Effects of Various Oxygenation Conditions on the Enhancement by Fluosol-DA of Melphalan Antitumor Activity

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ABSTRACT

The cytotoxicity of melphalan toward exponentially growing FSaIC fibrosarcoma cells under hypoxia, normal aeration, hyperoxygenation, and stationary phase normally oxygenated cells was examined. Through 4 logs of cell kill by melphalan, there was no difference in survival of FSaIC cells under any of the four conditions. In the fifth and sixth logs of cell kill, melphalan was most cytotoxic to normally aerated cells. DNA alkaline elution was performed in FSaIC cells treated for 1 h with melphalan under the various atmospheres. Both upon immediate elution and after a 6-h delay period the greatest number of DNA cross-links were formed in the normally oxygenated cells. Tumor growth delay studies of the FSaIC fibrosarcoma treated with melphalan were performed under four levels of oxygenation. From air breathing to 100% oxygen at 3 atm, the tumor growth delay produced by melphalan increased from about 3 days to about 9 days. With the addition of Fluosol-DA, there was an increase in tumor growth delay by melphalan from about 6.5 days with air breathing to about 13 days with 100% oxygen at 3 atm (1 h). When FSaIC fibrosarcoma tumors were treated with melphalan, and tumor cell survival was measured by colony formation in culture, increasing doses of melphalan produced increasing levels of tumor cell kill in a relatively log linear manner. The addition of Fluosol-DA to treatment with melphalan produced approximately 1 log greater tumor cell kill than melphalan and air breathing under the various oxygenation conditions. There was approximately a 4-fold increase in toxicity to bone marrow granulocyte-macrophage colony-forming units under both extended carbogen breathing conditions (6 h) and hyperbaric oxygenation conditions (100% oxygen, 1 h, 3 atm). The response of the spleen to these various treatment regimens appeared to be immediate and short-lived. Necrotic cells were seen on day 1 posttreatment, with a substantial reduction by day 4 posttreatment. Mitotic figures were essentially absent from the liver on day 1 posttreatment, but by day 4 were significantly increased in treatment groups receiving Fluosol-DA, with the largest number seen in the melphalan/Fluosol-DA with carbogen-breathing group. In conclusion, Fluosol-DA and 1 h of carbogen breathing significantly increases the antitumor activity of melphalan without a concomitant increase in normal tissue toxicity. Although increasing the oxygenation level increased the response of the tumor, normal tissue toxicity was also increased.

INTRODUCTION

Fluosol-DA with carbogen (95% oxygen and 5% carbon dioxide) breathing can increase the efficacy of melphalan treatment of the FSaIC murine fibrosarcoma (1, 2). Fluosol-DA influences the pharmacokinetics of melphalan and probably the bioavailability of the drug. Moreover, from tumor growth delay experiments, tumor excursion assays and alkaline elution studies of DNA cross-linking, it appears that oxygen breathing combined with Fluosol-DA produces a marked enhancement in melphalan cytotoxicity. Tumor growth delay results obtained with Intraplrip suggest that the lipophilic nature of Intraplrip and Fluosol-DA contributes to the enhancement of melphalan antitumor activity. However, it is the oxygen-carrying capacity of Fluosol-DA which leads to its superiority over Intraplrip (2).

Unlike the slope change seen in the enhancement of melphalan antitumor activity by high doses of misonidazole (3-6), the survival curves of both melphalan and melphalan/Fluosol-DA with carbogen breathing are parallel, which suggests that the addition of Fluosol-DA and carbogen breathing to treatment with melphalan is allowing the drug to be cytotoxic to an additional subpopulation of cells.

Perfluorochemicals have excellent oxygen- and carbon dioxide-carrying capacity (7-9). To fully utilize the oxygen-carrying capacity of these materials, high partial pressures of oxygen are used. Unlike hemoglobin, where oxygen is chelated to the molecule, the solvent action of the perfluorochemicals for oxygen does not involve any kind of chemical or chelating process. The amount of oxygen carried by the perfluorochemicals increases linearly with the partial pressure of oxygen; therefore, the perfluorochemical emulsion will carry more oxygen under hyperbaric conditions (10).

In these studies we have further explored the effects of oxygen on melphalan cytotoxicity in vitro and in vivo. The effects of various atmospheres on the cytotoxicity and DNA cross-linking of melphalan were assessed. In an attempt to define the limits of the oxygen and Fluosol-DA/oxygen effect on melphalan antitumor activity, tumor growth delay and tumor cell survival of the FSaIC fibrosarcoma were measured in animals exposed to hyperbaric oxygen (1 h) and in animals exposed to extended carbogen breathing (6 h). Fluosol-DA is taken up by the cells of the reticuloendothelial system of the liver and spleen (7-9). In using Fluosol-DA in combination with chemotherapeutic agents which may be sequestered in the lipophilic perfluorochemical particles, it is important to examine effects on these normal tissues. We have examined by histopathology the liver and spleen tissues of animals treated with melphalan/Fluosol-DA/carbon on days 1 and 4 posttreatment. The response of bone marrow in animals treated with melphalan/Fluosol-DA and hyperbaric oxygen or extended carbogen breathing has been assessed by granulocyte-macrophage colony-forming units and compared to that of animals exposed to carbogen breathing for 1 h post-drug treatment.

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 perfluoropropiolamine; 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 x 10^5 cm^2/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 (8). Melphan (l-phenylalanine mustard) was a gift from Burroughs Wellcome Co. (Research Triangle Park, NC).  

In Vivo Survival Studies. FSaIC murine fibrosarcoma cells grow as
monolayers in αMEM (Grand Island Biological Co., Grand Island, NY) supplemented with 10% FBS (Sterile Systems, Logan, UT). For experiments, FSAlIC cells were grown in plastic culture flasks and used when in exponential growth or were grown to confluent monolayers for experiments. To produce hypoxia or hyperoxia, flasks were fitted with sterile rubber sleeve serum stoppers and exposed to continuously flowing 95% nitrogen/5% CO₂ or 95% oxygen/5% CO₂ (carbogen) humidified atmosphere for 4 h at 37°C prior to drug treatment (12, 13). Parallel flasks were maintained in humidified 95% air/5% CO₂. At this time, melphalan or vehicle was added to the flasks by injection through the rubber stoppers without disturbing the controlled atmosphere. After exposure to the drug for 1 h at 37°C, the cells were washed with 3 ml of sterile PBS, suspended by treatment with 0.01% trypsin in PBS for 15 min, plated in replicate 100-mm dishes at 3 dilutions in αMEM plus 10% FBS, and the surviving fraction of cells was measured by colony formation. No difference existed between the survival of untreated or vehicle-treated cells maintained under the controlled atmosphere used; the plating efficiency for these control cultures was 25–30%. Colonies which grow to countable size (>50 cells) in 10–12 days were visualized with crystal violet and counted manually. Each survival curve was determined in three independent experiments.

Alkaline Elution. Alkaline elution was performed by standard procedures (14). FSAlIC cells (2.75 x 10⁵) were labeled with 1.25 »Ci of [³²P]thymidine (53.2 mCi/mmol; New England Nuclear, Boston, MA) in αMEM with 10% FBS for approximately three doublings, after which the [³²P]thymidine was removed and the cells were washed. Melphalan (100 μM) was added to the cells under each of the controlled atmospheres as described above and incubated for 60 min at 37°C. The cells were either washed and suspended by treatment with 0.1% EDTA or washed with pregassed PBS, after which fresh pregassed medium plus 10% FBS was added and incubation at 37°C was resumed for 6 h. Cells were then suspended by treatment with 0.1% EDTA and DNA alkaline elution was performed as previously described (2). Each point was measured in 3 independent experiments. Cross-linking factor (CLF) was calculated as

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\text{CLF} = \frac{\log \text{(irradiated control)}}{\log \text{(irradiated drug)}}
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Tumor. The FSAl fibrosarcoma (15) adapted for growth in culture (FSAlIC) (16) was carried in male C3H/FeJ mice (The Jackson Laboratory, Bar Harbor, ME). For the experiments, 2 x 10⁴ tumor cells prepared from a brei of several stock tumors were implanted i.m. into the legs of 8- to 10-week old male C3H/FeJ mice.

Tumor Growth Delay Experiments. 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% O₂/5% CO₂ for 1 or 6 h or were placed in a hyperbaric chamber filled with 100% oxygen at 2 or 3 atm for 1 h and then removed to air. Mice were sacrificed 24 h after treatment to allow for full expression of drug cytotoxicity and repair of potentially lethal damage and then soaked in 95% ethanol. The tumors were excised under sterile conditions and single cell suspensions were prepared for the colony forming assay (2). One week later the plates were stained with crystal violet and colonies of more than 50 cells were counted. The untreated tumor cell suspensions had a plating efficiency of 9–12%. The results are expressed as the surviving fraction ± SE of cells from treated groups compared to untreated controls.

Bone Marrow Toxicity. Bone marrow was taken from the same animals used for the tumor excision assay and a colony forming assay was carried out as described previously (2). Colonies of at least 50 cells were scored on a Acculite colony counter (Fisher, Springfield, NJ). The results from 3 experiments, in which each group was measured in triplicate, were averaged. The results are expressed as the surviving fraction of treated groups compared to untreated controls.

Hepatic and Splenic Toxicity. On day 0, C3H/FeJ mice (8 animals per group) were treated with melphalan (10 mg/kg) by i.p. injection, or with Fluosol-DA (0.3 ml) i.v. followed by melphalan. Animals were then allowed to breathe air or were maintained in carbogen (95% O₂/5% CO₂) for 1 h (oxygen-treated group). Control animals were given i.p. saline and allowed to breathe air. On days 1 and 4 posttreatment, four animals from each group were sacrificed by cervical dislocation, and longitudinal 1-mm slices of spleen, liver, kidney, lung, pancreas, and small bowel were fixed in neutral buffered 10% formalin. The tissues were embedded in paraffin, sectioned at 10 μm, and stained with hematoxylin-eosin and Masson trichrome stains by standard methods.

Slides were initially reviewed unblinded in order to identify patterns of tissue toxicity induced by this protocol. There was no evidence of histological toxicity in lung, kidney, pancreas, or small bowel at 1 or 4 days. Following melphalan treatment, cellular depletion of both the white and red pulp of the spleen was noted, regardless of whether Fluosol-DA or oxygen treatment was included. Fluosol-DA treatment resulted in prominent accumulation of foamy macrophages in the splenic red pulp, which was unaffected by melphalan or oxygen treatment. In the liver, occasional sinusoidal foamy macrophages were noted following Fluosol-DA administration. Two prominent features were noted, however, in these two organs. While occasional necrotic cells were present in spleen white and red pulp in control animals, they were marked increased in number in treated animals. In addition, hepato-cellular mitotic figures were generally absent in livers from control animals, but were more frequently seen in livers from experimental animals. The identification of variability in the number of necrotic cells in the spleen and mitotic figures in the liver parenchyma prompted an objective correlation of these parameters with treatment modalities. Histological grading was carried out blinded on randomized coded tissue sections, using an American Optics microscope. Liver sections were scored for mitotic figures in randomly selected high-power fields (×400). Splenic sections were scored for the presence of karyorrhectic cells, indicative of recent cell necrosis. Scoring randomly selected high-power fields provided an overall determination of the number of necrotic cells in the spleen. Sections were then scored separately for the number of necrotic cells per high-power field in the white pulp and in the red pulp. The data presented are the means of at least 10 high-power fields in each tissue section for each parameter scored.

RESULTS

Our previous in vivo findings of the enhancement of FSAlIC tumor growth delay and tumor cell killing by melphalan and Fluosol-DA with carbogen breathing (1, 2) led us to examine in vitro the cytotoxicity of melphalan toward exponentially

parisons were made with the Dunn multiple comparisons test.

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% O₂/5% CO₂ for 1 or 6 h or were placed in a hyperbaric chamber filled with 100% oxygen at 2 or 3 atm for 1 h and then removed to air. Mice were sacrificed 24 h after treatment to allow for full expression of drug cytotoxicity and repair of potentially lethal damage and then soaked in 95% ethanol. The tumors were excised under sterile conditions and single cell suspensions were prepared for the colony forming assay (2). One week later the plates were stained with crystal violet and colonies of more than 50 cells were counted. The untreated tumor cell suspensions had a plating efficiency of 9–12%. The results are expressed as the surviving fraction ± SE of cells from treated groups compared to untreated controls.

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growing FSaIC fibrosarcoma cells under hypoxia, normal aeration, and hyperoxygenation, as well as stationary phase normally oxygenated cells. Fig. 1 shows the survival of FSaIC cells under these four different conditions over the melphalan concentration range of 1–500 μM. Through four logs of cell kill by melphalan, there is no difference in survival of exponentially growing FSaIC cells under any of the three levels of cellular oxygenation or in normally oxygenated stationary phase. In the fifth and sixth logs of cell kill, however, a small difference emerges. Melphalan is most cytotoxic toward normally aerated cells and somewhat less cytotoxic toward hypoxic cells, hyperoxygenated cells, and stationary phase cells.

DNA alkaline elution was performed on FSaIC fibrosarcoma cells treated for 1 h with melphalan (100 μM) under hypoxia, normal aeration, and hyperoxygenation (Fig. 2). Both upon immediate elution and after a 6-h delay period to allow for full expression of cross-link formation, the greatest number of DNA cross-links were formed in the normally oxygenated cells and somewhat fewer DNA cross-links were seen in the hypoxic and hyperoxygenated cells. Therefore, the level of DNA cross-linking correlated well with the in vitro cytotoxicity observed.

In an effort to determine the limits of enhancement in melphalan antitumor activity which can be achieved with Fluosol-DA and/or oxygen breathing, tumor growth delay studies of the FSaIC fibrosarcoma were performed under four levels of oxygenation: (a) air breathing (20% oxygen); (b) carbogen breathing for 1 h (95% oxygen; 1 atm); (c) 100% oxygen breathing for 1 h (2 atm); and (d) 100% oxygen for 1 h (3 atm) immediately after drug treatment with melphalan or melphalan/Fluosol-DA (Fig. 3). Neither oxygen nor Fluosol-DA alone have any effect on the growth of this tumor (7, 8). There was a continued increase in the tumor growth delay observed with melphalan and increasing levels of oxygenation both in the presence and absence of Fluosol-DA. From air breathing to 100% oxygen at 3 atm, the tumor growth delay increased from about 3 days to about 9 days. With the addition of Fluosol-DA, there was an increase in tumor growth delay from about 6.5 days with air breathing to about 13 days with 100% oxygen at 3 atm (1 h). Melphalan/Fluosol-DA and 1 h of carbogen breathing (95% oxygen; 1 atm) produced approximately the same level of tumor growth delay as melphalan followed by 100% oxygen for 1 h (3 atm), indicating a greater level of oxygen carrying capacity by Fluosol-DA than by plasma.

When FSaIC fibrosarcoma tumors were treated with melphalan or melphalan/Fluosol-DA under various oxygenation conditions in vivo, then excised 24 h later and tumor cell survival was measured by colony formation in culture, the

![Fig. 1](cancerres.aacrjournals.org) Survival of exponentially growing normally oxygenated (○), hypoxic (□), hyperoxygenated (■), and stationary phase (SP) normally oxygenated (□) FSaIC cells treated for 1 h with various concentrations of melphalan in vitro. Points, means of three independent determinations; bars, SE.

![Fig. 2](cancerres.aacrjournals.org) DNA cross-linking in the FSaIC cells immediately after (○) and 6 h post-treatment (□) with melphalan (100 μM) for 1 h under hypoxic, normally oxygenated, and hyperoxygenated conditions. The data shown are derived from three independent experiments.

![Fig. 3](cancerres.aacrjournals.org) Growth delay of the FSaIC fibrosarcoma produced by a single dose of melphalan (10 mg/kg) (○), or Fluosol-DA (FDA) (0.3 ml) immediately followed by melphalan (10 mg/kg) (□) under various conditions of oxygenation for 1 h post-drug treatment and then maintained in air. Points, means of three independent experiments with seven animals/group (21 animals/point); bars, SE.
Significantly different from melphalan with air breathing. When melphalan was followed by 1 h of 100% oxygen at 3 atm, there was approximately a 6-fold increase in tumor cell killing over the dosage range of 5–15 mg/kg of melphalan. The addition of Fluosol-DA to treatment with melphalan over the dosage range of 5–15 mg/kg produced approximately 1 log greater tumor cell kill than did melphalan and air breathing under all three oxygenation conditions. There was no difference in the tumor cell kill seen with melphalan/Fluosol-DA with carbogen breathing for 1 or 6 h or with 100% oxygen at 3 atm for 1 h. At the highest melphalan dose of 25 mg/kg, the effect of Fluosol-DA and carbogen breathing appears to be diminishing.

Although no increased tumor cell kill was seen 24 h posttreatment in the tumor cell survival assay, there was approximately a 4-fold increase in toxicity to bone marrow granulocyte-macrophage colony forming units both under extended carbogen-breathing conditions (6 h) and under hyperbaric oxygenation conditions (100% oxygen for 1 h at 3 atm) (Fig. 5). The presence or absence of Fluosol-DA did not influence the toxicity of melphalan to the bone marrow under these oxygenation conditions.

Fluosol-DA is retained in liver and spleen at a level that is proportional to the dose of Fluosol-DA injected (11). In chronically treated animals, the histological changes in these tissues consist of proliferated Kupffer cells and reticular cells which show enlargement with fairly abundant, coarsely vacuolated or “foamy” cytoplasm (16, 17). Melphalan can be sequestered in the lipophilic perfluorochemical particles of Fluosol-DA; therefore, a histopathological study was carried out on these organs in animals treated with melphalan with and without Fluosol-DA under air breathing and carbogen breathing conditions (Fig. 6).

Mitotic figures were essentially absent from the liver on day 1 posttreatment, but by day 4 were significantly increased in treatment groups receiving Fluosol-DA, with the largest number seen in the melphalan/Fluosol-DA with carbogen-breathing group. The response of the spleen to these various treatment regimens appears to be more immediate and short-lived. Significant numbers of necrotic cells were seen on day 1 posttreatment, with a substantial reduction by day 4 posttreatment in all of the experimental groups. The administration of Fluosol-DA alone led to an increased number of necrotic cells in the red pulp on day 1 (F group), which was reduced slightly by carbogen breathing (FO group). The number of necrotic cells in the white pulp was modestly increased by Fluosol-DA, with no further influence of carbogen breathing. Melphalan treatment alone induced modest but comparable increases in necrotic cells in both red and white pulp (M group), which was also slightly reduced by carbogen breathing (MO group). While melphalan given with Fluosol-DA did not potentiate the effect of melphalan/Fluosol-DA in the red pulp, there was a substantial increase in the number of necrotic cells in the white pulp (MF group). The further addition of carbogen breathing (MFO) slightly reduced the toxicity of melphalan and Fluosol-DA in the white pulp, but increased to the toxicity slightly in the red pulp.

Results on day 1 posttreatment for the spleen generally reflected the scoring indices obtained for the white pulp, with a modest influence from the red pulp. In particular, combined treatment with melphalan and Fluosol-DA (MF group) produced a substantial increase in necrotic cells observed in the spleen, largely the result of increased toxicity in the white pulp. In all but one circumstance (red pulp in the MFO group), the addition of carbogen breathing reduced or did not alter the toxic effect of melphalan and Fluosol-DA. By day 4 posttreatment the number of necrotic cells in both splenic red and white pulp was substantially reduced, with persistent elevation most notable in Fluosol-DA treated animals. Animals treated with
melphalan either alone or with carbogen breathing essentially returned to normal by day 4 posttreatment.

**DISCUSSION**

The enhancement of melphalan antitumor activity seen *in vivo* with the addition of Fluosol-DA and breathing an atmosphere of high oxygen content results from a combination of effects upon this drug. Melphalan can be taken up into the emulsion and thereby be protected from metabolism in the phalan for 1 h under hypoxia, normal aeration, and hyperoxy-
generation and were able to find an effect due to level of cellular oxygenation only in the fifth and sixth logs of cell kill. This is a very small effect, and probably is not a perfect model for occurrence *in vivo*, since these are exponentially growing cells, and all of the groups were exposed to air immediately after drug exposure. However, the results do support the notion that oxygen is not directly a factor in the cytotoxic action of melphalan. There does, however, seem to be an optimal level of cellular oxygenation for melphalan action, since at relatively high drug concentrations both hypoxic and hyperoxic conditions led to less oxygenation and fewer DNA cross-links in cells exposed to the drug *in vitro*.

An inconsistency was seen in the results obtained in the tumor growth delay study and the tumor cell survival assay. Whereas tumor growth delay continued to increase as the level of oxygenation was increased from air breathing to hyperbaric oxygen for 1 h at 3 atm both in the presence and absence of Fluosol-DA, this effect was seen in the tumor excision assay only in the absence of Fluosol-DA. The tumor cell recovery from tumors for the excision assay in the various treatment groups was the same. This dichotomy between results by these two standard assay methods has occurred before (15), and suggests that Fluosol-DA must have other effects on the tumor-host which operate at greater than 24 h posttreatment, and that these other effects, such as alterations in tumor cell population kinetics, are influenced by the level of oxygenation immediately after drug administration.

The tumor cell survival results indicate that the direct cytotoxic effect of melphalan(Fluosol-DA) may be maximal with carbogen breathing (1 h) with 1 log greater cell kill than with air breathing at the therapeutic dose of the drug, since neither extended carbogen breathing (6 h) nor hyperbaric oxygen (1 h at 3 atm) produced increased tumor cell killing. Hyperbaric oxygenation in the absence of Fluosol-DA did produce a significant increase in tumor cell killing compared to air breathing but did not reach the level of tumor cell killing by Fluosol-DA and carbogen. Both extended breathing and hyperbaric oxygenation produced a small increase in cytotoxicity to bone marrow granulocyte-macrophage progenitors, but Fluosol-DA did not appear to be involved in that effect.

Fluosol-DA is a factor in the toxicity of the combination of melphalan(Fluosol-DA)/carbogen breathing to both the liver and the spleen. Fluosol-DA is retained in the liver and spleen at a level that is proportional to the dose of Fluosol-DA injected (9, 11). In chronically treated animals, the histological changes consist of proliferated Kupffer cells and reticular cells with abundant, coarsely vacuolated foamy cytoplasm (9, 16, 17). The maintenance of organ cellularity and preservation of cellular ultrastructure have suggested that there is no intrinsic toxicity of perfluorochemicals (17). Sustained melphalan treatment induces splenic atrophy, bone marrow depression, and petechial hemorrhages in the skin and gastrointestinal tract (18, 19).

In Fig. 6 we have demonstrated acute toxicity of both Fluosol-DA and melphalan in the spleen through careful documentation of necrotic cells present in tissue sections. Concomitant with the accumulation of foamy macrophages in the red pulp of the spleen following Fluosol-DA administration is a marked increase in necrotic cells in the red pulp, which was not affected by melphalan treatment. Administration of both Fluosol-DA and melphalan led to a marked increase in necrotic cells in the white pulp, suggesting that the perfluorochemical acts as a vehicle for enhanced delivery of melphalan to the white pulp. Additional treatment with carbogen ameliorated the apparent toxicity of both Fluosol-DA and melphalan. The effects of
Fluosol-DA and melphalan on the spleen were largely dissipated by day 4 posttreatment. Thus, the histological toxicity of these agents is short-lived. Our observations, however, provide a basis for understanding the atrophy of the spleen induced by chronic melphalan treatment; the potential for chronic damage from Fluosol-DA remains to be explored.

In contrast, while there was no apparent damage to the liver following Fluosol-DA and melphalan treatment, a regenerative response was induced, as shown by an increase in hepatocellular mitotic figures. While the mechanisms are not clear, this regenerative response may be a reflection of increased metabolic activity, particularly following administration of Fluosol-DA, melphalan, and carbogen. Most importantly, these results indicate that carbogen breathing does not increase the endogenous toxicity of a combined melphalan/Fluosol-DA regimen. While the enhancement of melphalan toxicity in the splenic white pulp by Fluosol-DA is of concern, the effect is short-lived.

Increasing the levels of oxygenation in vivo by combining Fluosol-DA administration with hyperbaric oxygenation produced an increase in the antitumor effect of melphalan when the tumors remained in situ. This finding was not reflected in an increased tumor cell killing when tumors were excised 24 h posttreatment; therefore, this treatment must produce additional tumoricidal effects which occur at greater than 24 h posttreatment. Treatment with melphalan/Fluosol-DA with air or carbogen breathing produced small increases in toxicity to the liver and spleen compared to Fluosol-DA alone. Finally, Fluosol-DA and 1 h of carbogen breathing significantly increases the antitumor activity of melphalan without a significant increase in normal tissue toxicity. Increasing the oxygenation period to 6 h or the oxygenation level to 3 atm for 1 h results in increased tumor response; however, normal tissue toxicity was correspondingly increased. The other tumor-host effects of Fluosol-DA continue to be explored.

REFERENCES


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