Targeting Hypoxia in Head and Neck Cancer

Lester J. Peters

From the Peter MacCallum Cancer Institute, Melbourne, Australia

Correspondence to: L. J. Peters, Peter MacCallum Cancer Institute, Locked Bag 1, A'Becket Street, VIC 8006 Melbourne, Australia. E-mail: lpeters@petermac.unimelb.edu.au

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The idea of 'targeting' hypoxia stems from recognition of the fact that oxygen (or its lack) is central to the practice of radiation oncology. Targeting embraces the alternative goals of trying to overcome hypoxia on the one hand and trying to exploit it on the other. This presentation briefly reviews these two approaches with major emphasis on the latter.

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As early as 1907, the observation was made that interference with the blood supply of an irradiated part reduced radiation skin reactions. However, it was not until the late 1940s that the presence of molecular oxygen at the time of radiation, rather than the metabolic state of irradiated cells, was shown to determine their radiosensitivity (1). The mechanism by which oxygen exerts its radiosensitizing effect can be thought of in terms of its interaction with free radicals produced by radiation to 'fix' (make permanent) the damage. To exert its radiosensitizing effect, oxygen has to be present at the time of radiation because of the very short lifetime of free radicals. The oxygen effect is biologically ubiquitous and all cells, normal or malignant, are rendered relatively radioresistant by hypoxia.

Throughout her career at the Gray Laboratory, Julie Denekamp was associated with research related to hypoxia. It was she who popularized the concept of the disordered vasculature of tumours as being a 'two-edged sword'—at once the cause of radiobiological hypoxia and a target for anti-tumour therapy (2, 3).

STRATEGIES TO OVERCOME HYPOXIA

That the radiocurability of human tumours might be limited by hypoxia was first proposed by Thomlinson and Gray in 1955 (4) on the basis of pathological observations of the patterns of necrosis in tumours in relation to identifiable blood vessels. They reasoned that at the interface between viable tumour cells and necrosis there must be radioresistant tumour cells by virtue of the limited diffusion range of oxygen from the tumour capillaries. This mechanism is well established as the cause of 'chronic' hypoxia in tumours. However, it is now recognized that transient but profound 'acute' hypoxia is also experienced in tumours by virtue of the intermittent shutdown of blood flow through the disordered vasculature (5, 6).

Although the evidence that hypoxic cells might well be responsible for failure to achieve tumour control with radiotherapy and was at first circumstantial, it has now been verified in a number of studies using direct measurements of intra-tumoural oxygen concentration (7, 8). Furthermore, evidence has been adduced to show that hypoxia in tumours is associated not only with radioresistance but also with progression towards a more malignant phenotype (9).

Since the 1950s, an enormous effort has been expended in basic, pre-clinical and clinical research to develop strategies to overcome the radioresistance of hypoxic cells. These include:

- breathing hyperbaric oxygen to increase the partial pressure of oxygen in the blood
- use of erythropoietin (EPO) or artificial blood products such as perfluorocarbons to improve the oxygen carrying capacity of blood
- use of drugs such as RSR 13 to reduce the binding of oxygen to hemoglobin
- use of vasodilators and carbogen (ARCON)
- use of electron affinic drugs to substitute radiochemically at the molecular level for oxygen
- treatment with high LET radiation which is less dependent on oxygen for its cytotoxicity.

Although none of these strategies has achieved general acceptance, a 1996 meta-analysis of all the trials performed

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using hypoxic cell sensitizers or hyperbaric oxygen by Overgaard and Horsman (10) showed a small but statistically highly significant benefit in terms of both local control and survival. Preliminary results from some of the newer strategies, such as ARCON and EPO, are promising and need to be further tested.

STRATEGIES TO EXPLOIT HYPOXIA

An important conceptual shift in the strategy for dealing with hypoxic cells came with the idea of selectively *killing* hypoxic cells rather than sensitizing them. Several strategies have been developed to achieve this goal:

Mitomycin C and its analogues

Differential hypoxic cell cytotoxicity was first suggested as a rationale for the use of combined chemoradiotherapy with mitomycin C because this drug requires metabolic reduction of its benzoquinone ring to produce the cytotoxic agent. Subsequent small randomized clinical trials have suggested that the addition of mitomycin C to radiotherapy does have a therapeutic benefit. However, a large International Atomic Energy Agency (IAEA) sponsored trial failed to show a benefit from giving a single dose of 15 mg/m^2 at the end of the first week of radiotherapy (11).

Hyperthermia

An approach not aimed specifically at hypoxia but more towards the low intra-tumoural pH associated with hypoxia is the use of hyperthermia. This is because cells at low pH are more vulnerable to killing by heat than are cells at physiologic pH. Although there is no question that heating to temperatures of $\geq 42°C$ enhances the effects of radiation, most clinical trials of hyperthermia have been compromised by poor quality control and uncertain achievement of therapeutic temperatures. In the head and neck region, hyperthermia has been abandoned by most centres as an adjunct to radiotherapy, largely because of the practical problems associated with heating in the presence of bone, air cavities and large blood vessels.

Anaerobic bacterial or immunologic vectors

It was originally proposed that Clostridium species, as obligate anaerobes, could be used to target tumour hypoxia and selectively deliver cytotoxic agents. Subsequently, it has been shown that attenuated strains of Salmonella typhimurium selectively replicate in transplanted solid tumours even though the bacterium is not an obligate anaerobe. Both vectors have been shown in experimental systems to produce antitumoural effect when 'armed' with a prodrug-activating enzyme (12). Another indirect approach that exploited the presence of necrotic material in tumours was the development of radioactive-labelled antibodies against the products of tumour necrosis. Although this approach was shown to be effective in experimental systems, it has not been tested clinically.

Bioreductive drugs

The most successful strategy yet devised for exploiting tumour hypoxia is through the bioreductive release of diffusible cytotoxins. Several pro-drugs have been developed which become cytotoxic under hypoxic conditions. The best known agent in this class is tirapazamine, a drug discovered in the mid-1980s as a byproduct of research aimed at rationally synthesizing an improved radiosensitizer (13). It was found that tirapazamine (or SR4233 as it was then called) killed hypoxic cells at much lower concentrations than those needed to kill well-oxygenated cells, with a HCR (hypoxic cytotoxicity ratio) in the range of 50–300 for various different cell lines.

The mechanism for selective hypoxic cytotoxicity by tirapazamine is its reduction under hypoxic conditions to produce a highly reactive free radical, which in turn produces both single- and double-strand DNA breaks. Under aerobic conditions, the tirapazamine radical is back-oxidated to its non-toxic parent, with the production of a superoxide radical that is degraded by the naturally occurring enzyme superoxide dismutase. The differential hypoxic cytotoxicity of tirapazamine results from the fact that the tirapazamine radical is much more cytotoxic than the superoxide radical.

Of significant interest is that tirapazamine has also been shown to potentiate the cytotoxicity of cisplatin in hypoxic cells (14). Potentiation depends on the tirapazamine exposure being at oxygen concentrations below about 1%. It appears to be mediated via inhibition of repair of cisplatin-induced DNA interstrand cross links. Because the level of hypoxia required for this interaction is found only in tumours, a differential therapeutic effect is therefore possible.

CLINICAL STUDIES WITH TIRAPAZAMINE

After it was demonstrated in several in vivo preclinical tumour models that tirapazamine could enhance the therapeutic effect of both radiation and cisplatin, a number of clinical studies have been undertaken at the Phase I and II level using both combinations. In addition, one Phase III trial of tirapazamine plus cisplatin has been completed in patients with metastatic non-small cell lung cancer. These trials established maximum tolerated doses (MTD) of tirapazamine for 12 doses given concurrently with radiotherapy to be approximately 160 mg/m^2. When given with cisplatin, 75 mg/m^2 every 3 weeks, the MTD of tirapazamine was approximately 390 mg/m^2.

Although the therapeutic results observed in a Phase II trial of tirapazamine plus radiation in head and neck cancer were encouraging (15), it was the advent of clinical testing using the triple combination of tirapazamine, cisplatin and radiation that has led to the most impressive early results.

THE PETER MACCALLUM HEAD AND NECK EXPERIENCE

The first clinical trial of the triple combination of concurrent tirapazamine, cisplatin and radiotherapy was undertaken in patients with advanced head and neck cancer. Between January 1997 and March 1998, we conducted a Phase I trial in 16 patients with very advanced disease (all stage IV, 10 with T4 and/or N3 disease) (16). Patients were treated according to the following protocol: tirapazamine 290 mg/m^2 was given 1 h prior to cisplatin 75 mg/m^2 followed immediately by radiotherapy in the 1st, 4th and 7th weeks of a conventionally fractionated regimen (70 Gy in 35 Fx); tirapazamine alone was given at a dose of 160 mg/m^2 immediately before radiotherapy three times a week initially in weeks 2, 3, 5 and 6, but for the last 10 patients in weeks 2 and 3 only (Fig. 1). The dose-limiting toxicity of the triple combination was unexpectedly febrile neutropenia, which was overcome by omitting the tirapazamine doses in weeks 5 and 6. Radiation reactions were enhanced to the extent consistent with concurrent chemotherapy using the more standard cisplatin 5FU combinations and were generally manageable.

The tumour responses achieved were remarkably good, with an overall complete response rate of 81%. With a median potential follow-up time of 32 months (minimum 24 months), only 2 patients have failed in the local or region site, yielding an actuarial 3-year local regional progression-free rate of 88%. Actuarial 3-year survival was 67% (Fig. 2). As a corollary of this study, PET scanning was performed to track changes in hypoxia and tumour metabolic activity during and after therapy. Fourteen out of 15 patients studied had demonstrable hypoxia on their initial PET scans with F18 misonidazole, 13 of whom showed substantial reduction or elimination of imagable hypoxia during treatment. The patient who failed to resolve his hypoxia had local disease persistence.

In an almost identical Phase I trial conducted in Calgary by Craighead et al. (17), similarly impressive results were obtained in patients with advanced cervical cancer.

As a sequel to the Peter MacCallum trial, a randomized phase II study was initiated under the auspices of the Trans-Tasman Radiation Oncology Group (TROG), in which the triple combination of tirapazamine, cisplatin and radiotherapy was evaluated vis-à-vis chemoradiotherapy using concurrent cisplatin and 5FU as a chemo-boost during the last 2 weeks of radiotherapy. This trial was originally designed to select one of the two regimens as the experimental arm for a future Phase III trial against radiotherapy alone as the control. However, treatment with concurrent chemoradiotherapy with cisplatin and 5FU has now evolved into a standard of care (18) and the decision was made to continue the randomized study as a mini Phase III to accrue a total of 120 patients. Based on an interim analysis of the first 63 patients entered onto this trial through January 2000, it has been decided by the IND holder of tirapazamine Sanofi-Synthelabo to proceed with a definitive international Phase III registration trial.

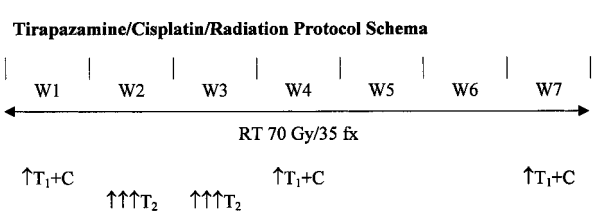

Fig. 1. Schema depicting the schedule for administration of tirapazamine, cisplatin and radiotherapy derived from the Peter MacCallum Cancer Institute Phase I trial. T_1 = tirapazamine, 290 mg/m^2; T_2 = tirapazamine, 160 mg/m^2; C = cisplatin, 75 mg/m^2.

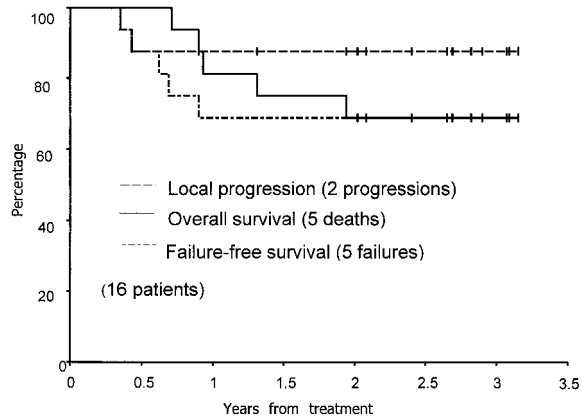

Fig. 2. Actuarial plots of local regional control, disease-free survival and overall survival from the Phase I study.

MAKING TUMOURS MORE HYPOXIC?

As Julie Denekamp recognized, a somewhat counter-intuitive conclusion emerges from the strategy of exploiting hypoxia in that the therapeutic index of combined chemoradiotherapy could actually be improved by making tumours more hypoxic. The idea of vascular targeting of tumours to achieve a therapeutic effect by eliciting infarction is an old one which is currently undergoing renewed study (19). One example is the drug DMXAA, a vascular targeting agent that is currently in clinical trial on the basis of its demonstrated anti-tumoural effect in pre-clinical models, thought to be attributable to local induction of TNFa. The same agent has been shown to potentiate the effect of bioreductive drugs like tirapazamine in vivo but has not yet been tested with radiotherapy (20).

THE FUTURE

After nearly 50 years of effort, the ability of hypoxia to frustrate the curability of solid human cancers is still a major challenge for all head and neck oncologists. Nonetheless, the prospects of reducing the impact of hypoxic cells has never looked brighter (21). First, the ability

to detect and quantify tumour hypoxia allows identification of patients in whom this is likely to be cure-limiting. Second, new therapeutic strategies designed either to reduce hypoxia or to take advantage of it to achieve differential cytotoxicity are emerging. Preliminary results show great promise, but much remains to be done. Testing of new leads from the laboratory requires well-conducted clinical trials (22). Our responsibility as radiation oncologists is to support these trials whenever possible and to implement new therapeutic strategies into our practice when they are shown to be superior to conventional methods.

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