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ORIGINAL ARTICLE

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Allosteric effectors of hemoglobin as modulators of chemotherapy and radiation therapy in vitro and in vivo

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Abstract *Introduction*: A series of molecules designed to be allosteric effectors of hemoglobin were examined for their potential as radiation sensitizers in vitro and in vivo and for their potential as chemosensitizers in vivo as well as for their antimetastatic effect. *Results*: At a concentration of 100 μM for 1 h prior to, during and for 1.5 h after radiation exposure, the allosteric effectors decreased the shoulder of the radiation survival curve of normally oxygenated EMT-6 cells and increased the slope of the radiation survival curves of hypoxic EMT-6 cells resulting in dose-modifying factors of 1.8 to 2.1. In vivo the allosteric effectors had antitumor activity against the Lewis lung carcinoma and produced primarily additive tumor growth delay when administered along with fractionated radiation therapy. When administered on days 4 through 18 after tumor implantation, the allosteric effectors, especially JP-7, RSR-13 and RSR-4, were highly effective antimetastatic agents in animals bearing Lewis lung carcinoma. In cell culture, simultaneous exposure to the allosteric effectors (at 100 μM) effectively sensitized EMT-6 cells to the effects of 4-hydroperoxycyclophosphamide, thiotepa and carboplatin. The allosteric effectors were not very cytotoxic toward EMT-6 tumor cells from tumors treated in vivo with single doses of each molecule nor were these agents very cytotoxic toward bone marrow CFU-GM taken from the same animals. *Conclusions*: It is likely that the allosteric effectors have a molecular target in addition to hemoglobin. Other possible targets include hydroxymethyl-glutaryl-CoA reductase or microsomal cytochrome b_5.

Key words Radiosensitization · Allosteric effectors · Chemosensitization · Combined modality therapy

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Introduction

RSR-13, [2,4-[[(3,5-dimethylanilino)carbonyl]methyl] phenoxy]-2-methyl propionic acid] has been identified as an allosteric effector of hemoglobin. RSR-13 binds to the hemoglobin molecule thus altering its configuration so that the bound oxygen is released more readily [1–3, 16, 37]. Intravenous administration of RSR-13 to rats bearing the 13762 mammary carcinoma results in a decrease in the hypoxic fraction (percent of pO_2 readings < 5 mmHg) of the tumor to an extent that is dependent upon the RSR-13 dose and is maximal at 30 min after RSR-13 administration. Breathing carbogen (95% oxygen/5% carbon dioxide) along with RSR-13 administration markedly increases tumor oxygenation. Intravenous infusion of RSR-13 (200 mg/kg) over 60 min results in maximal tumor oxygenation at 40 min into the infusion. By 30 min after completion of the infusion, tumor oxygenation has returned to baseline.

Administration of RSR-13 to animals bearing the Lewis lung carcinoma prior to each fraction of a fractionated radiation therapy regimen results in dose modification with dose modifying factors up to 1.7. RSR-13 administration also results in decreased numbers of lung metastases in these same animals. When RSR-13 is administered on days 4 through 18, the modification of fractionated radiation remains the same but the number of lung metastases is decreased further. In animals bearing the MB-49 bladder carcinoma, administration of RSR-13 results in increased responses to both fractionated radiation therapy and chemotherapy and marked decreases in lung metastases. RSR-13 is effective in decreasing the number of lung colonies formed by intravenously injected tumor cells if administered

on the day prior to, on the same day as or on the day after the tumor cells.

The current study was undertaken to assess the ability of RSR-13 and seven congeners to act as radiation sensitizers in vitro and in vivo and to act as chemosensitizers in vitro. Antimetastatic effects were assessed in animals bearing the Lewis lung carcinoma and tumoricidal effects and bone marrow toxicity were assessed in animals bearing the EMT-6 murine mammary carcinoma.

Materials and methods

Drugs

RSR-13, [2-4-[[(3, 5-dimethylanilino)carbonyl]methyl]phenoxy]-2-methyl propionic acid], and the congeners JP-7, RSR-4, RSR-46, KDD-86, GSJ-60, GSJ-61 and KDD-167 were gifts from Allos Therapeutics (Denver, Col.) (Fig. 1). 4-Hydroperoxycyclophosphamide was a gift from MGI Pharma (Minneapolis, Minn.). Melphalan and carboplatin were purchased from Sigma Chemical Co. (St. Louis, Mo.). Triethylenethiophosphoramide (thiotepa) was purchased from the Dana-Farber Cancer Institute pharmacy.

Cell line

EMT-6 mouse mammary tumor cells have been widely used for the study of hypoxia [21–23]. Cultures were maintained in exponential growth in Waymouth's medium (I.S.I. Corporation, Chicago, Ill.), supplemented with 15% newborn calf serum, 100 IU/ml penicillin and 100 μg/ml streptomycin (Grand Island Biological Co., Grand Island, N.Y.). The doubling time of cultures growing at 37 °C in a humidified atmosphere of 5% CO_2/95% air was 16–19 h [20]. In vitro plating efficiencies of the control culture ranged from 65% to 80%.

Radiation sensitization

To produce hypoxia, plastic flasks containing exponentially growing monolayers in complete medium plus serum were fitted with sterile rubber septa and exposed to a continuously flowing gas mixture comprising 95% N_2/5% CO_2. After 4 h, the drug or vehicle was added to the flasks by injection through the rubber septum without disturbing the hypoxia. Parallel flasks were maintained under normally oxygenated conditions of a humidified atmosphere comprising 95% air/5% CO_2. EMT-6 cells were exposed to 100 μ*M* of an allosteric effector or vehicle for 1 h prior to, during and for 1.5 h after, radiation delivery. Radiation (2.5, 5, 10, 15 or 20 G) was delivered using a Gamma Cell 40 (0.88 Gy/min; Atomic Energy of Canada, Ottawa, Ontario).

Chemosensitization

EMT-6 cells in exponential growth were exposed simultaneously for 1 h to 100 μ*M* of an allosteric effector and various concentrations of 4-hydroperoxycyclophosphamide (4-HC), melphalan, thiotepa or carboplatin. The cells were washed free of drug using phosphate-buffered 0.9% saline.

Colony formation

Cell viability was measured by the ability of single cells to form colonies in vitro as described elsewhere [20, 24]. Each experiment was repeated three times and each data point per experiment represents the results of three different dilutions of cells plated in triplicate [28].

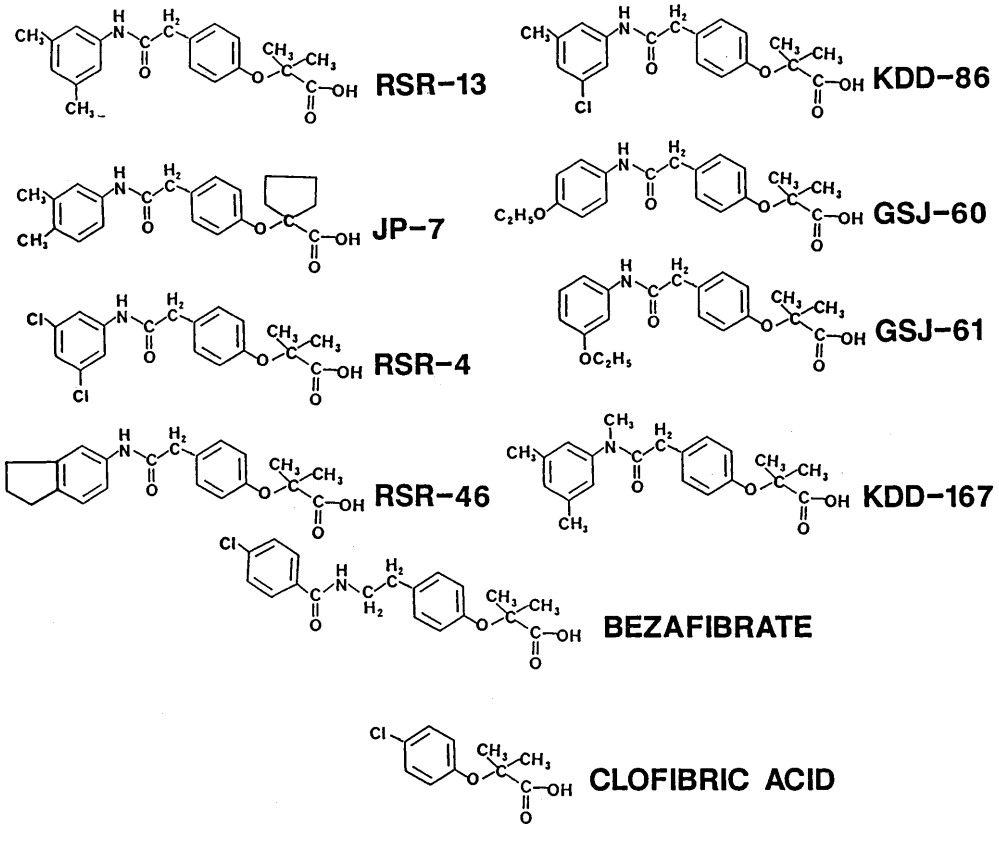

Fig. 1 Chemical structure of the allosteric effectors as well as bezafibrate and clofibric acid

Tumor growth delay experiments

The Lewis lung tumor [26, 29, 30] was carried in male C57BL mice (Taconic Farms, Germantown, N.Y.). For experiments, 2×10^6 tumor cells prepared from a brei of several stock tumors were implanted subcutaneously into a hind leg of male mice at 8–10 weeks of age.

Animals bearing the Lewis lung carcinoma were treated with an allosteric effector (100 mg/kg, except RSR-4 50 mg/kg) administered intraperitoneally daily on days 4 through 18 after tumor cell implantation when the tumors were about 100 mm^3 in volume. The allosteric effector was administered immediately before radiation therapy. Radiation therapy was delivered in fractions of 0, 2, 3 or 4 Gy daily for 5 days locally to the tumor-bearing limb on days 7 through 11 (Gamma Cell 40; dose rate 0.88 Gy/min).

The progress of each tumor was measured thrice weekly until it reached a volume of 500 mm^3. Tumor growth delay was calculated as the days taken by each individual tumor to reach 500 mm^3 compared with the untreated controls. Each treatment group had six animals and each experiment was repeated three times. The days of tumor growth delay are the mean values ± SE for the treatment group compared with the control.

Lung metastases

The number of external lung metastases from animals treated as described above on day 20 after tumor implantation were counted manually and scored as ≥3 mm or less in diameter. The data are shown as the mean values obtained for 6–12 pairs of lungs. Parentheses indicate the number of metastases that were large.

Tumor cell survival assay

The EMT-6 murine mammary carcinoma which is an in vivo-in vitro tumor system was used for these experiments [22, 23]. The EMT-6 tumor was carried in female Balb/C mice (Taconic Farms). For the experiments, 2×10^6 tumor cells prepared from a brei of several stock tumors were implanted intramuscularly into both hind-legs of female Balb/C mice at 8 to 10 weeks of age. On day 8 after tumor cell implantation when the tumors were about 150 mm^3 in volume, an allosteric effector (100, 300 or 500 mg/kg) was administered as a single dose by intraperitoneal injection. The mice were killed 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, and single-cell suspensions were prepared [32, 33]. The untreated tumor cell suspensions had a plating efficiency of 8% to 12%. Each treatment group consisted of two animals and each experiment was repeated three times. The results are expressed as the surviving fraction ± SEM of cells from treated groups compared with untreated controls.

Bone marrow toxicity

Bone marrow was taken from the same animals used for the tumor excision assay. A pool of marrow from the femurs of two animals was obtained by gently flushing the marrow through a 23-gauge needle and colony formation was carried out as described previously [32, 33]. Colonies of at least 50 cells were scored on an Acculite colony counter (Fisher, Springfield, N.J.). The results from three experiments, in which each group was measured at three cell concentrations in duplicate, were averaged and are expressed as the surviving fraction of treated groups compared with untreated controls.

Results

The chemical structures of the allosteric effectors along with the structure of bezafibrate and clofibric acid are shown in Fig. 1. The major variations of the congeners are on the substituents of the anilino-carbonyl moiety, on the dimethyl moiety of the clofibric acid (JP-7) and on the anilino-carbonyl nitrogen (KDD-167). Exposure of EMT-6 cells in culture to 100 μM of each of the allosteric effectors for 3 h under normally oxygenated and hypoxic conditions was not toxic toward the cells (Fig. 2). Exposure of normally oxygenated EMT-6 cells to the allosteric effectors prior to, during and after, irradiation resulted in a diminution of the shoulder on the radiation survival curve indicating inhibition of the repair of sublethal radiation damage [9, 31]. The effects of the allosteric effectors on the slope of the radiation survival curves ranged from no effect (JP-7, RSR-13 and KDD-86) to a slope increase (dose modifying factor) of 1.5 (RSR-13 and GSJ-61). The allosteric effectors were all radiation sensitizers of hypoxic EMT-6 cells. The most effective hypoxic cell radiation sensitizer was RSR-13 which produced a radiation dose-modifying factor of 2.6. The other allosteric effectors produced radiation dose-modifying factors ranging from 1.8 to 2.1 with no significant difference amongst them.

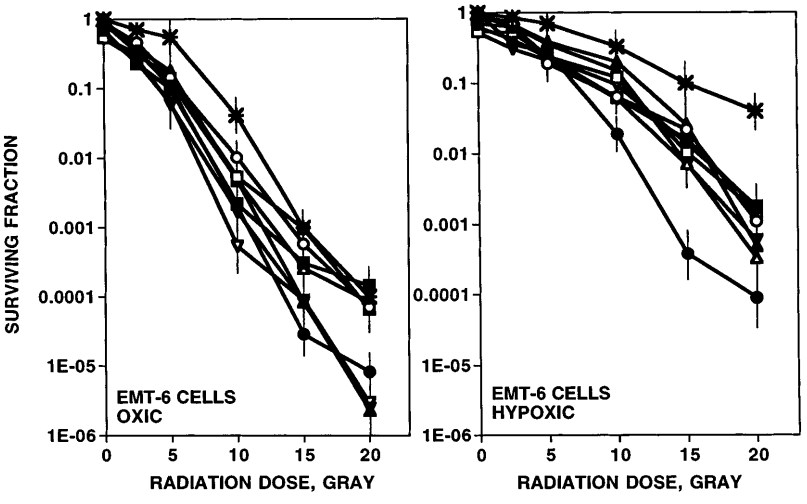

Fig. 2 Survival of EMT-6 murine mammary carcinoma cells exposed under normally oxygenated or hypoxic conditions to radiation alone (*) or along with an allosteric effector of hemoglobin for 1 h prior to, during and for 1.5 h after, radiation delivery (● RSR-13, ○ JP-7, ■ RSR-4, □ RSR-46, ▲ KDD-86, △ GSJ-61, ▼ KDD-167, ▽ GSJ-60). The data are the means of three experiments; *bars* are SEM

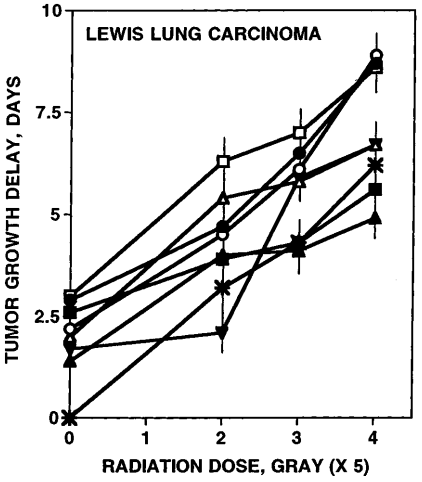

Fig. 3 Growth delay of the murine Lewis lung carcinoma after treatment of the tumor-bearing limb with fractionated radiation therapy (2, 3 or 4 Gy) daily for 5 days beginning on day 7 after tumor cell implantation alone (∗) or along with 100 μ*M* of an allosteric effector of hemoglobin (100 mg/kg) administered by intraperitoneal injection daily on days 4 through 18 after tumor cell implantation (● RSR-13, ○ JP-7, ■ KDD-86, □ RSR-4, ▲ RSR-46, △ GSJ-61, ▼ KDD-167). The data are the means of three experiments; *bars* are SEM

Treatment of mice bearing the Lewis lung tumor with fractionated radiation therapy in the presence and absence of treatment with an allosteric effector was used to assess the effect of these agents along with radiation therapy in vivo (Fig. 3). The Lewis lung carcinoma is a highly hypoxic tumor as determined by polarographic oxygen electrode measurements [34]. The allosteric effectors were administered to the animals by intraperitoneal injection once per day on days 4 through 18 after tumor cell implantation. All of the allosteric effectors were administered at a dose of 100 mg/kg without toxicity except RSR-4 which was administered at the tolerated dose of 50 mg/kg. The allosteric effectors all had antitumor activity producing tumor growth delays ranging from about 3 days for RSR-13, RSR-4 and KDD-86 to about 1.5 days for RSR-46, KDD-167 and GSJ-61. In vivo the allosteric effectors produced an effect with fractionated radiation that, in general, reflected additivity of the two treatment modalities.

The Lewis lung carcinoma metastasizes avidly to the lungs of animals from subcutaneously implanted primary tumor. The number of lung metastases present on day 20 was used to determine the effect of the allosteric effectors on systemic disease (Table 1). The allosteric effectors that were most effective in decreasing lung metastases were JP-7, RSR-13 and RSR-4; the least effective was KDD-167.

Small molecules that have been shown to be effective hypoxic cell radiation sensitizers are also often effective chemosensitizers. To begin to study the chemosensitizing potential of the allosteric effectors, EMT-6 cells in exponential growth were exposed simultaneously to an allosteric effector (100 μ*M*) and to a cytotoxic anticancer drug for 1 h or to the cytotoxic anticancer drug for 1 h alone, and their survival determined by colony formation (Fig. 4). 4-Hydroperoxycyclophosphamide (4-HC) is a derivative of cyclophosphamide which is active in cell culture. 4-HC killed EMT-6 tumor cells in a dose-dependent manner reaching a maximum of 2 logs at a 4-HC concentration of 250 μ*M* for 1 h. Simultaneous exposure of the cells to 4-HC and each of the allosteric effector molecules markedly increased cell killing by the drug. The most effective sensitizers were KDD-167, GSJ-61 and RSR-4 which increased the killing of EMT-6 cells by 4 or more logs at a 4-HC concentration of 100 μ*M*. Even the least effective sensitizers KDD-86 and RSR-46 were highly effective sensitizers increasing the killing of EMT-6 cells 1.5 to 2.5 logs. Melphalan (phenylalanine mustard) was a potent cytotoxic agent toward exponentially growing EMT-6 tumor cells. A 1-h exposure to 100 μ*M* of the drug killed 4.5 logs of cells. Simultaneous exposure of the EMT-6 cells to 100 μ*M* of

Table 1 Growth delay of the Lewis lung carcinoma and number and percent large lung metastases on day 20 after long-term treatment with various allosteric effectors of hemoglobin and fractionated radiation therapy. Radiation was delivered locally to the tumor-bearing limb on days 7 through 11. Drugs were administered by intraperitoneal injection

Treatment group	Tumor growth delay (days)	Number of lung metastases (% large)
Controls	–	25 (60)
5 × 2 Gy	3.2 ± 0.3	21 (56)
5 × 3 Gy	4.3 ± 0.4	20 (52)
5 × 4 Gy	6.2 ± 0.6	18 (52)
RSR-13 (100 mg/kg) days 4–18	2.9 ± 0.3	10 (34)
+ 5 × 2 Gy	4.7 ± 0.5	8 (44)
+ 5 × 3 Gy	6.5 ± 0.8	6 (30)
+ 5 × 4 Gy	8.7 ± 0.9	5 (18)
JP-7 (100 mg/kg) days 4–18	2.2 ± 0.3	8 (25)
+ 5 × 2 Gy	4.5 ± 0.4	4 (31)
+ 5 × 3 Gy	6.1 ± 0.5	3 (25)
+ 5 × 4 Gy	8.9 ± 0.8	2.5 (40)
KDD-86 (100 mg/kg) days 4–18	2.6 ± 0.3	14 (35)
+ 5 × 2 Gy	3.9 ± 0.3	13 (39)
+ 5 × 3 Gy	4.3 ± 0.4	12 (43)
+ 5 × 4 Gy	5.6 ± 0.5	9 (29)
RSR-4 (50 mg/kg) days 4–18	3.0 ± 0.3	12 (46)
+ 5 × 2 Gy	6.3 ± 0.5	11 (33)
+ 5 × 3 Gy	7.0 ± 0.5	10 (35)
+ 5 × 4 Gy	8.6 ± 0.7	3 (50)
RSR-46 (100 mg/kg) days 4–18	1.4 ± 0.3	13 (35)
+ 5 × 2 Gy	4.0 ± 0.3	13 (35)
+ 5 × 3 Gy	4.1 ± 0.4	12 (35)
+ 5 × 4 Gy	4.9 ± 0.4	8 (40)
GSJ-61 (100 mg/kg) days 4–18	2.0 ± 0.3	15 (25)
+ 5 × 2 Gy	5.4 ± 0.5	12 (35)
+ 5 × 3 Gy	5.8 ± 0.6	10 (35)
+ 5 × 4 Gy	6.7 ± 0.6	7 (35)
KDD-167 (100 mg/kg) days 4–18	1.7 ± 0.3	17 (44)
+ 5 × 2 Gy	2.1 ± 0.3	16 (34)
+ 5 × 3 Gy	5.9 ± 0.5	16 (34)
+ 5 × 4 Gy	6.7 ± 0.6	14 (32)

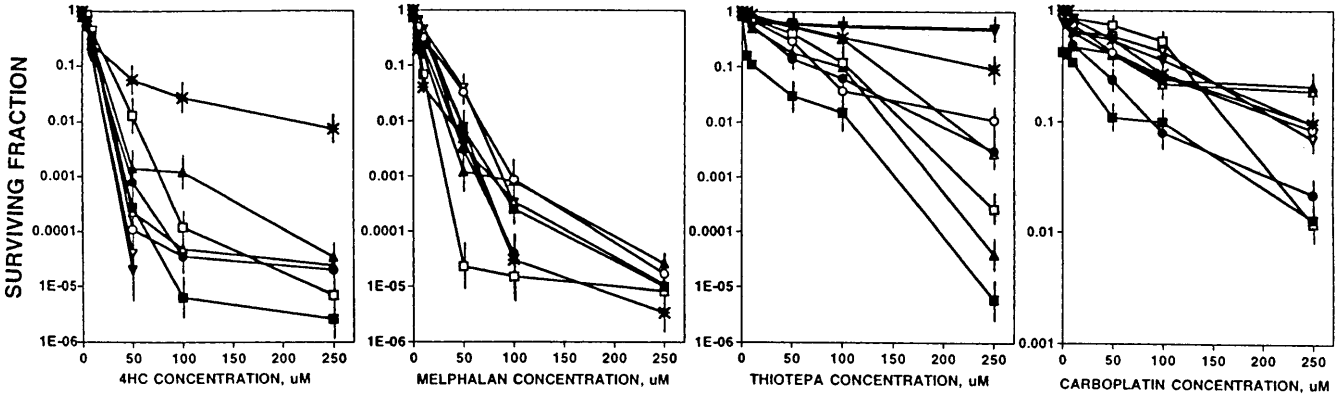

Fig. 4 Survival of EMT-6 murine mammary carcinoma cells exposed to various concentrations of 4-hydroperoxycyclophosphamide (4-HC), melphalan, thiotepa or carboplatin for 1 h alone (*) or along with 100 μ*M* of an allosteric effector of hemoglobin (● RSR-13, ○ JP-7, ■ RSR-4, □ RSR-46, ▲ KDD-86, △ GSJ-61, ▼ KDD-167, ▽ GSJ-60). The data are the means of three experiments; *bars* are SEM

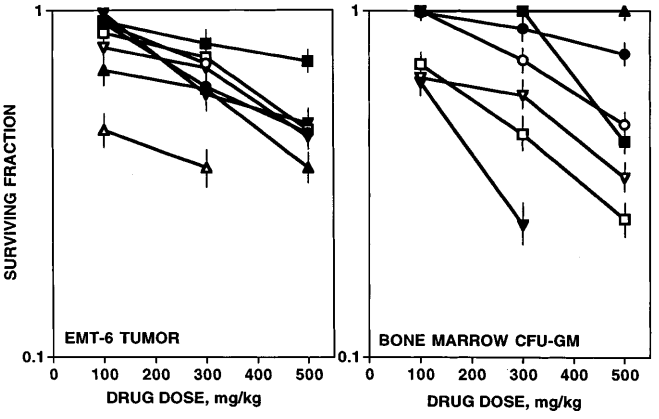

Fig. 5 Survival of EMT-6 murine mammary carcinoma cells from tumors treated in vivo and bone marrow CFU-GM from the same animals after treatment of the tumor-bearing animals with single doses (100, 300 or 500 mg/kg) of the allosteric effectors of hemoglobin by intraperitoneal injection (● RSR-13, ○ RSR-4, ■ RSR-46, □ GSJ-61, ▲ GSJ-60, △ JP-7, ▼ KDD-86, ▽ KDD-167). The data are the means of three experiments; *bars* are SEM

an allosteric effector along with melphalan did not increase cell killing by the drug. In fact a 1-h exposure to all the allosteric effectors together with 100 μ*M* melphalan decreased cell killing to 3.5 logs, except RSR-13 which did not alter melphalan cell killing and RSR-46 which increased cell killing by lower concentrations of melphalan and did not alter cell killing by higher concentrations of melphalan.

A 1-h exposure to 250 μ*M* thiotepa (triethylenethiophosphoramide) killed about 1 log of EMT-6 cells. Simultaneous exposure to RSR-4, KDD-86 or RSR-46 together with thiotepa resulted in markedly increased cell killing. Thus, at a thiotepa concentration of 250 μ*M* cell killing was increased from 1 log to 3.5 to 5.5 logs. GSJ-60, RSR-13 and JP-7 moderately increased, and KDD-167 decreased, cell killing by thiotepa.

Carboplatin was also an effective cytotoxic agent toward EMT-6 cells. A 1-h exposure to 250 μ*M* of the drug killed 2 logs of cells. Simultaneous exposure to RSR-4 or RSR-13 together with carboplatin increased cell killing by almost 1 log. Simultaneous exposure to GSJ-61 or JP-7 together with carboplatin did not alter cell killing and simultaneous exposure to KDD-86 or GSJ-60 together with carboplatin did not alter or slightly diminished cell killing.

The tumor cell survival and bone marrow CFU-GM survival assays were used to assess the cytotoxicity of the allosteric effectors toward EMT-6 cells grown as a tumor in the syngeneic host and toward bone marrow CFU-GM as a representative sensitive normal tissue from those same animals. The allosteric effectors were each administered to the animals as single doses (100, 300 or 500 mg/kg) by intraperitoneal injection. The allosteric effector that was most cytotoxic toward the EMT-6 tumor cells in vivo was GSJ-60 (Fig. 5). GSJ-60 was lethal at a dose of 500 mg/kg but was not cytotoxic toward the bone marrow CFU-GM. Interestingly, RSR-4 which was more toxic than the other allosteric effectors when administered on a daily schedule for 2 weeks (Table 1) was the least cytotoxic allosteric effector toward EMT-6 tumor cells in vivo. The allosteric effectors KDD-167, RSR-46 and GSJ-61 were more cytotoxic toward the bone marrow CFU-GM than toward the EMT-6 tumor cells in vivo. Overall, however, the allosteric effectors were not very cytotoxic, killing only a maximum of 50% of the EMT-6 tumor cells at a dose of 500 mg/kg.

Discussion

Siemann and Macler [27] have demonstrated that the acute elevation of 2,3-diphosphoglycerate (2,3-DPG) levels in the red blood cells of KHT sarcoma-bearing mice significantly enhances the response of the tumors to single doses of radiation. This improvement is primarily the result of a reduction in the fraction of hypoxic tumor cells from about 15% to about 3%. These results demonstrate that the manipulation of erythrocyte 2,3-DPG levels may be an effective approach to improving the tumor response to radiotherapy. In the early 1980s it was recognized that the antilipidemic fibric acid analogs,

clofibrate, bezafibrate and gemfibrozil, decrease the affinity of hemoglobin for oxygen in vitro [1, 19, 39]. Hirst et al. [13] has shown that the administration of clofibrate to RIF-1 tumor-bearing mice is able to markedly sensitize the tumor to single-dose radiation therapy. Building on this knowledge, Abraham and colleagues have prepared and characterized a series of fibric acid derivatives that are potent allosteric effectors of oxygen binding to hemoglobin and that pass freely in and out of red blood cells [2, 3, 16, 37]. Thus, the allosteric effectors belong to a broad class of molecules with the known biologic activity of lowering serum cholesterol, triglycerides and low-density lipoproteins.

The allosteric effector molecules were effective radiation sensitizers of hypoxic EMT-6 cells in culture, effective inhibitors of sublethal radiation damage in normally oxygenated EMT-6 cells in culture and in some cases highly effective chemosensitizers of EMT-6 cells in culture exposed to cytotoxic anticancer drugs. Clearly, these effects must be a result of a biological action of these molecules that is not dependent on hemoglobin binding. The radiation sensitization of hypoxic cells in culture by these molecules was greatest at the highest radiation doses which may indicate that this 'direct' sensitization effect may not be as important in vivo as the enhanced oxygen delivery occurring from the allosteric effect of these molecules on hemoglobin. The allosteric effectors do not have the nitro-aromatic structural moiety found in the classical radiation sensitizers [27, 32]. The mechanism of antilipidemic action of clofibrate has not been definitively established; however, clofibrate circulates mainly as a protein-bound molecule and has been found to block cholesterol biosynthesis at a step prior to mevalonate formation. The other major class of hypolipidemic agents represented by lovastatin, simvastatin and pravastatin are believed to act through inhibition of the enzyme hydroxymethyl-glutaryl-CoA (HMG-CoA) reductase [4, 14, 35, 36]. The structures of HMG-CoA reductases from several organisms have been elucidated [6, 7, 17, 38].

The formation of mevalonic acid is an important control point in the biosynthesis of cholesterol. Mevalonic acid is formed by the condensation of three molecules of acetyl-CoA. Acetyl-CoA and acetoacetyl-CoA are joined by the enzyme hydroxymethylglutaryl-CoA synthase to form β-hydroxy-β-methylglutaryl CoA, which is then reduced by the enzyme β-hydroxy-β-methylglutaryl-CoA reductase to form mevalonic acid. While the most widely recognized role of this pathway is in the biosynthesis of cholesterol, isoprenyl units formed from mevalonic acid such as geranyl-PP, farnesyl-PP and geranylgeranyl-PP are critical to signal transduction through the RAS pathway [25]. Inhibition of this signal transduction pathway could slow or prevent DNA damage repair.

Another possible enzymatic target of the allosteric effectors may be microsomal cytochrome b_5 and the cytochrome b_5-oxygenase/reductase system. These enzymes, like hemoglobin, bind molecular oxygen in their normal function. This pathway utilizes the reduction of molecular oxygen to convert saturated fatty acids to unsaturated fatty acids. Of the essential fatty acids produced by this pathway arachidonic acid is the most abundant and is a precursor to the prostaglandins, thromboxanes, leukotrienes and prostacyclin which are important signal-transducing molecules. Many hemoglobins and hemoglobin-like molecules have been characterized in non-erythrocyte cell types [5, 10]. The cytochrome b_5 pathway has been implicated in drug metabolism in cells that do not express cytochrome P-450.

It has been recognized for several years that anticoagulants, antilipidemic agents and related molecules can alter tumor growth and especially tumor spread or metastasis. The allosteric effectors are members of the fibric acid class of molecules which is known to have hypolipidemic activity by increasing triglyceride-rich lipoprotein catabolism through increased lipoprotein lipase activity [8]. Early theories regarding the antimetastatic actions of these molecules centered on the survival of tumor cells in the circulating blood and the ability of these tumor cells to extravasate from circulation to implant in distant tissues [11, 12, 18]. Recently, it has been shown that treatment of mice with 50 mg/kg lovastatin, a naturally occurring hypolipidemic agent that inhibits cholesterol biosynthesis, three times per week beginning on the same day as intravenous injection of 10^6 B16 melanoma cells decreases the number of lung colonies formed [15]. Lovastatin has no effect on subcutaneous B16 melanoma growth but in cell culture decreases the attachment, the motility and the invasion activities of B16 cells [15]. The allosteric effectors were effective inhibitors of metastasis formation in animals bearing the Lewis lung carcinoma but did not cause cells to detach from the cell culture dishes under the conditions of these radiation sensitization or chemosensitization studies.

One may speculate that the allosteric effectors have more than one biologic target. In cell culture in isolated malignant cells these molecules while not cytotoxic themselves augmented the cytotoxicity of radiation and some anticancer drugs perhaps through inhibition of DNA repair. In vivo, it has been shown that RSR-13 can increases tumor oxygenation, making the tumor more susceptible to the cytotoxic action of radiation therapy. In vivo, these molecules were not very cytotoxic toward EMT-6 tumor cells even at very high doses, but did decrease the number of lung metastases formed in animals bearing the Lewis lung carcinoma. While the biologic targets of these molecules have not been fully elucidated, their activity in multiple cancer models makes them worthy of further investigation.

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