Novel Radiation Sensitizers Targeting Tissue Hypoxia

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By Eric K. Rowinsky, MD [2]

That hypoxic tissues are more resistant to the effects of radiation than well-oxygenated tissues has been known for many decades, and repeated in vitro demonstrations have confirmed that to achieve the same degree of cytotoxicity, hypoxic cells require about three times the radiation dose that well-oxygenated cells need. Hypoxic cell sensitizers enhance the tissue response to standard radiation, generally by mimicking the effects of oxygen, which induces the formation and stabilization of toxic DNA radicals. Although many hypoxic cell sensitizers like the nitroimidazoles have been evaluated in combination with radiation, these agents have had no or only minimal therapeutic impact due to either their limited potency or their toxicity at biologically relevant concentrations. This article reviews several new modalities that either increase oxygen delivery or sensitize hypoxic tissues. These modalities, all currently in early clinical evaluations, include: (1) tirapazamine, a bioreductive agent; (2) gadolinium texaphyrin, a hypoxic cell sensitizer with biolocalization properties using magnetic resonance imaging; (3) RSR13, an allosteric modifier of hemoglobin; and (4) bovine hemoglobin modified by the attachment of polyethylene glycol polymers.[ONCOLOGY 13(Suppl 5):61-70,1999]

Introduction

Classic experiments performed in the early part of this century first established that the absence of oxygen diminished the lethal effects of radiation therapy. In general, under anaerobic conditions, the radiation dose must be increased by a factor of 2.5 to 3 to achieve the same degree of cytotoxicity that occurs under oxygenated conditions. The radiosensitivity of cells increases as the partial pressure of oxygen increases from 0 to 20 to 40 mmHg. Cells at oxygen tensions of 20 to 40 mmHg demonstrate radiosensitivities that are nearly equivalent to those of cells exposed to 100% oxygen. Therefore, increasing oxygen pressure beyond this minimal level is not necessarily beneficial.[1]

Hypoxic conditions are present in tumors and, based on experimental studies, hypoxia appears to be a major cause of treatment failure with radiation therapy and chemotherapy. In animal models, 10% to 20% of tumor cells are generally found to be hypoxic.[2,3] Direct oxygen measurements in human tumors have confirmed tumor hypoxia in glioblastoma multiforme and in carcinomas of the breast, uterine cervix, and head and neck.[2,3] Potential mechanisms of chronic or transient hypoxia include obstruction of blood flow, inadequate or defective (malignant) angiogenesis, and failure of cellular growth control, allowing the cell population to outstrip the capacity of the capillary blood supply. In general, tumor cells are oxygenated up to a distance of about 150 mm from capillaries; beyond this distance, tumor cells become oxygen-depleted and either die or survive in a hypoxic state.[4-8] Since hypoxic cells are substantially more resistant to radiation than are oxygenated cells, even a small hypoxic fraction in a tumor will dominate the overall response to radiation by increasing the probability that some viable tumor cells will survive the treatment. Conversely, few hypoxic cells exist in normal tissues. Therefore, therapies that increase the delivery of oxygen to hypoxic cells are not expected to increase the toxicity of radiation to normal tissues.

Several clinical studies have demonstrated that tumors with low median partial pressures of oxygen have a higher in-field failure rate after radiation therapy. For example, compared with well-oxygenated tumors of similar size and stage, tumors of the uterine cervix have been found to have a higher rate of recurrence if the median partial pressure of oxygen in tissue is < 10 mmHg.[9] A similar phenomenon has been noted in patients with head and neck cancer.[10]

In glioblastoma multiforme, a significant fraction of hypoxic cells may partially account for the poor clinical results with radiation therapy. In vitro, glioblastoma cells are as radiosensitive as cells from tumors that radiation can cure, and yet local recurrence of glioblastoma is universal. Therefore, the clinical radioreistance of glioblastoma in vivo has been postulated to result from factors in the tumor microenvironment, particularly hypoxia.[11] In addition, experiments with rat 9L gliosarcoma have demonstrated that tumor oxygen delivery is directly associated with the efficacy of fractionated
radiation.[12]
Intraoperative oxygen measurements in patients with glioblastoma have confirmed significant tumor hypoxia. The median partial pressure of oxygen in the tumor was 7.4 mmHg; 25% of all recorded measurements were < 2.5 mmHg.[13] Although reoxygenation of hypoxic areas can occur during fractionated radiation therapy, this process does not eliminate the problem of hypoxia.[1,14,15] Measures to increase tumor oxygenation are thus worthy of evaluation, particularly in patients with glioblastoma multiforme.

Several therapeutic modalities intended to reduce tumor hypoxia in humans have been evaluated in preclinical and clinical trials, and the results of these investigations suggest that reducing the hypoxia fraction does improve the efficacy of radiation therapy. Hyperbaric oxygen in conjunction with radiation therapy resulted in greater local tumor control and longer survival compared with conventional therapy for squamous cell carcinoma of the head and neck and of the uterine cervix.[16-19] Treatment with a fluorocarbon emulsion and breathing carbogen (95% oxygen/5% carbon dioxide) during radiation therapy was associated with an enhanced response rate to radiation in patients with advanced squamous cell carcinoma of the head and neck.[20] In addition, a study of patients with high-grade brain tumors who received the fluorocarbon emulsion and breathed 100% oxygen during radiation therapy yielded an encouraging proportion of long-term survivors.[21] Interestingly, these clinical studies have not demonstrated a significant increase in the normal tissue complications of radiation, although high rates of acute mucosal and skin reactions were noted. Each of these measures has suffered from limitations that have hampered its use in larger clinical trials where significant numbers of patients could be enrolled, however. For example, hyperbaric oxygen chambers are too expensive and cumbersome to use with radiation therapy, and effective doses of hyperbaric oxygen are toxic.

Radiation sensitizers mimic the effects of oxygen to increase radiation damage. The most common class of radiation sensitizer that has been evaluated in clinical studies is the nitroimidazoles (eg, misonidazole). However, their major limitation is neurotoxicity, which has prevented the delivery of effective doses with conventional daily fractionated radiation. One randomized trial suggested improved survival when the radiosensitizer nimorazole was used to treat head and neck cancer,[22] but thus far, radiation sensitizers have not led to consistent improvements in the therapeutic index compared with optimal fractionation schedules of radiation used alone.[23]

This report will consider four novel and highly specific therapeutics designed to increase oxygen delivery or sensitize hypoxic tissues during radiation therapy. These modalities, which are all in early clinical investigations, include: (1) tirapazamine, which is a hypoxia-selective cytotoxin that maintains its differential toxicity relative to aerobic cells at higher oxygen concentrations than do other bioreductive agents; (2) gadolinium texaphyrin, an easy to reduce metallotexaphyrin that is readily capable of capturing hydrated electrons and thus increasing the concentration of hydroxyl radicals available after exposure to a given dose of ionizing radiation; (3) RSR13, an allosteric modifier of human hemoglobin that facilitates oxygen unloading at low partial pressures of oxygen; and (4) bovine hemoglobin modified with polyethylene glycol (PEG) to enhance the oxygen-carrying capacity of human blood.

**Agents that Sensitize Hypoxic Tissue**

**Tirapazamine**

Tirapazamine is the first and only representative of a unique class of hypoxic-selective cytotoxins. This agent has a high selective hypoxic cytotoxicity (20-fold to 300-fold for different cell lines) and, compared with other bioreductive agents, maintains its differential toxicity relative to aerobic cells at oxygen concentrations that are approximately 10-fold higher.[24] The hypoxic cytotoxicity ratio (ratio of equitoxic doses under aerobic and hypoxic conditions) of tirapazamine is 1 to 2 orders of magnitude greater than nitroimidazoles, mitomycin C (Mutamycin), or porfiromycin. This could be an important reason for its excellent activity in preclinical models with both radiation and some anticancer drugs, particularly since cells at intermediate oxygen levels may be more important than extremely hypoxic cells in governing tumor response to fractionated radiation.[25]

Figure 1 illustrates the mechanism of the selective toxicity of tirapazamine. The toxic species has been identified as the radical formed by the one-electron reduction of tirapazamine. The radical induces DNA double-strand breaks, presumed to be the principal mechanism of cell killing under hypoxic conditions.[25,26] Recently, the principal activating enzymes that result in DNA damage have been demonstrated to be located in the cell nucleus, probably associated with the nuclear matrix.[26-29] Under aerobic conditions, oxygen can remove the additional electron from the
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tirapazamine radical, thereby oxidizing it back to the nontoxic parent with the concomitant production of superoxide radical.[28]
The toxic species of tirapazamine is thought to be the one-electron reduction product, an oxidizing radical anion that causes extensive single-strand and double-strand breaks in DNA. Mouse liver microsome studies have identified cytochrome P450 as the most likely mediator of free radical production under hypoxic conditions. The action of tirapazamine is distinct from the action of other radiosensitizers like the nitroimidazoles. Unlike these agents, tirapazamine does not form macromolecular adducts. In addition, to be effective nitroimidazoles must be present under hypoxic conditions when radiation is given, whereas tirapazamine is intrinsically cytotoxic to hypoxic cells and may accentuate radiation damage by inhibiting DNA repair. In essence, in addition to preferential killing of hypoxic cells both in vitro and in vivo, tirapazamine radiosensitizes aerobic cells in vitro if the cells are exposed to the drug under hypoxic conditions, either before or after radiation. The combined effects of tirapazamine and radiation have been studied in a large number of tumor models using various dose schedules in many animal species. In studies in which F5Asc murine fibrosarcoma was treated with tirapazamine and single high doses of radiation (10, 20, and 30 Gy), the slope of the radiation dose-dependent curve of delayed tumor growth was not altered, but a significant additive increase in tumor growth delay was noted.[30] In other studies of the effects of tirapazamine and varying schedules of fractionated radiation in SCCVII tumors in mice, tirapazamine in a fractionated radiation treatment regimen similar to that used in human radiotherapy (8 × 2.5 Gy over 4 days) greatly enhanced radiation response when administered either before or after radiation, with many long-term survivors.[30,31]
The hypothesis that tirapazamine is selective killing of hypoxic cells might exploit the fluctuating hypoxia in tumors was further evaluated in different transplantable mouse tumors by using a multidose regimen of tirapazamine with multiple small radiation fractions.[31-33] The tirapazamine-induced enhanced cell killing was greater than that produced by a large dose of the hypoxic cell-sensitizer etanidazole given before each radiation treatment, thereby demonstrating the superiority of using a hypoxic cytotoxic agent rather than a radiosensitizer in fractionated radiation protocols. In addition, tirapazamine has been demonstrated to be effective at sensitizing tumors to fractionated radiation with no increase of sensitivity to normal tissues.[32,33]

Tirapazamine is also effective in increasing tumor cell killing without increasing the toxicity of a number of commonly used anticancer drugs, particularly cisplatin (Platinol) and carboplatin (Paraplatin).[34,35] This potentiation can be quite large, up to the equivalent of exposing the tumor to five times more cisplatin but without the increased systemic toxicity this massive cisplatin dose would entail.

Based on the results of preclinical studies, phase I and II studies evaluated the feasibility and efficacy of tirapazamine with radiation and with chemotherapy.[36-41] The principal toxicities of tirapazamine include muscle cramping that is ameliorated with quinine, as well as ototoxicity, nausea, vomiting, and fatigue. The half-life of tirapazamine is approximately 50 minutes. The majority of phase I and early phase II studies of tirapazamine combined with radiation have evaluated drug administration thrice weekly for 12 doses with daily radiation.[36-38] Feasibility and encouraging activity have been demonstrated in phase I and II trials in patients undergoing radiation therapy for brain tumors, head and neck cancer, or melanoma, and trials of radiation with concurrent cisplatin and tirapazamine are planned.[36-38]

In patients with advanced non-small-cell lung cancer, the combination of tirapazamine and cisplatin, which as a single agent has an objective response rate of only 10%, produced responses in approximately 30% of patients. [39,40] In addition, the preliminary results of a randomized trial of cisplatin vs cisplatin plus tirapazamine in patients with advanced non-small-cell lung cancer indicate that the combination regimen is superior, and another prospective study of cisplatin plus etoposide (VePesid) vs cisplatin plus etoposide and tirapazamine is ongoing.[41] In vitro studies of tirapazamine combined with oxaliplatin and paclitaxel (Taxol) or carboplatin and paclitaxel have also demonstrated substantial reductions in well-established human lung cancer xenografts.[42,43]

Gadolinium (III) Texaphyrin
Efforts to develop gadolinium texaphyrin (Gd[III]-tex; NSC659238; Pharmacocycles Inc.) emanated from the ineffectiveness of previous radiosensitizers to adequately sensitize the entire cell population of tumors. In addition, most of these agents lack the preferential localization in tumors required to increase the therapeutic index, although radiation therapy itself can be localized to some extent. Halogenated pyrimidines, which depend on incorporation into replicating DNA, also have limited efficacy since many tumors contain low fractions of cells in the S phase of the cell cycle. The impetus to develop Gd(III)-tex was based on the expectation that effective radiation sensitizers
should (1) potentiate the activity of the administered radiation in the tumor but not in the surrounding tissues; (2) operate via a mechanism that is active against oxygenated and hypoxic cells and is independent of DNA incorporation; and (3) have minimal inherent toxicity.

Texaphyrins are large planar porphyrin-like macrocycles that are capable of coordinating a range of large cations, including gadolinium (III) (Gd(III)) and other members of the trivalent lanthanide series.[44,45] In general, the complexes formed are stable, with a 1:1 metal to ligand stoichiometry.[45] In addition, the complexes are also easily reduced. The porphyrin-like complex has a high electron affinity and forms a long-lived and highly reactive p-radical cation upon exposure to hydrated electrons, reducing ketyl radicals, or superoxide ions.[46] This facile reduction process, coupled with a potential to localize selectively to certain animal tumor models,[47] led to a consideration that these species could function as radiation sensitizers.[48] The hypothesis was that, like molecular oxygen, the easy to reduce metalloc texaphyrins would be able to capture hydrated electrons and thus increase the concentration of hydroxyl radicals available after exposure to a given dose of ionizing radiation.

Gd(III)-tex complexes were demonstrated to readily accomplish these objectives.[46] Gd(III)-tex, which is a pentadenylate aromatic metalloporphyrin, represents a new class of compounds known as the texaphyrins (Figure 2). Magnetic resonance imaging (MRI) was recognized to be able to detect certain paramagnetic texaphyrin complexes, such as Gd(III)-tex, and it was thought that this visualization would provide a means to determine directly the biolocalization properties (both temporal and spatial) of this series of this new class of putative radiation sensitizers. It was believed that MRI monitoring of the selective accumulation of Gd(III)-tex in neoplasms would enable treatment planning and subsequent monitoring of the response of cancers to radiation therapy.

Like many other naturally occurring porphyrins, Gd(III)-tex has selective biolocalization in tumors and the potential to form long-lived radicals by accepting solvated electrons generated by ionizing radiation in oxic or anoxic conditions.[46] Preclinical studies have demonstrated that under pulse radiolysis conditions, Gd(III)-tex undergoes a one-electron reduction to form a relatively stable radical anion.[46] This radical anion undergoes protonation relatively slowly in neutral solution, with a half-life of several hundred microseconds, which translates into the formation of a long-lived reactive ion radical species. The binding of the solvated electron also allows the hydroxyl radical to survive longer. In addition, the formation of this reduced texaphyrin radical is not dependent on oxygen.

In vitro experiments using several cell lines have demonstrated that Gd(III)-tex causes DNA-strand scission during radiolysis. In these experiments, concentrations of Gd(III)-tex from 0.1 to 100 μM increased double-stranded DNA strand breakage in a dose-dependent manner. Additional experiments in cell-free systems have demonstrated that the reduced Gd(III)-tex radical can covalently modify cytosine bonds. In vivo studies have also demonstrated dose-dependent radiation sensitization of human cancer cell lines.[46] The sensitization enhancement ratio (SER) has been studied in the mouse L1210 leukemia line, demonstrating SERs up to 3.2.

In subsequent studies, Gd(III)-tex (Figure 2, compound 1) was found to be an effective radiation sensitizer for human HT29 colon cancer cells, with a SER of 1.92, which led to further in vivo studies. In addition, the results of these studies were independent of the specific complex employed (Figure 2, compounds 1 and 2), suggesting that the sensitization effect of Gd(III)-tex derives from the basic macrocyclic structure and properties (eg, ease of reduction) rather than a judicious choice of exocyclic substituents.

In general, in vivo studies with Gd(III)-tex have demonstrated dose-dependent radiation sensitization, which resulted in improved survival of tumor-bearing animals.[46] Gd(III)-tex accumulates in tumor tissue with a selective sparing of normal surrounding tissue. Animals studies using 56Gd or 14C radiolabeled Gd(III)-tex injected into tumor-bearing animals demonstrated rapid clearance of the drug from blood and normal tissues, with delayed clearance from tumors, resulting in up to eightfold higher concentrations in tumors compared with surrounding tissues. Because Gd(III)-tex contains the paramagnetic metal ion Gd(III), its selectivity has been demonstrated by MRI of tumor-bearing animals, which shows enhancement of tumors but not normal surrounding tissues. This selective enhancement persists for up to 24 hours after a single administration.[47] Since disposition of the agent is also by both hepatic and renal means, liver and kidney enhancement has been observed as well.

MRI and radiation sensitization studies performed in mice bearing SMT-F and EMT6 mammary murine carcinomas have demonstrated enhancement of tumors, with maximal contrast enhancement noted immediately after injection; however, at least 30% enhancement of the tumor was observed up to 5 hours after injection.[46] Administration of Gd(III)-tex before a single fraction of radiation improved
survival significantly in SMT-F-bearing mice compared with animals receiving radiation alone. For animals receiving radiation at both 2 hours and 5 hours after administration of the agent, significant therapeutic effects on tumor size were observed; however, there were no differences in survival in the groups receiving Gd(III)-tex at 2 hours vs 5 hours before radiation. A significant radiation sensitization effect was also shown in the studies involving BALB/C mice bearing EMT-6 tumors in the right leg that were injected with Gd(III)-tex compound 1 or control solutions before 1-, 2-, or 4-Gy radiation.[46] Even after 1 Gy of radiation for 5 days, there was a significant difference between the Gd(III)-tex treatment and control groups. In addition, the results of studies evaluating radiation-induced toxicity in normal tissues (short-term erythema and long-term leg contracture) showed no enhanced radiation sensitivity of normal tissues when Gd(III)-tex complex 1 was present. In preclinical studies of Gd(III)-tex in animals, hepatotoxicity was the principal dose-limiting toxicity. Based on the results of tumor localization, MRI detection, and animal tumor radiosensitization studies, Gd(III)-tex compound 1 appeared to be a very efficient and unique radiation sensitizer.

Preliminary clinical investigations of Gd(III)-tex in combination with radiation have been performed.[49-51] In one of the earliest phase I studies, the feasibility of administering single doses of Gd(III)-tex 0.5 to 28 µmol/kg followed 2 hours later by radiation was evaluated.[47] The maximum tolerated dose was 19.4 µmol/kg, with reversible renal tubular toxicity precluding further dose escalation. Grade 3 or 4 toxicities that were probably drug related also included dyspnea, nausea, vomiting, and hyperbilirubinemia. One patient with a G6PD deficiency developed hemolytic anemia. In subsequent patient cohorts beginning at the 8.3 µg/kg dose level, antiemetics prevented nausea and vomiting. Transient greenish discoloration of skin and urine was seen at Gd(III)-tex doses ≥ 6.2 µmol/kg. Tumor selectivity of Gd(III)-tex was established using MRI, which showed selective uptake in primary and metastatic tumors but not in normal tissues. Normal tissues showed no evidence of radiation toxicity, and the treatments were well tolerated. In a phase I/II multidose trial of Gd(III)-tex in patients with brain metastases, patients received 10 daily intravenous injections of Gd(III)-tex, each followed by whole brain irradiation (total of 10 fractions, 30 Gy).[50,51] The daily dose of Gd(III)-tex was escalated in five patient cohorts from 0.25 to 8.4 mmol/kg without dose-limiting toxicity. Overall, clinical and biological tolerability was excellent. The maximum tolerated dose was reached at 6.3 mg/kg per injection, principally because of reversible grade 3 or 4 elevations in hepato cellular function tests and/or total bilirubin. One patient experienced grade 3 diarrhea. Two patients with pre-existing liver abnormalities had transient elevations in hepatic transaminases. However, there was no evidence of radiation toxicity. Tumor selectivity of Gd(III)-tex was established using MRI, which showed selective drug uptake in the metastases but not in the normal brain in nine of 10 patients. Gd(III)-tex accumulated with the 10 repeated administrations in 14 of 18 lesions. Median survival of the whole cohort was greater than 6 months.

These encouraging results have led to an ongoing phase III randomized trial of Gd(III)-tex vs placebo in patients with brain metastases undergoing whole brain radiation (10 fractions of 3 Gy administered over 10 days). In the study, patients are randomized to treatment either with Gd(III)-tex 5 mg/kg intravenously 2 to 5 hours before each of 10 whole-brain radiation treatments of 3 Gy or with the same radiation therapy alone. Primary and secondary parameters that will be assessed and compared include time to death, cause of death, response rate (determined by MRI), time to progression of brain metastases using MRI scanning at predetermined intervals, time to deterioration of neurocognitive function, quality of life, and toxicity.

**Agents Enhancing Tumor Oxygenation**

**RSR13, an Allosteric Modifier of Hemoglobin**

Allosteric modifiers of hemoglobin are molecules that alter the oxygen affinity of hemoglobin to enhance oxygen delivery to target tissues. Naturally occurring allosteric modifiers of hemoglobin include hydrogen ions (H+), carbon dioxide, organic phosphates, and 2,3-diphosphoglycerate, which is the most important allosteric modifier for human hemoglobin. These natural molecules shift the oxygen equilibrium curve of hemoglobin to modulate oxygen use in the tissues under various physiologic conditions. Each hemoglobin molecule, which is a tetrameric protein comprised of two pairs of symmetrically related α-globin and β-globin chains, is capable of binding four oxygen molecules, and the percentage of oxygen-binding sites of hemoglobin that are bound with oxygen defines the fractional oxygen saturation and the oxygen content of blood. The concentration of oxygen and the oxygen affinity of the binding site determine the percent saturation. Since oxygen is
a gas, the partial pressure of oxygen that it produces in solution describes its concentration. The partial pressure of oxygen that results in 50% saturation of hemoglobin is defined as the p50.

RSR13 (2-[4-[[3,5-dimethylanilino] carbonyl][methyl]phenoxy]-2-methylpropionic acid; Figure 3) is a small, synthetic, organic molecule that readily crosses the erythrocyte membrane. RSR13 binds to hemoglobin in the central water cavity and shifts the allosteric equilibrium between oxyhemoglobin and deoxyhemoglobin toward deoxyhemoglobin by noncovalent interactions with three subunits of the deoxyhemoglobin tetramer.[52-55] This interaction stabilizes deoxyhemoglobin by preventing narrowing of the water cavity, thereby reducing the oxygen affinity of hemoglobin. In essence, RSR13 decreases the oxygen affinity of hemoglobin and augments oxygen unloading in the microvasculature. This effect increases the oxygen diffusion gradient to the tissues. The therapeutic effect of [turbocharging] oxygen unloading from hemoglobin to tissues mimics and amplifies the physiologic tissue oxygenation. This approach has potential application in clinical situations characterized by tissue hypoxia due to reduced blood flow (regional or global), and reduced oxygen-carrying capacity and/or increased tissue oxygen demand. These therapeutic targets include cardiovascular, surgical, and critical care indications. In addition, by increasing tissue oxygenation, RSR13 may reduce tumor hypoxia and enhance the cytotoxic effects of radiation. An obvious advantage of RSR13 over other hypoxic radiation sensitizers is that it does not have to be delivered directly to tumor cells to exert its principal effects.

Since tissue oxygenation may play a role in responsiveness to some chemotherapeutic agents, particularly cytotoxic drugs that are [radiomimetic] in that their putative mechanisms of action mimic single-strand DNA breaks similar to radiation (eg, bifunctional alkylating agents, actinomycin D, anthracyclines, fluoropyrimidines, and several nucleosides), RSR13 may also enhance the cytotoxic effects of chemotherapy.

RSR13 has been evaluated in several rodent tumor models.[9,55-57] In a rat mammary adenocarcinoma 13762 model in vivo, RSR13 increased the median partial pressure of oxygen in the tumor and decreased the hypoxic fraction of tumor cells. Carbogen breathing, which was used to maximally saturate whole blood with oxygen, enhanced the effect of RSR13 at 150 mg/kg when measurements were made immediately after treatment. This dose significantly decreased the hypoxic fraction of tumor cells from 36% to 15% and increased the median partial pressure of oxygen in the tumor from 19.6 to 35.7 mmHg. When measurements were made 30 minutes after RSR13 treatment, this dose also produced the greatest effect.

In air-breathing animals, the median partial pressure of oxygen was 21.5 mmHg and there were 15% hypoxic readings. In carbogen-breathing animals, the median partial pressure of oxygen was increased to 67 mmHg and there were no detectable hypoxic readings. By 1 hour posttreatment, the tumor oxygenation returned to near pretreatment values for both air-breathing and carbogen-breathing animals. The time-response data suggest a window when tumors were most susceptible to the radioenhancing effects of RSR13. Results from this study demonstrated that the optimal dose of RSR13 in this model was approximately 150 mg/kg, and a peak reduction in tumor hypoxia occurred approximately 30 minutes after treatment.

In combination with radiation in the murine Lewis lung carcinoma model in vivo, RSR13 delays tumor growth and decreases metastatic tumor spread.[55] At doses of 100 and 200 mg/kg before each radiation dose, RSR13 treatment increased the delay in tumor growth and resulted in radiation dose-modifying factors of 1.25 and 1.63, respectively. Delaying radiation delivery until 30 minutes after RSR13 increased the tumor response to radiation at both doses of RSR13 such that the dose-modifying factors were 1.55 and 1.66, respectively. With regard to metastatic disease, administering RSR13 100 mg/kg alone decreased both the number of lung metastases and the percentage of large metastases, whereas radiation alone was ineffective. Moreover, combining RSR13 and radiation proved much more effective than RSR13 alone. RSR13 also augments the delay in tumor growth when it is used in conjunction with fractionated radiation therapy in a murine FSa-II fibrosarcoma model.[57]

The initial studies in humans involved healthy volunteers who received RSR13 as single intravenous doses of 10 to 100 mg/kg in a randomized, double-blind, placebo-controlled manner.[58] In addition to standard phase I objectives, the study sought to determine the dose of RSR13 required to increase the partial pressure of oxygen by 10 mmHg. The principal toxicity of RSR13 was pain proximal to the infusion site. There was a clear dose-response increase in p50. RSR doses of 75 and 100 mg/kg infused over 90 minutes resulted in mean reductions in arterial oxygen saturation of 6.3% ± 1.2% and 9.3% ± 1.5%, respectively.

The pharmacodynamic effect, measured both as the increase in p50 and the reduction in arterial oxygen saturation, reached a maximal response within 0 to 15 minutes postinfusion and returned to
near baseline by approximately 12 hours after an RSR dose of 100 mg/kg. The pharmacokinetic and functional pharmacodynamic half-lives were 3 to 5 hours.

RSR13 is currently being evaluated in patients undergoing radiation, in patients undergoing elective surgery involving general anesthesia, and in patients with myocardial ischemia. In the study involving patients receiving general anesthesia for elective surgery, p50 was affected in a dose-responsive manner.[58] At an RSR dose of 100 mg/kg over 60 minutes, the mean maximum increase in p50 was 11.8 mmHg.

In a phase Ib study in patients undergoing palliative radiotherapy, RSR13 was well tolerated at doses up to 100 mg/kg administered daily for up to 10 days during 2 to 3 weeks of radiation.[59] No severe drug-related toxicities occurred, and several patients with large bulky tumors had complete or partial responses. A subsequent phase Ib study is being performed in patients with newly diagnosed glioblastoma multiforme who are undergoing radiation.[60] The first and second dose levels involved RSR13 at a dose of 100 mg/kg given every other day (level 1) and every day (level 2) during cranial irradiation (60 Gy in 30 fractions). Supplemental oxygen by nasal cannula (4 L/min) has been given during RSR13 and continued until after radiotherapy.

At dose level 1, toxicity was similar to radiation alone, and accrual to dose level 2 is ongoing. The mean p50 level at the end of the infusion was significantly increased by more than 8.6 mmHg from the baseline value of 26.6 mmHg, indicating a substantially increased effect of oxygen unloading. In addition, studies of intratumoral partial pressure of oxygen in individual patients indicate that supplemental oxygen can significantly increase the intratumoral partial pressure of oxygen in patients treated with RSR13.[61] With a follow-up of 4 to 9 months in the nine patients treated at dose level 1, three patients have undergone reoperation, with pathologic effects of necrosis/radiation effect in two patients and mixed atypical glioma cells and necrosis/radiation effect in the other. A multi-institutional phase II study in previously untreated patients with glioblastoma multiforme is planned.

PEG-Hemoglobin

Since the 1930s, anemia has been known to lead to resistance to the effects of radiation in both neoplastic and normal tissues. After the hematocrit increases, oxygen delivery to tissues is a compromise between the higher oxygen-carrying capacity afforded by an increased number of red blood cells and the increased viscosity of the blood, which may lead to decreased blood flow. There may be an optimal hematocrit value for any given tumor under specific conditions of oxygen use.[62,63] For example, hematocrit values above the normal range have been demonstrated to be optimal for oxygen delivery to several murine tumors, whereas hemodilution has been shown to improve oxygen delivery in a rat tumor.[64-66] Nevertheless, the effect of an increased hematocrit is likely to be transient because adaptation to altered oxygen delivery always occurs, and while it may be feasible to exploit adaptation to anemia by transfusing to optimal values before radiation, the therapeutic benefit afforded by this approach has been demonstrated to be very short-lived for single doses of radiation, at least in mouse tumors.[64-68]

Since cancer patients are usually anemic and evidence suggests that anemia may be an important prognostic factor with regard to treatment results, pursuing studies designed to determine whether increasing hemoglobin levels into the normal range can improve the prognosis and treatment outcome for patients given radiation therapy is rational.[6] The partial pressure of oxygen and the pH are important factors in the transport of oxygen. In the lungs, where the partial pressure of oxygen is high (> 110 mmHg) and the pH is relatively high (7.60), hemoglobin tends to be maximally saturated with oxygen (> 96%), whereas the oxygen tension and pH are low in peripheral tissues (< 50 mmHg and pH 7.20), resulting in less avid binding of oxygen to hemoglobin and off-loading of oxygen to a level of about 65% saturation.

Bovine blood is a readily available source of hemoglobin, and the molecular structure of bovine hemoglobin is similar to that of human hemoglobin.[69-72] However, bovine hemoglobin has a higher p50 than does human hemoglobin (26 ± 3 mmHg vs 16 ± 3 mmHg).[69] The oxygen affinity of bovine hemoglobin is regulated by chloride ions, whereas the oxygen affinity of human hemoglobin is regulated by the presence of 2,3-diphosphoglycerate.[70,73] In addition, in contrast to human hemoglobin, bovine hemoglobin has low concentrations of organic phosphates and more pronounced Haldane (carbon dioxide) and Bohr (pH) effects.[69]

In preclinical studies, ultrapurified bovine hemoglobin has also been shown to have low antigenicity among mammals,[71,72] but the administration of unmodified bovine hemoglobin to patients as an oxygen delivery agent would undoubtedly be problematic because of the rapid breakdown of the tetrameric protein.

Derivatization with PEG has been found to be a highly biocompatible method to improve the
therapeutic benefit of exogenously administered proteins. Coupling with PEG increases the circulating half-life, decreases the immunogenicity and antigenicity, and increases the solubility of proteins.[74-79] The number and molecular weight of PEG molecules that are bound, as well as the coupling moiety, can be varied to suit the specific protein. PEG-adenosine deaminase (Adagen) and PEG-L-asparaginase (Oncofex) are currently in clinical use, thus confirming the value of this approach. PEG-hemoglobin (PEG-Hb; Figure 4) was prepared by coupling PEG (molecular weight, 5,000 daltons) to a succinimidyl carbonate linker to the ε-amino group of lysine, resulting in a product with a molecular weight range of 120,000 to 130,000 daltons.[80] PEG-Hb is prepared from bovine blood collected aseptically from a controlled herd of cattle. The hemoglobin is isolated, modified with PEG, and subjected to ion-exchange chromatographic purification. PEG-Hb is effectively a conjugate of stroma-free bovine hemoglobin and multiple units of monomethoxypolyethylene glycol (m-PEG, molecular weight, 5,000 daltons). The p50 of PEG-Hb is 14 mmHg when measured under standard hemoxanalyzer conditions. The half-life of PEG-Hb ranges from 15 to 17 hours in rats and 49 to 53 hours in dogs.[80]

In several animal species, PEG-Hb has been demonstrated to substantially increase tissue oxygenation and radiosensitize tumors.[80-82] In a variety of subcutaneous tumor models, including the hypoxic rat osteogenic sarcoma URM-106, murine Lewis lung carcinoma, and rat gliosarcoma 9L, with tumor surface oxygen tension measured by several methods that use phosphorescence quenching, a time-dependent increase in oxygen tension in all tumors was noted.[82] The maximum tissue oxygen tensions were observed 2 hours after treatment with PEG-Hb, and the effect was sustained for at least 2 hours. Although the study was terminated at that point, the increased oxygen tension is thought likely to follow the circulatory half-life of PEG-Hb. Following a single 4-Gy dose of radiation, osteogenic sarcoma tumors in the PEG-Hb–treated group regressed dramatically, with complete regressions occurring in 100% of high-dose PEG-Hb–treated rats compared with animals treated with control solutions. In the same series of studies, all PEG-Hb–plus radiation-treated nude rats bearing human prostate carcinoma PC-3 showed significant delays in tumor growth compared with animals treated with radiation alone.[82]

In other studies involving rats bearing 13762 mammary carcinoma using a partial pressure of oxygen histogram, administration of PEG-Hb decreased the percent of the partial pressure of oxygen readings ≤ 5 mmHg in animals breathing 28% oxygen or carbogen, respectively.[81] A single administration of PEG-Hb was able to increase tumor oxygenation for at least 3 days; under these conditions, PEG-Hb administration along with fractionated radiation (2, 3, or 4 Gy × 5) resulted in radiation dose-modifying factors of 1.2, 1.4, and 1.5, respectively.[81] With the EMT-6 mouse mammary carcinoma cell survival assay, administration of PEG-Hb along with fractionated radiation (2, 3, or 4 Gy × 5) resulted in dose-modifying factors of 1.2, 1.5, and 1.7, respectively, when the animals breathed 28% oxygen or carbogen before and during the delivery of each radiation fraction. In EMT-6 tumor growth delay, administration of PEG-Hb (6 mL/kg) along with fractionated radiation therapy (2, 3, or 4 Gy × 5) resulted in dose-modifying factors of 1.3 ± 0.2, 1.5 ± 0.2, and 1.7 ± 0.2 when the animals breathed air, 28% oxygen, or carbogen, respectively, before and during the delivery of radiation. Administration of PEG-Hb alone on the first treatment day of a 5-day fractionated radiation therapy regimen resulted in dose-modifying factors of 1.3 ± 0.2 and 1.4 ± 0.2 if the animals breathed 28% oxygen or carbogen, respectively, before and during each radiation fraction delivery.

These impressive results have been confirmed in other studies employing PEG-Hb in dogs with naturally occurring canine nasal tumors.[83] In this study, six of eight animals receiving multiple PEG-Hb doses and radiation exhibited striking reductions in tumor size. Although none of these studies address precisely how PEG-Hb sensitizes hypoxic tumors, elevated oxygen tension is believed to play a pivotal role. However, in the cascade of the tumoricidal events that occur following radiation, there could be some additional factors acting as catalysts. In particular, the iron within the heme may act as a catalyst for a cytotoxic mechanism that involves oxygen and radiation.[84] The ability of PEG-Hb to increase tumor oxygen content and improve the effectiveness of radiotherapy served as the rationale for a phase Ib clinical study of PEG-Hb in patients with solid malignancies who were undergoing fractionated radiation treatment. A prior phase Ia study in 34 normal volunteers demonstrated that single doses of PEG-Hb up to 8.33 mL/kg were well tolerated except for gastrointestinal spasm (principally esophageal spasm). This effect is presumed to be secondary to the nitrous oxide-scavenging properties of PEG-Hb, resulting in spasm that was effectively prevented and well managed with an atropine-like antispasmodic agent (levesinex). To date, patients have been treated with PEG-Hb administered weekly for 3 weeks at doses of 2 mg/kg, 4 mg/kg, and 6 mg/kg, with 100% oxygen breathing for 1 hour before and during radiation (15
2.5-Gy fractions over 3 weeks). The predominant toxicities have been transient, mild systolic hypertension and esophageal spasm that has been noted infrequently following institution of the antispasmodic premedication. Pharmacologic studies in human subjects have shown that the half-life of PEG-Hb is approximately 43 hours. Disposition is principally via the liver and reticuloendothelial organs, and about 1% of PEG-Hb is excreted in the urine. In contrast to non-pegylated hemoglobin preparations, no significant renal toxicity has been noted in either preclinical or clinical studies. In addition, the induction of antibodies to bovine hemoglobin has been negligible and, although clotting times have been shortened, no clotting or bleeding has occurred. Phase II studies in patients with high-grade astrocytomas undergoing primary radiation are planned.

**Conclusion**

Although tissue hypoxia has been recognized as a major reason for the ineffectiveness of radiation in some tumors for some time, the majority of maneuvers to increase the oxygen content of blood and early oxygen-mimetic radiosensitizers have been associated with small, albeit real, clinical efficacy in some situations. Nevertheless, early hypoxic cell radiosensitizers have been associated with insurmountable technical difficulties, pharmacologic properties resulting in ineffective perfusion of deep hypoxic tissues, and/or intolerable toxicity. Over the last several years, there has been renewed interest in hypoxic cell radiosensitizers, and a diverse range of these rational agents, directed at a variety of mechanisms to improve tissue oxygenation or radiosensitization, is being evaluated in preliminary clinical studies. The evaluation of these modalities in prospective, randomized, well-controlled clinical trials is the most important challenge awaiting clinical investigators.

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