Modulating the oxygen tension in tumours by hypothermia and hyperbaric oxygen

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Summary
Hypothermia is associated with reduced metabolism of tissues and especially reduced oxygen consumption by tumours. If the blood supply to a hypothermic tumour can be maintained then the hypoxic fraction of cells should be reduced and the radiation response increased. This hypothesis has been tested with radiation under hyperbaric oxygen and increased tumour response has been demonstrated.

Introduction

The oxygen tension in some cells in some tumours is lower than the physiological range, and the sensitivity of such cells to radiotherapy may be two to three times less than that of well oxygenated cells. This radio-resistance of hypoxic tumour cells is one of the problems that radiotherapists face and clinical radiobiologists try to overcome[1]. Cells remain sensitive to radiation until the oxygen tension has fallen to a partial pressure of less than 10 mm of mercury. For all intents and purposes, therefore, normal tissues do not exhibit any significant radio-resistance, because their oxygen tension will very rarely fall below such a partial pressure.

In the case of some solid tumours, however, the blood supply becomes abnormal as a consequence of rapid growth and pressure and there are some areas with a very low oxygen tension. Necrotic foci can be seen in histological sections, as described by Thomlinson and Gray[2]. Tumour cells at the edge of such necrotic foci will remain viable, but be very hypoxic and very radio-resistant. There is a steep gradient of effect between normal radiosensitivity and radio-resistance, which may be just a few cell layers thick. This is the region where modulation of the oxygen tension may be achieved by a variety of means.

Radiotherapists have learned that anaemia reduces the effectiveness of X-ray treatment and this is usually corrected by blood transfusion. On the other hand, one series of anaemic patients with carcinoma of the cervix stage III, showed a dramatic increase in local control when they were irradiated under hyperbaric oxygen[3]. One explanation for this may be that anaemic blood is less viscous and that, provided the oxygen content of the plasma is increased by hyperbaric oxygen, this less viscous blood will diffuse more readily to the hypoxic areas of the tumour. Hyperbaric oxygen is less beneficial in patients who are not anaemic because the oxygen carrying capacity of a normal level of haemoglobin cannot be increased; only the plasma content can be increased by hyperbaric oxygen.

Hyperbaric oxygen has other effects upon the blood supply due to vasoactive phenomena. Vasoconstriction of the vessels supplying a tumour will reduce the advantage of the increased oxygen carrying capacity of the blood. A vasodilating drug might therefore be thought to be useful but the opposite results may be obtained due to the fact that it will be the vessels supplying the *normal* tissue which will be dilated rather than the vessels supplying the tumour. Hydralazine has been used in this way and has sometimes been found to have almost the same effect on the tumour blood supply as the placing of a clamp[4]. The vasoactive effects of anaesthesia have also to be considered, and different anaesthetics have different pharmacological effects in this respect.

When anaesthesia is used with experimental mice, thermoregulation is disrupted and hypothermia occurs in these animals[5]. We now report the effects of hypothermia and anaesthesia on the oxygen tension in tumours, and the consequent change in radiation response of such tumours. A working hypothesis is proposed and data are reported which substantiate it:

Hypothermia
(1) Reduced oxygen consumption by all tissues.
(2) Same blood supply to tumours.

Hyperbaric oxygen and hypothermia
(1) Reduced oxygen tension in normal tissue.
(2) Increased oxygen tension in tumours.
(3) Reduced hypoxic fraction in tumours.
(4) Increased radiosensitivity in tumours.

We now report the results of some recent studies which support this hypothesis when considered in conjunction with our earlier studies in this field. The recent studies involve oxygen polarography *in vitro*, ^{86}rubidium extraction of tumours and normal tissues *in vivo*, and regrowth delay of tumours in irradiated mice.

Materials and methods

Female inbred C3H/He SPF-derived mice bred in our Medical School, 13-17 weeks old were used for this work. The tumour was C3H mammary adenocarcinoma which has been serially transplanted for some years[6]. The methods of transplanation: ketamine and diazepam anaesthesia, the irradiation technique, and the hyperbaric oxygen technique, have all been described by Tozer *et al.*[7]. The temperature measurement of the mice and the temperature control of the hyperbaric oxygen chamber have been described by Nias *et al.*[5].

The rate of oxygen consumption by a stirred tumour cell suspension in a sealed vessel was measured polarographically using the Clark electrode system.

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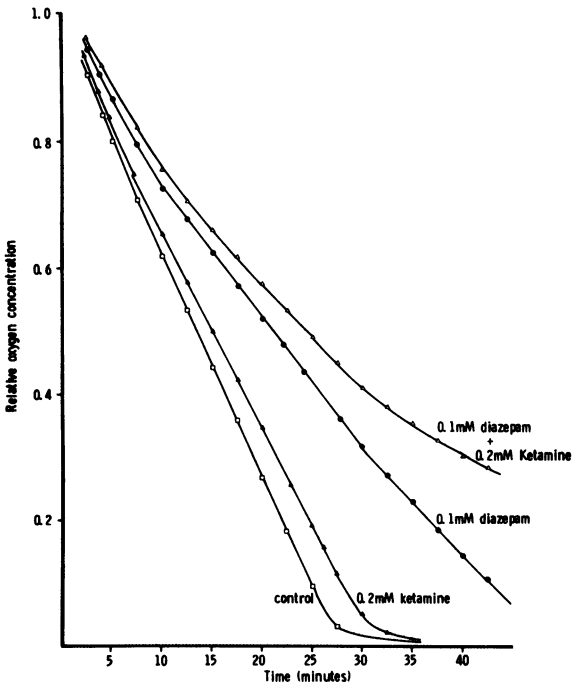

Figure 1. Rate of oxygen consumption by a stirred tumour cell suspension measured by the Clark electrode system

^{86}Rubidium extraction by mouse tissues was undertaken using the technique of Sapirstein[8], the details are described by Photiou[9] who used RIF tumours in that study. Regrowth delay of the mouse mammary tumour was estimated from tumour volume measurements made every other day on the basis of the time taken for the volume to increase to 3.5 times the volume at the time of treatment (which was 160-220 mm^3).

Results

Figure 1 shows that the rate of oxygen consumption is reduced when a tumour cell suspension is exposed to nontoxic doses of ketamine and diazepam (equivalent to those used *in vivo*). Ketamine alone reduced the rate only to 93%, diazepam alone reduced it to 58%. The combination of ketamine and diazepam (as used *in vivo*, reduced the rate of oxygen consumption to 51% of the control value. These measurements were made at 37°C. In another study (data not shown) when tumour cells were cooled from 37°C to 29°C (without anaesthesia) the rate of oxygen consumption was reduced to 58%.

Figure 2 shows the ^{86}rubidium extraction by various tissues in the mouse. Anaesthesia increases the relative perfusion of the visceral organs, reduces the perfusion of muscle but has no effect on the relative perfusion of tumour.

Figure 3 shows tumour regrowth delay. Mice were irradiated in air or under hyperbaric oxygen under various conditions. With one exception a dose of 25 Gy given under hyperbaric oxygen conditions increased the regrowth delay compared with 25 Gy given under aerated conditions. The exception was when the blood supply to the tumour was clamped and the mice were anaesthetized so that the temperature fell to 29°C. The maximum regrowth delay was seen in similar mice when the tumour was not clamped. At the control temperature of 36°C there was less regrowth delay and the use of a vasodilator Hydralazine did not reduce regrowth delay to any great extent. There was slightly more regrowth delay in hyperbaric oxygen when the tumours were clamped and the temperature was maintained at 36°C in unanaesthetized mice.

Discussion

The working hypothesis begins with the statement that hypothermia will reduce the oxygen consumption of tissues. This is only to be expected since metabolism is reduced by 6% for every °C fall in temperature[10]. When experimental mice are anaesthetized their temperature falls quickly. This is because of their poor thermoregulation. They are no longer moving around nor are they able to remain in close proximity with their neighbours. The temperature falls rapidly from 38°C to 32°C within 10 min[5]. The data obtained with the Clark electrode system (Figure 1) showed that oxygen consumption is reduced both by anaesthesia alone and also by hypothermia alone. In both cases the rate of oxygen consumption was reduced by about 50%. These data were obtained with tumour cells *in vitro*.

The ^{86}rubidium extraction data were obtained *in vivo* and provided a comparison of the blood flow to tissues in unanaesthetized mice and mice that were anaesthetized and therefore hypothermic. Whereas there was a significant increase in the relative perfusion of the gut, kidneys and liver in hypothermic anaesthetized mice, and a significant decrease in muscle, there was no significant difference in the relative perfusion of tumour as a result of anaesthesia and hypothermia.

The working hypothesis in this paper required confirmation of the effect of hypothermia in reducing oxygen consumption of tissues. Figure 1 showed that anaesthesia (and hypothermia) reduced oxygen

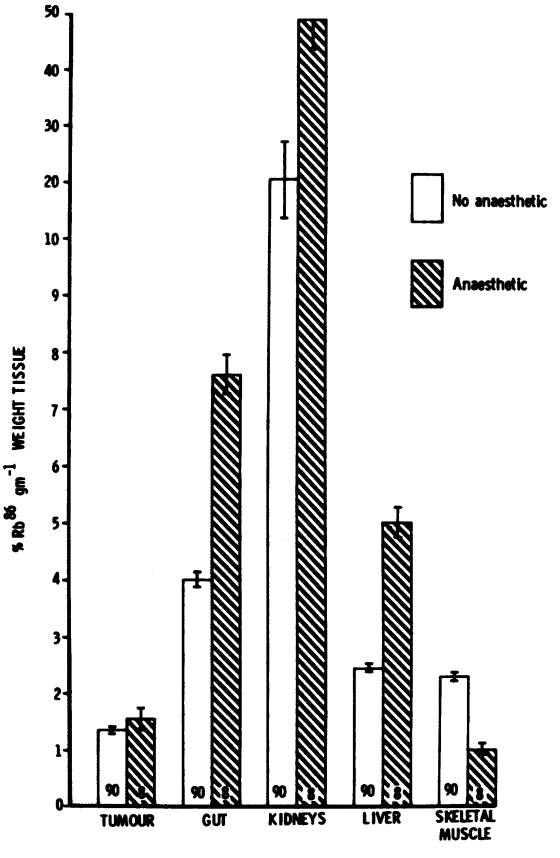

Figure 2. ^{86}Rubidium extraction by various mouse tissues with and without anaesthesia

consumption at the cellular level. The second statement in the hypothesis was that under these conditions of hypothermia the blood supply to tumours is maintained. Assuming that cardiac output is not reduced by anaesthesia, this was confirmed by the data in Figure 2. Whereas the relative perfusion of normal tissues was either increased or decreased, the perfusion of tumour remained unchanged. These are the conditions when mice are to be irradiated under normal aerated conditions. Previous studies using the ^{133}xenon clearance technique had also shown no reduction in tumour blood flow under hypothermic conditions[5].

Under conditions of hyperbaric oxygen there will be an increased provision of oxygen due to saturation of the plasma, but the blood supply to a tissue is dependent upon a number of factors including heart rate, cardiac output, degree of vasodilatation or vasoconstriction and other physiological variables, all of which will be affected by hypothermia[11]. The vasoactive effect of an anaesthetic will also influence the blood supply to a tumour during irradiation, as will hyperbaric oxygen. Many of these factors have opposing effects, but the working hypothesis was that under conditions of hypothermia and hyperbaric oxygen there would be a reduction in oxygen tension in normal tissues, and an increase in oxygen tension in tumours. This was confirmed by Tozer[12] using oxygen polarography and the data were published by Nias *et al.*[5]. These show that with anaesthesia (and therefore hypothermia) there was a relative increase in oxygen concentration in the tumour, and a relative decrease in oxygen tension in subcutaneous tissue. Hyperbaric oxygen increased the absolute levels in addition to the differences just described.

The working hypothesis has now been confirmed to the extent that hypothermia will reduce oxygen consumption by tissues, but that the blood supply to a tumour may be maintained under aerated conditions and under hyperbaric oxygen conditions the oxygen tension in a tumour is increased. The radiobiological problem of solid tumours is that there are areas of hypoxia which are radioresistant. This problem may therefore be overcome to some extent under the conditions now being described. If oxygen tension is maintained and oxygen consumption is reduced then the net effect should be a *reduction* in hypoxic fraction of a tumour and therefore an increase in the net radiosensitivity of that tumour.

The data in Figure 3 confirm that there is indeed increased radiosensitivity of tumours under conditions of anaesthesia and hypothermia combined with hyperbaric oxygen during irradiation. There is the maximum regrowth delay under these conditions. Hyperbaric oxygen alone, without anaesthesia, with the mice maintained at 36°C has less effect. The full extent of the benefit of this combination is shown by comparing the amount of regrowth delay with that obtained under clamped conditions with anaesthesia at 29°C. Under those two conditions the tumour should be completely hypoxic, on the one hand and the benefit of the reduced oxygen metabolism 29°C coupled with an unrestricted oxygen supply is maximal on the other.

The vasodilating drug hydralazine was used in these studies because hyperbaric oxygen has a vasoconstrictive effect, and it was hoped that the drug would counter this. In the event the drug had little or no effect since the regrowth delay is little different

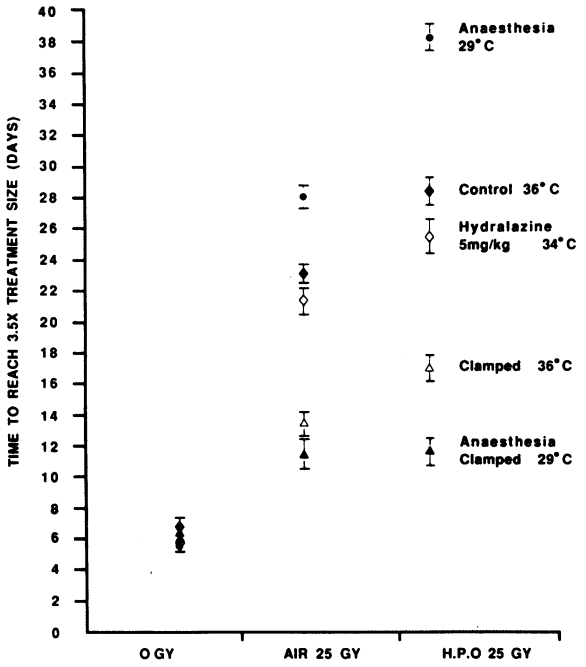

Figure 3. Regrowth delay of C_3H mouse mammary tumour irradiated under various conditions

from that of control animals in both air and HPO groups. Hydralazine has been shown to reduce the blood supply to tumour, presumably by some 'stealing' mechanism due to vasodilatation of the vascular supply to normal tissues[4,13]. This selective action of such a vasoactive drug is by no means consistent[14], however, and the suggestion that the action of hydralazine is equivalent to clamping off the tumour blood supply has obviously not been confirmed in our work.

The bottom two sets of data from tumours irradiated in air, show that clamping with anaesthesia at 29°C and clamping at 36°C reduces the radiation effect to the same extent. This is to be expected since the blood supply should be completely occluded and the hypoxic fraction of the cells in such tumours should approach 100%. The effect of hypothermia should therefore be negligible. The same effect should also be shown in the tumours irradiated under HPO, and there is indeed the same effect with anaesthesia at 29°C. It is not clear why there is more regrowth delay in clamped tumours maintained at 36°C in HPO unless some oxygen can diffuse into the tumour through the skin.

In conclusion these studies have shown that oxygen tension to tumours can be modulated by hypothermia and hyperbaric oxygen to increase the radiation response. The combination is clinically feasible[15], and the working hypothesis that hypothermia reduces oxygen consumption, and that hyperbaric oxygen maintains the oxygen supply has been confirmed. In consequence, reduction in the hypoxic fraction in a tumour and increase in its radiation response has been shown to be a practicable way of achieving therapeutic gain.

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