Short Communication

Relationship between radiobiological hypoxia and direct estimates of tumour oxygenation in a mouse tumour model

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Abstract

CDF1 mice were allowed to breathe either oxygen, carbogen or different concentrations of carbon monoxide gas. These treatments significantly altered the percentage of clonogenic hypoxic cells in a C3H mouse mammary carcinoma and these changes were found to correlate with direct measurements of tumour oxygenation obtained using an Eppendorf pO_2 electrode.

Key words: CDF1 mice; C3H mammary carcinoma; Gas breathing; Hypoxic fraction; pO_2 measurements

1. Introduction

Radioresistant hypoxic cells identified in most animal and human solid tumours [11,17] are believed to compromise the success of clinical radiotherapy [13]. Numerous attempts have been made to identify those human tumours which contain hypoxic cells and therefore are most likely to benefit from therapies which can overcome hypoxia (for review see Ref. 6). These techniques have included measurements of tumour vascularization, cryospectrophotometric estimates of intravascular haemoglobin oxygen saturations, tumour metabolic activity, the binding of radioactive or fluorescently labelled nitroimidazole compounds, and the determination of oxygen partial pressure (pO_2) distributions using electrodes. Of these procedures, probably the most clinically applicable direct method involves measurements of tumour oxygenation with electrodes (for review see Ref. 17). In fact, several clinical studies have used electrodes to estimate pO_2 in human tumours and reported that the less well oxygenated tumours showed the poorest response to radiotherapy [1,3,8,10,14]. However, until now there has been no direct correlation between electrode measurements of tumour oxygenation and the percentage of clonogenic hypoxic cells in tumours. In the present study we have modified the normal level of radiobiological hypoxia in a murine tumour, simply by allowing the mice to breathe different gas mixtures, and then studied the relationship between these results and tumour oxygenation measurements obtained with an Eppendorf oxygen electrode.

2. Materials and methods

Male and female CDF1 mice between 10–14 weeks of age were implanted in the right rear foot with a C3H mammary carcinoma as previously described [12]. All treatments were carried out when tumours had reached a volume of approximately 200 mm^3, which generally occurred 2–3 weeks after challenge. Non-anaesthetized mice were restrained in lucite jigs, with their tumour-bearing legs exposed and loosely attached to the jig with tape, without impairing the blood supply to the foot. Mice were then gassed with either oxygen, carbogen (95% O_2 + 5% CO_2) or different concentrations of carbon monoxide (CO). The gas flow rate was 2.5 l/min and the period of gas exposure prior to any subsequent treatment was 5 min for oxygen or carbogen and 35–45 minutes for CO, with the flow being maintained during subsequent treatments.

Radiation was given with a conventional therapeutic 250 kV X-ray machine at a dose rate of 2.3 Gy/min, dosimetry being accomplished by use of an integrating chamber. Tumours only
were irradiated, the remainder of the animal being shielded by 1 cm of lead. To secure homogeneity of the radiation dose, tumours were immersed in a water bath at room temperature with about 5 cm of water between the X-ray source and the tumour. Irradiation of hypoxic tumours involved constricting the blood flow, using a rubber tube tightened around the tumour-bearing leg, for 5 min before and during the irradiation period. Tumours were observed at weekly intervals after treatment and the percentage of animals at each radiation dose showing local tumour control 90 days after treatment, recorded. Hypoxic fractions were determined from direct analysis of the radiation dose-response data, obtained under clamped and unclamped conditions, as described previously [2], with an average of 322 mice being used to obtain each dose response curve.

In additional experiments, a fine needle autosensitive electrode probe (Eppendorf, Hamburg, Germany) was inserted up to a depth of 1 mm into the tumour of similarly gas-treated but non-irradiated mice. It was moved automatically through the tissue in 0.7 mm increments, followed each time by a 0.3 mm backward step prior to measurement. Response time was 1.4 s. After making measurements along one track in the tumour the probe was removed and repeated parallel insertions were made until a total of 30–90 measurements were obtained. An average of 6 mice were used for each treatment condition, with an average of 52 measurements per tumour. The relative frequency of the pO2 measurements was automatically calculated and displayed as a histogram. From the raw data various parameters may be selected, but we chose to use the percentage of pO2 values ≤5 mmHg because our preliminary studies suggested that this parameter showed the strongest correlation with hypoxia [7].

3. Results

The relationship between the percentage of pO2 values ≤5 mmHg and the radiobiological hypoxic fraction measured under all the various treatment conditions is illustrated in Fig. 1. In air-breathing mice the normal hypoxic fraction was around 12% and some 40% of the pO2 measurements were at 5 mmHg or less. Clamping tumours results in 100% radiobiological hypoxia and almost 100% of the pO2 values are now ≤5 mmHg. Carbon monoxide breathing increases both tumour hypoxia and the number of low pO2 values measured, but this effect was dependent on the concentration of carbon monoxide in the gas. Carbogen and oxygen breathing improved tumour oxygenation, and this is seen in the figure by both the decrease in the number of pO2 values ≤5 mmHg as well as a reduction in the hypoxic fraction.

4. Discussion

Extensive detailed studies by Vaupel and colleagues using tumours of different sizes have always strongly suggested that electrode measurements of tumour oxygenation might be a good indicator of radiobiological hypoxia in tumours [9,15,16,18]. It has also been suggested from several small clinical studies in which electrode measurements of tumour oxygenation were made in cancers of the cervix, head and neck, breast, lung, bladder and lymphomas [1,3,8,10,14]. In those studies it was found that the tumours that were the least responsive to radiation therapy were in fact those that showed the lowest levels of oxygenation. We have now directly demonstrated this correlation using the same mouse tumour model, assayed in situ, in which we attempted to change tumour oxygenation simply by varying the oxygen status of the mouse blood. With oxygen and carbogen breathing there was an improvement in tumour oxygen state that probably resulted from an increase in the amount of physically dissolved oxygen in the blood [4]. On the other hand, carbon monoxide decreased tumour oxygenation and while this can primarily be attributed to an increase in carboxyhaemoglobin reducing the amount of oxygen transported to the tissues, there is evidence to suggest that at least part of this effect may be the result of a decrease in tumour blood flow [5].

Whatever the mechanisms involved we have clearly shown that electrode measurements of pO2 are an excellent indicator of the percentage of clonogenic hypoxic cells in a particular tumour. Although these results need to be confirmed using other animal and human tumour models, they do add further support to those clinical studies currently being performed in which pO2 measurements with electrodes are being made in an effort to identify those human tumours which contain hypoxic cells and thus resistant to radiation therapy.

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