HYPERTHERMIA CLASSIC ARTICLE

Tumour oxygenation is increased by hyperthermia at mild temperatures

C. W. SONG, A. SHAKIL, J. L. OSBORN, & K. IWATA

Department of Therapeutic Radiology, University of Minnesota Medical School, Minneapolis, MN, USA

(Received 11 September 1995; Revised 16 November 1995; Accepted 1 December 1995)

Abstract

The effects of hyperthermia on the oxygenation status in R3230 AC tumours of Fischer rats were measured using a polarographic oxygen electrode system. The median $pO_2$ in about 10 mm diameter tumours grown s.c. in the leg of rats was $3.7 \pm 0.3$ mm Hg and it significantly increased upon heating at modest temperatures. For example, the tumour $pO_2$ measured within 10–15 min after heating for 30 min at 42.5°C was about three-fold greater than that in the control tumours. About 62% of $pO_2$ values measured in control tumours were $<5$ mm Hg. After heating at 42.5°C for 30 min, 37% of $pO_2$ values were $<5$ mm Hg. Such an increase in tumour oxygenation or reoxygenation of hypoxic cells appeared to result from an increase in tumour blood flow caused by the mild temperature hyperthermia. The presence of hypoxic cells in tumours is believed to be a major factor in limiting the effectiveness of radiotherapy, certain chemotherapy drugs and phototherapy. Hyperthermia at mild temperatures easily achievable with the use of presently available clinical hyperthermia devices may be an effective means to overcome the hypoxic protection in the treatment of human tumours.

Keywords: Tumour $pO_2$, hyperthermia, R3230 AC tumour

Introduction

Hypoxic cells are about three-fold radioresistant relative to oxygenated cells, and hypoxic cells are also resistant to certain chemotherapy drugs as well as phototherapy. Indications are that fractions of cells in human tumours are hypoxic and that the presence of such hypoxic cells may be one of the limiting factors for the complete control of human tumours by radiotherapy [1–6].

In recent years, the effectiveness of hyperthermia as an adjuvant to radiotherapy has been demonstrated [7–8]. Experiments with laboratory animal tumours demonstrated that heating for 30–60 min at relatively high temperatures, i.e. $>43$–$44^\circ$C, causes damage to tumour blood vessels and kills tumour cells [9–12]. In addition, hyperthermia causes direct cellular radiosensitization [13–15]. Unfortunately, with most of the currently used clinical hyperthermia devices, it is not an easy task to raise the human tumour temperature high enough to cause vascular damage, kill the tumour cells, including hypoxic cells, and also to radiosensitize the tumour cells. Oleson [16] recently pointed out that the thermal doses in past clinical trials in which hyperthermia was found to be effective were too small for inducing significant direct cell killing as well as thermal radiosensitization. Oleson thus hypothesized that indirect radiosensitization through an increase in blood flow and ensuing reoxygenation of hypoxic cells rather than thermal cytotoxicity and direct thermal radiosensitization could have been the reasons for the increase in tumour response to the combined treatment of hyperthermia and radiotherapy. As Koch et al. [17] suggested, improvement of tumour oxygenation by hyperthermia is a compelling hypothesis which warrants experimental investigation. We report here that hyperthermia at modest temperatures, e.g. 41–43°C, increases blood flow.
and increases oxygenation in a transplantable rat tumour suggesting that hyperthermia at modest temperatures may indeed be an effective means to overcome the hypoxic protection in radiotherapy.

Materials and methods

Tumours

The R3230 adenocarcinoma (R3230 AC tumour) (originally obtained from Dr M. Dewhirst, Duke University) of Fischer rat was used. Stock tumour cells stored frozen in liquid nitrogen are periodically thawed, cultured in vitro and injected subcutaneously (s.c.) into the right flank of male Fischer rats. Tumours are then maintained by serial transplantation of small viable tumour pieces to the right flank of other rats. For the present study, tumours grown in the flank of rats were minced to about 1.5 mm³ pieces and transplanted s.c. into the right hind legs of 250–300 g male Fischer rats. Tumours grown to about 10 mm in diameter, which took 20–30 days, were used for the experiments.

Heating

Tumours grown in the leg of rats were heated with a water bath as we described in detail elsewhere [18]. Briefly, the rats were anaesthetized by an i.p. injection of a mixture of ketamine (87 mg/kg) and xylazine (13 mg/kg) in about 0.3 ml isotonic saline. Hairs on the tumour-bearing right legs were clipped with an electric clipper. The tumour-bearing legs were immersed into the preheated water through 2.5 cm diameter holes on a plexiglas board. The tumours were heated at 40.5–43.5 °C for 30 or 60 min. During the heating, the temperature in the tumour periphery underneath the skin, as measured with 29-gauge needle-type thermocouples, was close to the water temperature while the temperature in the deeper regions was 0.2–0.3 °C lower than the water temperature. The rectal temperature rose from control temperature (36°C) by <1.0–1.5°C during the tumour heating.

Measurement of tumour oxygenation

The partial oxygen tension (pO₂) in the tumours was measured with an Eppendorf potentiographic oxygen electrode system (Eppendorf, Hamburg, Germany). The principle and technical details of this equipment have been described by Vaupel et al. [4]. The electrodes were calibrated using isotonic saline solution equilibrated alternatively with air and 100% nitrogen gas immediately before and after measurement of pO₂ in each tumour. Immediately after the tumours were heated, the rats were placed on a plexiglas board, the tumour-bearing right leg was gently stretched and affixed by taping the foot to the board. The body temperature (rectal temperature) was maintained at 36–37°C by placing a heating pad under the board. Commercially available self-adhering reference electrodes (SynCor™ National ECG Electrode) were attached to the surface of the shaved dorsal surface of the rats. A small incision was made in the skin on the distal side of tumours and a pO₂ sensitive needle-type electrode (300μm diameter) was inserted and horizontally advanced about 1.5 mm deep so that the electrode tip was inside the tumour tissue. The computer-controlled electrode was automatically advanced by about 0.7 mm forward steps, immediately withdrawn by 0.3 mm to reduce the compression pressure, and the pO₂ was read. The same procedures were repeated resulting in a 0.4 mm net forward advancement of the electrodes after each pO₂ measurement. The length of each track was predetermined according to the geometry of the tumours. The pO₂ was measured along 4–5 parallel tracks in the lower plane and 3–4 tracks in the upper plane of each tumour. Along each track, 10–15 pO₂ readings were made resulting in 80–120 readings in each tumour. Measurement of pO₂ in each tumour was completed within 10–15 min after tumour heating. The measured pO₂ value was corrected for tumour temperature measured with a needle-type thermocouple immediately after the pO₂ measurement. The pO₂ in unheated control tumours was measured 30–60 min after the host rats were anaesthetized.

Data analysis

The information on pO₂ values recorded in the Eppendorf histogram was transferred to an IBM personal computer and the data was analysed. The mean and median pO₂ values in each tumour were obtained and their average for each group was calculated. The % of pO₂ readings <5 mm Hg in each tumour was also obtained and averaged for each experimental group. The non-parametric Mann-Whitney U-test was used for the determination of statistical significance of the differences between the experimental groups. The Wilcoxon Rank Sum Test was used when the sample numbers exceeded 20 in either of the comparing groups.

Results

pO₂ parameters in R3230 AC tumours determined immediately after heating at 40.5–43.5 °C for 30 or 60 min are shown in Table I. The mean and median pO₂ in 25 control tumours were 9.8±0.6 mm Hg and 3.7±0.3 mm Hg respectively. A significant increase in tumour pO₂ occurred upon heating at temperatures as low as 40.5°C for 30 min,
as indicated by the increase in median pO2 to 5.4 mm Hg (p < 0.02). Figure 1 shows the histograms of pO2 distribution in tumours heated at 42.5–43.5°C for 30–60 min. A marked increase in pO2 occurred when the tumours were heated at 42.5°C for 30 min; the median pO2 increased to 12.2 ± 1.8 mm Hg, which was about 3-fold greater than the median pO2 in the control tumours (p < 0.001). However, the tumour pO2 returned to control level when the heating at 42.5°C was prolonged to 60 min. The mean and median pO2 in tumours heated at 43.5°C for 30 min also increased to the level similar to those caused by heating at 42.5°C for 30 min. On the other hand, prolonging the heating at 43.5°C to 60 min resulted in tumour pO2 to be significantly lower than the pO2 in control tumours (p < 0.001). Figure 2 shows the changes in the fraction of pO2 readings <5.0 mm Hg, which probably corresponds to about one-half the pO2 for a radiobiologically oxygenated condition. About 62% of all the pO2 readings in 25 control tumours were <5.0 mm Hg and about 37 and 30% of the pO2 readings were below 5 mm Hg when the tumours were heated at 42.5 and 43.5°C for 30 min respectively.

Table I. pO2 values in R3230 AC tumours heated for 30 or 60 min at 40.5–43.5°C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>n</th>
<th>Mean pO2*</th>
<th>Median pO2*</th>
<th>%pO2 &lt; 5.0 mm Hg*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25</td>
<td>3431</td>
<td>9.8 ± 0.6</td>
<td>3.7 ± 0.3</td>
<td>62.0 ± 2.1</td>
</tr>
<tr>
<td>40.5°C, 30 min</td>
<td>20</td>
<td>2099</td>
<td>14.4 ± 0.9</td>
<td>5.4 ± 0.7</td>
<td>55.4 ± 2.7</td>
</tr>
<tr>
<td>40.5°C, 60 min</td>
<td>21</td>
<td>2009</td>
<td>14.6 ± 1.1</td>
<td>4.2 ± 0.4</td>
<td>56.9 ± 1.9</td>
</tr>
<tr>
<td>41.5°C, 30 min</td>
<td>25</td>
<td>2228</td>
<td>14.9 ± 0.8</td>
<td>5.9 ± 0.6</td>
<td>52.7 ± 2.0</td>
</tr>
<tr>
<td>41.5°C, 60 min</td>
<td>26</td>
<td>3156</td>
<td>13.4 ± 0.8</td>
<td>6.5 ± 0.8</td>
<td>50.3 ± 2.8</td>
</tr>
<tr>
<td>42.5°C, 30 min</td>
<td>21</td>
<td>1938</td>
<td>20.2 ± 1.4</td>
<td>12.2 ± 1.8</td>
<td>37.1 ± 2.9</td>
</tr>
<tr>
<td>42.5°C, 60 min</td>
<td>18</td>
<td>2514</td>
<td>10.4 ± 0.8</td>
<td>3.7 ± 0.5</td>
<td>60.5 ± 3.0</td>
</tr>
<tr>
<td>43.5°C, 30 min</td>
<td>17</td>
<td>1889</td>
<td>20.5 ± 2.1</td>
<td>12.6 ± 1.8</td>
<td>29.3 ± 3.5</td>
</tr>
<tr>
<td>43.5°C, 60 min</td>
<td>13</td>
<td>1418</td>
<td>6.3 ± 0.5</td>
<td>2.1 ± 0.2</td>
<td>78.8 ± 1.7</td>
</tr>
</tbody>
</table>

N = number of tumours studied; n = number of pO2 values measured. The mean and median pO2 values in each tumour were obtained and their average for each experimental group ±1 SE is shown.

Figure 1. Frequency distribution of pO2 in R3230 AC tumours following heating at 42.5 and 43.5°C for 30 or 60 min.
tumour cells. These results are in agreement with our observations in the present study that the pO2 in R3230 AC tumours markedly increased upon heating at 41.5–43.5°C for 30 min. We have previously observed that hyperthermia at 40–42°C for 1 h markedly improved the oxygenation in FSa II tumours of C3H mice [23].

The increase in tumour pO2 by hyperthermia may result either from a decrease in oxygen consumption or an improvement of oxygen supply through an increase in tumour blood flow [24]. However, oxygen consumption in tumour cells has been reported not to decrease but increase upon heating at 40–42°C [25–26, 12]. Therefore, it would be reasonable to conclude that a decline in oxygen consumption was not the main factor for the improvement of oxygenation in R3230 AC tumours upon heating at 40.5–42.5°C although the possibility that heating at 43.5°C caused cellular injury and decreased oxygen consumption may not be ruled out. It has been known that the newly formed tumour vasculatures are vulnerable to heating [9–12]. However, the tumour blood flow tends to increase upon heating at relatively mild temperatures. We have indeed observed that heating at 42.5–5°C for 30 min significantly increases the blood flow in R3230 AC tumours [27] indicating that the increase in pO2 in R3230 AC tumours heated at 42.5°C for 30 min in the present study resulted mainly from an increase in tumour blood flow. The decline in tumour pO2 after heating for 60 min at 42.5 or 43.5°C (Table I and Figure 1) may be caused either by a decrease in tumour blood perfusion or an increase in oxygen consumption. Experiments are in progress to elucidate the relationship between pO2, blood flow, and oxygen consumption in heated tumours. As shown in Figure 2, the pO2 readings <5.0 mm Hg in the tumours significantly decreased upon heating at 40.5–43.5°C for 30 min, indicating significant fractions of the radiobiologically hypoxic cell populations are reoxygenated. The vascular response in human tumours to mild temperature hyperthermia may not be the same as that in rodent tumours. Nevertheless, Waterman et al. [28] reported that the blood flow in human tumours was increased by amounts varying from 15 to 250% during the first 20–50 min heating at 41–45°C suggesting that modest thermal dose hyperthermia may improve oxygenation also in human tumours.

In conclusion, hyperthermia at mild temperatures, i.e. 41.5–42.5°C, which are achievable with presently available hyperthermia technology is an effective means to overcome the hypoxic protection in radiotherapy by reoxygenating the hypoxic cells. Recent results strongly suggest that prolonged heating at mild temperatures also increases the radiosensitivity of tumour cells [13–14]. The potential usefulness

Discussion

The results obtained in the present study unequivocally demonstrated that hyperthermia at modest temperatures significantly improves tumour oxygenation. Although the effect of hyperthermia on tumour oxygenation has been sporadically reported in the past [19–20, 12, 21], this potentially important phenomenon has not been systematically investigated. Bicher et al. [19] reported that the pO2 in a C3H mouse mammary adenocarcinoma increased as a result of an increase in tumour blood flow when the tumours were heated at temperatures below $41^\circ$C, but it decreased as the heating temperature was raised $>41^\circ$C. Vaupel et al. [22] observed that the oxygenation status in DS-carcinoma of rats significantly improved upon heating at $40^\circ$C while it declined when the heating temperature was raised to $43^\circ$C. More recently, Hetzel et al. [21] reported that the pO2 in a C3H mouse mammary adenocarcinoma grown in the flank increased 24 h after an exposure of the tumour to a TCD10 dose (dose which can cure 10% of tumours in 30 days) of heating at $43.5^\circ$C. When the heating dose was increased to TCD 60 (dose which can cure 60% of tumours in 30 days) of heating at $43.5^\circ$C, the tumour pO2 decreased at 4 h but increased again at 48 h. The investigators concluded that the improvement of tumour oxygenation after heating with the TCD10 dose resulted probably from an increase in tumour blood flow and that the improvement of tumour oxygenation following the heating at the TCD60 dose resulted mainly from a decrease in oxygen consumption as a result of heat damage to the

![Figure 2. The % frequency of pO2 readings below 5 mm Hg in R3230 AC tumours heated at different temperatures for 30 min are shown. An average for each group $\pm 1$SE is shown.](image-url)
of mild temperature hyperthermia in combination with irradiation in treating human tumours warrant careful examination.

Acknowledgements

The authors would like to thank Dr Dorothy Aeppli for her assistance with the statistical analysis, and Ms Peggy Evans for her assistance in the preparation of the manuscript. This research was supported by NCI grant numbers CA13353 and CA44114.

References