Body Distribution of Free, Liposomal and Nanoparticle-Associated Mitoxantrone in B16-Melanoma-Bearing Mice

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ABSTRACT

B16-melanoma-bearing mice were treated with four different formulations containing equivalent doses of the highly effective antineoplastic drug mitoxantrone. The formulations were: A mitoxantrone solution, a negatively charged liposome preparation (small unilamellar vesicles), a 14C-labeled polybutylcyanoacrylate-(PBCA) nanoparticle suspension, and a suspension of poloxamine 1508-coated 14C-PBCA-nanoparticles. After 1, 4 and 24 hr, three animals of each group were killed and the mitoxantrone concentrations in the blood, tumor, liver, spleen, heart and bone marrow were determined using a high performance liquid chromatography technique. Additionally, the concentrations of PBCA particles in the same tissues were measured by scintillation counting to compare the mitoxantrone distribution with the corresponding PBCA nanoparticle distribution. Each formulation led to a different body distribution profile of the drug. Liposomes drastically increased the blood level of mitoxantrone even after 24 hr, although free drug was cleared quickly. Liposomes also raised the concentration in the liver and spleen, but not the drug level in the tumor. PBCA-nanoparticles considerably increased the mitoxantrone concentrations in tumor, heart and spleen. However, the increase in tumor concentrations was not statistically significant due to the high variability. Nevertheless, the tumor growth was reduced significantly (P < .05) compared to both, the liposome and the solution preparation. The nanoparticle polymer concentrations did not completely mirror those of the drug concentrations. Especially in the heart, where no nanoparticle polymer radioactivity was found, the particle concentration did not completely correspond to the mitoxantrone concentration, revealing that a part of the drug was lost from the particles. These pharmacokinetic results correspond to parallel therapeutic effects obtained with mitoxantrone-loaded nanoparticles and liposomes in the B16 melanoma.

Mitoxantrone (Novantrone) is a clinically well-established anticancer drug with a high antitumor activity especially for the treatment of breast cancer, acute leukemia, malignant lymphomas and hepatocellular carcinoma (Smith, 1983; Shenkenberg and Von Hoff, 1986; Ehhinger et al., 1990; Faulds et al., 1991; Schleyer et al., 1994). The mechanism for its antiviral, antibacterial and anticancer activity is probably due to inhibition of DNA and RNA synthesis through DNA intercalation (Durr, 1984) and possibly an electrostatic interaction with different cellular membranes (Burns et al., 1988). The dose-limiting toxicity of the anthracenedione derivative is the myelosuppression combined with a weak cardiotoxicity (Faulds et al., 1991).

A way to overcome the toxic side effects of some drugs is to change their pharmacological behavior by the use of colloidal delivery systems. A wide variety of anticancer drugs has been incorporated in or associated with drug carrier systems such as liposomes or nanoparticles (Gregoriadis 1993; Lasic, 1993; Kreuter, 1994). The first of them are now under investigation in clinical trials (phase I-III) (Pestalozzi et al., 1992; Schwen-dener, 1993).

Both carrier systems have advantages and disadvantages. Polyalkylycyaooacrylate nanoparticles are solid, porous spheres, and the drug is attached by sorptive processes (Couvreur et al., 1979; Kreuter, 1994). In liposomes, the drugs are mostly encapsulated or strongly associated with the lipid bilayer. The body distribution of both carrier systems is mainly influenced by particle size and surface properties such as charge and hydrophilicity (Crommelin and Schreier, 1994; Kreuter, 1994). The main target organs for colloidal delivery systems are organs of the reticuloendothelial system, especially the liver and spleen. The body distribution may be altered by changes in the size and surface properties of both systems.

For the study mitoxantrone was encapsulated in negatively charged SUV or associated with nanoparticles pre-
pared by emulsion polymerization using PBCA. These nanoparticles represent a well-characterized, biodegradable system with a good bioavailability (Couvreur, 1979b, Kreuter, 1994). Poloxamine 1508 is a surfactant that was first investigated in combination with polyalkylcyanoacrylate nanoparticles by Tröster et al. (1992) and Beck et al. (1993). This surfactant is able to increase the blood circulation time and to decrease the liver uptake of the nanoparticles.

Materials and methods

Mitoxantrone (Novantrone) was kindly provided by GERMED (ASTA, Dresden, Germany). Water for HPLC was prepared by filtering doubly distilled water through Norganic cartridges (Millipore, Eschborn, Germany). Acetonitrile, LiChrosorb and ammonium dihydrogen phosphate were obtained from Merck (Darmstadt, Germany). Sodium-1-octane sulfate was obtained from Waters Assoc. (Eschborn, Germany). Columns (Bakerbond SPE 1 ml, no. 7121–01 with bottom fritt in base, 20 μm) were obtained from J.T. Baker Inc., (Phillipsburg, NJ) and XAD-2 absorbent, research grade, particle size 0.05 to 0.1 mm were from Serva (Heidelberg, Germany).

The following chemicals were obtained: methanol (Merck, Darmstadt, Germany), butylcyanoacrylate monomer (Sichel-Werke, Hanover, Germany), 15N monomer (3.4 Mbo/1ml) (Amershams Radiochemical Centre, Amersham, Buckinghamshire, UK), dextran 70 (Fluka, Buchs, Switzerland), poloxamer 188 (Pluronic PE6800) (Erlsloh, Dusseldorf, Germany), poloxamine 1508 (Tetronic 1508) (Erlsloh), hydrogenated egg phosphatidylcholin (our production), cholesterol (Merck), diethylphosphate (Serva, Heidelberg, Germany), Sephadex G 50 (Pharmacia, Freiburg, Germany) and Beckmann Tissue Solubilizer-450 (Beckmann, Fullerton, CA).

PBCA-nanoparticles were prepared by emulsion polymerization (Beck et al., 1993) using 1% butylcyanoacrylate monomer, 1% dextran 70 and 0.2% poloxamer 188 in 0.01 N HCl. After 4 hr of stirring, the suspensions were adjusted to a pH of 6.0 with 1 and 0.1 N sodium hydroxide solution. Stirring was continued for 1 hr to complete polymerization. Agglomerates were removed by filtration through a glass filter no. 3.

Two different methods were used for drug loading. First, the incorporation method, by which mitoxantrone was dissolved in the polymerization medium before the monomer was added. Second, the adsorption method, by which mitoxantrone was added to a previously polymerized and neutralized particle suspension and then stirred for another 24 hr (Beck et al., 1993). In both cases, the mitoxantrone concentrations varied from 0.1 to 4 mg/ml. For the in vivo tests, suspensions with a drug concentration of 1 mg/ml were chosen. Nonencapsulated or nonadsorbed mitoxantrone was not removed. Another series of suspensions was prepared by addition of 1% poloxamine 1508 as a coating surfactant to the resulting suspensions (Beck et al., 1993).

The mitoxantrone loading was measured by ultrafiltration (Ultra-sart 10, Sartorius, Göttingen, Germany) through cellulose nitrate membranes with a pore size of 20 nm by using a magnetic stirrer (500–600 rpm) and nitrogen at a pressure of 1 kPa/cm2. After spectrometric determination at 612 nm (Specord UV/VIS, Carl Zeiss, Jena, Germany) the amount of drug bound to the particles was calculated as the difference between the initial drug concentration and the drug concentration in the filtrate. Drug loss by adsorption to the filter membranes could be avoided by inserting them into a drug solution leading to a saturation of the membrane surface.

Preparation of liposomes. The liposomes used were negatively charged SUV. The lipid components hydrogenated egg phosphatidylcholin (neutral at pH 7.4) (Hauser, 1984), cholesterol and dicetylphosphate (anionic lipid at pH 7.4) at a molar ratio 1:1:0.25 were dissolved in chloroform. The organic solvent was evaporated on a rotary evaporator (water bath 35°C). Multilamellar vesicles were prepared by adding phosphate-buffer solution at pH 7.4 containing 0.823 mg/ml mitoxantrone to the lipid film and shaking for 24 hr at room temperature (lipid concentration 21.2 mg/ml). Multilamellar vesicles were transformed into SUV by sonication (Branson sonifier B 15, 6 times for 4 min, 50% pulse input at 55°C bath temperature) under nitrogen. Remaining free mitoxantrone was separated by column chromatography on Sephadex G 50. Encapsulated mitoxantrone was determined spectrometrically after 1:100 dilution with ethanol:chloroform 1:1 at 612 nm and by HPLC, according to Van Belle et al. (1985).

HPLC. The mitoxantrone concentration was determined using ion pair HPLC and a Waters system 484 (Millipore, Milford, MA) supplied with an automated sample processor (WISP 710B), a UV-VIS absorbance detector to 700 nm (SPD-6AV, Shimadzu, Duisburg, Germany) and a model 720 system controller. The solid phase consisted of a Nova-Pac C18 column (150 × 3.9 I.D., 4-μm particle size) equipped with a Nova-Pac C18 guard column (20 × 2.1 mm I.D., 10-μm particle size).

The optimal chromatographic separation of mitoxantrone in biological samples was achieved using isocratic elution conditions [acetonitrile-ammonium dihydrogen phosphate-buffer solution (pH 2.7) containing 25 mM sodium-1-octane sulfonate as an ion pair forming reagent] at a flow rate 1 ml/min and room temperature. The evaluation of the peak areas was done using the external standard method and the Maxima Software 820 (version 3.30). Mitoxantrone was detected at 658 nm with a sensitivity of 3 ng/ml.

Sample clean-up procedure. A total of 150 mg of XAD-2 beads suspended in 1 ml of methanol was packed into the small Bakerbond columns. Afterward, columns were washed with distilled water and then with 10 ml phosphate-buffered saline at pH 7.4, and finally with 6 ml 0.05 M ammonium dihydrogen phosphate solution pH 2.7 (adjusted with phosphoric acid).

The serum and bone marrow samples were applied directly to the XAD-2 columns. Liver, spleen, heart and tumor samples were first homogenized after the addition of 4 ml of water. After that, the suspensions were extracted with 6 ml of a solution containing acidic chloroform and methanol (2:1), and finally centrifuged at 7000 min−1 for 10 min. The water phase was extracted twice more with 4 ml chloroform, centrifuged and the water phase containing the mitoxantrone was applied to the XAD-2 columns. Then the column was washed with 0.2 ml of 2-propanol-0.05 M ammonium dihydrogen phosphate pH 2.7 (30.70, v/v) and the mitoxantrone was eluted with another 0.8 ml of this solution into glass microvials (Waters no. 9298). A 0.1-ml aliquot of the eluate was injected into the HPLC system.

Particle size determination. The particle size was determined by photon correlation spectroscopy using a BI-200SM Goniometer Ver. 2.0 (Brookhaven Instruments Corp., Holtsville, NY). For these measurements the original suspensions were diluted with distilled and filtered water until a slight turbidity remained. The particle sizes were 253 ± 5 nm for the uncoated and 266 ± 4 nm for the poloxamine 1508-coated nanoparticles.

Liposome size distribution was checked by electron microscopy after staining with uranyl acetate and by light scattering (Coulter particle sizer N4). An average vesicle diameter of 122 ± 20 nm was determined.

Mice and tumor models. Mice were purchased in specific patho-
gene free quality (Biomodelle GmbH, Schoenwalde, Germany) and held under low-germ conventional conditions (22°C room temperature, 60% relative humidity). They received autoclaved standard diet and tap water ad libitum.

B16-melanoma was inoculated as 1:3 diluted tumor homogenate (0.5 × 106 cells/mouse) i.m. into female B6D2F1/Shoe mice on day 0. Mice were treated i.v. on days 1, 8 and 15 with the corresponding mitoxantrone preparations. Twice a week the tumor diameters were measured by caliper and the corresponding volumes (V) calculated for the ellipsoid by the formula V = 0.52 × L × W2. The tumor volumes were calculated as the difference between the initial tumor volume and the tumor volume on day 15.
according to the equation (1):

\[ V = width^2 \times length \]

Tumor volume on day 22 was expressed as a percentage of the tumor volume of control (i.e., untreated) animals.

The mitoxantrone dose in each experiment was 5 mg/kg body weight; the injection volume for the saline solutions was 0.2 ml/20 g body weight; and for liposomal (nonencapsulated mitoxantrone was removed); and nanoparticle preparations (nonassociated mitoxantrone was not removed) 0.1 ml/20 g body weight. Body weight changes in percentages between day of treatment and 3 to 4 days later, as well as leukocyte counts measured on day 4, were used for the estimation of toxicity in each experiment.

Data obtained were analyzed for significant differences using Mann and Whitney’s U test for two random variables (Mann and Whitney 1947, Sachs 1984 (see table 2).

In the pharmacokinetic study mitoxantrone-loaded nanoparticles or liposomes were administered i.v. on day 10 in a dose of 10 mg/kg to B16 melanoma bearing mice. Then 1, 4 and 24 hr after injection mice were killed by cervical dislocation, and serum, tumor, liver, bone marrow, heart and spleen were removed. The organs were kept frozen until determination by HPLC or liquid scintillation counting.

Data analysis. All data in table 1 and in the figures are expressed as mean \pm S.D. The significance of distribution between the test groups was analyzed by analysis of variance with Tukey-Kramer HSD and Student’s t tests. P < 0.05 was the criterion for statistical significance.

Results

In our study, the body distributions in mice after i.v. injection of mitoxantrone in the form of four different preparations were compared. The four preparations consisted of two PBCA nanoparticle preparations, with and without additional coating of poloxamer 1508, one liposomal preparation and one aqueous solution. The results of the mitoxantrone distribution are presented in \( \mu g \) per g of tissue and serum in table 1 and in percentage of injected dose in figure 1 to 3. Due to differences in the sizes of the organs, especially the tumors, these two types of concentration levels may differ. The results show that the body distribution after i.v. injection of mitoxantrone can be greatly affected by association with nanoparticles or encapsulation in liposomes.

By far the highest concentration of mitoxantrone was observed in the serum after injection in liposomal form (\( P < .05 \)). Fourteen percent of the total dose remained in the blood after 1 hr and decreased to 1% after 24 hr (see fig. 3). With all other preparations, very low serum concentrations were observed.

The tumor concentrations of mitoxantrone after 1 hr were highest with the nanoparticles. At this time uncoated nanoparticles yielded the highest drug concentrations in \( \mu g/g \) tissue (table 1). However, due to the different sizes of the tumors, much higher statistically significant percentual total

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>Serum</th>
<th>Tumor</th>
<th>Liver</th>
<th>Bone Marrow</th>
<th>Heart</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hr</td>
<td>A</td>
<td>2.2 ± 0</td>
<td>1.248 ± 1.87</td>
<td>0.054 ± 0.09</td>
<td>0.19 ± 0.01&lt;sup&gt;g&lt;/sup&gt;</td>
<td>6.204 ± 1.77&lt;sup&gt;g&lt;/sup&gt;</td>
<td>3.57 ± 1.55</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.63 ± 0</td>
<td>0.99 ± 1.02</td>
<td>0.493 ± 0.66</td>
<td>0.17 ± 0.02</td>
<td>4.52 ± 1.12</td>
<td>4.825 ± 2.33&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>524.2 ± 68.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.106 ± 0.09</td>
<td>0.592 ± 0.30</td>
<td>0.114 ± 0.03</td>
<td>3.42 ± 1.87</td>
<td>1.48 ± 1.07</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>1.38 ± 1.29</td>
<td>0.094 ± 0.16</td>
<td>0.07 ± 0.03</td>
<td>0.136 ± 0.02</td>
<td>3.37 ± 0.63</td>
<td>0.705 ± 0.93</td>
</tr>
<tr>
<td>4 hr</td>
<td>A</td>
<td>1.069 ± 1.85</td>
<td>0.456 ± 0.49</td>
<td>0.120 ± 0.12</td>
<td>0.229 ± 0.03</td>
<td>2.256 ± 0.27</td>
<td>4.84 ± 0.67&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.206 ± 0.36</td>
<td>0</td>
<td>0.027 ± 0.03</td>
<td>2.15 ± 0.48</td>
<td>3.85 ± 0.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>21.25 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.126 ± 0.07</td>
<td>1.09 ± 1.05</td>
<td>1.69 ± 0.03&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.353 ± 1.11</td>
<td>1.75 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0.901 ± 1.31</td>
<td>0.210 ± 0.30</td>
<td>0.14 ± 0.08</td>
<td>0.100 ± 0.12</td>
<td>1.367 ± 0.96</td>
<td>1.11 ± 0.55</td>
</tr>
<tr>
<td>24 hr</td>
<td>A</td>
<td>0.11 ± 0.19</td>
<td>0</td>
<td>0.63 ± 0.41</td>
<td>0.465 ± 0.10</td>
<td>5.25 ± 4.12</td>
<td>5.02 ± 0.89</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0</td>
<td>0.270 ± 0.38</td>
<td>1.055 ± 0.51</td>
<td>0.353 ± 0.06</td>
<td>5.87 ± 5.35</td>
<td>12.6 ± 0.14&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>13.34 ± 8.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.094 ± 0.05</td>
<td>1.85 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.54 ± 0.06&lt;sup&gt;i&lt;/sup&gt;</td>
<td>2.67 ± 2.54</td>
<td>2.62 ± 0.86</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0.366 ± 0.04</td>
<td>0.013 ± 0.02</td>
<td>0.686 ± 0.53</td>
<td>0.21 ± 0.01</td>
<td>3.29 ± 2.18</td>
<td>3.93 ± 3.25</td>
</tr>
</tbody>
</table>

<sup>a</sup> Calculated to 1.3 ml whole blood.
<sup>b</sup> A against C and D, P < .05.
<sup>c</sup> A against C and D, P < .05.
<sup>d</sup> B against D, P < .05.
<sup>e</sup> Significant P < .05.
<sup>f</sup> A against C and D, P < .05.
<sup>g</sup> C against B and D, P < .05.
<sup>h</sup> C against D, P < .05.
<sup>i</sup> C against B and D, P < .05.
<sup>j</sup> B against A, C, and D.
drug concentrations were observed in the group that had obtained the poloxamine 1508-coated nanoparticles. The increased drug uptake with nanoparticles was combined with an increased efficacy against the same tumor (table 2) (Beck et al., 1993). With the liposomes, the tumor concentration in the B16 melanoma was even lower than with free drug. This also paralleled the therapeutic efficacy of the different preparations in this tumor model (table 2). In all cases the tumor concentrations decreased quite rapidly (figs. 1–3).

The mitoxantrone concentrations in the liver and in the spleen were higher with the colloidal drug carriers, nanoparticles and liposomes, than with the free solution. Significant differences in liver and spleen concentrations of the drug were observed between the two nanoparticle preparations. After 4 and 24 hr, liver concentrations of liposomal mitoxantrone were significantly ($P < .05$) higher than with the nanoparticles. Interestingly, the spleen concentrations of the drug in $\mu g/g$ tissue (table 1) were higher than the liver concentrations in all cases. This also led to higher percentual concentrations despite the fact that the liver is larger than the spleen. The situation is somewhat different when comparing the nanoparticle polymer concentrations by measuring radioactivity. Similar $^{14}$C-concentrations were observable on a $\mu g$ per $g$ tissue basis resulting in a higher percentual liver uptake due to the larger liver size.

The nanoparticle polymer distribution did not mirror the drug distribution. This is especially pronounced in the liver where the highest polymer concentrations were observed although comparatively little drug was transported into this organ by this carrier.

**Discussion**

The objective of our study was to compare the body distribution after i.v. injection of mitoxantrone bound to two types of nanoparticle carriers with that of a liposome preparation and a solution of the free drug. These preparations were already tested in an earlier investigation by Beck et al. (1993) for their efficacy in two different tumor models, a P388 leukemia as well as a B16 melanoma as in our study. The preparations showed very pronounced differences in efficacy. Although in the leukemia the liposomes performed best, followed by the solution and third, the nanoparticles, the picture was reversed in the B16 melanoma. The nanoparticles were the most efficient preparation, followed by the drug solution although the liposomes were performing poorest. Our report attempts to correlate the drug distribution with the drug efficacy in the melanoma model. In addition, the nanoparticle polymer was labeled with $^{14}$C in the polymer chain to determine the carrier’s body distribution. Mitoxantrone was bound to previously produced $^{14}$C-labeled nano-

**TABLE 2**

Therapeutic efficacy of different mitoxantrone preparations on the tumor model B16 melanoma

<table>
<thead>
<tr>
<th>Group</th>
<th>Preparation</th>
<th>Body Weight Change (%)</th>
<th>Leucocyte Count ($1 \times 10^9$/liter)</th>
<th>Tumor Volume on Day 22 T/C (%)</th>
<th>No. of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Mitoxantrone NP, adsorpt*</td>
<td>-2</td>
<td>4.8$^c$</td>
<td>$^{14}$C$v$</td>
<td>10</td>
</tr>
<tr>
<td>B</td>
<td>Mitoxantrone NP, adsorpt + poloxamine$^b$</td>
<td>0</td>
<td>3.4$^c$</td>
<td>$^{14}$C$v$</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>Mitoxantrone liposomes$^b$</td>
<td>0</td>
<td>3.3$^c$</td>
<td>$^{33}$$^c$</td>
<td>10</td>
</tr>
<tr>
<td>D</td>
<td>Mitoxantrone solution$^b$</td>
<td>-2</td>
<td>3.5$^c$</td>
<td>$^{24}$$^c$</td>
<td>10</td>
</tr>
<tr>
<td>E</td>
<td>Unloaded NP$^a$</td>
<td>+2</td>
<td>7.9</td>
<td>93</td>
<td>10</td>
</tr>
<tr>
<td>F</td>
<td>Unloaded NP + poloxamine$^a$</td>
<td>+1</td>
<td>7.6</td>
<td>103</td>
<td>10</td>
</tr>
<tr>
<td>G</td>
<td>Mitoxantrone NP, incorp$^a$</td>
<td>0</td>
<td>3.8$^c$</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>H</td>
<td>Mitoxantrone NP, incorp + poloxamine$^a$</td>
<td>-1</td>
<td>3.4$^c$</td>
<td>$^{18}$C$v$</td>
<td>10</td>
</tr>
<tr>
<td>I</td>
<td>Control group (NaCl solution 0.9 per cent)$^a$</td>
<td>+1</td>
<td>8.5</td>
<td>100</td>
<td>10</td>
</tr>
</tbody>
</table>

* Data from previous experiment by our group (Beck et al., 1993).

$^a$ These groups are included in the body distribution experiments.

$^b$ Significantly different from control groups (according to U test).

$^c$ Significantly different from mitoxantrone solution (U test).

$^d$ Significantly different from mitoxantrone liposomes (U test).
particles by adsorption. Although this preparation method led to binding of only 10% of the total mitoxantrone (Beck et al., 1993), it had to be used because the 14C-labeled monomer is not stable but polymerizes within a few minutes. As a consequence of this rapid polymerization due to the radioactivity, more efficient entrapment and binding methods could not be used in our study. However, the use of radiolabeled polymer enabled the simultaneous observation of the body distribution of the nanoparticle polymer and of the drug mitoxantrone. With the liposomes, 78% of the mitoxantrone was incorporated in the liposomal bilayer.

As shown in table 1 and figures 1 to 3 the body distribution was changed considerably by association with nanoparticles or encapsulation in liposomes. Moreover, PBCA nanoparticles without coating showed a slightly, but in most cases not statistically, different distribution in comparison to particles coated with poloxamine 1508. It is noteworthy that the difference in body distributions between the nanoparticle preparations, the liposomes and the aqueous solution occurred despite the fact that in the case of the nanoparticles, only 10% of the drug was bound whereas 90% was in free form. Nevertheless, the relatively low portion of drug that was bound to the nanoparticles most likely was the reason for the observed difference in polymer and drug distribution. This is quite apparent in the case of the liver where about 35% of the polymer radioactivity was found after 1 hr, but comparatively very little drug.

The low binding of the drug to the nanoparticles also did not decrease the in vivo performance of these carriers against the B16 melanoma. Both nanoparticle preparations yielded the highest tumor concentrations of mitoxantrone (table 1, figs. 1–3) and led to the highest tumor efficacy (table 2). Although the differences in the drug concentrations were not statistically significant due to the high variability of the tumor concentrations, their efficacy in tumor growth reduction achieved a statistically significant level compared to the drug solution and the liposomes. It is interesting to note that the high efficacy was obtained despite the rapid disappearance of the drug from the tumor site. However, it can be anticipated that higher tumor concentrations and antitumoral efficacy can be achieved with a better binding of antitumoral drugs to the nanoparticles; however, this strongly depends on the chemical and physicochemical nature of the drug and may require major changes in carrier composition (Kreuter, 1994). These changes in turn may unfavorably influence the body and tumor distribution. In addition, as our results with liposomes that exhibit excellent binding of mitoxantrone show, strong binding is not important if the carrier cannot deliver the drug to the tumor.

Another major problem aside from efficacy of antineoplastic drugs is their toxicity. Indeed, nanoparticles in some cases were shown to improve efficacy but to increase toxicity of some anticancer drugs (Kreuter, 1994). This apparently does not occur with mitoxantrone. As shown in table 2, no significant increase in toxicity was observed with the nanoparticles in comparison to the other mitoxantrone formulations.

Liposomes by far achieved the highest serum levels. As discussed above, this had no influence on their antitumoral behavior against the B16 melanoma. Nanoparticles and the drug solution yielded only low serum concentrations. Interestingly, the drug concentration in the serum after adsorptive binding of mitoxantrone to the uncoated particles was higher than after binding to the poloxamine 1508-coated particles (figs. 1–3). The same result was seen when comparing the concentrations of the radiolabeled polymers (figs. 4–6). It contrasts earlier observations of Tröster and Kreuter (1992) who demonstrated using PMMA nanoparticles that coating with poloxamine 1508 keeps the nanoparticles in circulation for prolonged times. However, this polymer, PMMA, is much more hydrophobic than PBCA. As a consequence, PMMA as well as polystyrene, which has been used by Illum et al. (1987), bind surfactants better than a more hydrophilic polymer such as PBCA. The latter polymer probably desorbed poloxamine 1508 very rapidly resulting in faster clearance of the decoated particles from the serum. A similar finding, that PBCA nanoparticles coated with poloxamine 908 were not retained in the blood circulation for prolonged times, was already seen by Douglas et al. (1986).

A considerable amount of the drug was distributed into the heart. Surprisingly, the heart concentration was much higher with the nanoparticles. This difference was statistically different after only 1 hr for the uncoated nanoparticles compared to the liposomes and the drug solution. The high mitoxantrone heart concentration with nanoparticles contrasts earlier findings of Verdun et al. (1990) with a related drug, doxorubicin, in which a significantly reduced heart accumulation was observed after binding to nanoparticles. A reason for this may be the fact that doxorubicin binds much better (>90%) to this type of nanoparticle than mitoxantrone. The reduced heart accumulation of doxorubicin was combined with a much lower toxicity (Couvreur et al., 1982) and a better tolerance in patients (Kattan et al., 1992). It is presently not known if the higher heart accumulation of mitoxantrone as observed in our study leads to a higher cardiac toxicity. Nevertheless, this possibility has to be kept in mind when considering nanoparticles as carriers for this drug.

Conclusions

Our study showed that despite of the low loading efficacy of mitoxantrone in case of nanoparticles, a significantly different distribution of drugs may be achieved in comparison to free drug and other drug formulations. Especially relevant was the considerably enhanced mitoxantrone concentration in tumors.
after injection of the two nanoparticle preparations in the tumor compared to the liposomes and to the free drug solution. Although these enhanced concentrations were only for coated nanoparticles (4 hr) statistically significant (due to the high concentration variabilities), they led to important and statistically significant decreases in tumor growth after an observation period of 22 days without an increase in toxicity, again compared to the liposomes and the solution formulations of the drug. Different preparations, nanoparticles and liposomes, with different surface properties of the formulation, therefore, may enable the specific targeting of different types of tumors. The slower biodegradability of the nanoparticles may enhance the accumulation in the vasculature of solid tumor tissue such as the B16 melanoma. In contrast, the longer persistence of the liposomes in the blood enables a better treatment of circulating tumor cells as could be shown with the P388. A part of the problem of cancer, i.e., the dissemination of cancerous cells throughout the body and the resulting induction of metastases, may possibly be attacked by a combination of both types of drug carriers. Nanoparticles may be more efficient against the tumor in its solid form whereas the liposomes may be more efficient against leaking and circulating tumor cells. Therefore, the suggested combination may lead to synergistic effects; this in turn may improve the therapeutic efficacy and may result in a more specific tumor therapy.

References


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