Combination Chemotherapy and Photodynamic Therapy with N-(2-Hydroxypropyl)methacrylamide Copolymer-bound Anticancer Drugs Inhibit Human Ovarian Carcinoma Heterotransplanted in Nude Mice

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ABSTRACT

This study characterizes the efficacy and toxicity of: (a) free Adriamycin and N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-Adriamycin conjugate (P-A); (b) free and HPMA copolymer-meso-chlorin e6 monoethylenediamine disodium salt (Mce6) conjugate (P-C) and light-induced photodynamic therapy; and (c) combinations of the HPMA copolymer conjugates (P-A and P-C) in the destruction of human epithelial ovarian carcinoma heterotransplanted in the nude mouse (OVCAR-3). Eight-week-old female nu/nu mice were injected in both flanks with 0.04–0.05 cm3 OVCAR-3 solid tumor dispersed in media. When bilateral tumors reached a minimum volume of 0.18 cm3 (one axis, 2.0-mm minimum) and demonstrated consistent growth, the experiments were initiated. Drugs were given i.v. unless otherwise noted. Tumor-bearing mice were allocated to the following protocols: (a) Adriamycin at 1 mg/kg, P-A at 30 mg/kg (2.2 mg/kg Adriamycin equivalent), and controls (n = 6 each); (b) Mce6 and light (2 h after administration; 650 nm light for 15 min to deliver 220 J/cm2) at 1.25, 2.5, and 5 mg/kg (n = 6 each), 2.5 mg/kg i.p. (n = 4), and controls (n = 6); (c) P-C at 12.5, 25, and 75 mg/kg (1.5, 2.9, and 8.7 mg/kg Mce6 equivalent, respectively) with light (18 h after administration); 650 nm light for 15 min to deliver 220 J/cm2), P-C at 25 mg/kg (2.9 mg/kg Mce6 equivalent) with no light administration, and controls (n = 7 each); and (d) a combination of P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) and P-C (12.5 and 75 mg/kg, 1.5 mg/kg and 8.7 mg/kg Mce6 equivalent, respectively) with and without light (n = 7 each; 18 h after administration; 650 nm light for 15 min to deliver 220 J/cm2) and controls (n = 12). Tumor volumes and animal weights were assessed for significant differences from the treated and control groups by Student’s t test. Adriamycin (1 mg/kg) and P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) caused less than a 10% weight loss, and treated tumor volumes (day 10–32) were significantly less than those of controls (all P < 0.045). Mce6 (2.5–10 mg/kg i.v.), caused tumor regression in 80% of tumors and a shock syndrome in 17–83%. i.p. dosing (2.5 mg/kg) was uniformly fatal. Mce6 at 1.25 mg/kg did not show reproducible efficacy. P-C with light (25 and 75 mg/kg, 2.9 and 8.7 mg/kg Mce6 equivalent, respectively) demonstrated significant tumor destruction (P < 0.003) but not complete ablation. The combinations of P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) plus P-C (12.5 and 75 mg/kg, 1.5 mg/kg and 8.7 mg/kg Mce6 equivalent, respectively) resulted in complete tumor ablation. Free Mce6 demonstrates a narrow margin of safety, which is extended by incorporation into HPMA copolymers. P-A demonstrates safety and efficacy in vivo. The combined chemotherapy and photodynamic therapy of P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) with P-C and light (12.5 and 75 mg/kg, 1.5 and 8.7 mg/kg Mce6 equivalent, respectively) was nontoxic and allowed us to attain a significant improvement in tumor cures than those obtained by P-A or P-C with light alone.

INTRODUCTION

The anticancer agent Adriamycin (doxorubicin) is associated with two significant nonspecific toxicities: cardiotoxicity and bone marrow suppression (1, 2). Adriamycin reversibly stabilizes covalent adducts between topoisomerase II and DNA, resulting in DNA breaks and subsequent cell death (3). Adriamycin also generates free radicals, which damage cellular membranes (4). Reports suggest that the use-limiting cardiotoxicity of Adriamycin is mediated by oxygen radical formation (damaging mitochondrial membranes and decreasing superoxide dismutase activity with further generation of reactive oxygen species), resulting in cellular injury (5). Recently, the potential of water-soluble synthetic polymers as anticancer drug carriers has been recognized (6, 7). HPMA copolymer-anticancer drug conjugates containing as drug attachment and release sites oligopeptide side chains have been designed (8) and studied in detail (for review, see Ref. 9). For example, HPMA copolymer-bound Adriamycin (Fig. 1) demonstrated a longer intravascular half-life (15-fold) and a 100-fold lower peak initial cardiac level compared with free drug, thereby reducing nonspecific toxic reactions (10). Adriamycin also demonstrated the capacity to act in a cooperative fashion with the photosensitizer meso-chlorin e6 monoethylenediamine against ovarian cancer in vitro (11). The combination of Adriamycin and photodynamic therapy has proven to be effective against the surface-spreading mesothelioma in both in vivo and in vitro models (12).

PDT in the treatment of cancer is also associated with the nonspecific toxicity of diffuse photosensitivity. PDT with the hematoporphyrin derivative Photofrin II has been used to eradicate human ovarian epithelial carcinoma in the musculoperitoneum of the nude mouse (13). The mechanism of destruction appears to depend on microcirculatory damage caused by arteriolar constriction and venous thrombosis (14, 15). Cell membrane and mitochondrial damage are concomitant with these events (16) and are attributed to singlet oxygen formation.

Mce6 is a photoactivatable chemical that exhibits pharmacological and photophysical properties better suited to PDT (17). To date, it has shown promise in ovarian cancer applications in combination with...
Adriamycin in vitro (11), but the combination has not been evaluated in vivo.

Both of these powerful anticancer compounds are relatively low molecular weight chemicals with high pharmacokinetic volumes of distribution. Distribution of these compounds into the cell is normally through passive diffusion. By binding these anticancer drugs to polymeric carriers, uptake is limited to endocytosis (pinocytosis; Refs. 9 and 18). The conjugates are lysosomotropic. Through the resulting volume of distribution alterations and cellular processing limitations provided by the HPMA copolymer, an increased margin of safety is usually noted (9, 19). The potential for the combination of P-A administered with P-C has been demonstrated in vivo in the treatment of experimental Neuro 2A neuroblastoma (20) and for the treatment of ovarian cancer in vitro.4

This report characterizes the efficacy and toxicity of free Adriamycin, P-A, free Mce6, with light-induced PDT, P-C with light-induced PDT, and a combination of the HPMA copolymer conjugates (P-A and P-C with light) in the destruction of human epithelial OVCAR-3 cells heterotransplanted in the nude mouse. This documentation justifies full-dose escalation studies of each HPMA copolymer-drug conjugate alone and in combination in vivo and will be correlated with our in vitro predictions in future communications.

MATERIALS AND METHODS

P-A and P-C were prepared as described previously (20). Their structure is shown in Fig. 1. In brief, the conjugates were synthesized using a two-step procedure. In the first step, the polymer precursor was prepared by radical precipitation copolymerization of HPMA and N-methacryloylglucylphenylalanineureglycine p-nitrophenyl ester (19, 20). The polymer precursor contained 7.8 mol% active ester groups ($M_n$ 19,000; $M_w$ : $M_n$ 1.5). In the second step, Adriamycin or Mce6 was bound to the polymer precursor by aminolysis (20, 21). The conjugates were dissolved in methanol and purified on an LH 20 column. The copolymer band was collected, evaporated to dryness, dissolved in deionized water, frozen, and lyophilized. P-A contained 2.4 mol% (7.4 weight%) bound Adriamycin ($\epsilon_{488}$ 1.19 $\times$ 10^4 liter/mole/cm in water). P-C contained 3.3 mol% (11.6 weight%) of bound Mce6 ($\epsilon_{930}$ 1.58 $\times$ 10^4 liter/mole/cm in methanol).

The guidelines for animal care in our institution were followed under an approved protocol from our Institutional Animal Care and Use Committee. Eight-week-old female nu/nu mice were injected (14-gauge trocar) in both flanks with 0.04—0.05 cm3 OVCAR-3 solid tumor dispersed in media. Tumor was obtained from a single donor, which had been previously implanted with 2 million cells from tissue culture in each flank.

Growth of the heterotransplanted tumor was monitored every 2—4 days. Tumor volume was calculated using the formula $V = \frac{4}{3} \pi R_1^2 R_2$, with $R_1$ being the smallest axis measured, as described previously (13). Consistent growth rates and a minimum tumor volume of 0.18 cm^3 (one axis, 2.0-mm minimum) was required before experiments were initiated (day 0). All subsequent tumor and weight measurements were standardized to the day 0 tumor volume (100%). For all experimental groups, each mouse had at least one tumor nodule on each side (33% had more than one). The percentage of tumor volume (day 0, 100%) and weight was determined, and a mean ± SE was derived for each day measured. These volumes and weights were then assessed for significant differences from the control mean tumor volumes by Student’s t test after 25 days.

To evaluate Adriamycin compared with P-A, tumor volumes and animal weights of 1 mg/kg Adriamycin (i.v.), 30 mg/kg P-A (2.2 mg/kg Adriamycin equivalent i.v.), and controls (n = 6 each) were compared.

Free Mce6, with light was administered (i.v. by tail vein) at 1.25, 2.5, 5, and 10 mg/kg (n = 6 each group) on day 0 (light:argon-pumped dye laser (model 600; Cooper Medical Corp., Santa Clara, CA) at 650 nm for 15 min to deliver 220 J/cm^2; the fiber was a flat-cut silicone optical fiber 400 µm in diameter. The light beam size ranged from 7 to 8.6 mm in diameter, as measured with a caliper. The power setting was power density, 244 mW/cm^2; energy density, 220 J/cm^2. Mce6 was activated at 650 nm for 15 min. Controls (n = 6) received PBS (diluent) and no laser light. Four mice received 2.5 mg/kg Mce6, i.p. with light. Two h after injection of Mce6, the mice were anesthetized (sodium pentothal, 95 mg/kg i.p.), and the tumor on one side of each mouse was illuminated. The contralateral tumor of each animal served as an additional no-light control.

A dose-response study of P-C with light (18 h after P-C administration, illumination at 650 nm for 15 min to deliver 220 J/cm^2) at 12.5, 25, and 75 mg/kg (1.5, 2.9, and 8.7 mg/kg Mce6 equivalent, respectively) was compared with a study of control animals who received 25 mg/kg P-C but no light (n = 7 each).

The combination of P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) with P-C (12.5 and 75 mg/kg, 1.5 and 8.7 mg/kg Mce6 equivalent, respectively) and light (18 h after P-C administration, illumination on one side at 650 nm for 15 min to deliver 220 J/cm^2; the fiber was a flat-cut silicone optical fiber 400 µm in diameter. The light beam size ranged from 7 to 8.6 mm in diameter, as measured with a caliper. The power setting was power density, 244 mW/cm^2; energy density, 220 J/cm^2. Mce6 was activated at 650 nm for 15 min. Controls (n = 6) received PBS (diluent) and no laser light. Four mice received 2.5 mg/kg Mce6, i.p. with light. Two h after injection of Mce6, the mice were anesthetized (sodium pentothal, 95 mg/kg i.p.), and the tumor on one side of each mouse was illuminated. The contralateral tumor of each animal served as an additional no-light control.

A dose-response study of P-C with light (18 h after P-C administration, illumination at 650 nm for 15 min to deliver 220 J/cm^2) at 12.5, 25, and 75 mg/kg (1.5, 2.9, and 8.7 mg/kg Mce6 equivalent, respectively) was compared with a study of control animals who received 25 mg/kg P-C but no light (n = 7 each).

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RESULTS

Both Adriamycin (1 mg/kg) and P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) inhibited the growth of s.c. human OVCAR-3 tumors compared with control tumors (all $P < 0.045$; Fig. 2). Both...
were no deaths, and weights remained stable throughout the experiment.

Mce₆ (2.5 mg/kg) followed by light (650 nm light for 15 min, 220 J/cm²) caused ablation of 80% of tumors (Fig. 4). This was statistically significant compared with control tumors (all P < 0.01). Continued tumor growth in nonresponding tumors (20%) was evident from day 21 until the completion of the study. Death occurred in one of six mice (Fig. 5). i.p. dosing at 2.5 mg/kg resulted in 100% mortality or sacrifice within 24 h after light administration because of the shock syndrome.

The administration of 5 and 10 mg/kg Mce₆ (i.v., followed 2 h later with 650 nm light for 15 min, 220 J/cm²) resulted in mortality in five

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**Fig. 2.** Inhibition of OVCAR-3 tumors heterotransplanted in nude mice by Adriamycin (1 mg/kg; ○) and P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent; ●) compared with controls (△). Tumor volumes from day 10 forward were significantly less than controls (all P < 0.045). There was no significant difference between P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) and Adriamycin (1 mg/kg). Although tumor growth was inhibited, there were no cures. Bars, SE.

**Fig. 4.** Percentage of tumor volume in nude mice treated with Mce₆ and light (650 nm, 220 J/cm²): 1.25 mg/kg (○), 2.5 mg/kg (□), 5 mg/kg (+), and 10 mg/kg (■) i.v., 2.5 mg/kg with no light (△), and controls (△). The lack of a significant response to 1.25 mg/kg Mce₆ and light (○) by human ovarian epithelial carcinoma is demonstrated. With Mce₆ and light at 2.5 mg/kg (□), the human OVCAR-3 tumor was destroyed (all P < 0.01 compared with controls). This dose caused mortality (shock syndrome) to one of six mice (17%). Eighty percent of tumors responded to therapy with regression of tumor volume. Continued growth in nonresponding tumors became evident by day 21, but mean tumor volumes remained significantly less than control tumors (all P < 0.01). Mce₆ with light at 5 mg/kg (+) and 10 mg/kg (■) caused mortality in five of six (83%) mice. A shock syndrome developed within 24 h after irradiation. Autopsy specimens revealed Mce₆ aggregation in the liver and lungs without evidence of acute toxicity or hemorrhagic necrosis. The surviving mice (5 and 10 mg/kg) had complete ablation of treated tumors. Bars, SE.

**Fig. 3.** Weight changes in mice receiving free Adriamycin (1 mg/kg; △) and P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent; ■). Weight losses were less than 8% for both drugs. There were no statistically significant differences between the two drug forms. Bars, SE.

**Fig. 5.** Mortality plot for Mce₆ administered at 1.25 mg/kg (■), 2.5 mg/kg (○), 5 mg/kg (□), and 10 mg/kg (●) i.v. and 2.5 mg/kg (△) given i.p. followed by light (650 nm, 220 J/cm²). Mortality (shock syndrome) was noted in doses of ≥2.5 mg/kg.
of six mice who died or were sacrificed within 24 h of light (83%) in both groups (Fig. 5). A shock syndrome was induced similar to that described by Veenhizen et al. (22). In these mice, heart, lung, liver, intestine, spleen, kidney, muscle, skin, and tumor sections were obtained. Aggregates of Mce6 were seen in the lung and liver, but no acute hepatotoxic or alveolar/interstitial reactions were noted. Platelet aggregates and fibrin deposition were also noted in these organs. In the surviving mice, treated tumors were destroyed. Responding tumors in treated animals initially showed blanching, followed by eschar formation within 2–5 days after light was administered. The eschar resolved in all cases by day 10.

P-C with light at 12.5 mg/kg (1.5 mg/kg Mce6 equivalent) was ineffective in inhibiting the growth of heterotransplanted OVCAR-3 tumors and was nontoxic (no deaths, significant weight changes, or activity changes; Fig. 6). P-C with light at 25 and 75 mg/kg (2.9 and 8.7 mg/kg Mce6 equivalent, respectively) significantly inhibited growth (P < 0.003) compared with controls and P-C with light at 12.5 mg/kg (1.5 mg/kg Mce6 equivalent; P = 0.02). P-C with light at 25 mg/kg (2.9 mg/kg Mce6 equivalent) also resulted in two toxic deaths attributed to the shock syndrome described for free Mce6 (2.5–10 mg/kg). Postmortem examinations revealed that the mice that developed this toxic effect had tumor implants overlying the liver, which were illuminated. The shock syndrome was not seen with P-C and light at 75 mg/kg (8.7 mg/kg Mce6 equivalent); however, illuminated tumors were well away from the liver.

The results of the combination chemotherapy and PDT are shown in Fig. 7. In the seven mice receiving the combinations of P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) with P-C (12.5 mg/kg, 1.5 mg/kg Mce6 equivalent) without light, P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) with P-C (12.5 mg/kg, 1.5 mg/kg Mce6 equivalent) with light, P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) with P-C (75 mg/kg, 8.7 mg/kg Mce6 equivalent) without light, and P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) with P-C (75 mg/kg, 8.7 mg/kg Mce6 equivalent) with light (all P < 0.001). The tumor volumes in the group treated with the combination of P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) and P-C (12.5 mg/kg, 1.5 mg/kg Mce6 equivalent) with light were significantly less than the tumors volumes of the controls on day 11 (all P < 0.002), those treated with P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) on day 11 (P < 0.03), and those treated with both P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) and P-C (12.5 or 75 mg/kg, 1.5 or 8.7 mg/kg Mce6 equivalent, respectively) without light (both P < 0.03); the tumor volumes were significantly greater than in those treated with the combination of P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) and P-C (75 mg/kg, 8.7 mg/kg Mce6 equivalent) and light on day 15 (all P < 0.02).

Further comparisons of the tumor volumes in the various experimental groups revealed the tumor volumes in the group treated with P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) plus P-C (12.5 mg/kg, 1.5 mg/kg Mce6 equivalent) and light (650 nm, 220 J/cm²) were not significantly different from the tumor volumes of those treated with P-C (25 and 75 mg/kg, 2.9 and 8.7 mg/kg Mce6 equivalent, respectively) plus light at any measurement. The tumors treated with P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) plus P-C (12.5 mg/kg, 1.5 mg/kg Mce6 equivalent) without light, P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) plus P-C (75 mg/kg, 8.7 mg/kg Mce6 equivalent) without light, and P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) alone were also statistically equivalent. The combined treatment groups with P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) as the active cytotoxic agent (P-C but no light given) had tumor volumes that were significantly less than control...
DISCUSSION

There is an urgent need to design carrier systems capable of overcoming physiological barriers and to deliver anticancer drugs into tumor tissue (23). The potential of soluble polymers as targetable lysosomotropic carriers was suggested (24). In recent years, HPMA copolymers were systematically developed as anticancer drug vehicles (6, 7, 9, 20, 25). P-A conjugates were shown to be effective against a number of tumors, including M5076, P388, B16 melanoma, Walker carcorna, LS174T, and L1210 leukemia (26, 27). A P-A conjugate (6, 25) underwent Phase I clinical trials (28). The results (28) demonstrated the low nonspecific toxicity of the HPMA copolymer conjugate. The starting dose was 20 mg/m² as an i.v. infusion every 3 weeks (six doses total). This dose was increased to 320 mg/m² without demonstrating substantial side effects. The conjugate contained about 8 weight% of Adriamycin. Consequently, the patients were administered up to 35 g copolymer conjugates (in six doses), indicating the biocompatibility of the HPMA copolymer carrier.

Adriamycin has proven to be cytotoxic in mouse and human ovarian epithelial carcinoma (11, 29). In this study, P-A demonstrated potential activity against ovarian epithelial cancer cells. Our goal is to develop combinations of chemotherapy and PDT for ovarian cancer that are delivered via HPMA copolymer-based drug delivery systems to increase their safety and, potentially, their efficacy. The alteration of body distribution of low molecular weight anthracycline antibiotics (Adriamycin and daunomycin) via macromolecular drug carriers has increased their therapeutic index in multiple studies (30–33). This study also suggests the potential for an increased safety margin of P-A when compared with Adriamycin, which will be confirmed through dose escalation studies. In vitro studies have been completed that confirm the in vivo findings and may prove to be helpful in defining appropriate combinations. The improved safety is attributed to the limitation of nonspecific uptake of the HPMA copolymer-bound drug. The latter enters cells via endocytosis. The macromolecules that gain entry into cells are then channeled to the lysosomal compartment (9).

Adriamycin conjugated to the HPMA copolymer through a lysosomal cleavable tetrapeptide spacer (Gly-Phe-Leu-Gly) is then released from the carrier, followed by diffusional transport to the cytoplasm and nucleus, allowing the Adriamycin to intercalate with the DNA. This sequence of subcellular trafficking of P-A has been visualized by confocal fluorescence microscopy (34).

We next investigated the efficacy and safety of the photosensitizer Mce₆. Photosensitizers represent one class of agents that allow targeting of the PDT through the controlled administration of light. This method of targeting is particularly applicable to peritoneal-spreading ovarian epithelial carcinoma. The major side effects of such therapy are prolonged skin photosensitivity, which requires patients to remain out of sunlight for prolonged periods (6–8 weeks) and, in the case of ovarian carcinoma, i.p. organ toxicity from nonspecific uptake.

PDT usually uses photosensitizers activatable with wavelengths between 600 and 900 nm, because interference from endogenous biomolecules is minimal in this range (35). Mce₆ represents a "second-generation" photosensitizer with a major absorption peak at 650 nm, which is associated with greater tissue penetration at this wavelength.

Tochner et al. (36) demonstrated the efficacy of PDT in the treatment of a murine ascites-creating ovarian carcinoma (embryonal ovarian carcinoma). Using in vitro models, other investigators have shown responsiveness of various ovarian cancer cell lines to PDT (37, 38). Using the model described in this report, we reported on the efficacy of the hematoporphyrin derivative Photofrin II in treating s.c. ovarian carcinoma (13). In that report, we also noted nontoxic uptake of the photoactivating agent, increasing the potential for nonselective toxicity.

Other studies highlight properties that must be considered in using Mce₆ as a photosensitizer for ovarian cancer. First, Kostenich et al. (39) noted that the antitumor effect of chlorin e₆ was directly proportional to the dosage and light energy exposure and inversely proportional to the time interval between photosensitizer administration and irradiation. They had 10–60% cure rates at doses of 1–10 mg/kg, respectively, in other animal models. In EMT-6 tumors growing in BALB/c mice, chlorin e₆ was not retained in greater amounts by tumor tissues relative to normal tissues (40). McMahon et al. (41) demonstrated direct chondrosarcoma tumor cytotoxicity and vascular stasis in rats. Blood flow stasis was the result of platelet aggregation and mechanical obstruction of flow rather than vessel constriction. Platelet aggregation and damage, endothelial cell injury, and arachidonic acid metabolite release have also been reported in other studies (42–44). The shock syndrome noted in our study and in a previous study of another photosensitizer may be related to massive prostaglandin release (22). The clinical observation of the syndrome occurrence in mice with tumors overlying the liver indicates the need for targeted illumination and organ shielding, which can be accomplished in humans.

The extremely narrow margin of safety for Mce₆ in this animal model and former studies showing prolonged retention and tumor nonspecificity highlight the need for alternative delivery methods for the administration of photosensitizers (13, 40). Attachment of a drug (photosensitizer) to a water-soluble polymeric carrier decreases its nonspecific toxicity and immunogenicity (9, 20). These effects may be attributed mainly to the change in body distribution, intracellular processing in membrane-limited organelles, and lag time between the delivery of the drug and activation by light (20, 45). For example, Mce₆ was conjugated to HPMA copolymers (20, 45, 46), and the photoproperties and biological properties were studied in detail. Binding of Mce₆ to HPMA copolymer carriers significantly increased its resistance to photobleaching and had little effect on the spectrum or triplet lifetime of Mce₆ but reduced the quantum yield of singlet oxygen production by illuminated Mce₆ (45). The body distribution of P-C was different when compared with free Mce₆ (20). In agreement with the hypothesis of Maeda et al. (47) and Matsumura and Maeda (48) on the "enhanced permeability and retention effect" of polymer conjugates in solid tumors, we have observed an increased concentration of P-C in the Neuro 2A neuroblastoma tumor in A/J mice when compared with free Mce₆ (20). It appears that alternative drug delivery systems (9) in general, and targetable, water-soluble P-C conjugates in particular (46, 49), may provide a greater margin of safety and an improved therapeutic effect. This article documents a severalfold increase in the margin of safety and improved therapeutic effects of P-C with light (12.5–75 mg/kg, 1.5–8.7 mg/kg Mce₆ equivalent) compared with free Mce₆ (2.5 mg/kg).

The potential to further reduce Mce₆ toxicity was demonstrated by combining P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) with P-C and light at 12.5 and 75 mg/kg (1.5 and 8.7 mg/kg Mce₆ equivalent, respectively). These combinations resulted in tumor destruction without nonspecific toxicity not obtained by equal doses of P-A or P-C alone.

This animal model demonstrated the efficacy of P-A at 30 mg/kg (2.2 mg/kg Adriamycin equivalent), which was comparable with that of Adriamycin at 1 mg/kg (both less than maximal tolerable doses). Free Mce₆ with light (>2.5 mg/kg) demonstrated a very narrow safety margin, which was significantly improved after conjugation with HPMA copolymers (P-C). The combination of P-A (30 mg/kg, 2.2 mg/kg Adriamycin
equivalent) and P-C (75 mg/kg, 8.7 mg/kg Mce equivalent) demonstrated complete tumor ablation and safety in vivo. Combining P-C (12.5 or 75 mg/kg, 1.5 or 8.7 mg/kg Mce equivalent, respectively) with P-A (30 mg/kg, 2.2 mg/kg Adriamycin equivalent) allowed us to attain a significant improvement in tumor destruction than that obtained by P-A or P-C alone. These in vivo studies substantiate prior in vitro predictions of efficacy by in vitro studies. Recently, photodynamic properties of Adriamycin were noted in vitro (50). If this property has been elicited in vivo in conjunction with P-C, further increases in efficacy and safety may be attainable. Experiments are underway to determine whether we have obtained a photodynamic effect of Adriamycin in addition to its chemotherapeutic effect. We continue to explore methods to increase the therapeutic index of these agents alone and in combination with monoclonal antibody targeting (49) and other targeting methods. Phase I (28) and II studies have been initiated in the United Kingdom for a P-A conjugate and P-A with galactosamine as a targeting moiety. These clinical trials will assist in the development of similar agents for the treatment of ovarian cancer.

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