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Comments and Critique

Overcoming Tumour Radiation Resistance Resulting from Acute Hypoxia

RADIORESISTANT HYPOXIC cells, found to exist in animal and human solid tumours [1, 2], are believed to compromise the success of clinical radiotherapy [3]. Hypoxia was originally only considered to be long-term or chronic in nature, arising as a result of a diffusion limitation of oxygen (Fig. 1) [4]. More recently it was suggested that hypoxia in tumours could also be acute [5]. This was later confirmed and shown to result from transient fluctuations in microregional blood flow [6]. Efforts to eliminate hypoxia have involved either sensitising hypoxia cells to radiation or preferentially killing them, and this can be achieved using treatments like radiosensitising/bioreductive drugs [7] or hyperthermia [8]. Alternatively, methods have been studied in which the amount of oxygen available to tumours is actually increased prior to irradiation. This can occur following oxygen or carbogen breathing under normobaric or hyperbaric conditions [9], or using perfluorochemical emulsions [10], haemoglobin–oxygen affinity modifiers [11] and calcium antagonists [12].

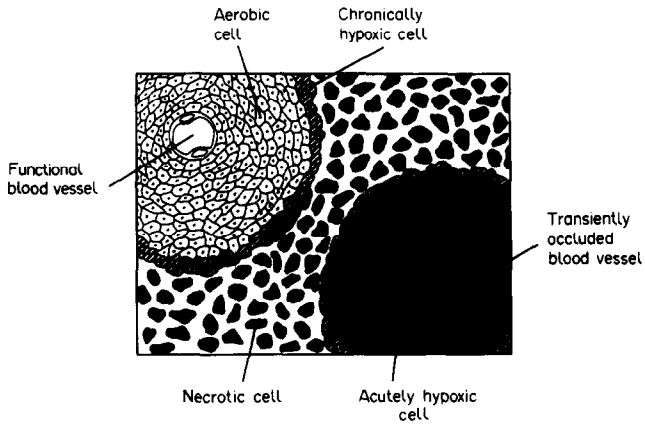

Fig. 1. Schematic representation of a tumour cross-section illustrating both chronic and acute hypoxia. Viable tumour cells are seen growing around a functional blood vessel. Oxygen diffuses from the vessel and is consumed by the aerobic cells. Beyond the diffusion distance of oxygen, typically around 150 μm, cells are necrotic. Immediately prior to the necrotic zone is a layer of cells which are oxygen deprived yet viable. These cells are the result of a diffusion limitation of oxygen and remain hypoxic until they become either reoxygenated or die, hence are referred to as chronically hypoxic cells. If blood flow through a vessel is transiently stopped then all the normally aerobic cells down-stream of the occlusion are suddenly made hypoxic. These cells are considered acutely hypoxic because they only remain hypoxic as long as the occlusion continues, becoming oxygenated again when blood flow resumes.

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While these procedures work extremely well against chronic hypoxia, most of them have little or no influence on hypoxia that is acute. Increasing the oxygenation status of blood, for example, will be of little benefit if blood flow is inhibited. Similarly, for hyperthermia the increase in cellular sensitivity to heat under hypoxic conditions is not due to hypoxia *per se*, but is rather a consequence of the cellular metabolic changes resulting from prolonged oxygen deprivation [8] and it is unlikely that acutely hypoxic cells are hypoxic for a time period sufficient to induce such changes. So, how do we overcome the problem of acute hypoxia in tumours? One possible method is to use radiosensitising drugs or certain bioreductives, since these agents can kill or radiosensitise any hypoxic cell whether it be acute or chronic [13]. The ability of these drugs to influence acute hypoxia is also illustrated in experiments in which tumours are treated with radiation and heat, a combination which can effectively kill the aerobic and chronically hypoxic cells, but not those that are acutely hypoxic. If, however, misonidazole, nimorazole or SR-4233 are introduced into this treatment protocol then a substantial improvement in tumour response is observed [14–16]. Drugs of this type have undergone extensive clinical testing, and although they can improve response to radiation therapy the results are far from ideal, primarily because of dose-limiting toxicity [3, 13]. However, this might not be such a problem if the drugs are used to specifically overcome acute hypoxia and combined with a treatment against chronic hypoxia. In fact, the tumour radiosensitisation produced by high doses of misonidazole or nimorazole can be improved even if the doses are reduced by as much as 50% provided the tumours are subsequently heated [14, 15].

An alternative approach to the acute hypoxia problem would be to make these cells chronically hypoxic and then to attack them with a treatment that specifically kills such cells. There are a number of agents which can substantially decrease blood flow to tumours and can maintain this reduction for extensive periods [17]. This includes physiological modifiers of blood flow like hydralazine and vascular damaging agents such as flavone acetic acid. These reductions in tumour blood flow are sufficient to lead to the development of 100% radiobiological hypoxia and this can result in a significant enhancement of the response of the tumours to heat [17]. Proof that it is not just the aerobic tumour cells which are made chronically hypoxic, but also those that are acutely hypoxic, comes from the finding that if mice are injected with hydralazine several hours after local tumour irradiation (a sequence which has no influence on radiation response alone) and the tumours are subsequently heated, then the resulting response of the tumour is greater than that seen with radiation and heat alone [17]. While many of these blood-flow-reducing agents have been or are being established in the

clinic, they have the disadvantage that under certain conditions many of them can also increase tumour blood flow. In addition, the vascular damaging agents tend to result in blood flow reductions that can last for at least 24 h and this would seriously reduce the effectiveness of any conventional fractionated radiation treatment [17].

Probably the best method for overcoming acute hypoxia would be to actually prevent it from occurring in the first place. Several studies have shown that nicotinamide, angiotensin II and flunarizine are all capable of preventing the transient fluctuations in tumour blood flow that result in the development of acute hypoxia [18–20]. How these agents do this is not entirely clear, primarily because the mechanisms responsible for this intermittent tumour blood flow have not been established. Suggestions for the causative factors include: vessel plugging by white blood cells, red blood cells or circulating tumour cells [21]; collapse of vessels in regions of high tumour interstitial pressure [22]; and spontaneous vasomotion in incorporated host arterioles affecting flow in downstream capillaries [23]. Flunarizine can increase red blood cell deformability [24] and thus may reduce vessel plugging. On the other hand, angiotensin II increases arterial blood pressure [19] which could decrease the likelihood of vessel collapse due to high interstitial pressures in tumours. The mechanism responsible for the nicotinamide effect is unknown. It is unlikely to be the same as that for angiotensin II because at high doses nicotinamide decreases arterial blood pressure in mice [25]. Regardless of the mechanisms responsible, all three agents which can prevent the development of acute hypoxia will also enhance radiation damage in tumours [18, 19, 26]. Nicotinamide has also been combined with treatments that specifically overcome chronic hypoxia including hyperthermia [18, 27], perfluorochemical emulsions [28] and oxygen/carbogen breathing [29], and in all instances a significant improvement in tumour radiation response was observed compared to each individual treatment.

In conclusion, there appears to be at least three different methods for overcoming acute hypoxia in tumours, all of which are clinically applicable. Of these nicotinamide probably has the greatest clinical potential, since it shows low toxicity in humans [30], with an oral dose of 6 g being considered acceptable for prolonged administration and perhaps even higher doses possible with shorter treatment times. More importantly, the combination of treatments which can attack both acute and chronic hypoxia may be the way forward and by so doing hypoxia may eventually cease to be an obstacle in radiotherapy.

Michael R. Horsman and Jens Overgaard
Danish Cancer Society
Department of Experimental Clinical Oncology
Nörrebrogade 44
DK-8000 Aarhus C
Denmark

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