Approaches to Defining the Mechanism of Enhancement by Fluosol-DA 20% with carbogen of Melphalan Antitumor Activity

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ABSTRACT

Fluosol-DA with carbogen (95% oxygen and 5% carbon dioxide) breathing can increase the efficacy of melphalan. Addition of Fluosol-DA to treatment with melphalan leads to a greater increase in tumor growth delay under conditions of air breathing and carbogen breathing than does the fat emulsion Intralipid. The ability of melphalan to kill tumor cells increased with dose over the range of drug examined. At the lower doses of drug there is some increase in tumor cell killing seen with the addition of carbogen breathing or Fluosol-DA and air breathing; however, at the highest dose of the drug this difference disappeared. Throughout the melphalan dosage range examined there is approximately 1 log greater tumor cell kill observed with the addition of Fluosol-DA and carbogen breathing compared to the drug treatment alone. There was no significant difference in the survival of bone marrow cells under any of the treatment conditions. Fluosol-DA itself with air or carbogen breathing produced no detectable cross-links in DNA from tumors treated in vivo. The cross-linking factors for melphalan with air or carbogen breathing and for melphalan plus Fluosol-DA and air breathing were similar; when carbogen breathing was added to the treatment combination, the cross-linking factor increased almost 3-fold. When melphalan was dissolved in Fluosol-DA, the melphalan moved quickly into the lipophilic perfluorochemical particles so that after 1 h 60% of the drug was in the perfluorochemical layer. At 24 h, 85–90% of the melphalan was sequestered in the perfluorochemical particles. The pharmacokinetics of [14C]melphalan alone, [14C]melphalan plus Fluosol-DA, and [14C]melphalan prepared in Fluosol-DA were studied in several tissues of F344 rat fibrosarcoma-bearing mice. In general, the tissue absorption and distribution t1/2 for melphalan were shortened in the presence of Fluosol-DA (except for kidneys). Shifting the t1/2 for absorption and distribution to shorter times produces a much sharper and earlier peak in the drug exposure of the tumor. Fluosol-DA provides a relatively nontoxic means of increasing oxygen delivery to tumors and a therapeutically meaningful way of improving melphalan antitumor activity.

INTRODUCTION

Hypoxia has long been known to protect cells from the cytotoxic effects of radiation. The level of cellular oxygenation is an important factor in the action of many antineoplastic agents. Reactive free radicals of oxygen may be responsible for the cleavage of DNA by several agents including bleomycin, procarbazine, streptonigrin, actinomycin D, and Adriamycin. The cellular state of oxygenation may also have a strong influence on the cytotoxicity of antimetabolites and Vinca alkaloids such as vincristine, which are most lethal to cells which are progressing through the cell cycle. Cells which are hypoxic may be either noncycling or slowly progressing through the cell cycle (1, 2). Several antineoplastic agents have been classified by their selective toxicities toward oxygenated or hypoxic tumor cells (3).

Compounds which mimic the effect of oxygen in cells such as the radiosensitizer misonidazole have been shown to enhance the cytotoxicity of several antitumor alkylating agents including melphalan, cyclophosphamide, 1,3-bis(2-chloroethyl)-1-nitrosourea, and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea in vitro and in vivo. This phenomenon has been termed chemosensitization or chemopotentiation (1). The potentiation of the antitumor effect of these drugs by misonidazole in vivo was seen without a similar increase in normal tissue toxicity. The presence of hypoxic cells in solid tumors may account for the preferential effect, since chemosensitization in vitro occurs only when cells are exposed to misonidazole under hypoxic conditions, i.e., conditions in which reduction of misonidazole through formation of oxygen-mimicking free radicals can occur.

Like most bifunctional alkylating agents, melphalan exerts cytotoxic effects through interaction with DNA by forming either interstrand, intrastrand, or DNA-protein cross-links. For the most simple bifunctional alkylating agents, maximum levels of cross-linking are observed immediately following drug exposure (4). The formation of DNA cross-links in murine L1210 leukemia cells by melphalan increased most rapidly during the first 5 h following drug exposure (5, 6). The delay is most probably due to a slow conversion of monoadducts to cross-links. Although the molecular details of misonidazole enhancement of melphalan antitumor activity remain to be elucidated, it has been established that misonidazole pretreatment results in increased levels of melphalan-induced cross-links in DNA (7–10). Alterations in drug pharmacokinetics and in intracellular non-protein sulfhydryl levels may also play a role in the chemosensitization effects observed (9, 11–13).

Perfluorochemicals have excellent oxygen- and carbon dioxide-carrying capacity (14–16). To fully utilize the oxygen-carrying capacity of these materials, high partial pressures of oxygen are used. Unlike hemoglobin, where oxygen is bound to the molecule, the solvent action of the perfluorochemicals for oxygen does not involve any kind of chemical or chelating process. The gas molecules situate themselves in the spaces between the molecules (14, 16). The uptake and release of oxygen from perfluorochemical emulsions are completely reversible. At least 90% of the emulsion particles in the preparation which we used are less than 0.2 μm in diameter, much smaller than RBCs (average diameter, 5–10 μm). The perfluorochemical emulsion, Fluosol-DA, in combination with breathing a 100 or 95% oxygen atmosphere has been shown to enhance the response of several solid rodent tumors to single-dose and fractionated radiation treatment (17–21) with little influence on normal tissues (22, 23). As a result of these studies, Fluosol-DA plus oxygen breathing has recently entered clinical trials in combination with radiotherapy in advanced head and neck cancer (24).

Fluosol-DA with carbogen (95% oxygen and 5% carbon dioxide) breathing can also increase the efficacy of melphalan treatment of tumors (25). The perfluorochemical particles of Fluosol-DA form a highly lipophilic environment; thus, part of the melphalan may be dissolved in Fluosol-DA, the melphalan moved quickly into the lipophilic perfluorochemical particles so that after 1 h 60% of the drug was in the perfluorochemical layer. At 24 h, 85–90% of the melphalan was sequestered in the perfluorochemical particles. The pharmacokinetics of [14C]melphalan alone, [14C]melphalan plus Fluosol-DA, and [14C]melphalan prepared in Fluosol-DA were studied in several tissues of F344 rat fibrosarcoma-bearing mice. In general, the tissue absorption and distribution t1/2 for melphalan were shortened in the presence of Fluosol-DA (except for kidneys). Shifting the t1/2 for absorption and distribution to shorter times produces a much sharper and earlier peak in the drug exposure of the tumor. Fluosol-DA provides a relatively nontoxic means of increasing oxygen delivery to tumors and a therapeutically meaningful way of improving melphalan antitumor activity.
in the presence of the perfluorochemical emulsion particles. In this study, we aim to approach the question of the relative contributions of oxygen and drug distribution into a lipophilic environment through studies using Intralipid, a fat emulsion, and we begin to explore the mechanism by which Fluosol-DA and carbogen breathing enhance the antitumor activity of melphalan.

MATERIALS AND METHODS

Drugs. Fluosol-DA 20% (Green Cross Corp., Osaka, Japan) was obtained from Alpha Therapeutics Corp. (Los Angeles, CA). The stem emulsion consists of 25% (w/v) perfluorochemicals: 7 parts perfluorodecalin; 3 parts perfluorotripropylamine; Pluronic F-68 (2.7%, w/v); yolk phospholipids (0.4%, w/v) as emulsifiers; and glycerol (0.8%, w/v) as a cryoprotecting agent. The annex solution (electrolyte/bicarbonate solution) furnishes the preparation with physiological osmolality. The stem emulsion particles provide a surface area of 1.82 × 10^6 cm²/liter available for oxygen diffusion (about 100 times the surface area of the RBC in whole blood). The half-life of Fluosol-DA in vivo is about 12 h (5). Intraplalid 20% (KabiVitrum, Inc., Alameda, CA) was obtained from the Dana-Farber Cancer Institute pharmacy.

Melphalan (l-phenylalanine mustard) was a gift from Burroughs Wellcome Co. (Research Triangle Park, NC) and [chloroethyl-2-14C]-melphalan (specific activity, 20 mCi/mmol) was a gift from Dr. John A. Kepler, Research Triangle Institute (Research Triangle Park, NC).

Tumor. The FSAl fibrosarcoma (26) adapted for growth in culture (FSAlIC) (18) was carried in C3H/Be/FcJ male mice (The Jackson Laboratory, Bar Harbor, ME). For the experiments, 2 × 10^5 tumor cells prepared from a brei of several stock tumors were implanted i.m. into the legs of C3H/Be/FcJ male mice 8–10 weeks of age.

Tumor Growth Delay Experiments. When the tumors were approximately 50 mm³ in volume, the perfluorochemical emulsion Fluosol-DA (0.3 ml) was injected via the tail vein. Immediately afterward the drug was administered by i.p. injection. Melphalan was dissolved in 2% N HCl/ethanol solution and then diluted in sterile 0.9% PBS³ or diluted into Fluosol-DA or Intralipid. The animals were then allowed to breathe air or were placed in a circulating atmosphere of 95% O₂ and 5% CO₂ (carbogen) for 1 or 2 h and then removed to air. The progress of each tumor was measured 3 times weekly until it reached a volume of 500 mm³. Tumor growth delay was calculated as the days taken by each individual tumor to reach 500 mm³ compared to the untreated controls. Each treatment group had 7 animals and the experiment was repeated 3 times. Days of tumor growth delay are the mean ± SE for the treatment group compared to the control.

Tumor Excision Assay. When the tumors were approximately 50 mm³ in volume (about 1 week after tumor cell implantation) the perfluorochemical emulsion Fluosol-DA (0.3 ml) was injected via the tail vein. Immediately afterward melphalan was administered by i.p. injection. The animals were then allowed to breathe air or were placed in a circulating atmosphere of 95% O₂ and 5% CO₂ (carbogen) for 1 or 2 h and then removed to air. Mice were sacrificed and blood was obtained by cardiac puncture. The blood was allowed to clot at room temperature and then centrifuged at 2000 × g for 10 min at 4°C followed by filtration under sterile conditions (28). The bone marrow cell cultures were incubated for 7 days in a humidified 5% CO₂ atmosphere at 37°C. The colony-stimulating activity-containing supernatant was obtained by centrifugation of the medium at 10,000 × g for 10 min at 4°C followed by filtration under sterile conditions (28). The bone marrow cell cultures were incubated for 7 days in a humidified 5% CO₂ atmosphere at 37°C and then fixed with 10% glacial acetic acid.

Fluosol-DA/CARBGEN ENHANCEMENT OF MELPHALAN ACTIVITY

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The abbreviations used are: PBS, phosphate-buffered saline; FBS, fetal bovine serum.

log(irradiated control)  
\[ CLF = \frac{\text{control}}{\text{log(irradiated drug)} \text{ control}} \]
Pharmacokinetics Study. FSA1IC fibrosarcoma-bearing mice, as described above, were given injections of 6 μCi of [ethylthioethyl-1,2,14C] melphalan (10 mg/kg; 7.84 mCi/mmol) with Fluosol-DA (12 ml/kg, 0.3 ml) and melphalan alone. Animals were sacrificed at 0.25, 0.5, 0.75, 1.00, 1.25, 3.00, 3.25, 6.00, 6.25, 24.00, and 24.25 h posttreatment. Known wet weights of tumor, liver, spleen, kidney, heart, lungs, skin, fat, gut, skeletal muscle, and blood were dissolved in a tissue solubilizer (Protosol; New England Nuclear), then counted by liquid scintillation in Aquasol (New England Nuclear). The data are expressed as μg 14C equivalents per g tissue wet weight. The data were fitted to a triexponential equation of the form

\[ C = Ae^{-kt} + Be^{-kt} + Ce^{-kt} \]

which allowed derivation of the n0,5 for absorption, distribution, and elimination of the drug as well as calculation of the area under the concentration versus time curve (31, 32).

Melphalan and Fluosol-DA Interaction Studies. A high-performance liquid chromatography–UV system was used to measure the levels of melphalan in the aqueous portion of Fluosol-DA. The mobile phase for high-performance liquid chromatography was 1 m ammonium acetate, 0.7% acetic acid, and 40% ethanol delivered at a rate of 1.5 ml/min using a Spectra Physics pump (33, 34) and Brownlee C18 reverse phase column. Separation was effected isocratically at room temperature with detection at 254 nm. Quantitation was made by construction of a standard curve of peak height versus melphalan standards of known concentration. The calibration curve was linear on a log-log plot from 0.25–15 μg/ml melphalan.

In the first experiment, concentrations of melphalan of 5, 10, and 15 μg/ml were dissolved in Fluosol-DA and after allowing equilibration for 0.25, 0.50, 1.00, 4.00, and 24.00 h, aliquots were removed from each tube. The Fluosol-DA containing melphalan was centrifuged for 10 min in an Eppendorf microcentrifuge which separated the aqueous portion from the denser perfluorochemical portion of the Fluosol-DA. Ten μl of the supernatant were used to measure the amount of melphalan under each condition. The experiment was repeated 3 times.

In the second experiment, 15 μg/ml melphalan were dissolved in Fluosol-DA and allowed to equilibrate for 1 h. Aliquots were removed and diluted 2-, 5-, or 10-fold with PBS and allowed to equilibrate once again for 0.25, 0.5, 1.0, 4.0, or 24.0 h. Then, as described above, aliquots were removed, centrifuged, and the amount of melphalan in the aqueous layer was measured. The experiment was repeated 3 times.

### RESULTS

The influence of Fluosol-DA and Intralipid, a fat emulsion, with and without carbogen breathing, on the tumor growth delay of the FSA1IC fibrosarcoma caused by a single dose of 10 mg/kg melphalan was examined (Table 1). Under air breathing, Fluosol-DA produced a 2-fold increase in tumor growth delay compared to melphalan alone. Intralipid had no influence on the drug’s effect. When carbogen breathing was added to the protocol, there was a 3-fold increase in tumor growth delay with Fluosol-DA and only a 1.6-fold increase in tumor growth delay with Intralipid. Preparing melphalan in Fluosol-DA produced a 4-fold increase in tumor growth delay with air breathing and a 10-fold increase in tumor growth delay with carbogen breathing. Preparing melphalan in Intralipid produced a 1.5-fold increase in tumor growth delay with air breathing and a 3.7-fold increase in tumor growth delay with carbogen breathing. Therefore, addition of Fluosol-DA to treatment with melphalan leads to a greater increase in tumor growth delay under both conditions of air and carbogen breathing than does Intralipid.

When FSA1IC tumors were excised 24 h after treatment with melphalan or melphalan plus Fluosol-DA with air or carbogen breathing and tumor cell survival was measured by colony formation, the results shown in Fig. 1 were obtained. Throughout the melphalan dosage range examined there is approximately 1 log greater tumor cell kill observed with the addition of Fluosol-DA and carbogen breathing compared to the drug treatment alone. In the mouse, 10 μg/kg of melphalan is a therapeutic dose, producing a FSA1IC tumor growth delay of 2.7 ± 0.3 (SE) days (25). The ability of melphalan to kill tumor cells increases with dose over the range of drug examined. At the lower doses of drug (5 and 10 μg/kg) there is some increase in tumor cell killing seen with the addition of carbogen breathing or Fluosol-DA and air breathing; however, at the highest dose of the drug, as shown by the near coincidence of the points, this difference disappears.

The survival of bone marrow cells from these same animals was measured by the colony-forming ability of granulocyte-macrophage colony-forming units in culture. The killing of bone marrow cells increases linearly over the dosage range examined at a level consistent with previous reports (Fig. 2; Refs. 35 and 36). There was no significant difference in the survival of bone marrow cells under any of the treatment conditions.

Following the methodology of Murray and Meyn (7) the alkaline elution of cells from FSA1IC tumors treated in vivo was used to examine the effect of these treatment conditions on the cross-linking or DNA in tumor cells. In the mouse, 10 mg/kg of melphalan is a therapeutic dose, producing a 3.7-fold increase in tumor growth delay with carbogen breathing or Fluosol-DA and air breathing; however, at the highest dose of the drug, as shown by the near coincidence of the points, this difference disappears.

The cross-linking factors for melphalan (10 mg/kg) was 1.95 and 1.99 with air and carbogen breathing, respectively.
1. Fluorosol-DA and carbogen enhancement of melphalan activity

2. Survival of bone marrow cells as measured by the granulocyte-macrophage colony-forming unit assay from mice treated with various doses of melphalan (M) with air breathing (Θ), melphalan followed by carbogen breathing for 1 h (C), melphalan and Fluorosol-DA (F) with air breathing (Θ), or melphalan and Fluorosol-DA followed by carbogen breathing for 1 h (C). Bars, SE.

3. Table 2 DNA cross-linking factors from alkaline elution of FSaIC tumors treated in vivo

Fluosol-DA was administered i.v. at a dose of 12 ml/kg (0.3 ml). Melphalan was given as a single dose i.p. of 10 mg/kg.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Cross-linking factor</th>
<th>Air</th>
<th>Carbogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluosol-DA</td>
<td>1.07*</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Melphalan</td>
<td>1.95</td>
<td>1.99</td>
<td></td>
</tr>
<tr>
<td>Melphalan + Fluosol-DA</td>
<td>2.02</td>
<td>5.82</td>
<td></td>
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</table>

* Cross-linking factor = 1.00 indicates no cross-links.

4. Fig. 3. Fraction of melphalan in the aqueous portion of Fluosol-DA (FDA) over time standing at room temperature. Total μg of melphalan per ml of Fluosol-DA with (Θ) 5, (C) 10, and (Θ) 15 μg/ml. Bars, SE.

5. Fig. 4. Amount of melphalan (μg) in the aqueous portion of Fluosol-DA after equilibration of 15 μg/ml melphalan (1 h) followed by dilution with phosphate-buffered 0.9% saline and reequilibration. Bars, SE.

6. DISCUSSION

Solid tumors may be refractory to a cytotoxic agent for several reasons, including (a) the limited penetration of the periods of time (Fig. 4). Shortly after dilution, the melphalan was increased in the aqueous portion of the Fluosol-DA. However, after long periods of reequilibration, the melphalan moved back into the lipophilic perfluorochemical. The immediate effect of an increase of melphalan in the aqueous phase indicates that melphalan sequestered in the perfluorochemical can be accessed into a more hydrophilic environment.

The pharmacokinetics of [14C]melphalan alone, [14C]melphalan plus Fluosol-DA, and [14C]melphalan prepared in Fluosol-DA were studied in several tissues of FSaIC fibrosarcoma-bearing mice (Table 3). In general, the tissue absorption and distribution parameters for melphalan were shortened in the presence of Fluosol-DA (except for the kidneys). In the blood, the half-life for elimination for melphalan alone was only 20 min; however, with Fluosol-DA that time increased to 6.7 h, and when the drug was prepared in Fluosol-DA the half-life for elimination was 5.2 h. In the liver (and most other tissues) melphalan is absorbed and distributed much more rapidly in the presence of Fluosol-DA. The elimination time is also shortened and in general the area under the concentration versus time curve for exposure to the drug is smaller. In the kidneys, however, the half-time parameters for absorption, distribution, and elimination are all longer when Fluosol-DA is present, giving a 3.6-fold increase in the area under the curve for that tissue.

The melphalan concentration versus time curves for the 3 treatment conditions over the first 6 h are shown in Fig. 5. As can be seen, in the presence of Fluosol-DA there is a shifting of the half-lives for absorption and distribution to shorter times which produces a much sharper and earlier peak in the drug exposure of the tumor. Since the drug in the presence of Fluosol-DA is eliminated from the tumor more slowly, the overall effect is to produce essentially no change in the area under the drug exposure curve for the tumor.
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Table 3 Pharmacokinetic parameters for [14C]melphalan administered i.v. to mice bearing FSaIC tumors
These parameters were derived from curves based on 11 data points over 24 h. The data were analyzed via a computer program based on a 2-compartment model (21, 32). Data for blood, spleen, brain, heart, lungs, skin, fat, and gut are not shown. Melphalan was administered at a dose of 10 mg/kg. Fluosol-DA was administered at a dose of 12 ml/kg (0.3 ml). For melphalan in Fluosol-DA, melphalan (10 mg/kg) was prepared in Fluosol-DA (0.3 ml) and administered as a single injection.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment group</th>
<th>$t_{1/2}$ absorption (min)</th>
<th>$t_{1/2}$ distribution (min)</th>
<th>$t_{1/2}$ elimination (h)</th>
<th>Area under curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>Melphalan</td>
<td>34</td>
<td>10.3</td>
<td>19.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Melphalan + Fluosol-DA</td>
<td>20</td>
<td>27.8</td>
<td>16.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Melphalan in Fluosol-DA</td>
<td>12</td>
<td>26.1</td>
<td>16.8</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>Melphalan</td>
<td>34</td>
<td>37.2</td>
<td>56.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Melphalan + Fluosol-DA</td>
<td>8.5</td>
<td>28.9</td>
<td>16.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Melphalan in Fluosol-DA</td>
<td>11</td>
<td>24.0</td>
<td>14.9</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>Melphalan</td>
<td>3.6</td>
<td>2.3</td>
<td>44.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Melphalan + Fluosol-DA</td>
<td>16</td>
<td>14.8</td>
<td>108.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Melphalan in Fluosol-DA</td>
<td>40</td>
<td>39.7</td>
<td>158.0</td>
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</table>

Fig. 5. FSaIC tumor levels of [14C]melphalan (M) (10 mg/kg) alone (•), with Fluosol-DA (F) (12 ml/kg; 0.3 ml) (○), or prepared in Fluosol-DA (0.3 ml) (△).

The increase in tumor growth delay and the decrease in tumor cell survival obtained with Fluosol-DA and air breathing represent the contribution to the therapeutic effect of melphalan treatment resulting from the alteration in the drug pharmacokinetics of the drug caused by Fluosol-DA. Melphalan (and probably many other lipophilic drugs) readily moves into the lipophilic environment of Fluosol-DA. This lipophilic environment may protect the drug from hydrolysis and nonspecific binding in circulation and thereby allow delivery of a greater proportion of active drug to the tumor. Similar results were obtained when melphalan was entrapped in small neutral liposomes (42). Although the area under the concentration versus time curve for melphalan in the presence of Fluosol-DA did not change, the shortened absorption and distribution $t_{1/2}$ would tend to support the notion that more of the drug arriving at the tumor is the active agent, thus leading to enhanced antitumor activity. At the highest melphalan dose (15 mg/kg), no additional cell killing was seen upon addition of Fluosol-DA and air breathing, indicating that increasing the drug dose to a certain level can compensate for drug loss in the absence of Fluosol-DA.

The details of the involvement of oxygen in the antitumor activity of melphalan in combination with Fluosol-DA remain to be elucidated. The findings reported here demonstrate a relatively nontoxic means of increasing the delivery of oxygen to tumors, resulting in a therapeutically meaningful improvement in melphalan antitumor activity.

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REFERENCES

3. Teicher, B. A., Lazo, J. S., and Sartorelli, A. C. Classification of antineo-
22. Mason, K. A., Withers, H. R., and Steckel, R. J. Acute effects of a perflu-
23. Rockwell, S., Mate, T. P., Irvin, C. G., and Nierenberg, M. Reactions of
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