COMMENTARY

A potential solution for eliminating hypoxia as a cause for radioresistance

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Despite the clear evidence that hypoxia is deleterious for achieving local tumor control and better survival after radiotherapy, there is no established method for reducing or eliminating it. In PNAS, Benej et al. (1) describe a class of hypoxic radiosensitizers that decreases hypoxia by blocking mitochondrial complex 1 to inhibit oxygen consumption rate. The lead compound is papavarine, a drug approved by the Food and Drug Administration to treat erectile dysfunction and vasospasm. Importantly, the activity of the drug in inhibiting complex 1 is distinct from the phosphodiesterase activity associated with vasodilation. This feature permitted the development of derivatives that maintain the mitochondrial activity but lack the phosphodiesterase activity.

It is well established that hypoxic cells are two to three times more resistant to radiation than aerobic cells (2). Theoretically, therefore, hypoxia could be a cause for treatment failure after radiotherapy, because the radiation dose required to sterilize all tumor cells would exceed the normal tissue tolerance dose. In 1955, Thomlinson and Gray (3) published the first paper to suggest that hypoxia could be a cause for radioresistance in human tumors. This landmark paper clearly demonstrated that tumor cords in lung rarely became larger than 180 μm, regardless of the size of any tumor lesion. Beyond 180 μm from the vasculature, the tissue was necrotic. Thomlinson and Gray modeled oxygen transport using available laboratory data and, from that, speculated that cells at the border between the feeding vasculature and the necrosis would be radiobiologically hypoxic [partial oxygen pressure (pO_2) < 10 mmHg]. Following this prediction, many clinical studies have reported that human tumors are hypoxic and that hypoxia reduces the likelihood for local tumor control after radiotherapy or chemoradiotherapy (4). The first tests of associations between hypoxia and radiotherapy or radiochemotherapy outcome used invasive polarographic electrodes. As an example, in a metaanalysis of head and neck cancer patients treated with radiotherapy or chemoradiotherapy, hypoxic fraction was independently associated with survival (5).

A multitude of studies at the preclinical level and in human clinical trials evaluated dozens of putative methods to reduce or eliminate hypoxia during radiotherapy (6). In an extensive review of the clinical literature, Overgaard and Horsman reported a meta-analysis of 83 randomized trials involving over 10,000 patients (6). The common goal was to improve local tumor control and survival after radiotherapy or radiochemotherapy by reducing hypoxia. The clinical trials focused on three approaches: (*i*) increasing oxygen delivery, (*ii*) using drugs that mimic the radiosensitizing properties of oxygen, or (*iii*) using drugs that selectively kill hypoxic cells. The overall benefit of these strategies was not significant in most trials. The most convincing result came from metaanalysis evidence that reduction in hypoxia was beneficial after radiotherapy in head and neck cancer (6, 7).

The key question, then, is why the randomized trials failed overall to demonstrate an impact on improving tumor control after radiotherapy. Many of the trials were underpowered, which contributed to lack of potential significance (6, 7). However, there is no doubt that three other mitigating issues contributed to lack of success:

i) Not all human cancers are hypoxic. Extensive studies using oxygen electrodes and imaging methods show that the extent of hypoxia varies widely and that not all human tumors are hypoxic (4). Design of the randomized clinical trials referred to above included the tacit assumption that all tumors had radiobiologically significant hypoxia. It is highly likely that a subset of tumors was not hypoxic and, therefore, would not benefit from hypoxia modification. The presence of nonhypoxic tumors in the overall population would reduce the power to detect differences between control and hypoxia modification groups. If hypoxia measurement were used as a selection tool, it is highly likely that these trials would have shown a

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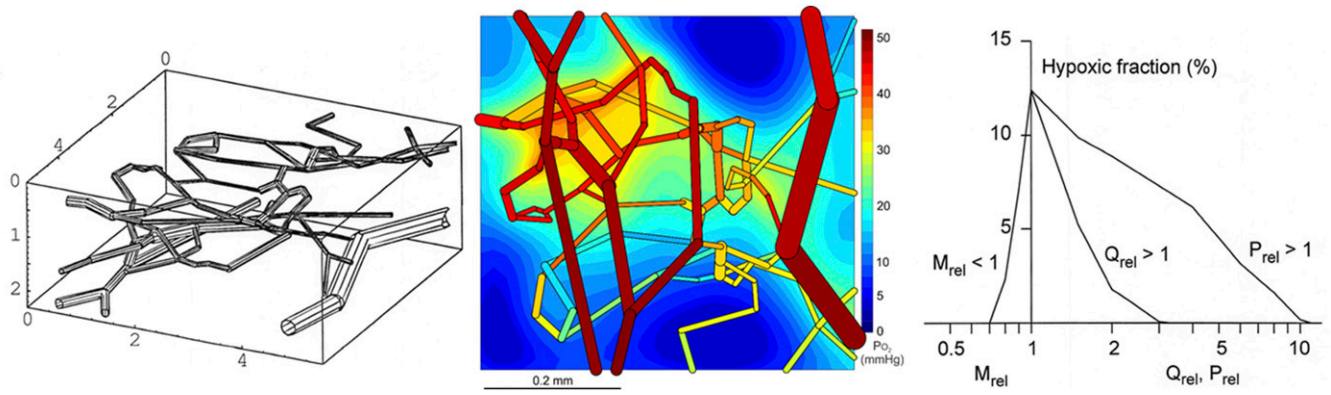

Fig. 1. Sensitivity study examining three methods to reduce tumor hypoxia. This sensitivity study used oxygen transport modeling with Green's functions to compare three methods for eliminating hypoxia in tumors. The Green's function method allows for input of actual experimental data: (*i*) vascular geometry; (*ii*) flow direction, flow velocity, and vascular pO_2 of the vascular segments within the network; and (*iii*) oxygen consumption rate. The Green's function computes the pO_2 at each location within the field, assuming O_2 diffusion from the microvessels. This simulation used confocal imaging to obtain vascular geometry from a tumor growing in skin fold window chamber (*Left*). Baseline oxygen consumption rate of 1.5 cm^3 O_2·100 g^{-1}·min^{-1} was measured previously. *Center* depicts the network from the bottom, where the feeding vessels are located (vascular pO_2 = 50 mmHg). The baseline condition for the central plane of the 500-μm × 500-μm × 200-μm tissue cube is 125 μm from the feeding vessels. Note that the pO_2 of this plane is not influenced by the feeding vessels because distance between the plane and these vessels is near the diffusion distance of oxygen. The pO_2 distribution depicts radial gradients of oxygen concentration from the vasculature, with significant variation, from near zero, to a maximum close to that of the feeding vessels (40 mmHg). The sensitivity study addressed the question of what relative change in vascular pO_2 (P_{rel}), flow rate (Q_{rel}), or oxygen consumption rate (M_{rel}) would be required to reduce the hypoxic fraction to zero. *Right* shows the predictions. At baseline, the hypoxic fraction (percent volume <1 mmHg) is ∼12%. Increasing vascular pO_2 required a 10× increase in O_2 content of breathing gas, above air breathing conditions to eliminate hypoxia. This translates to hyperbaric treatment at 2 atm O_2. A threefold increase in flow velocity is of intermediate efficiency in eliminating hypoxia. However, the most efficient means to eliminate hypoxia is by reducing oxygen consumption rate by 30%. M_{rel}, P_{rel}, and Q_{rel} all compared to the baseline condition, as depicted in *Center*. *Left* and *Right* reprinted with permission from ref. 14, *Springer Nature*: *Advances in Experimental Medicine and Biology*, copyright (1998). *Center* has not been published previously.

greater benefit (8). Methods to measure hypoxia were not available at the time of many early trials, but this is not the case now. For example, PET and single-photon emission computed tomography (SPECT) tracers (9), certain MRI and CT endpoints (9), and optical spectroscopy (10, 11) effectively predict or directly assess the extent or severity of hypoxia, respectively. Future trials should include measurement of extent of hypoxia as an entry criterion for trial accrual.

ii) Attempts to reduce hypoxia by increasing delivery are not efficient. The vast majority of approaches to reduce tumor hypoxia focused on means to increase delivery (12). This is the least efficient option, however. We compared the efficiencies of methods to reduce hypoxia, using Green's function calculations that were based on detailed microcirculatory measurements (13, 14). Reduction in oxygen consumption rate was predicted to be 30-fold more efficient in reducing hypoxia than increasing oxygen delivery by methods such as breathing hyperbaric oxygen or carbogen (Fig. 1). Further, we predicted that only a 30% reduction in oxygen consumption rate would be sufficient to eliminate hypoxia. Here, Benej et al. (1) demonstrate that a 30 to 50% reduction in oxygen consumption rate is achievable in the clinically relevant papavarine dose range (1 to 10 μM). Thus, papavarine or its derivatives should effectively reduce hypoxia at clinically achievable doses. To verify this, however, it would be advisable to include hypoxia measurement before and after drug administration to test the efficiency of hypoxia reduction. If necessary, oxygen or carbogen breathing could further reduce hypoxia when combined with inhibition of oxygen consumption. We previously reported that the combination of transient hyperglycemia (which transiently reduces oxygen consumption rate) and oxygen breathing synergistically increased tumor pO_2, with the majority of measurements exceeding the 10-mmHg threshold for radiobiologic hypoxia (15).

iii) Some hypoxia modification strategies were toxic. Key examples include the 2-nitroimidazole hypoxia mimics. These drugs mimicked the activity of oxygen by interacting with DNA adducts in irradiated cells (12). Despite very promising preclinical results, the lead drugs caused peripheral neurotoxicity in human trials. This toxicity contributed to failure in many randomized trials, because of drug dose-schedule limitations (16). One drug of this class, nimorazole, demonstrated less toxicity and is a standard of care in Denmark in combination with radiotherapy (12). Designed to selectively kill hypoxic tumor cells, derivatives of the classic 2-nitroimidazole compounds kill hypoxic cells after prolonged exposure (16). The classic example is tirapazamine. In clinical trials, this drug caused muscle toxicity, which also limited the drug dose schedule (17). Although early trials were promising, tirapazamine ultimately failed in phase III trials (18, 19). Newer classes of the hypoxic cytotoxins, including prodrugs converted to toxic intermediaries in hypoxic conditions, may yield a therapeutic advantage because they minimize muscle toxicity and have superior transport properties to enhance delivery to hypoxic tumor regions (20–22).

The toxicities of the hypoxia mimics and the hypoxic cytotoxins occurred because of accumulation of drug into normal tissues. A rapid onset of O_2 transport inhibition, combined with a relatively short half-life and rapid clearance, provides a strong rationale to use papavarine and its derivatives with radiotherapy. Inhibition is required only while radiotherapy is given. It is also likely that these drugs will have less normal tissue toxicity because of favorable pharmacokinetics. The pharmacokinetics are an essential feature

of papavarine or its derivatives, in comparison with others in this class (1). Nevertheless, it will be imperative to rule out the possibility of cumulative toxicity in preclinical studies, preferably before conducting clinical trials.

In summary, the paper by Benej et al. (1) represents a potential landmark in the six-decade-old quest to eliminate hypoxia as a cause for radiotherapy treatment failure. To avoid the pitfalls of prior trials, hypoxia-imaging methods should be used before and after drug administration to verify that patients have hypoxic tumors before treatment and that the drug effectively reduces it. Further, careful examination is required to verify that drug toxicity does not compromise the optimal trial design, which should include drug administration with every radiation dose fraction.

Finally, one needs to consider the issue of whether improving local tumor control by selectively killing hypoxic cells in combination with radiotherapy will ultimately improve survival. Because hypoxia promotes more aggressive tumor behavior (23), it is possible that local control would be improved, but survival would not be impacted. In head and neck cancer, it has been definitively shown that hypoxia modification with radiotherapy impacts both local tumor control and increases survival (7). However, head and neck cancer has a low metastatic rate. Patients with other cancers with a greater tendency toward metastasis may not have improved survival as a result of improving local tumor control.

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