Cancer Chemother Pharmacol (1989) 25: 99–102

Cancer Chemotherapy and Pharmacology



# The influence of Fluosol-DA and carbogen breathing on the antitumor effects of cyclophosphamide in vivo*

**Gwi E. Kim**, and Chang W. Song**

University of Minnesota Medical School, Department of Therapeutic Radiology-Radiation Oncology, Harvard Street at East River Road, Box 494 UMHC, Minneapolis, MN 55455, USA

**Summary.** The effects of Fluosol-DA, an oxygen-carrying perfluorochemical emulsion, and carbogen breathing alone or in combination on the antitumor activity of cyclophosphamide (CTX) in vivo were investigated. The addition of 12 ml/kg Fluosol-DA immediately prior to CTX treatment exerted no effect on the antitumor effect of CTX on the RIF-1 tumor in C3H mice. On the other hand, carbogen breathing alone for 8 h significantly enhanced the antitumor effect of CTX, with a dose-modification factor of $1.29 \pm 0.07$. The combination of Fluosol-DA and carbogen breathing further increased the effect of CTX, with a dose-modification factor of $1.63 \pm 0.05$. There was no significant difference in animal lethality within the treatment groups. It was concluded that Fluosol-DA in combination with carbogen breathing may be useful for the enhancement of CTX chemotherapy of human neoplasms

## Introduction

It has long been known that parts of malignant tumors tend to become hypoxic due to poor vascularization and insufficient oxygen supply and that the presence of hypoxic cells is probably a limiting factor in the complete control of tumors by radiotherapy. A number of divergent means have been proposed and tested to overcome hypoxic protection in radiotherapy. Notably, oxygen mimics such as misonidazole have been extensively studied in recent years but have been found to be ineffective in clinical settings. During the past several years, the potential usefulness of perfluorochemicals in overcoming the hypoxic problem in radiotherapy has been investigated, exploiting the interesting ability of perfluorochemicals to carry large amounts of oxygen and increase tumor oxygenation. Fluosol-DA is one of the perfluorochemical emulsions that has been reported to be effective in enhancing the response of various rodent tumors to radiation [6, 7, 8, 9, 11, 13, 14, 16, 17]. Clinical trials of Fluosol-DA are now in progress [12].

It has also been demonstrated that the presence of hypoxic cells or hypoxic areas in tumors renders the latter refractory to certain chemotherapeutic drugs. An interesting observation is that Fluosol-DA in combination with carbogen breathing can enhance the effect of some antitumor drugs, although the underlying mechanism is not clear [18]. A series of studies by Teicher et al. [18–21] unequivocally demonstrated that Fluosol-DA with carbogen breathing could significantly enhance the effect of bleomycin, melphalan, busulfan, and nitrosoureas to suppress the growth of FSaII fibrosarcoma in C3H mice. Teicher et al. [21] reported that treatment of mice bearing FSaII tumors with 100 mg/kg cyclophosphamide (CTX) in combination with Fluosol-DA at 0.1, 0.2, or 0.3 ml/mouse and 2–6 h carbogen breathing five times on alternate days significantly delayed the tumor growth. However, Fluosol-DA with carbogen breathing for 1–2 h instead of 6 h [21] was ineffective in enhancing the antitumor effect of CTX. This was probably due to the fact that CTX is a prodrug, which has to be converted to an alkylating compound through a complex metabolic process lasting several hours after its administration. Since it is quite likely that the effectiveness of CTX in combination with Fluosol-DA and carbogen breathing is dependent on the CTX dose and the tumor, we investigated the effect of various doses of CTX on RIF-1 tumors in C3H mice in combination with Fluosol-DA and carbogen breathing.

## Materials and methods

*Tumor and animals.* The RIF-1 tumor was grown subcutaneously in the right leg of female C3H mice (Jackson Laboratory, Bar Harbor, Me). The characteristics of the RIF-1 tumor and techniques for animal inoculation as well as the assessment of tumor growth have been described in previous papers [6, 12, 14]. Briefly, about $1 \times 10^5$ cells from culture were suspended in 0.05 ml RPMI 1640 culture medium without supplement and injected s.c. into the right leg of 8- to 10-week-old female C3H mice. Tumors were subjected to various treatments when their diameters reached 6–8 mm. Mice with or without tumors were also used to study the lethality of CXT treatment.

*Drugs.* Fluosol-DA 20% (Alpha Therapeutic Corp., Los Angeles, Calif) is an emulsified preparation of a mixture of perfluorochemicals consisting of perfluorodecalin (14% w/v, perfluorotripropylamine (6% w/v) and other com-

* This work was supported by NCI grant CA13353

** *Current address:* Yonsei University Medical College, Department of Radiation Oncology, Yonsei Cancer Center, Seoul, Korea

*Offprint requests to:* C. W. Song

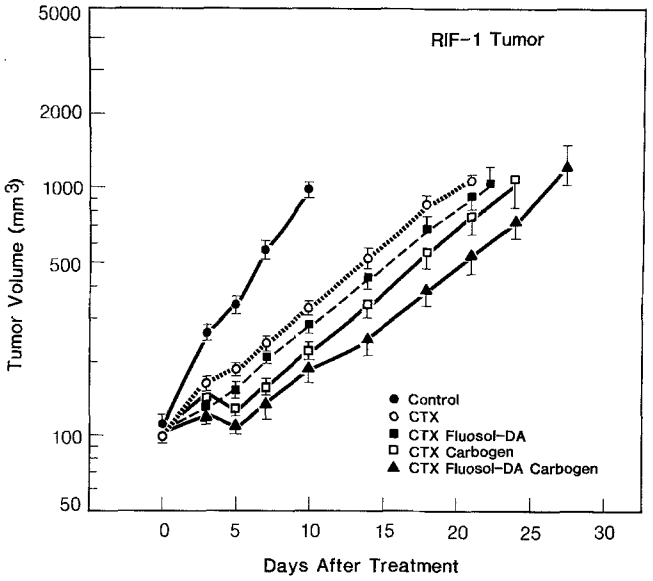


**Fig. 1.** Changes in the growth rate of RIF-1 tumors in mice treated with 200 mg/kg CTX with or without Fluosol-DA and carbogen breathing. Tumor values are shown as a function of days after various treatments. Data points represent the means ±SE of 8–16 tumors

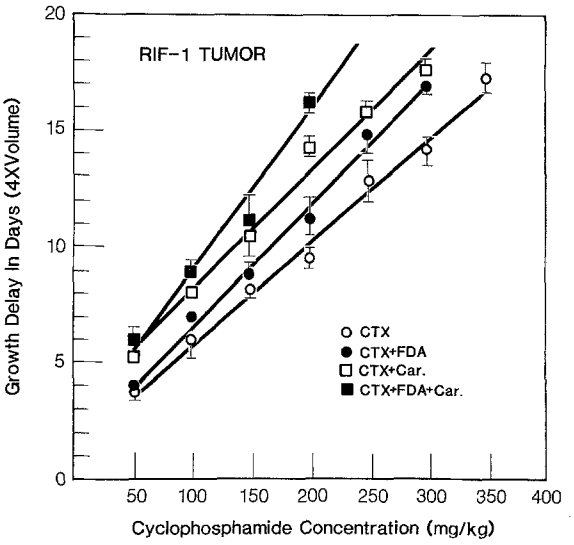


**Fig. 2.** Growth delay (in days) of RIF-1 tumors in mice given various treatments. The best-fitting lines were obtained by linear-regression analysis. *CTX,* cyclophosphamide; *FDA,* Fluosol-DA; *Car,* carbogen. Data points represent the means ±SE of 8–16 tumors

ponents in Krebs-Ringer's bicarbonate solution. The characteristics of Fluosol-DA emulsion have been described in previous papers [6, 13, 14]. The frozen stock emulsion was thawed and prepared for i. v. injection immediately before each treatment [6, 13 14]. Cyclophosphamide monohydrate (CTX) was purchased from Sigma Chemical Company (St. Louis, Mo). CTX was dissolved in ethyl alcohol and then diluted in sterile saline to the desired concentrations.

*Treatment and tumor response.* CTX solutions of varying concentrations were injected via the tail vein. Fluosol-DA 20% emulsion was oxygenated by gassing with carbogen for 30 min and was given through the tail vein at 12 ml/kg (about 0.3 ml/mouse). For the gassing of mice with carbogen, the animals were placed in a Plexiglas box (15 × 20 × 30 cm), which was continuously gassed for 8 h with carbogen through a hole in the cover. The treatments included: (a) CTX in air, (b) CTX + Fluosol-DA, (c) CTX + carbogen, (d) CTX + Fluosol-DA + carbogen, and (e) no treatment.

Tumor growth was followed by measuring the two perpendicular diameters of the tumors 2–3 times/week with a caliper; the tumor volume was calculated with the use of the formula: $V = 0.4\ ab^2$, where a and b are, respectively, the longer and shorter diameters. Experiments were repeated 2–3 times, and 7–8 mice were used for each experimental group.

## Results

The changes in the growth of RIF-1 tumors after various treatments are shown in Fig. 1. The growth of RIF-1 tumors was significantly delayed by an injection of CTX alone at 200 mg/kg. Combinations of CTX with Fluosol-DA at 12 ml/kg were slightly more effective than CTX alone, and combinations of CTX with carbogen breathing for 8 h were significantly more effective in delaying tumor growth than CTX alone. The greatest tumor growth delay was observed when CTX was combined with both Fluosol-DA and carbogen breathing. For example, although it took about 6 days for control tumors to grow 4 times larger, in mice treated with CTX in combination with Fluosol-DA and carbogen breathing, the size of tumors increased 4-fold in about 22 days.

The relative effectiveness of the different treatments was calculated in terms of growth delay by subtracting the

**Table 1.** Influence of Fluosol-DA 20% and carbogen breathing on the toxicity of CTX

| Treatment group | | Proportion of deaths per CTX dose: | | |
|---|---|---|---|---|
| | | 50 mg/kg | 300 mg/kg | 600 mg/kg |
| Air breathing | CTX | 0/12 (0%) | 6/18 (33%) | 11/14 (79%) |
| | CTX + FDA | 0/12 (0%) | 4/22 (18%) | 14/14 (100%) |
| Carbogen breathing | CTX | 0/12 (0%) | 6/19 (32%) | 11/13 (85%) |
| | CTX + FDA | 0/12 (0%) | 3/12 (25%) | 11/14 (79%) |

FDA, Fluosol-DA 20%

number of days required for the untreated tumors to increase 4-fold in size from the corresponding days for the treated tumors. Figure 2 shows the growth delay of RIF-1 tumors treated with various doses of CTX with or without Fluosol-DA treatment and carbogen breathing. Dose-modification factors (DMF) for the adjuvant treatment were calculated by dividing the dose of CTX that resulted in a tumor growth delay of 15 days by the dose that caused the same effect with the adjuvants. The DMF for Fluosol-DA treatment with air breathing was $1.18 \pm 0.06$, and that for carbogen breathing alone was $1.29 \pm 0.07$. The DMF for Fluosol-DA treatment with carbogen breathing increased to $1.63 \pm 0.05$. Animal lethality following various combinations of the treatments is summarized in Table 1. Increases in the CTX dose led to increased animal mortality. The addition of Fluosol-DA alone or in combination with carbogen breathing exerted no effect on CTX-induced mortality in mice.

## Discussion

The present experiments demonstrated that carbogen breathing alone for 8 h could significantly enhance the antitumor effect of CTX on RIF-1 tumors in mice. Moreover, the addition of Fluosol-DA in combination with carbogen breathing for the same duration markedly increased CTX antitumor activity.

The mechanism by which the antitumor activity of CTX was enhanced by Fluosol-DA injections and carbogen breathing is unclear. It has repeatedly been demonstrated that the administration of Fluosol-DA with carbogen breathing significantly enhances the response of tumors in vivo to radiation [6, 7, 8, 9, 11, 13, 14, 16, 17]. Such a radiosensitizing effect has been attributed to an increase in tumor $pO_2$ and the resultant reoxygenation of hypoxic cells [3, 14]. Thus, it would be reasonable to conclude that the enhancement of CTX effects by Fluosol-DA with carbogen breathing is also due to an improvement in tumor oxygenation. In this connection, Dixon et al. [2] reported that CTX was less effective in killing hypoxic cells relative to oxic cells in an experimental rat tumor. However, the effect of CTX on murine tumor cells has also been shown to be independent of the oxygenation status of tumors [1, 15].

Indications are that the marked increase in the antitumor effects of a number of drugs brought about by Fluosol-DA injection and carbogen breathing was, at least in part, due to changes in pharmacokinetics. Being a prodrug, CTX is not directly cytotoxic and must be activated to toxic metabolites by hepatic microsomal oxidase. Juma et al. [5] have demonstrated that the half-life of plasma alkylating activity in man is 7.7 h. The plasma level of alkylating activity has been maintained for at least 6 h after CTX administration in man [21]. Teicher et al. [19] have suggested that Fluosol-DA may protect lipophilic drugs from hydrolysis or binding in circulation, thereby increasing the delivery of some drugs, such as melphalan, to the tumor.

We have previously observed that carbogen breathing alone or Fluosol-DA injection alone slightly increases the tumor blood flow (Lee and Sorg, unpublished data). Recently, Hiraga et al. [4] demonstrated that a perfluorochemical emulsion could increase local cerebral blood flow in intracranial Walker 256 tumors in rats. Therefore, it would be reasonable to conclude that an increase in the delivery of cytotoxic metabolites of CTX to tumors as a consequence of an increase in blood flow is at least partially responsible for the observed enhancement of CTX antitumor activity by Fluosol-DA in combination with carbogen breathing.

Our observation that Fluosol-DA and carbogen breathing did not increase the toxicity of CTX sufficiently to kill mice is in agreement with the report by Teicher et al. [21] that the toxicity of CTX to bone marrow was not influenced by Fluosol-DA and/or carbogen breathing. If the increased antitumor activity of CTX caused by Fluosol-DA and carbogen was attributable to a change in pharmacokinetics, i.e., increased delivery of the drug to tissues, it is not clear why the combination of Fluosol-DA and carbogen breathing did not increase the effect of CTX on normal tissues. Perhaps an increase in oxygen delivery is the major mechanism by which Fluosol-DA and carbogen enhance the effect of CTX in vivo, and the effect of CTX on the well-oxygenated normal tissues is thus not affected by these adjuvants.

In conclusion, Fluosol-DA combined with carbogen breathing enhanced the effect of CTX to suppress the growth of RIF-1 tumors in C3H mice. The death of C3H mice due to high doses of CTX was not affected by Fluosol-DA or carbogen alone or in combination. Although the mechanism underlying this chemosensitization remains unclear, our results as well as the observations of other investigators strongly indicate the potential usefulness of perfluorochemical emulsion combined with carbogen or oxygen breathing in enhancing the antitumor activity of CTX.

## References


1. Begg AC, Shriev DC, Smith KA, Jerry NHA (1985) Effect of hypoxia, pH and growth state on cell killing in Chinese hamster V79 cells in vitro by activated cyclophosphamide. Cancer Res 45: 3454–3459
2. Dixon B, Moore JV, Speakman H (1978) Radiobiological hypoxia of a transplanted rat tumor and the effect of treatment with cyclophosphamide. Eur J Cancer 14: 1383–1387
3. Hasegawa T, Rhee JG, Levitt SH, Song CW (1987) Increase in tumor $pO_2$ by perfluorochemicals and carbogen. Int J Radiat Oncol Biol Phys 13: 569–574
4. Hiraga S, Klubes P, Owens ES, Cysyk RL, Blasberg G (1987) Increases in brain tumor and cerebral blood flow by blood-perfluorochemical emulsion (Fluosol-DA) exchange. Cancer Res 47: 3296–3302
5. Juma FD, Roger HJ, Trounce JR (1979) Pharmacokinetics of cyclophosphamide and alkylating activity in man after intravenous and oral administration. Br J Clin Pharmacol 8: 209–217
6. Lee I, Levitt SH, Song CW (1987) Effects of Fluosol-DA 20% and carbogen on the response of SCK tumors and skin of A/J mice. Radiat Res 112: 173–182
7. Mason KA, Withers HR, Steckel RJ (1985) Acute effects of a perfluorochemical oxygen carrier on the normal tissues of the mouse. Radiat Res 104: 387–394
8. Moulder JE, Fish BL (1988) Intermittent use of a perfluorochemical emulsion and carbogen breathing with fractionated irradiation. Int J Radiat Oncol Biol Phys 15: 1193–1196
9. Rockwell S (1985) Use of a perfluorochemical emulsion to improve oxygenation in a solid tumor. Int J Radiat Oncol Biol Phys 11: 97–103

10. Rockwell S, Irvin CG, Nirenburg M (1986) Effect of a perfluorochemical emulsion on the development of artificial lung metastases in mice. Exp Clin Metast 4: 45–50
11. Rockwell S, Irvin CG, Kelly M (1988) Preclinical studies of a perfluorochemical emulsion as an adjunct to radiotherapy. Int J Radiat Oncol Biol Phys 15: 913–920
12. Rose C, Lustig R, McIntosh N, Teicher B (1986) A clinical trial of Fluosol-DA 20% in advanced squamous cell carcinoma of the head and neck. Int J Radiat Oncol Biol Phys 12: 1325–1327
13. Song CW, Zhang WL, Pence DM, Lee I, Levitt SH (1985) Increased radiosensitivity of tumor by perfluorochemicals and carbogen. Int J Radiat Oncol Biol Phys 11: 1833–1836
14. Song CW, Lee I, Hasegawa T, Rhee JG, Levitt SH (1987) Increase in $pO_2$ and radiosensitivity of tumors by Fluosol-DA (20%) and carbogen. Cancer Res 47: 442–446
15. Tannock IF (1982) Response of aerobic and hypoxic cells in a solid tumor to Adriamycin and cyclophosphamide and interaction of the drugs with radiation. Cancer Res 42: 4921–4926
16. Teicher BA, Rose CM (1984) Perfluorochemical emulsions can increase tumor radiosensitivity. Science 223: 934–936
17. Teicher BA, Rose CM (1984) Oxygen-carrying perfluorochemical emulsion as an adjuvant to radiation therapy in mice. Cancer Res 44: 4285–4288
18. Teicher BA, Holden SA (1987) Survey of the effect of adding Fluosol-DA 20%/$O_2$ treatment with various chemotherapeutic agents. Cancer Treat Rep 71: 173–177
19. Teicher BA, Holden SA, Jacobs JL (1987) Approaches to defining the mechanism of Fluosol-DA 20% with carbogen enhancement of melphalan antitumor activity. Cancer Res 47: 513–518
20. Teicher BA, Crawford JM, Holden SA, Cathcart KNS (1987) Effects of various oxygenation conditions on the enhancement by Fluosol-DA of melphalan antitumor activity. Cancer 47: 5036–5041
21. Teicher BA, Herman T, Holden SA, Cathcart KNS (1988) The effect of Fluosol-DA and oxygenation status on the activity of cyclophosphamide in vivo. Cancer Chemother Pharmacol 21: 286–291

Received 13 March 1989/Accepted 6 June 1989