Efaproxiral (RSR13) Plus Oxygen Breathing Increases the Therapeutic Ratio of Carboplatin in EMT6 Mouse Mammary Tumors

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Carboplatin, a member of the platinum family of alkylating agents, is often used in combination with radiotherapy. Some studies, including a recent publication from our laboratory, have suggested that the cytotoxic effects of platinum compounds may be altered by changes in the post-treatment oxygenation. The study reported here assessed whether post-treatment changes in tumor oxygenation caused by oxygen breathing alone or in combination with efaproxiral (RSR13) altered the effects of carboplatin. Efaproxiral, which allosterically modifies hemoglobin-oxygen binding to increase tumor pO_2, has been shown to increase the effects of radiation in animal tumor models and is in a second, confirmatory phase III clinical trial as an adjuvant to radiotherapy. These studies with EMT6 tumors in BALB/c Rw mice used clonogenic assays to assess tumor cell survival and tumor growth studies to assess antineoplastic activity and treatment-related toxicity. Efaproxiral plus oxygen breathing for 5 hrs after carboplatin treatment significantly increased the antineoplastic effects of carboplatin. The increased antineoplastic effects of carboplatin produced by efaproxiral plus oxygen breathing occurred without a concomitant increase in host toxicity. These findings suggest that the increases in tumor oxygenation produced by Efaproxiral plus oxygen breathing increased the therapeutic ratio of carboplatin.

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Introduction

Efaproxiral (RSR13; Allos Therapeutics, Inc., Westminster, CO), a synthetic allosteric modifier of hemoglobin-oxygen binding affinity, has been shown to bind reversibly to hemoglobin, stabilizing the deoxyhemoglobin tetramer conformation to reduce its affinity for oxygen (1–4). Efaproxiral combined with oxygen breathing increases tumor and tissue oxygenation in several rodent models (5–10) and increases whole blood p50 in humans (11). Efaproxiral is being tested in clinical trials as an adjuvant to radiotherapy and may also be used in combination with chemotherapy and combined modality therapy (12).

The alkylating agents cisplatin and carboplatin are widely used for the treatment of solid malignancies. The literature contains conflicting evidence regarding the effect of hypoxia on the cytotoxicity of these drugs (13–21). The effects of oxygen on the cytotoxicity of platinum compounds appear to be complex; both the oxygenation at the time of treatment and the post-treatment oxygen levels may be important. In our previous studies (13), simultaneous 2-hr treatments and prolonged pretreatments with oxygen breathing or with efaproxiral plus oxygen breathing did not alter the cytotoxicity of cisplatin or carboplatin to EMT6 tumor cells *in vivo*. However, we found lower tumor cell survivals when mice were treated with oxygen or with efaproxiral plus oxygen during and for 5 hr after injection of carboplatin. We hypothesized that the lower tumor cell survivals reflected an improvement in oxygenation during the period when the carboplatin cross links and mono-adducts are being formed, a process which may require several hours. The studies described below are an extension of these studies. The initial experiments confirmed the previous findings with greater statistical precision and included an additional control group designed to assess the possibility, left open in the previous studies, that the 2-hr interval between carboplatin injection and assay was too short for drug cytotoxicity to be fully manifest and that the longer delay before plating was at least partially responsible for the greater effect observed with the 5-hr post-treatment

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oxygen breathing or efaproxiral plus oxygen breathing regimens. Subsequent experiments used a tumor growth delay assay to confirm the observed improvement in response using a clinically relevant endpoint.

Materials and Methods

Drugs. Efaproxiral (RSR13, 2-[4-[2-[(3,5-dimethylphenyl)amino]-2-oxoethyl]phenoxy]-2-methylpropanoic acid monosodium salt) was provided by Allos Therapeutics. Efaproxiral and carboplatin (Sigma-Aldrich, St. Louis, MO) were dissolved in sterile, pyrogen-free physiologic saline and injected ip.

Animals and Tumor Models. Experiments were performed using male BALB/c Rw mice 2.5–3.5 months of age. EMT6 tumors (subline Rw) were implanted by injecting 2×10^5 cells, harvested from exponentially growing cell cultures, intradermally into the flank, and were treated approximately 2 weeks later at volumes of about 100 mm^3. The origins and characteristics of the EMT6 line and the techniques used to propagate and assay the cells and tumors are described elsewhere (13, 22–24). All protocols were reviewed and approved by the Yale Institutional Animal Care and Use Committee. Experiments were performed in full compliance with governmental, Association for the Assessment of Laboratory Animal Care, and institutional regulations and with the principles outlined in the U.S. Public Health Service Guide. Euthanasia was always performed using carbon dioxide.

Mice receiving oxygen were placed in gassing boxes, which were flushed continuously with 100% oxygen for 15 mins before and for 2 or 5 hrs after injection of carboplatin. To examine the effects of prolonged treatments with efaproxiral plus oxygen, 150 mg/kg efaproxiral was administered 15 mins before, as well as 1.5 and 3.5 hrs after the carboplatin injection (450 mg/kg cumulative dose); mice were treated with oxygen throughout this period. In human patients, efaproxiral is generally given as an infusion over 30–60 min and has a half life of 3–4.5 hrs (12). The three-injection regimen in mice (which have a much shorter drug half life), therefore, models a clinical regimen achievable with a single treatment. Air-breathing mice were kept in standard cages. Carboplatin was given at a dose of 150 mg/kg for the tumor cell survival assays; 100 mg/kg carboplatin generally was used for the tumor growth delay assays because the delayed (bone marrow) toxicity of 150 mg/kg carboplatin was found to be unacceptable in pilot studies. Control groups included both untreated mice and mice treated with efaproxiral plus oxygen alone, without carboplatin.

Tumor cell survival was assayed using a colony formation assay. Tumors were explanted, and a single cell suspension was created by mincing and trypsinization as detailed previously (24). Cells were counted under a phase contrast microscope using trypan blue to identify cells damaged during the suspension process. Cells were plated at low densities into petri dishes (Corning, Corning, NY) containing Waymouth's medium (Invitrogen, Carlsbad, CA), supplemented with 15% serum (a 1:1 mixture of fetal bovine serum [Gemini, Woodland, CA] and fetal clone [HyClone, Logan, NY]), penicillin/streptomycin (Gemini), fungizone (Invitrogen), and gentamycin (Invitrogen), and incubated at 37°C in a humidified atmosphere of 95% air/5% CO_2 for 14 days. Cultures were fixed with methanol and stained with crystal violet. Colonies containing more than 50 cells were scored. Surviving fractions were calculated using the plating efficiencies of cells from untreated control tumors plated the same day.

For the tumor growth delay assays, mice were randomized by tumor volume into treatment groups when tumors were roughly 100 mm^3. Tumors were measured three times per week by a blinded observer. The length (L), width (W), and height (H) of the tumor were measured using vernier calipers, and volume was calculated using the formula of a hemiellipsoid: $(L \times W \times H \times \pi)/6$. Mice were monitored for signs of drug toxicity (including behavioral changes, failure to groom, and appearance) and weighed three times per week throughout of the study. Each mouse was euthanized when its tumor volume reached 1000 mm^3. A necropsy was performed on each animal to assess toxicity and lung metastases (25, 26). Lungs were removed, cleaned of extraneous tissues, fixed in Bouin's, washed with 95% ethanol, and stored in ethanol until they were counted. Lungs were coded and blinded, and tumor nodules on the lung surfaces were counted using a dissecting microscope.

A Kaplan-Meier analysis was performed by scoring as an event the time that the tumor reached four times (4×) its initial treatment volume. The statistical significance of differences between groups in the tumor growth delay assay was determined using the general t test and log-rank (χ^2) test; the Shapiro-Wilk's test was used to ensure that data were normally distributed. The statistical significance of differences between groups in the tumor cell survival assay and lung metastases assay was tested using the Mann-Whitney U test because some data sets were not normally distributed. Significance was set at 95% ($P = 0.05$) for all analyses.

Results

Tumor Cell Survival Assay. Figure 1 shows the effects of efaproxiral plus oxygen breathing on the survival of cells from EMT6 tumors in mice treated with 150 mg/kg carboplatin. The present experiments repeated some groups from our prior studies. Because there were no statistically significant differences between the survivals determined in these and previous studies (13), the data were combined to provide greater statistical power. Mice were treated with the following regimens: carboplatin, assayed 2 or 5 hrs after injection; oxygen breathing from 15 mins before carboplatin injection until assay 2 or 5 hrs after injection; 150 mg/kg efaproxiral 15 mins before carboplatin injection plus oxygen

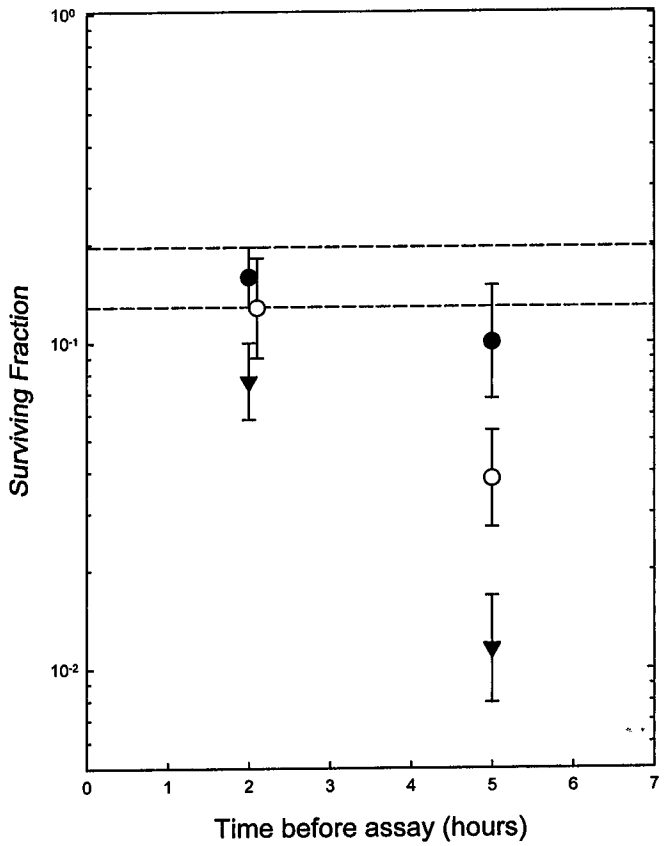

Figure 1. Effects of efaproxiral plus oxygen breathing on the survival of cells in EMT6 tumors treated with 150 mg/kg carboplatin. ●: carboplatin only, air-breathing mice. ○: carboplatin plus oxygen breathing. ▼: efaproxiral plus oxygen breathing from the first efaproxiral injection until the time of assay. Points are geometric means ± SEM of 5 to 11 independent determinations.

breathing from 15 mins before until assay at 2 hrs after injection; three doses of 150 mg/kg efaproxiral (15 mins before, 1.5 hrs after, and 3.5 hrs after carboplatin injection) plus oxygen breathing from 15 mins before carboplatin until assay at 5 hrs after carboplatin injection; three doses of efaproxiral given on the same schedule plus oxygen breathing for 5.25 hrs (no carboplatin).

Treatment with carboplatin killed approximately 90% of the tumor cells (Fig. 1); the survival of cells from tumors in air-breathing mice assayed 2 hrs and 5 hrs after treatment was not significantly different. There was no statistically significant differences in the survival of tumor cells from air-breathing mice, mice breathing oxygen, or mice receiving efaproxiral plus oxygen when tumors were assayed 2 hrs after the injection of carboplatin.

Treatment of mice with oxygen from 15 mins before until 5 hrs after the carboplatin injection reduced the survival of tumor cells below that in air-breathing mice assayed 5 hrs after treatment, but this difference was not statistically significant ($P = 0.07$). The survival of tumor cells from mice treated with oxygen or efaproxiral plus oxygen for 5 hrs after carboplatin injection was significantly lower than those for mice assayed 2 hrs after carboplatin injection ($P < 0.05$), suggesting that carboplatin damage continued to develop during this period. When tumors were assayed 5 hrs after carboplatin injection, the treatment regimen using three doses of efaproxiral combined with oxygen breathing produced a greater decrease in tumor cell survival than that seen in mice breathing air ($P < 0.01$) or oxygen ($P < 0.05$) for the same period but not treated with efaproxiral. Three doses of efaproxiral plus oxygen breathing for 5.25 hrs had no significant effect on the viability of tumor cells in the absence of carboplatin (data not shown).

Tumor Growth Delay Assay. Figure 2 shows the effects of efaproxiral plus oxygen breathing on the growth of EMT6 tumors in animals treated with 100 mg/kg carboplatin. The findings are from two independent experiments; these experiments were in good agreement, and the data were, therefore, pooled. Tumor growth delays were analyzed by determining the time from the day of treatment to the time the tumor reached four times its treatment volume. Mice were treated with one of three regimens: 100 mg/kg carboplatin in air-breathing mice; oxygen breathing from 15 mins before until 5 hrs after 100 mg/kg carboplatin injection; three doses of 150 mg/kg efaproxiral (given 15 mins before, 1.5 hrs after, and 3.5 hrs after carboplatin injection) plus oxygen breathing from 15 mins before until 5 hrs after 100 mg/kg carboplatin injection.

Treatment with carboplatin alone produced a small but statistically significant delay in the growth of the tumors, increasing the mean time to 4× from the value 11.4 ± 0.4 days for the untreated control group to 14.7 ± 0.7 days ($P < 0.001$) and producing a growth delay of 3.3 ± 0.7 days (Table 1). Oxygen breathing did not increase the effects of carboplatin. Treatment with efaproxiral plus oxygen breathing produced a growth delay of 5.7 ± 0.7 days; this was

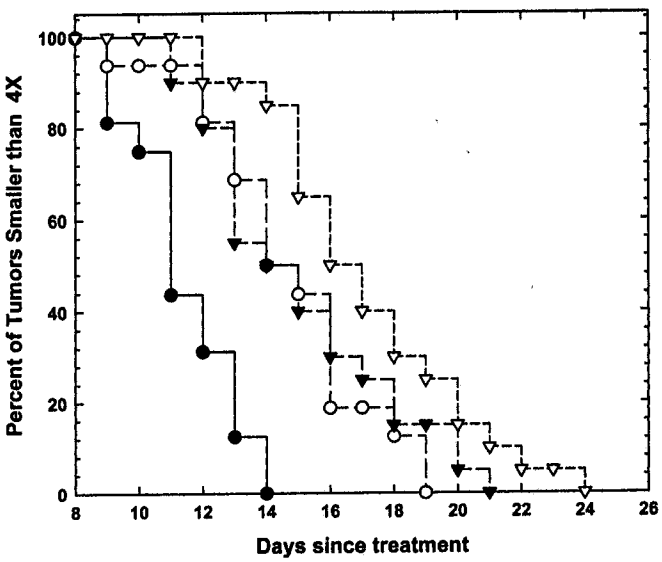

Figure 2. Kaplan Meyer plot of the time at which tumors reached four times the initial treatment volume. ●: Control, no treatment ($n = 16$). ○: 100 mg/kg carboplatin ($n = 16$). ▼: 100 mg/kg carboplatin plus oxygen breathing ($n = 20$). ▽: 100 mg/kg carboplatin plus efaproxiral plus oxygen breathing ($n = 20$).

Table 1. Comparison of Tumor Growth Delays

Treatment	Growth delay
Control	0
Carboplatin	3.3 ± 0.7*
Carboplatin + O_2	3.6 ± 0.7*
Carboplatin + efaproxiral + O_2	5.7 ± 0.7**

* $P < 0.001$ relative to control.
** $P < 0.001$ relative to control, and $P < 0.05$ relative to carboplatin in air-breathing or oxygen-breathing mice.

significantly different ($P < 0.05$) from the growth delays for mice treated with carboplatin in air-breathing or oxygen-breathing mice. The statistical comparisons resulting from the Kaplan-Meier analysis (Fig. 2), made using the log-rank (χ^2) test were the same for all comparisons mentioned above, except that the difference between carboplatin plus oxygen plus efaproxiral and the carboplatin plus oxygen groups was not significant when this test was used ($P = 0.11$). However, the relative effect ratio for the carboplatin plus oxygen plus efaproxiral treatment calculated during the log-rank analysis indicated that the addition of efaproxiral was beneficial, which is in agreement with the *t* test (relative effect for carboplatin plus efaproxiral plus oxygen = 1.6 vs. carboplatin plus oxygen, 1.9 vs. carboplatin only, and 4.4 vs. control).

The dose of carboplatin that can be administered is limited by the toxicity of the drug to the bone marrow; 100 mg/kg was the highest dose that could be given without producing severe toxicities. A dose of 150 mg/kg produced unacceptable toxicity: two of seven mice were euthanized at the time of hematologic crises, and the surviving mice showed severe weight loss (4.6 ± 0.5 g), became lethargic, failed to groom, and showed other signs of toxicity. To assess whether treatment with oxygen or efaproxiral plus oxygen increased the toxicity of 100 mg/kg carboplatin, mice in the tumor growth studies were monitored closely for evidence of increased toxicity. Measurements of body weight made over the course of the experiments showed no statistically significant differences between the three carboplatin-treated groups, (air breathing, oxygen breathing, and efaproxiral plus oxygen breathing), all of which showed average weight losses of ~1 g at the nadir. Observations of the condition of the animals (behavior, grooming, and general appearance) revealed no significant differences between the three carboplatin-treated groups at any time during the experiment. In addition, necropsy findings for mice at the time of euthanasia were generally unremarkable and revealed no differences between the three treatment groups.

The number of spontaneous lung metastases was quite variable, as expected. The number of lung metastases was significantly higher ($P < 0.05$) in untreated control animals (mean ± SD; 25 ± 13 visible lung metastases per mouse) than in mice treated with carboplatin alone (14 ± 11), carboplatin plus oxygen (18 ± 12), or carboplatin plus oxygen plus efaproxiral (15 ± 12). The carboplatin-treated groups were not significantly different.

Discussion

We reported previously (10) that efaproxiral plus oxygen breathing reduced the radiobiological hypoxic fraction of EMT6 tumors from the value of 24% found in both air-breathing and oxygen-breathing mice to 9% and improved the response of the tumors to radiation. In contrast, oxygen breathing (or carbogen breathing) alone produces only minimal effects on the oxygenation of EMT6 tumors (10, 22, 23, 27) and does not alter the response of tumors to radiation (10, 25, 27). More recently we reported (13) that post-treatment, but not pretreatment, changes in tumor oxygenation produced by efaproxiral plus oxygen breathing decreased tumor cell survival in EMT6 tumors treated with carboplatin. The present studies confirmed these findings and also showed that the lower tumor cell survival seen when mice were treated with oxygen or with efaproxiral plus oxygen for 5 hrs after the injection of carboplatin was not an artifact of delayed plating, allowing us to conclude that the prolonged improvement in tumor oxygenation produced by the efaproxiral plus oxygen breathing increased the cytotoxicity of carboplatin.

Treatment with 100 mg/kg carboplatin slowed tumor growth in air-breathing mice, producing a growth delay of 3.3 days. The effect of carboplatin was relatively small; this reflects the general resistance of EMT6 tumors to antineoplastic drugs. The surviving fractions and growth delays seen after treatment of EMT6 tumors with 100 mg/kg carboplatin in air-breathing mice are similar to those seen for ~8 Gy of x-rays (23) or 4 mg/kg mitomycin C (28) and greater than those obtainable with nonlethal doses of Adriamycin and Taxotere (data not shown). Posttreatment oxygen breathing did not increase the growth delay over that in air-breathing mice, producing only an insignificant 9% increase from carboplatin alone. Efaproxiral plus oxygen increased the growth delay to 5.7 days; this was 2.4 days (71%) greater than that for carboplatin alone and 2.1 days (57%) greater than that for carboplatin plus oxygen breathing.

The enhancement produced by treatment with efaproxiral plus oxygen can be estimated by comparing data from the present studies with data from our studies using higher doses of carboplatin alone. The surviving fraction of cells from tumors treated with 150 mg/kg carboplatin plus the 5-hr treatment with efaproxiral and oxygen (0.011; Fig. 1) was lower than the surviving fraction of 0.033 found previously (13) in air breathing mice for 200 mg/kg carboplatin; the limited solubility of carboplatin precluded testing higher doses. The growth delay of 5.7 days seen after treatment with 100 mg/kg carboplatin plus efaproxiral and oxygen was similar to the growth delay of 5.5 ± 1.4 seen with 150 mg/kg carboplatin in a pilot study. However, 150

mg/kg carboplatin produced unacceptable toxicity. The adjuvant treatment with efaproxiral plus oxygen breathing, therefore, improved the tumor growth delay obtained with 100 mg/kg carboplatin to or beyond that obtained with the highly toxic dose of 150 mg/kg carboplatin, but did so without increasing the toxicity beyond that seen with 100 mg/kg carboplatin in air-breathing mice. These comparisons suggest that the enhancement ratio for this adjuvant treatment is on the order of 1.5. A therapeutic gain of this magnitude in the clinic would be very significant.

The increase in tumor response observed using two different endpoints, without a concomitant increase in host toxicity, shows that the adjuvant treatment with efaproxiral plus oxygen breathing produced a significant increase in the therapeutic ratio of carboplatin in this tumor/host system. The dose of efaproxiral used in our studies is within the range being used in human studies (12). Because of the longer half life of efaproxiral in patients, a prolonged increase in tumor oxygenation could be achieved with a single infusion of drug. The regimen tested in our studies, therefore, could be translated readily to clinical use. The potential clinical value of such regimens merits further study in other animal models and, if confirmed, in clinical trials.

1. Abraham DJ, Wireko FC, Randad RS, Poyart C, Kister J, Bohn B, Liard JF, Kunert MP. Allosteric modifiers of hemoglobin: 2-[4-[[(3,5-disubstituted anilino)carbonyl]methyl]phenoxy]-2-methylpropionic acid derivatives that lower the oxygen affinity of hemoglobin in red cell suspensions, in whole blood, and in vivo in rats. Biochemistry 31: 9141–9149, 1992.
2. Randad RS, Mahran MA, Mehanna AS, Abraham DJ. Allosteric modifiers of hemoglobin, 1: design, synthesis, testing, and structure-allosteric activity relationship of novel hemoglobin oxygen affinity decreasing agents. J Med Chem 34:752–757, 1991.
3. Safo MK, Moure CM, Burnett JC, Joshi GS, Abraham DJ. High-resolution crystal structure of deoxy hemoglobin complexed with a potent allosteric effector. Protein Sci 10:951–957, 2001.
4. Wireko FC, Kellogg GE, Abraham DJ. Allosteric modifiers of hemoglobin, 2: crystallographically determined binding sites and hydrophobic binding/interaction analysis of novel hemoglobin oxygen effectors. J Med Chem 34:758–767, 1991.
5. Amorino GP, Lee H, Holburn GE, Paschal CB, Hercules SK, Shyr Y, Steffen RP, Choy H. Enhancement of tumor oxygenation and radiation response by the allosteric effector of hemoglobin, RSR13. Radiat Res 156:294–300, 2001.
6. Doppenberg EM, Rice MR, Alessandri B, Qian Y, Di X, Bullock R. Reducing hemoglobin oxygen affinity does not increase hydroxyl radicals after acute subdural hematoma in the rat. J Neurotrauma 16: 123–133, 1999.
7. Hou H, Khan N, O'Hara JA, Grinberg OY, Dunn JF, Abajian MA, Wilmot CM, Demidenko E, Lu S, Steffen RP, Swartz HM. Increased oxygenation of intracranial tumors by Efaproxyn (efaproxiral), an allosteric hemoglobin modifier, *in vivo* EPR oximetry study. Int J Radiat Oncol Biol Phys 61:1503–1509, 2005.
8. Hou H, Khan N, O'Hara JA, Grinberg OY, Dunn JF, Abajian MA, Wilmot CM, Makki M, Demidenko E, Lu S, Steffen RP, Swartz HM. Effect of RSR13, an allosteric hemoglobin modifier, on oxygenation in murine tumors: an *in vivo* electron paramagnetic resonance oximetry and bold MRI study. Int J Radiat Oncol Biol Phys 59:834–843, 2004.
9. Richardson RS, Tagore K, Haseler LJ, Jordan M, Wagner PD. Increased VO_2 max with right-shifted Hb-O2 dissociation curve at a constant O_2 delivery in dog muscle *in situ*. J Appl Physiol 84:995-1002, 1998.
10. Rockwell S, Kelley M. RSR13, a synthetic allosteric modifier of hemoglobin, as an adjunct to radiotherapy: preliminary studies with EMT6 cells and tumors and normal tissues in mice. Radiat Oncol Invest 6:199–208, 1998.
11. Wahr JA, Gerber M, Venitz J, Baliga N. Allosteric modification of oxygen delivery by hemoglobin. Anesth Analg 92:615–620, 2001.
12. Suh JH. Efaproxiral: a novel radiation sensitiser. Expert Opin Investig Drugs 13:543–550, 2004.
13. Donnelly ET, Kelley M, Rockwell S. Effects of RSR13 and oxygen on the cytotoxicity of cisplatin and carboplatin to EMT6 mouse mammary tumor cells *in vitro* and *in vivo*. Cancer Chemother Pharmacol 53:43–50, 2004.
14. Douple EB, Richmond RC. Radiosensitization of hypoxic tumor cells by cis- and trans-dichlorodiammineplatinum (II). Int J Radiat Oncol Biol Phys 5:1369–1372, 1979.
15. Grau C, Overgaard J. Effect of cancer chemotherapy on the hypoxic fraction of a solid tumor measured using a local tumor control assay. Radiother Oncol 13:301–309, 1988.
16. Koch S, Mayer F, Honecker F, Schittenhelm M, Bokemeyer C. Efficacy of cytotoxic agents used in the treatment of testicular germ cell tumours under normoxic and hypoxic conditions *in vitro*. Br J Cancer 89:2133–2139, 2003.
17. Korbelik M, Skov K. Inactivation of hypoxic cells by cisplatin and radiation at clinically relevant doses. Radiat Res 119:145–156, 1989.
18. Melvik JE, Pettersen EO. Oxygen- and temperature-dependent cytotoxic and radiosensitizing effects of cis-dichlorodiammineplatinum(II) on human NHIK 3025 cells *in vitro*. Radiat Res 114:489–499, 1988.
19. Skov KA, Adomat H, Farrell NP, Matthews JB. Assessment of toxicity of bis-platinum complexes in hypoxic and aerobic cells. Anticancer Drug Des 13:207–220, 1998.
20. Stratford IJ, Williamson C, Adams GE. Combination studies with misonidazole and a cis-platinum complex: cytotoxicity and radiosensitization *in vitro*. Br J Cancer 41:517–522, 1980.
21. Teicher BA, Lazo JS, Sartorelli AC. Classification of antineoplastic agents by their selective toxicities toward oxygenated and hypoxic tumor cells. Cancer Res 41:73–81, 1981.
22. Donnelly ET, Liu Y, Fatunmbi YO, Lee I, Magda D, Rockwell S. Effects of texaphyrins on the oxygenation of EMT6 mouse mammary tumors. Int J Radiat Oncol Biol Phys 58:1570–1576, 2004.
23. 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 10:695–712, 1984.
24. Rockwell S. *In vivo–in vitro* tumor systems: new models for studying the response of tumours to therapy. Lab Anim Sci 27:831–851, 1977.
25. Rockwell S, Irvin CG, Nierenburg M. Effect of a perfluorochemical emulsion on the development of artificial lung metastases in mice. Clin Exp Metastasis 4:45–50, 1986.
26. Rockwell S, Nierenburg M, Irvin CG. Enhancement of artificial lung metastases by misonidazole. Int J Radiat Oncol Biol Phys 10:1395–1398, 1984.
27. Rockwell S. Perfluorochemical emulsions and radiation therapy. Artif Cells Blood Substitutes and Immobil Biotech 22:1097–1108, 1994.
28. Rockwell S, Nierenburg M, Irvin CG. Effects of the mode of administration of mitomycin on tumor and marrow response and on the therapeutic ratio. Cancer Treat Rep 71:927–934, 1987.