Int. J. Cancer (Radiat. Oncol. Invest): **90,** 256–264 (2000)

Publication of the International Union Against Cancer

Pentoxifylline Improves the Oxygenation and Radiation Response of BA1112 Rat Rhabdomyosarcomas and EMT6 Mouse Mammary Carcinomas

David R. Collingridge, Ph.D., and Sara Rockwell, Ph.D.*
Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, Connecticut

SUMMARY Tumor hypoxia can significantly impact the efficacy of cancer therapy. Pentoxifylline, a methylxanthine derivative, can improve oxygen delivery to tissues and is widely used in the treatment of peripheral vascular disease and various cerebrovascular disorders. In this article, we show that pentoxifylline, combined with oxygen breathing, significantly improves the radiation response of two experimental tumors in vivo through improved tumor oxygenation. We also demonstrate that pentoxifylline does not directly radiosensitize EMT6 cells in vitro and does not modify the tumor radiation response when administered postirradiation to solid EMT6 tumors. Our findings confirm that preirradiation administration of pentoxifylline can improve radiation efficacy, but suggest that its role as a postirradiation modifier of treatment response, reported by others, may be tumor-specific. *Int. J. Cancer (Radiat. Oncol. Invest.) 90, 256–264 (2000).*

Key words: tumor oxygenation; radiosensitization; pentoxifylline

INTRODUCTION

The hypoxic and acidic microenvironments within tumors offer unique targets for anticancer intervention. Hypoxic tumor cells can be up to 3 times more resistant to radiation than their oxic counterparts [1]. Attempts to improve the efficacy of radiotherapy and chemotherapy by modifying tumor oxygenation has been an area of active research for many years [2–5]. Indeed, as early as 1930 Fischer-Wasels [6] was using carbogen breathing during radiotherapy to improve tumor oxygenation and radiation response in cancer patients.

One experimental combination therapy utilizes the improved blood-oxygen saturation achievable with carbogen breathing [7,8] in combination with the vitamin B3 analog, nicotinamide, which normalizes tumor blood flow [9] and may increase DNA damage following irradiation via DNA repair inhibition [10,11]. The combination of nicotinamide plus carbogen breathing can improve radiation response significantly in experimental animal tumors in vivo [12]. This finding has stimulated a number of clinical trials in tumors of the head and neck, bladder, and brain [13]. However, these trials have shown that the doses of nicotinamide required to achieve improved radiation response often induce nausea, vomiting, and headaches [13]. A low incidence of renal dysfunction has also been noted [13,14], which can be exacerbated when nicotinamide and carbogen are combined with other medications, for example, the anticonvulsant, phenytoin [13].

These side effects have stimulated an interest in evaluating other agents that might replace nico-

*Correspondence to: Sara Rockwell, Ph.D., Department of Therapeutic Radiology, Yale University School of Medicine, P.O. Box 208040, New Haven, CT 06520-8040. Phone: (203) 785-2693; Fax: (203) 785-7482; E-mail: sara.rockwell@yale.edu

Current address for David Collingridge, Ph.D.: PET Oncology Group, MRC Cyclotron Building, Imperial College School of Medicine, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK.

Received 18 February 2000; Revised 9 May 2000; Accepted 8 August 2000

tinamide. One possible candidate is the methylxanthine derivative pentoxifylline, which is currently in Phase I/II trials in both Europe and the USA [15,16]. This drug has been used for many years in the treatment of peripheral vascular disease, various cerebrovascular disorders, and in other conditions in which an amelioration of impaired or inadequate blood flow is beneficial [17,18]. Pentoxifylline is usually well-tolerated. Gastrointestinal symptoms have been recorded as a side effect, but the occurrence rate is not significantly different than that observed in patients receiving placebo [18].

Pentoxifylline has several modes of action: increased red cell deformability; reduced blood viscosity as a result of decreasing platelet aggregation and thrombus formation; vasodilation; increased leukocyte filterability; and various immunological effects [18–24]. These effects can reduce the incidence of acute hypoxia by improving blood delivery to tortuous vascular beds. Mouse studies have shown significant improvements in tumor oxygenation and perfusion, accompanied by increases in tumor radiation response [25–30]. Several investigators have also shown that, in vitro, administering pentoxifylline after irradiation can increase cell death by preventing the radiation-induced cell cycle block at G2/M [31,32], thereby forcing cells into premature, and fatal, cell division. Pentoxifylline has also been reported to potentiate the effects of certain antineoplastic drugs [33,34].

This article examines the effects of pentoxifylline on tumor oxygenation, measured with an Eppendorf pO_2 histograph, and on the radiobiological hypoxic fraction. We also investigated the effect of pentoxifylline administered after irradiation to look for effects on postirradiation proliferation and repair which might influence the radiation response of tumors in vivo.

MATERIALS AND METHODS

Animals and Tumor Models

Two tumors were used for these studies, the EMT6 mouse mammary carcinoma and the BA1112 rat rhabdomyosarcoma. The EMT6 mouse mammary carcinoma was grown in BALB/c Rw mice aged approximately 2.5 months. EMT6 tumors (subline EMT6-Rw) were implanted by injecting 2×10^5 cells, harvested from exponentially growing cell cultures, either intradermally in the skin of the flank for clonogenic assays, or subcutaneously into the rear dorsum for pO_2 measurements. The rear dorsal site was preferable for the pO_2 studies because it allowed easier access to the tumor with the pO_2 electrode. EMT6 tumors grown in the flank and rear dorsum do not exhibit significant differences in growth rate, clonogenicity, or oxygenation (data not shown). Tumors were selected for treatment when they had reached approximately 100 mm^3 in volume (approximately 2 weeks postinoculation).

BA1112 tumors were implanted by injecting 7,500 cells into the subcutaneous tissue between the ears of 8–10-week-old WAG/rij-Y rats. Tumors were maintained by inoculating single-cell suspensions prepared from solid tumors. Tumors were studied 3 to 4 weeks postinoculation, when they had reached approximately 300–400 mm^3 in volume.

All protocols used with experimental animals were reviewed and approved in advance by the Yale Animal Care and Use Committee. All experiments were performed in full compliance with governmental, AAALAC, and institutional regulations and with the principles outlined in the USPHS Guide.

Pentoxifylline

Pentoxifylline (Sigma, St. Louis, Mo) was prepared fresh for each experiment at a concentration of 12 mg/ml by dissolving the drug in sterile, pyrogen-free, physiologic saline. The drug was administered intraperitoneally (i.p.) at a dose of 50 mg/kg, 15 min prior to pO_2 measurement or irradiation. This schedule appeared optimal in preliminary experiments investigating the effect of a wide range of pentoxifylline dose–time combinations on the oxygenation of EMT6 tumors in vivo (data not shown). For experiments examining the effect of postirradiation administration of pentoxifylline, the drug was given i.p. at a dose of 50 mg/kg immediately after irradiation. Two additional injections of 50 mg/kg were administered, 1 and 2 hr later. This regimen was based on the clearance pharmacokinetics of Honess et al. [25] and was expected to produce plasma levels of ~50 μg/ml (0.2 mM) for at least 3 hr postirradiation.

Tumor Irradiation

Unanesthetized mice were placed in individual chambers of plastic gassing-irradiation boxes, and were allowed to breathe either air or 100% oxygen before and during irradiation. Similarly, unanesthetized rats were restrained in single-animal plastic restraint boxes, designed to localize the tumors in a defined position, and then gassed with the appropriate gas before and during irradiation. All irra-

diations were performed using a Siemans Stabilipan x-ray machine set at 250 kV and 15 mA. The mean dose rate for the mouse experiments was 1.3 Gy/min, and for the rat experiments, 2.4 Gy/min.

Measurement of Clonogenic Cell Survival

Full details of the procedures used with the EMT6 and BA1112 tumors have been reported previously [35,36]. Briefly, immediately following treatment tumors were excised, minced, and digested with trypsin. Cells were recovered by centrifugation, resuspended in cell culture medium and counted using Trypan blue exclusion. Known numbers of cells were plated in Petri dishes containing nutrient media and incubated at 37°C in a humidified incubator in an atmosphere of 95% air / 5% CO_2 for 14 days. The colonies were fixed, stained with crystal violet, and counted. The surviving fractions were calculated using the plating efficiencies of untreated controls plated on the same day. Pooled tumors from 3–5 animals per treatment group were used in clonogenic assay with EMT6 tumors. These experiments were repeated 3–9 times. Clonogenic assays with BA1112 tumors used one animal per treatment group and were repeated 6–13 times. The control plating efficiencies averaged 38 ± 3% for the EMT6 tumors and 47 ± 5% for the BA1112 tumors in these experiments.

Oxygen Measurements

Oxygen measurements were performed using an Eppendorf pO_2 histograph (Eppendorf KIMOC 6650, Hamburg, Germany). Full operational details for this machine have been described previously [37]. Oxygen measurements in EMT6 tumors were performed on unanesthetized mice restrained in plastic jigs, while, for practical reasons, measurements in BA1112 tumors were performed on rats anesthetized with an i.m. injection of 1 mg/kg acetylpromazine plus 50 mg/kg ketamine (Fort Dodge Animal Health, Fort Dodge, Iowa). A reference Ag/AgCl electrode (LMI Medical, Grand Rapids, Mich.) was attached to a shaved region on the back of each animal, and animals were positioned on a thermal barrier (Vetko, Colo.) to minimize decreases in core temperature under anesthesia. Animals breathed either atmospheric air or 100% oxygen delivered via a nose cone. Using a net step length of 0.6 mm, a total of 40–100 pO_2 measurements were made along six tracks through each tumor. All oxygen measurements were postcalibrated to account for barometric pressure and tumor temperature.

Effect of Pentoxifylline on EMT6 Cells in Culture

One million EMT6 cells were seeded into glass milk dilution bottles containing 10 ml of Waymouth's MB 752/1 medium (Gibco, Grand Island, NY), supplemented with 15% of a 1:1 mix of fetal bovine serum (Gibco) and Fetal Clone (Hyclone, Logan, Utah), fungizone (250 μg/ml; Gibco), penicillin/streptomycin (10000 U/ml penicillin, 10000 (μg/ml streptomycin; Gemini Bio-Products, Calabasas, Calif.), and gentamycin (50 mg/ml; Gibco). The cells were then incubated at 37°C in a humidified incubator gassed with 95% air / 5% CO_2. Cells were studied both in mid-exponential growth (3 days after seeding) and in plateau phase (6 days after seeding). Medium was changed daily for plateau phase cultures on days 4 through 6. Pentoxifylline was added 15 min prior to irradiation with 10 Gy of x-rays and cultures were incubated in the presence of pentoxifylline for an additional 24 hr postirradiation. Four drug concentrations were chosen: 0.2 mM (roughly equivalent to the peak plasma levels produced by an in vivo dose of 50 mg/kg) [25], 0.5 mM, 1 mM, and 2 mM (~10 times the peak in vivo concentration). Cells were suspended for clonogenic assay via trypsinization and were plated in Petri dishes in a manner identical to that described for the in vivo / in vitro clonogenic assays. Plating efficiencies in these experiments were 76 ± 5% for exponentially growing cultures and 55 ± 5% for plateau cultures.

Data Analysis

Surviving fractions for individual experiments were calculated relative to the contemporaneous plating efficiencies and are shown as geometric means with SEMs. From the pO_2 values obtained for each tumor a median and a percentage of readings less than 2.5 mmHg were calculated and then averaged for each treatment group. SEMs were also calculated and oxygen distributions, expressed as histograms, were computed from the pooled data. Mann-Whitney U-tests were used to compare oxygen tensions in different groups. Significance was set at 95% ($P = 0.05$) for all analyses. Survival data at a single radiation dose were compared using Mann-Whitney U-tests. Survival curves were fitted by regression analyses and compared at the midpoints of the curves using standard statistical techniques for comparing regression lines.

RESULTS

Figures 1 and 2 show the effect of oxygen breathing and pentoxifylline on the oxygenation of EMT6 and BA1112 tumors, respectively. The histograms

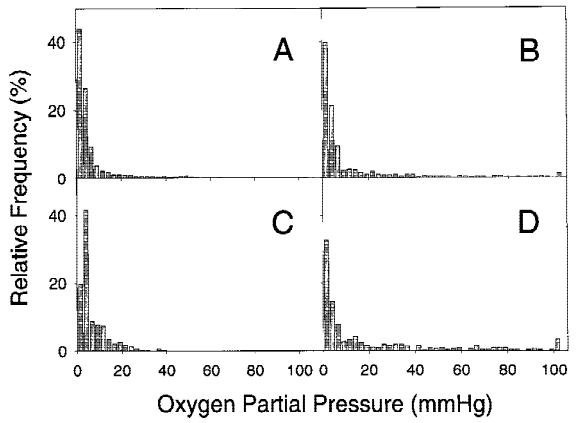

Fig. 1. Effect of pentoxifylline on the oxygenation of EMT6 tumors. **A**: Control (median pO_2 = 3 ± 0.3 mmHg; pO_2 readings <2.5 mmHg = 41 ± 4%). **B**: Oxygen breathing (median pO_2 = 4 ± 1 mmHg; pO_2 readings <2.5 mmHg = 38 ± 7%). **C**: Pentoxifylline only i.p., 50 mg/kg (median pO_2 = 4 ± 0.1 mmHg; pO_2 readings <2.5 mmHg = 17 ± 7%). **D**: Pentoxifylline plus oxygen breathing (median pO_2 = 8 ± 3 mmHg; pO_2 readings <2.5 mmHg = 30 ± 5%).

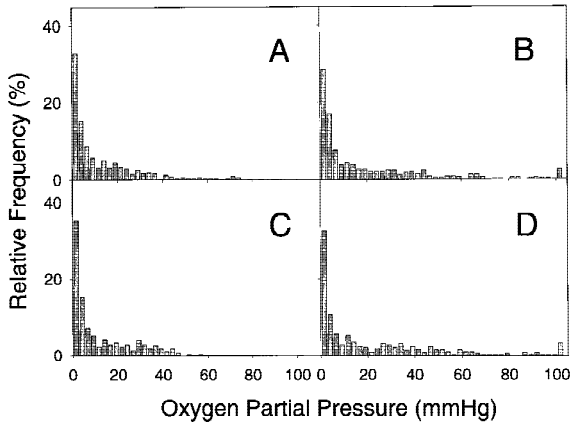

Fig. 2. Effect of pentoxifylline on the oxygenation of BA1112 tumors. **A**: Control (median pO_2 = 8 ± 3 mmHg; pO_2 readings <2.5 mmHg = 33 ± 6%). **B**: Oxygen breathing (median pO_2 = 9 ± 3 mmHg; pO_2 readings <2.5 mmHg = 28 ± 6%). **C**: Pentoxifylline only, i.p., 50 mg/kg (median pO_2 = 7 ± 2 mmHg; pO_2 readings <2.5 mmHg = 33 ± 6%). **D**: Pentoxifylline plus oxygen breathing (median pO_2 = 10 ± 3 mmHg; pO_2 readings <2.5 mmHg = 24 ± 5%).

for both tumors show significant proportions of readings less than 2.5 mmHg, i.e., at oxygen tensions which could induce radioresistance. In both tumors, the pO_2 histograms for animals breathing 100% O_2 showed increased proportions of readings at high pO_2's; however, there were no significant changes in the median pO_2 or in the percent of readings <2.5 mmHg ($P > 0.05$). Similarly, single injections of 50 mg/kg pentoxifylline did not significantly alter the oxygenation of either tumor, as-

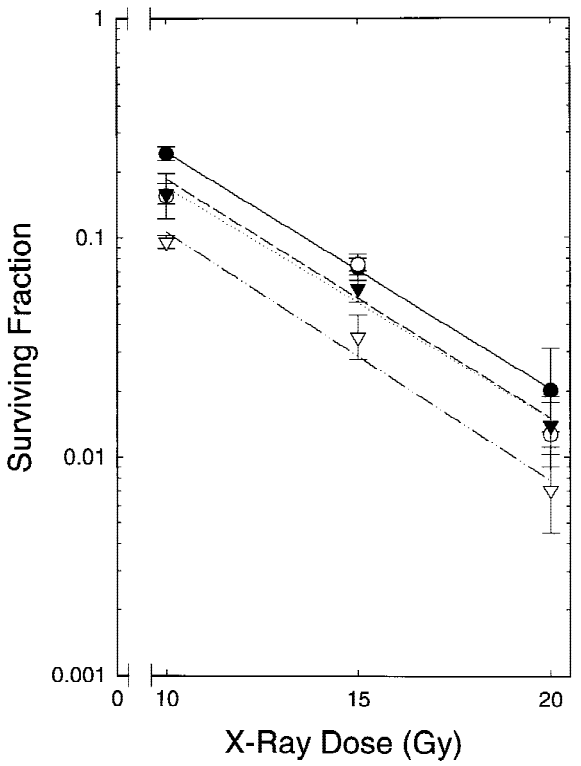

Fig. 3. Effect of pentoxifylline on the survival of cells in EMT6 tumors in vivo. ●: Tumors in air-breathing mice. ▼: Tumors in mice injected with pentoxifylline (i.p., 50 mg/kg; 15 min before irradiation), breathing air. ○: Tumors in oxygen-breathing mice. ▽: Tumors in mice receiving pentoxifylline plus oxygen. Points are geometric means ± SEMs for 3–9 independent determinations. Lines were fitted by regression analysis.

sayed 15 min after injection ($P > 0.05$). The combination of pentoxifylline and oxygen breathing produced greater changes in the oxygenation of both tumors. In the case of the EMT6 tumors, a significant increase in median pO_2 was seen ($P < 0.05$), although the decrease in the proportion of readings less than 2.5 mmHg did not quite reach significance ($P = 0.06$) (Fig. 1D). A similar trend was evident in the BA1112 data. Control tumors had a median pO_2 of 7.7 mmHg (Fig. 2A), which increased slightly to 8.9 mmHg when the animals breathed 100% oxygen (Fig. 2B), but further increased to 10.1 mmHg when animals breathed 100% oxygen in combination with pentoxifylline (Fig. 2D). The increases in tumor oxygenation seen with the electrode measurements were consistent with the changes in the survival curves, presented in Figures 3 and 4.

Figure 3 shows the effect of pentoxifylline alone and in combination with oxygen breathing, on the survival curve for cells from EMT6 tumors in vivo. The four survival curves are parallel. There

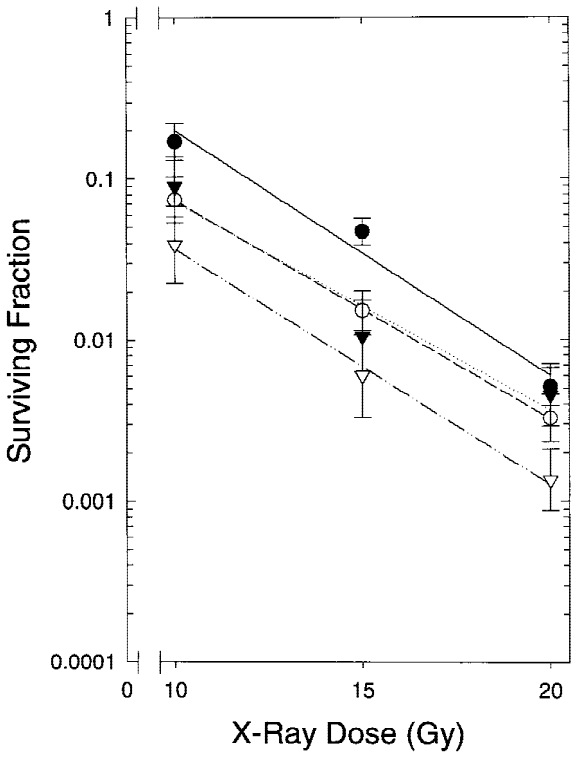

Fig. 4. Effect of pentoxifylline on the survival of cells in BA1112 tumors in vivo. ●: Tumors in air-breathing rats. ▼: Tumors in rats injected with pentoxifylline (i.p., 50 mg/kg; 15 min before irradiation), breathing air. ○: Tumors in oxygen-breathing rats. ▽: Tumors in rats receiving pentoxifylline plus oxygen. Points are geometric means ± SEMs for 6–13 independent determinations. Lines were fitted by regression analysis.

is a small decrease in the surviving fractions for tumors irradiated in animals treated with pentoxifylline alone or oxygen breathing alone. However, these curves were not significantly different from the curve obtained for tumors irradiated in air-breathing control animals ($P > 0.05$). In contrast, the survival curve obtained for tumors irradiated after treatment with both pentoxifylline and oxygen breathing showed a significant decrease in cell survivals relative to controls ($P < 0.05$). The change in the survival curves is of the type which would be expected theoretically from a decrease in the hypoxic fraction of the tumor [38,39].

The findings obtained with BA1112 tumors in vivo (Fig. 4) are very similar to those obtained for the EMT6 cells. Again, small decreases were seen in the surviving fractions of cells from tumors in rats irradiated after treatment with pentoxifylline alone ($P < 0.05$) or oxygen breathing alone ($P > 0.05$). A highly significant decrease in the surviving fractions of cells from irradiated BA1112 tumors was observed when both agents were administered concomitantly before irradiation ($P < 0.05$). Again, the parallel but offset survival curves showed the changes expected from decreases in the number of radiobiologically hypoxic tumor cells [38,39].

The effect of protracted pentoxifylline treatments, continuing after irradiation, was examined in the experiments presented in Table 1. Control tumors were excised 3.5 hr after irradiation with 15 Gy, to match the treatment and assay regimen used in the groups receiving triple doses of postirradiation pentoxifylline. As in the EMT6 cell survival curves in Figure 2, the surviving fractions of cells from tumors irradiated after treatment with pentoxifylline prior to irradiation, along with oxygen breathing before and during irradiation, were decreased relative to those for control tumors. The absolute values of the surviving fractions were slightly higher than those shown in Figure 2; this probably reflects the repair of potentially lethal damage occurring during the 3.5 hr that the irradiated tumors remained in situ prior to excision [40]. The surviving fractions of cells from EMT6 tumors treated with three additional postirradiation doses of pentoxifylline were not significantly different from those of cells from tumors treated with pentoxifylline before irradiation and excised 3.5 hr later ($P > 0.05$). Only preirradiation treatments with pentoxifylline altered the response of these tumors to radiation.

The effect of pentoxifylline on the viability, growth, and radiosensitivity of EMT6 cells was examined further using cells in culture. Figure 5 shows the effect of a 24-hr treatment with pentoxifylline on the viability and radiosensitivity of exponentially growing cells and of cells from plateau phase cultures. The pentoxifylline treatments produced only insignificant decreases in cell viability in the cultures ($P > 0.05$). The survivals of cells irradiated in the presence and in the absence of pentoxifylline were similar in both exponentially growing and plateau phase cultures. As expected, plateau phase cultures were slightly more sensitive to radiation than were exponentially growing cultures. These findings suggest that pentoxifylline did not alter either the radiosensitivity of the cells or the repair of sublethal or potentially lethal damage after irradiation. Additional studies (data not shown) showed that treatments over 1–4 days with pentoxifylline had no significant effects on the growth of EMT6 cells at concentrations of 0.2, 0.5, and 1.0 mM; a slight inhibition of growth was seen with 2 mM pentoxifylline (10 times the peak concentration expected in our studies in vivo). These

Table 1. Effect of Postirradiation Administration of Pentoxifylline on the Survival of Cells in EMT6 Tumors In Vivo

	Treatment	Surviving fraction[1]	*P*-value[2]
A	O_2 + 15 Gy, 3.5 hour delayed kill	0.098	
B	O_2 + pentoxifylline + 15 Gy, 3.5 hour delayed kill	0.062	B vs. A: $P = 0.5$
C	O_2 + 15 Gy + 3 postirradiation doses of pentoxifylline	0.078	C vs. A: $P = 0.3$
D	O_2 + Pentoxifylline + 15 Gy + 3 postirradiation doses of pentoxifylline	0.072	D vs. A: $P = 0.03$ D vs. B: $P = 0.5$

[1]Geometric means from seven independent determinations.
[2]Mann-Whitney U-test.

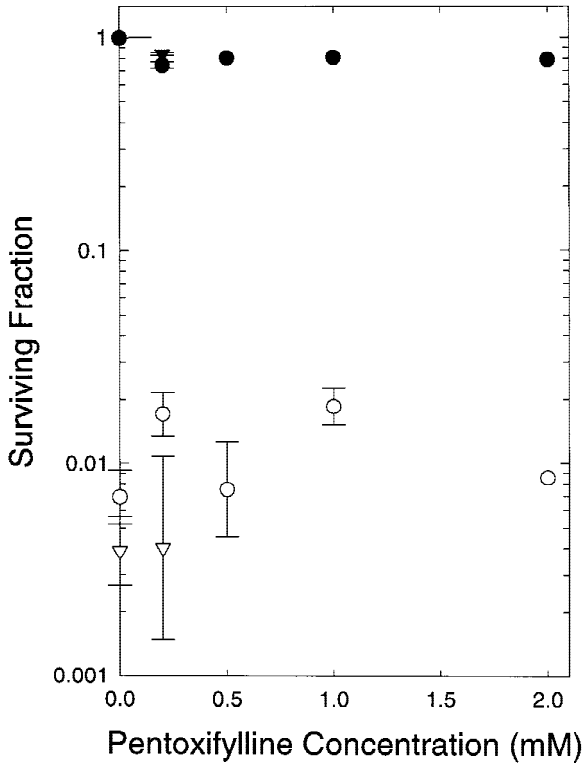

Fig. 5. Effect of pentoxifylline on survival of EMT6 cells in cell cultures. Solid symbols represent the surviving fractions of EMT6 cells exposed to pentoxifylline without irradiation. Open symbols represent the surviving fractions of EMT6 exposed to pentoxifylline during and for 24 hr after irradiation at a dose of 10 Gy. ● and ○: Exponentially growing cells. ▼ and ▽: Plateau phase cells. Points without error bars are the means from two experiments. Points with error bars are the geometric means ± 1 SEMs of data from at least three experiments.

cell culture studies provide no evidence for an effect of pentoxifylline on the proliferation of EMT6 cells at doses relevant to the use of this drug with radiation therapy.

DISCUSSION

The aim of this study was to examine the potential of pentoxifylline as an adjunct to radiotherapy. The data reported here demonstrate that the combination of pentoxifylline plus oxygen breathing has the potential to improve radiation response via increased tumor oxygenation. The oxygen tensions of the two experimental tumors examined here increased within 15 min following a single i.p. injection of pentoxifylline at a dose of 50 mg/kg and administration of O_2. It is well known that if molecular oxygen is present during irradiation, this molecule can participate in the chemical reactions that lead to the production of biologically significant radiation damage. A decrease in the number of hypoxic cells in the tumors therefore increases the radiation response of the neoplasms. The radiation dose–response curves for EMT6 and BA1112 tumors in animals treated with pentoxifylline and breathing oxygen before irradiation were parallel to those of cells irradiated in nondrug-treated air-breathing animals, but offset to lower survivals. This change is similar to that expected theoretically from a decrease in the hypoxic fraction [38,39]. This suggests that the improvements in tumor oxygenation have decreased the proportions of radiobiological hypoxic cells in both tumors and thereby increased the efficacy of the radiation treatment.

Our finding that pentoxifylline is an effective modifier of tumor oxygenation and tumor radiation response agrees with reports in other experimental tumor models [25–30]. Pentoxifylline has been shown to increase tumor perfusion [26] by increasing blood flow through vasoaction [20,21] and increased red and white cell flexibility [18,19]. These mechanisms could improve oxygen delivery to the tumor bed and provide a very plausible mechanism for the improved tumor responses shown in our studies.

Pentoxifylline, and other methylxanthine derivatives (e.g., caffeine and theophylline), have been reported to act on cells in G2 phase of the cell cycle to induce cells to undergo premature mitosis before completing repair of radiation-induced damage. Pentoxifylline has been shown to radiosensi-

tize several different types of tumor cells in vitro when administered after irradiation [31,32,41]. We asked whether this effect could be exploited in vivo to provide an additional radiation enhancement to complement, and improve upon, the radiation response achieved via preirradiation oxygen modification. We found no evidence that postirradiation treatments with pentoxifylline altered the radiation response of EMT6 tumors. However, our in vitro experiments with EMT6 tumors also showed that pentoxifylline was not cytotoxic to EMT6 cells, did not impair the growth of these cells, and, importantly, did not sensitize EMT6 cells to 10 Gy of x-rays when drug concentrations up to 10 times peak plasma levels expected in our in vivo studies (0.2 mM) [25] were maintained for 24 hr after irradiation. These data contrast with those reported by other investigators who have demonstrated radiosensitization of cells in vitro, particularly at concentrations approaching 2 mM [31,32,41]. Russell et al. [32] have shown that p53-deficient tumor cells are more sensitive to postirradiation pentoxifylline treatment. The G1 and G2 checkpoints are known to be controlled by p53 [42–44], while pentoxifylline acts on cells in G2; thus, p53 status may be an important factor influencing the efficacy of the postirradiation administration of pentoxifylline. The p53 status of the EMT6 cells is currently unknown.

The lack of radiosensitization of EMT6 cells by pentoxifylline in vitro explains, at least in part, the failure to observe additional decreases in surviving fractions of cells from solid EMT6 tumors grown in mice treated with pentoxifylline after irradiation. Animals were given three top-up doses of pentoxifylline at hourly intervals postirradiation. In mice, pentoxifylline serum levels decrease within 1 hr; thus, dosing at hourly intervals should maintain a constant serum concentration of approximately 0.2 mM during the 3.5 hr between irradiation and assay [25]. The experiments reported here examining postirradiation administration of pentoxifylline in vivo might not have fully explored the potential of this approach. It is possible that we did not expose the tumors to pentoxifylline at an effective concentration for a sufficient period of time postirradiation. However, the concentrations used in our in vitro studies (0.2–2 mM) are at or above the plasma level attainable in mice [25] and well above the maximally tolerated levels attainable in patients (10–30 μM) [32]. The use of a sustained release formulation of pentoxifylline for a period of time equal to at least one cell cycle might theoretically offer additional efficacy. However, no radiosensitization was observed in our in vitro studies, which used a postirradiation incubation time of 24 hr, approximately twice the cell cycle time of exponentially growing EMT6 cells in vitro. It is therefore highly unlikely that radioenhancement would have been attainable with EMT6 cells grown as tumors under any practicable treatment conditions.

CONCLUSION

In conclusion, pentoxifylline is an effective modulator of tumor oxygen tension in vivo, and can significantly enhance the radiation response of EMT6 mouse mammary carcinomas and BA1112 rhabdomyosarcomas. In these systems pentoxifylline appears to improve the efficacy of radiation therapy via an oxygen-modulation mechanism, rather than by directly altering the radiosensitivity or the repair capacity of the tumor cells.

ACKNOWLEDGMENTS

We thank Marianne Kelley, Jacqueline Mendes, Catherine Drost, and Lauren Farash for help with the experiments. We also thank Diana Fischer for assistance with the statistical analyses and regression analyses.

REFERENCES

1. Gray LH, Conger AD, Ebert M, Hornsey S, Scott OCA. The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. Br J Radiol 1953;26:638–648.
2. Butler SA, Wood PJ, Cole S, Williams C, Adams GE, Stratford IJ. Enhancement of bioreductive drug toxicity in murine tumors by inhibition of the activity of nitric oxide synthase. Br J Cancer 1997;76:438–444.
3. Chaplin DJ, Acker B. The effect of hydralazine on the tumor cytotoxicity of the hypoxic cell cytotoxin RSU-1069: evidence for therapeutic gain. Int J Radiat Oncol Biol Phys 1987;13:579–585.
4. Chaplin DJ, Horsman MR, Aoki DS. Nicotinamide, fluosol-DA and carbogen: a strategy to reoxygenate acutely and chronically hypoxic cells in vivo. Br J Cancer 1991;63:109–113.
5. Rockwell S, Kelley M, Irvin CG, Hughes CS, Porter E, Yabuki H, Fischer JJ. Modulation of tumor oxygenation and radiosensitivity by a perfluorooctylbromide emulsion. Radiother Oncol 1991;22:92–98.
6. Fischer-Wasels B. Die ergebnisse der gasbehandlung bsartiger geschwlste beim menschen. In: Verlag von JF, editor. Frankfurter zeitschrift fur pathologie. Berlin: Bergmann und Julius Springer; 1930. p 231–253.
7. Falk SJ, Ward R, Bleehen NM. The influence of carbogen breathing on tumor tissue oxygenation in man evaluated by computerized pO_2 histography. Br J Cancer 1992;66:919–924.
8. Hill SA, Collingridge DR, Vojnovic B, Chaplin, DJ. Tumor radiosensitization by high-oxygen-content gases: influence of the carbon dioxide content of the

inspired gas on pO_2, microcirculatory function and radiosensitivity. Int J Radiat Oncol Biol Phys 1998;40: 943–951.
9. Hill SA, Chaplin DJ. The effect of nicotinamide on microregional blood flow within tumors assessed using laser doppler probes. Acta Oncol 1995;34:401–404.
10. Olsson AR, Sheng Y, Pero RW, Chaplin DJ, Horsman MR. DNA damage and repair in tumor and non-tumor tissues of mice induced by nicotinamide. Br J Cancer 1996;74:368–373.
11. Rojas A, Denekamp J, Johns H, Kjellen E, Tsang R, Nilsson P, Stratford MRL, Dennis MF, Joiner MC. Nicotinamide as a repair inhibitor in vivo: studies using single and fractionated x-ray doses in mouse skin and kidneys. Radiat Res 1996;145:419–431.
12. Kjellen E, Joiner MC, Collier JM, Johns H, Rojas A. A therapeutic benefit from combining normobaric carbogen or oxygen with nicotinamide in fractionated x-ray treatments. Radiother Oncol 1991;22:81–91.
13. Saunders MI, Dische S. Clinical results of hypoxic cell radiosensitization from hyperbaric oxygen to accelerated radiotherapy, carbogen and nicotinamide. Br J Cancer 1996;74(Suppl. XXVII):S271–S278.
14. Kaanders JHAM, Pop LAM, Marres HAM, van der Maazen RWM, van der Kogel AJ, van Daal WAJ. Radiotherapy with carbogen breathing and nicotinamide in head and neck cancer: feasibility and toxicity. Radiother Oncol 1995;37:190–198.
15. Fuller BG. Phase I study of combined radiation response modifiers employing hydroxyurea and pentoxifylline for treatment of glioblastoma. NCI-sponsored trial (IDs: NCI-95-C-0069D and NCI-95-C-0069A), 1997. For details see NCI homepage at http://cancernet.nci.nih.gov/trialsrch.shtml, search using protocol ID number NCI-95-C-0069D. Accessed May 9, 2000.
16. Saunders MI. Phase I/II studies with pentoxifylline as a potential hypoxic cell modifier. In: Gray Laboratory Research Report 1998. For details see Gray Laboratory homepage at: http://www.graylab.ac.uk/lab/report98/mis/mis.pdf. Accessed May 2000. p 54.
17. Muller R. Pentoxifylline: a biochemical profile. J Med 1979;10:307–329.
18. Ward A, Clissold SP. Pentoxifylline: a review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic efficacy. Drugs 1987;34:50–97.
19. Honess DJ, Kitamoto Y, Rampling MR, Bleehen NM. Nicotinamide and pentoxifylline increase human leucocyte filterability: a possible mechanism for reduction of acute hypoxia. Br J Cancer 1996;74(Suppl. XXVII):S236–S240.
20. Kaapa P, Raj JU, Ibe BO, Anderson J. Effect of pentoxifylline on rabbit pulmonary circulation: influence of age and vasomotor tone. Am J Physiol 1991;261: H975–H981.
21. Kaye AD, Ibrahim IN, Kadowitz PJ, Nossaman BD. Analysis of responses to pentoxifylline in the pulmonary vascular bed of the cat. Crit Care Med 1996;24: 263–267.
22. Lissoni P, Ardizzoia A, Perego MS, Grassi MG, Arosio M, DíAmico P, Cazzaniga M, Crispino S, Tancini G, Barni S. Inhibition of tumor necrosis factor-alpha secretion by pentoxifylline in advanced cancer patients with abnormally high blood levels of tumor necrosis factor-alpha. J Biol Regul Homeost Agents 1993;7:73–75.
23. Reed WR, DeGowin RL. Suppressive effects of pentoxifylline on natural killer cell activity. J Lab Clin Med 1992;119:763–771.
24. Thompson JA, Lindgren CG, Benyunes MC, Bianco JA, Shields AF, Fefer A. The effects of pentoxifylline on the generation of human lymphokine-activated killer cell cytotoxicity. J Immunother. 1993;13:84–90.
25. Honess DJ, Dennis IF, Bleehen NM. Pentoxifylline: its pharmacokinetics and ability to improve perfusion and radiosensitivity in mice. Radiother Oncol 1993;28:208–218.
26. Honess DJ, Andrews MS, Ward R, Bleehen, NM. Pentoxifylline increases RIF-1 tumor pO_2 in a manner compatible with its ability to increase relative tumor perfusion. Acta Oncol 1995;34:385–389.
27. Lee I, Levitt SH, Song CW. Improved tumor oxygenation and radiosensitization by combination with nicotinamide and pentoxifylline. Int J Radiat Biol 1993;64: 237–244.
28. Lee I, Boucher TJ, Demhartner TJ, Jain RK. Changes in tumor blood flow, oxygenation and interstitial fluid pressure induced by pentoxifylline. Br J Cancer 1994; 69:492–496.
29. Song CW, Hasegawa T, Kwon HC, Lyons JC, Levitt SH. Increase in tumor oxygenation and radiosensitivity caused by pentoxifylline. Radiat Res 1992;130:205–210.
30. Teicher BA, Sotomayor EA, Robinson MF, Dupuis NP, Schwartz GN, Frei E III. Tumor oxygenation and radiosensitization by pentoxifylline and a perflubron emulsion/carbogen breathing. Int J Oncol 1993;2:13–21.
31. Li YX, Weber-Johnson K, Sun LQ, Paschoud N, Mirimanoff RO, Coucke PA. Effect of pentoxifylline on radiation-induced G2-phase delay and radiosensitivity of human colon and cervical cancer. Radiat Res 1998; 149:338–342.
32. Russell KJ, Wiens LW, Demers GW, Gallway DA, Le T, Rice GC, Bianco JA, Singer JW, Groudine M. Preferential radiosensitization of G1 checkpoint-deficient cells by methylxanthines. Int J Radiat Oncol Biol Phys 1996;36:1099–1106.
33. Ohsaki Y, Ishida S, Fujikane T, Kikuchi K. Pentoxifylline potentiates the antitumor effect of cisplatin and etoposide on human lung cancer cell lines. Oncology 1996;53:327–333.
34. Viladkar A, Chitnis M. In vitro effects of pentoxifylline and doxorubicin on cell survival and DNA damage in sensitive and MDR-P388 leukemia cells. Cancer Biother 1994;9:143–151.
35. Martin DF, Porter EA, Fischer JJ, Rockwell S. Effect of a perfluorocarbon emulsion on the radiation response of BA1112 rhabdomyosarcoma. Radiat Res 1987;112:45–53.

36. Rockwell S. In vivo-in vitro tumor systems: new models for studying the response of tumors to therapy. Lab Anim Sci 1977;27:831–851.
37. Collingridge DR, Young WK, Vojnovic B, Wardman P, Lynch EM, Hill SA, Chaplin DJ. Measurement of tumor oxygenation: a comparison between polarographic needle electrodes and a time-resolved luminescence-based optical sensor. Radiat Res 1997;147:329–334.
38. Moulder JE, Rockwell S. Hypoxic fractions of solid tumors: experimental techniques, methods of analysis, and a survey of existing data. Int J Radiat Oncol Biol Phys 1984;10:695–712.
39. Moulder JE, Rockwell S. Tumor hypoxia: Its impact on cancer therapy. Cancer Metastasis Rev 1987;5:313–341.
40. Hahn GM, Rockwell S, Kallman RF, Gordon LF, Frindel E. Repair of potentially lethal damage in vivo in solid tumor cells after x-irradiation. Cancer Res 1974; 34:351–354.
41. Kim SH, Khil MS, Ryu S, Kim JH. Enhancement of radiation response on human carcinoma cells in culture by pentoxifylline. Int J Radiat Oncol Biol Phys 1992; 25:61–65.
42. Kuerbitz SJ, Plunkett BS, Walsh WV, Kastan MB. Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc Natl Acad Sci USA 1992; 89:7491–7495.
43. Bunz F, Dutriaux A, Lengauer C, Waldman T, Zhou S, Brown J, Sedivy JM, Kinzler KW, Vogelstein B. Requirement for p53 and p21 to sustain G2 arrest after DNA damage. Science 1998;282:1497–1501.
44. Schwartz D, Almog N, Peled A, Goldfinger N, Rotter V. Role of wild type p53 in the G2 phase: regulation of the gamma-irradiation-induced delay and DNA repair. Oncogene 1997;15:2597–2607.