survival increased from 62.5 to 102 days (\( P = 0.015 \)). Photodynamic therapy with free chlorin_{e6} produced a smaller decrease in tumor weight and a smaller extension of survival, neither of which were statistically significant. A comparison of photoimmunotherapy with 10 J of light delivered at 30 or 300 mW showed that the higher fluence rate prolonged survival significantly more than the lower fluence rate. This may have been because the high fluence rate gave a contribution of laser-induced hyperthermia to the photodamage. Correlation studies showed that the amount of normal liver remaining at necropsy correlated best with survival. Photoimmunotherapy shows efficacy in destroying liver tumors, and future studies should maximize selectivity to minimize the destruction of normal liver.

INTRODUCTION

Colorectal cancer frequently metastasizes to the liver, where surgical resection can be difficult, and consequently other methods of locally attacking the tumors are being investigated. It has been proposed that PDT could fulfill a role in destroying hepatic metastases (1), but it has been more frequently argued that PDT is unsuitable for treating intrahepatic tumors for two reasons. Most PSs have a high tissue reduction (<20% of controls; \( P = 0.0035 \)) to compare the tumor response after the same light dose was delivered at two different fluence rates. Based on previous biodistribution studies that had shown that the photoimmunoconjugate with a polyanionic charge had both a higher absolute tumor chlorin_{e6} content and a greater tumor:normal liver ratio than those obtained with a photoimmunoconjugate bearing a polycationic charge, mice were treated 3 h after i.v. injection of the polyanionic 17.1A chlorin_{e6} conjugate or unconjugated photosensitizer. Red light was delivered into the liver tumor by an interstitial fiber, and tumor response end points were total tumor weight in the short term and survival in the long term. There was a highly significant reduction (<20% of controls; \( P = 0.0035 \)) in the weight of the tumors in the mice treated with photoimmunotherapy, and the median survival increased from 62.5 to 102 days (\( P = 0.015 \)). Photodynamic therapy with free chlorin_{e6} produced a smaller decrease in tumor weight and a smaller extension of survival, neither of which were statistically significant. A comparison of photoimmunotherapy with 10 J of light delivered at 30 or 300 mW showed that the higher fluence rate prolonged survival significantly more than the lower fluence rate. This may have been because the high fluence rate gave a contribution of laser-induced hyperthermia to the photodamage. Correlation studies showed that the amount of normal liver remaining at necropsy correlated best with survival. Photoimmunotherapy shows efficacy in destroying liver tumors, and future studies should maximize selectivity to minimize the destruction of normal liver.

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5 The abbreviations used are: PDT, photodynamic therapy; 17.1A-pl-c_{e6}-succ, conjugate between 17.1A monoclonal antibody and succinylated polyllysine chlorin_{e6}; c_{e6}, chlorin_{e6}; Mab, monoclonal antibody; PIC, photoimmunoconjugate; PIT, photodynamic therapy; PS, photosensitizer.

MATERIALS AND METHODS

Mice. All experiments were carried out with the approval of the Subcommittee on Research Animal Care of Massachusetts General Hospital and were...
in accord with the NIH Health Guide for the Care and Use of Laboratory Animals. Female Swiss nude mice (Cox Breeding Laboratories, Cambridge, MA; 2–3 weeks old; weighing 20–25 g) were kept in a barrier room under permanently sterile conditions to avoid any infections and had continual access to food and water ad libitum. Throughout the experiment, mice were housed in laminar flow racks under specific pathogen-free conditions and were monitored daily for general health status. Mice were sacrificed when their tumor burden interfered with normal living or when they became moribund.

**Cell Line and Mab.** HT29 tumor cell line derived from a human colorectal adenocarcinoma was obtained from ECACC (CAMR Centre for Applied Microbiology & Research, Wiltshire, UK) and used for all experiments. Cells were grown in DMEM/F12 (50/50 mixture) containing 15 mM HEPES and L-glutamine, were supplemented with 10% heat-inactivated fetal bovine serum (Whittaker Bioproducts, Walkersville, MD), 100 units/ml penicillin, and 100 μg/ml streptomycin, and were maintained in an incubator at 37°C in an atmosphere of 5% CO2. 17.1A murine Mab was a kind gift from Centocor (Malvern, PA).

**Preparation and Characterization of PIC.** This has been described previously (16). Briefly, 17.1A IgG was partially reduced with mercaptoethanol hydrochloride and reacted with a succinylated poly-l-lysine c6 conjugate, which had been derivatized with a heterofunctional reagent bearing a pyridylidihydriopropionamide group to form a disulfide bond between the IgG hinge sulfhydryl group and the succinylated poly-l-lysine c6 conjugate. There was an average of two poly-l-lysine chains and between eight and nine molecules of c6 per Mab, and the immunoreactivity of the PIC was demonstrated by two-color direct/indirect immunofluorescence and ELISA assays (16).

**Animal Model.** A xenograft model for liver metastases of colorectal cancer consisting of a discrete tumor within one lobe of the liver was used for the experiments. Mice were anesthetized by inhalation of Metofane (Pitman-Moore Inc, Mundelein, IL); 2 ml of liquid vaporized in a 500-ml closed container. Under aseptic conditions, mice were placed supine, and a 1-cm left median incision (starting from the subcostal region) was made through the skin and peritoneum to expose the left lateral lobe of the liver. That lobe was lifted out of the abdominal cavity and secured in place by positioning a sterile cotton-tipped stick inferior to the lobe. HT-29 cells (5 × 10⁶ or 20 × 10⁶) in 50 μl of sterile DMEM F/12 were injected between the upper surface of the lobe parenchyma and the liver capsule using a 30-gauge needle, and the lobe was then returned into the peritoneal cavity. The puncture wound in the capsule was sterilized with 100 μl of povidone iodine 10% (Clinidine Solution, Clinipad Corp., Guilford, CT). The peritoneum and abdominal wall were then closed with sterile Ethilon 4–0 monofilament nylon sutures (Ethicon Inc, Somerville, NJ), and the mice were monitored and kept warm until completely recovered.

**Intestinal Illumination.** PIT/PDT was performed 9 days after injection of 5 × 10⁶ HT29 cells, or 7 days after implantation of 20 × 10⁶ HT29 cells in the left lateral lobe of the liver. These time points were chosen because the tumor was still localized within the lobe and showed the appropriate size (5–9 mm in diameter) to perform intestinal PDT. Mice were anesthetized using Metofane and given i.v. injections (into the tail vein) of the PIC or PS dissolved in sterile filtered PBS. The dose was 0.25 mg/kg of body weight of the c6eq equivalent, which is equal to ~8.3 nmol of c6eq equivalent/mouse and in the case of the PIC, to 140 μg of IgG protein/mouse. The volume injected was ~40 μl of a solution of 200 μL c6eq equivalent, and the injection lasted 20–30 s for each mouse. Three h later, mice were again anesthetized, and the tumor-bearing lobe of the liver was exposed as described above. An argon-pumped dye laser (Coherent, Palo Alto, CA), focused through a ×10 microscope objective onto the proximal end of an optical fiber, was used to deliver 666 nm of light for the PIC and 654 nm of light for fixed c6 (interstitial via a 7.0 mm × 0.4-mm cylindrically diffusing fiberoptic tip (built in house). These wavelengths were previously shown (16) to correspond with the absorption maxima of the conjugate and free c6. The power output was measured by an integrating sphere and an oscilloscope. A 22-gauge catheter was introduced longitudinally into the tumor-bearing lobe so that the tip passed through the tumor and the entire diffusing tip was passed through the catheter, which was then withdrawn. The diffusing tip was then centered in the tumor using forces to detect its presence within the lobe. After treatment, the surgical wound was closed as described above.

**Phototoxicity and Extent of Liver Damage in Normal Mice.** This study was carried out with non-tumor-bearing mice. 17.1A-pl-c6eq-succ (0.25 mg/kg of c6eq equivalent) was injected as described, and illumination was performed after 3 h. Light was delivered as described above into the normal left lateral lobe of the mouse liver. Two fluence rates were used, 100 and 450 mW, and light doses of 20, 40, 60, 80, 100, 150, and 200 J total out of the fiber tip were used. These values corresponded to 143 and 643 mW/cm length of diffusing tip, and the fluences were 28, 57, 86, 114, 143, 214, and 286 J/cm of diffusing tip, respectively. The fluence rate of 450 mW was chosen to determine whether a high fluence rate with attendant possibility of causing thermal damage would produce toxicity in the mice. For an initial screening for toxicity, one mouse was treated at each light dose and fluence rate. Mice were carefully monitored after treatment and left for 4 days in the cages. At the 4th day, mice were sacrificed by carbon dioxide inhalation, and the whole liver was removed. Pieces (3-mm thick) of liver from the treated lobe, the border between treated and normal liver, and untreated liver (right lobe) were dissected and fixed for histology.

**Treatment Study 1.** This study was performed on mice implanted with 5 × 10⁶ HT29 cells which, after 9 days, led to liver tumors with a diameter ranging from 5 to 7 mm. Mice were injected with the 17.1A-pl-c6eq-succ or free c6eq as described above, and 3 h later, the tumor was illuminated with interstitial light. A total light dose of 80 J was delivered at a fluence rate of 100mW over a period of 13.3 min. This corresponded to 114 J at 143 mW/cm of diffusing tip. Three groups of controls were used. Control group 1 had interstitial insertion of the fiber into the tumor but no PS or light. Control group 2 had interstitial insertion of fiber and light delivered but no injection of PS. Control group 3 had an injection of 17.1A-pl-c6eq-succ or free c6eq and interstitial fiber insertion but no light injection. All experimental groups were divided into two subgroups. In the short-term subgroup, mice were sacrificed 9 days after PIT/PDT, and in the long-term subgroup, mice were followed for survival. In control groups 2 and 3, there were six mice in each short-term subgroup and two mice in each long-term subgroup, whereas control group 1 had six mice in the short-term subgroup and three mice in the long-term subgroup. The treatment group with free c6eq had six mice in the short-term subgroup and seven in the long-term subgroup. The treatment group with 17.1A-pl-c6eq-succ had seven mice in the short-term subgroup and nine mice in the long-term subgroup. No mortality occurred during the procedures. The short-term mice were sacrificed 9 days after treatment. The entire liver was removed and weighed. The liver was then dissected, and normal tissue and tumor were weighed separately.

**Treatment Study 2.** The following experiment was carried out to compare the effect of delivering a low light dose at two widely different fluence rates. Three groups of six mice had 20 × 10⁶ HT29 cells in 50 μl of PBS injected in the left lateral lobe of the liver as described previously. This number of cells was chosen to produce a tumor with a more uniform growth rate and hence a more predictable survival time among different control animals. Seven days later, when the tumors had a diameter of 7–9 mm, two groups of six mice were injected with 17.1A-pl-c6eq-succ (0.25 mg/kg of c6eq equivalent) in the tail vein as previously described. Three h later, all three groups were subjected to interstitial placement of the fiber in the tumor-bearing lobe of the liver. The control group received no light, whereas the two injected groups received 10 J of 666 nm light delivered at 30 mW (5.5 min) and 300 mW (33 s). These values correspond to 14 J at 43 or 430 mW/cm of diffusing tip. These mice were followed for long-term tumor response and survival.

**Survival Studies.** Mice for survival were monitored twice daily, and the end point was defined as death attributable to disease or tumor burden that affected the animal’s ability to move or feed normally. At necropsy, the mice were dissected, and the entire liver, hepatic tumor, normal liver, and any metastatic tumor deposits were removed and weighed. All extrahepatic tumor was dissected and weighed.

**Histology.** At necropsy, after weights of tumor and normal liver were obtained, pieces of tissue (200–300 mg) were removed from the right lobe, the treated area of the left lobe, and from any normal liver, which remained within the left lobe, and immediately placed in 10% formalin followed by routine processing for paraffin embedding and histological study. Sections were stained with H&E and examined by light microscopy.

**Statistics.** All values are expressed as ± SE. SEs of the ratios of two means were calculated in quadrature. Comparison between two means was carried out using a two-tailed Student’s t test assuming equal or unequal variances as appropriate. Survival analysis was performed using the Kaplan-Meier method. Survival curves were compared, and differences in survival were tested for significance using a log-rank test in the computer program GraphPad Prism.
**RESULTS**

**Phototoxicology.** No mortality occurred at any light dose. At 200 J and to a lesser extent at 150 J delivered at 450 mW, the mice took 6–12 h to recover completely after emerging from anesthesia. However, after 24 h, they were indistinguishable from mice receiving lower doses of PIT. There were no adverse effects at even the highest light dose of 200 J delivered either at 450 mW or at 100 mW. At 4 days after PIT, the histological examination of the sections taken from the treated left lobe showed extensive geographic zones of coagulative necrosis of liver, with residual viable liver tissue noted especially around portal triads (Fig. 1a). There were lymphocytic infiltrates at the well-defined interface of viable and necrotic liver and extensive coagulative necrosis of liver. Surprisingly, there was only a very weak light dose response in the degree of damage to the treated lobes seen by histology. The lobe treated at 20 J seemed to have slightly less damage than those treated with higher light doses (40–200 J). No abnormalities were seen in sections taken from the untreated right lobes of the livers.

**Short-term Treatment Study.** The mice tolerated the procedure well with no mortality or morbidity. At 9 days after the light treatment, the mice were sacrificed, and the livers were removed and weighed. They were carefully dissected, and the tumor and normal livers were weighed separately. There were three groups of controls comprising: (a) the fiber was inserted into the tumor, but without red light and PS; (b) tumors were treated with interstitial light with no PS injected; and (c) mice received either injections of free c₅₆ or PIC (three mice per drug) followed by the interstitial fiber but no light. There were no significant differences between the mean values for total liver weight, hepatic tumor weight, normal liver weight, or percent of liver replaced by tumor of any of these control groups (data not shown). These figures of the weights from the three control groups were then combined to give a single control group containing 18 mice.

The results of the two treatment groups versus the combined control group are shown in Table 1. Although PDT with free c₅₆ reduced the mean tumor weight to 43% of the control mean weight, this did not reach statistical significance ($P = 0.066$). The reduction in the mean tumor weight after treatment with the 17.1A-pl-c₅₆-succ (18% of control) was, however, highly significant ($P = 0.0035$). PDT with free c₅₆ led to a significant increase in the weight of normal liver (137% of control, $P = 0.044$), and in the case of PIT with 17.1A-pl-c₅₆-succ, the increase was highly significant (151% of control, $P = 0.001$). The mean of the total liver weights was reduced from 1.62 g in controls, to 1.44 g with c₅₆ PDT, to 1.34 g after PIT, but these differences were not significant. When the percentage of the total liver replaced by tumor was calculated, the reduction from 44% in controls to 26% after c₅₆ PDT was not significant ($P = 0.069$), whereas the reduction to 11% after PIT was highly significant ($P < 0.0001$).

The histology of the liver tumors after treatment and control is shown in Fig. 1. b-f. Fig. 1b (light but no PS) shows a moderately differentiated adenocarcinoma growing in and invading normal liver. Mucin droplets can be clearly seen, and there is no evidence of damage. Fig. 1c is from a tumor-bearing lobe after PDT with free c₅₆. Several nodules of viable adenocarcinoma are surrounded by fibrous stroma and at the periphery, necrotic tumor. In Fig. 1, d-f, all sections are from tumor-bearing lobes after PIT. Fig. 1d shows an island of viable metastatic adenocarcinoma, containing mucinous glands, surrounded by zones of hemorrhagic and necrotic tumor and necrotic liver tissue. Fig. 1e shows viable carcinoma surrounded by areas of extensive hemorrhagic necrosis and fibrosis.
PHOTOIMMUNOTHERAPY OF LIVER METASTASES

Mice were treated 9 days after tumor implantation and sacrificed after a further 9 days. The PS injection was 0.25 mg/kg of 17.1A-pl-c\textsubscript{e6} succ i.v. 3 h before treatment, and the light dose was 80 J delivered at 100 mW. Whole livers were removed and weighed and then dissected into normal liver and tumor, which were weighed separately. Values are means ± SE. Two-sided Ps are for differences between weights and the control weight.

### Table 1 Necropsy results of short-term tumor response experiment (5 × 10\textsuperscript{6} HT29 cells)

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Total liver (g)</th>
<th>Hepatic tumor (g)</th>
<th>Normal liver (g)</th>
<th>% liver replaced by tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 18)</td>
<td>1.62 ± 0.15</td>
<td>0.83 ± 0.20</td>
<td>0.79 ± 0.064</td>
<td>44 ± 5</td>
</tr>
<tr>
<td>c\textsubscript{e6} PDT (n = 6)</td>
<td>1.44 ± 0.099 (P = 0.35)</td>
<td>0.36 ± 0.14 (P = 0.066)</td>
<td>1.08 ± 0.10 (P = 0.044)</td>
<td>26 ± 7 (P = 0.069)</td>
</tr>
<tr>
<td>17.1A-pl-c\textsubscript{e6}-succ (n = 7)</td>
<td>1.34 ± 0.033 (P = 0.099)</td>
<td>0.15 ± 0.012 (P = 0.0035)</td>
<td>1.19 ± 0.036 (P = 0.001)</td>
<td>11 ± 1 (P &lt; 0.0001)</td>
</tr>
</tbody>
</table>

Experimental groups were as follows: control, 62.5 days; c\textsubscript{e6} PDT, 77 days; and 17.1A-pl-c\textsubscript{e6}-succ PIT, 102 days. The survival curves were compared by a log-rank test, and the curve for the c\textsubscript{e6} PDT group was not significantly different from that of the control group (P = 0.2), whereas the curves for the 17.1A-pl-c\textsubscript{e6}-succ PIT group and the control group were significantly different (P = 0.015).

At necropsy, the livers were removed and weighed, then dissected into tumor and normal liver and weighed again separately. The total extrahepatic tumor was collected from each mouse and weighed. The location of the extrahepatic tumor was largely s.c., with occasional tumor deposits in the pelvic cavity, including large intestine and stomach, and very occasionally in the axillae, thorax, and neck. The weights are given in Table 2. One mouse treated with 17.1A-pl-c\textsubscript{e6}-succ PIT survived for 144 days; at necropsy, it had no hepatic tumor, its liver weighed 1.36 g, and there was no extrahepatic tumor. There were few significant differences between treatment groups in any of the parameters measured. The mean weight of normal liver in the case of the c\textsubscript{e6} PDT group was significantly higher than the control (P = 0.009), but this difference became less in the case of 17.1A-pl-c\textsubscript{e6}-succ PIT (P = 0.082). The 17.1A-pl-c\textsubscript{e6}-succ PIT group had more than twice as much extrahepatic tumor as the other groups (5.26 g versus 2.05 and 1.66 g), but because of wide variations in individual mice, this did not reach statistical significance.

**PIT 2 Fluence Rate—Long-term Survival.** The second survival experiment used a tumor cell inoculum of 20 × 10\textsuperscript{6} HT29 cells (four times greater than the first experiment). Treatment was performed on the 7th day after injection when the tumor was somewhat larger (7–9 mm in diameter) than the first experiment. The effect of delivering the same relatively small light dose (10 J, one-eighth of the previous fluence) at two widely differing fluence rates (30 mW and 300 mW) was investigated. All of the mice tolerated the procedure well. The Kaplan-Meier curves are shown in Fig. 2B. Median survival times were as follows: control, 29.5 days; 17.1A-pl-c\textsubscript{e6}-succ PIT delivered at 30 mW, 34 days; and 17.1A-pl-c\textsubscript{e6}-succ PIT delivered at 300 mW, 56 days. The survival curve for the 300-mW group was significantly different from that of the control group (P = 0.028), but not from the 30-mW group (P = 0.079).

At necropsy, mice were dissected as before, and whole liver, liver tumor, normal liver, and total extrahepatic tumor were weighed as described. The results are given in Table 2. The 300-mW group had a significantly greater (P = 0.011) mean weight of normal liver than the control group, and the percentage of liver replaced by tumor was also significantly smaller (P = 0.049), whereas the total weight of extrahepatic tumor was significantly greater than controls (P = 0.036). There was also a significant increase (P = 0.045) over controls in total liver weight in the 30-mW group, which was not seen in the 300-mW group.

In Fig. 1, g–h, the histological appearance of sections from the treated liver lobes at necropsy are shown. Fig. 1g is taken from a mouse that was treated with PIT of 80 J delivered at 100 mW in the first survival study and which survived 92 days. It shows viable adenocarcinoma, surrounded by marked fibrosis and remnants of liver tissue; no significant necrosis is evident. Fig. 1h came from a mouse that had PIT with 10 J delivered at 30 mW and survived 39 days. It shows viable adenocarcinoma with large amounts of mucin, surrounded by liver tissue with nuclear pyknosis.

**DISCUSSION**

Hepatic metastases of colorectal cancer are only rarely thought to be eligible for surgical resection attributable to the common occurrence of multifocality, vascular involvement, and extrahepatic disease (18). Even when there is a single metastasis that is amenable to surgical removal, the patient may not be a candidate for surgery. Although systemic chemotherapy often has some initial effect, the metastases frequently develop resistance, and eventually, the tumors become resistant to all available drugs (19). At this point, some type of local therapy is often attempted to prolong life without seriously affecting its quality (20). Such therapies include regional chemotherapy via the hepatic artery (21), cryoablation (22), chemoembolization (23), percutaneous ethanol injection (24), and interstitial laser photocoagulation (25). Although PDT has been suggested for treatment of liver metastases (1), it has not found much support because of the relative lack of selectivity for tumor as opposed to normal liver that accumulates large amounts of clinically used PS. Because Mab con-
jugates have been proposed as targeting vehicles to increase the selectivity of PS for tumors (26), it was attractive to explore the ability of the Mab 17.1A, which is in clinical use for treating liver metastases of colorectal cancer (27), to target a PS to tumor cells while sparing normal liver.

The results from the previous biodistribution study showed that the anionic PIC gave not only higher absolute uptake of $c_\text{e6}$ in the tumor tissue, but also superior selectivity for the tumor over normal liver compared to the cationic 17.1A PIC. Free $c_\text{e6}$, however, gave only very low tissue uptakes, although the selectivity for the tumor was quite good. The 3-h time point after injection was chosen because the tumor: normal liver ratio for 17.1A-pl-$c_\text{e6}$-succ was better at 3 h than at 24 h, and also, the amount of free $c_\text{e6}$ in the tumor was much greater at 3 h.

There have been reports in the literature that PDT using unconjugated PS (especially in small rodents) can produce a systemic toxicity leading to death within 12–36 h after illumination (28–30). This resembles a shock-syndrome with systemic vascular permeability, hypotension, rhabdomyolysis, and death, and it has been shown to occur with PS administered both i.v. and i.p. (31). However, we have observed that with the use of conjugated PS, this systemic toxicity is much reduced or eliminated (32). To confirm this observation in the present study, an increasing series of fluences was interstitially delivered 3 h after i.v. injection of the anionic PIC in non-tumor-bearing mice. Even at the highest fluences (200 and 150 J delivered at 450 mW), the mice, although showing some temporary lethargy, recovered well after 12 h, and after 24 h, were indistinguishable from untreated mice. Histological slides taken 4 days after treatment showed well-confined focal areas of severe liver damage.

The short-term tumor response studies showed that the significant reduction in hepatic tumor found after PIT (18% of controls) was paralleled by a significant increase in the mean weight of normal liver. The percentage of total liver replaced by tumor was only 11% compared to the cationic 17.1A PIC. Free $c_\text{e6}$, however, gave only very low tissue uptakes, although the selectivity for the tumor was quite good. The 3-h time point after injection was chosen because the tumor: normal liver ratio for 17.1A-pl-$c_\text{e6}$-succ was better at 3 h than at 24 h, and also, the amount of free $c_\text{e6}$ in the tumor was much greater at 3 h.

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Table 2: Necropsy of long-term tumor response experiments

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Median survival (days)</th>
<th>Total liver (g)</th>
<th>Hepatic tumor (g)</th>
<th>Normal liver (g)</th>
<th>% liver replaced by tumor</th>
<th>Extrahepatic tumor (g)</th>
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<tbody>
<tr>
<td>First PIT experiment ($5 \times 10^6$ HT29 cells)</td>
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<tr>
<td>Control (n = 7)</td>
<td>62.5</td>
<td>2.07 ± 0.20</td>
<td>1.51 ± 0.29</td>
<td>0.55 ± 0.09</td>
<td>70 ± 8</td>
<td>2.05 ± 0.88</td>
</tr>
<tr>
<td>$c_\text{e6}$ (n = 7)</td>
<td>77 (P = 0.2)</td>
<td>2.72 ± 0.40</td>
<td>1.72 ± 0.34</td>
<td>1.01 ± 0.11 (P = 0.009)</td>
<td>61 ± 5</td>
<td>1.66 ± 0.86</td>
</tr>
<tr>
<td>17.1A-pl-$c_\text{e6}$-succ (n = 9)</td>
<td>102 (P = 0.015)</td>
<td>2.01 ± 0.20</td>
<td>1.25 ± 0.26</td>
<td>0.84 ± 0.12</td>
<td>56 ± 10</td>
<td>5.26 ± 1.65</td>
</tr>
<tr>
<td>Second PIT experiment ($20 \times 10^6$ HT29 cells)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 6)</td>
<td>25.5</td>
<td>1.94 ± 0.10</td>
<td>1.52 ± 0.095</td>
<td>0.41 ± 0.023</td>
<td>78 ± 1</td>
<td>1.37 ± 0.21</td>
</tr>
<tr>
<td>30 mW (n = 6)</td>
<td>34</td>
<td>2.72 ± 0.29 (P = 0.045)</td>
<td>2.17 ± 0.31</td>
<td>0.56 ± 0.80</td>
<td>78 ± 4</td>
<td>3.03 ± 0.8</td>
</tr>
<tr>
<td>300 mW (n = 6)</td>
<td>36 (P = 0.028)</td>
<td>2.04 ± 0.26</td>
<td>1.15 ± 0.32</td>
<td>0.89 ± 0.12 (P = 0.011)</td>
<td>51 ± 10 (P = 0.049)</td>
<td>6.22 ± 1.69 (P = 0.036)</td>
</tr>
</tbody>
</table>

Delivered to normal liver (although a fraction of those delivered to the tumor) were also several times higher for the PIC than for free $c_\text{e6}$.

The increase in survival (control 62 days compared to PIT 102 days) might at first seem disappointing, but considering that the HT29 tumor is fast growing in nude mice with a doubling time of the order of 2–3 days, the increase in survival of 39 days represents many tumor doublings. The second survival experiment studied PIT with light delivered to two widely differing fluence rates, i.e., 30 and 300 mW. The results show that delivering the same low light dose at the higher power gave a significant increase in survival compared to the lower power and that both gave a significant increase in survival over controls. This finding of increased PIT efficacy at a higher fluence rate in vivo contrasts with many reports in the literature that show that low fluence rates tend to give higher PDT efficacy. The explanation usually proposed for these findings is that PDT in vivo may consume most of the oxygen in the tissue, and in addition, lead to rapid onset of vascular shutdown that reduces the reoxygenation of the tissue by the blood flow. This oxygen depletion then sharply reduces the amount of reactive oxygen species generated and consequently reduces the efficacy of the treatment (33, 34). In the present case, an alternative explanation for the finding that higher power led to a significant increase in the efficacy of the treatment must be sought. Such an explanation might lie in the fact that the light delivery in the present study was by interstitial fiber into the liver, which is a particularly high-absorbing tissue (6). It may well be that this energy deposition led to a rise in temperature of a few degrees Celsius, which could cause sufficient hyperthermia to potentiate PDT, without causing any tissue damage when PS was not present. When the low fluence was used, the tissue cooling produced by the circulating blood may have been sufficient to remove any heat generated. This hypothesis could be tested in future work by measurement of the tissue temperatures by interstitial probes. There have been many reports in the literature of PDT being potentiated by mild hyperthermia (35, 36). The combination works best when the hyperthermia is administered after or during the PDT rather than before (37), and in some studies, the combination showed synergism (38). A report by Leunig et al. (39) compared Photofrin PDT of a hamster melanoma at 100 or 200 mW/cm$^2$ and found that the higher fluence that also raised the tissue temperature to 43°C produced a greater tumor response.

At necropsy, the weights of hepatic tumor, normal liver, and extrahepatic tumor were measured. Correlation plots were constructed using all of the mice from the long-term survival experiments to try to establish precisely what relationship the survival time had with weights of liver tumor, normal liver tissue, percentage of liver replaced with tumor, or total tumor burden. The correlation of survival with the amount of normal liver tissue ($r = 0.645$) was clearly the best and showed better correlation than that with the amount of liver tumor ($r = 0.249$) and with the percentage of liver replaced by tumor ($r = 0.544$). The correlation plot with total tumor burden ($r = 0.422$) actually showed a weak inverse correlation, i.e., the longer the mice lived, the more extrahepatic tumor they accumulated. The implication is that extrahepatic tumor is relatively harmless, and even hepatic tumor is probably not the main cause of mortality. If there is a correlation between the amount of normal liver tissue and survival, then too much PIT/PDT-mediated destruction in the liver may not only have destroyed the tumor, but also enough normal liver tissue to shorten life. Conversely, control mice with growing hepatic tumors have their normal liver rapidly replaced by tumor, which also reduces the amount of normal liver. The treated mice that died early with...
only small liver tumors may have had systemic disease from liver destruction. These conclusions reinforce the need for selectivity between normal liver and tumor.

Previous studies have been carried out to investigate the possibility of using PDT on animal models of hepatic metastasis. Nishiwaki et al. (40) used intra-arterial Lipiodol contrast medium to transport photophorbide a to VX-2 liver tumors in rabbits, and surface illumination of 200 J/cm² at a power density of 100 mW/cm². The surface temperature increased by 2.5°C, and they found selective necrosis of tumors with only slight damage to normal liver. Van Hillegersberg (1) used Photofrin (5 mg/kg) and interstitial light (100–1600 J/cm delivered at 200 mW/cm from a diffusing tip) to treat rats with a syngeneic colon cancer implanted in the liver. The best results were seen at 800 J/cm where 60% of the animals were cured. Svaneberg et al. (41) used i.v. 5-aminolevulanic acid (a precursor of protoporphyrin IX) to sensitize liver tumors in a similar rat model. They delivered 100 J/cm² surface illumination at 110 mW/cm² that produced no temperature rise and found a significant reduction in tumor growth rate.

In conclusion, we have demonstrated that a PIC derived from a Mab recognizing a colorectal cancer-associated antigen and bearing a polyanionic charge can efficiently target PS to a model of metastatic liver tumors in a similar rat model. They delivered 100 J/cm² surface illumination at 110 mW/cm² that produced no temperature rise and found a significant reduction in tumor growth rate.

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References

Experimental Photoimmunotherapy of Hepatic Metastases of Colorectal Cancer with a 17.1A Chlorin e6 Immunoconjugate

Marco Del Governatore, Michael R. Hamblin, Christopher R. Shea, et al.

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