Eur J Cancer Clin Oncol. Vol. 20, No. 5, pp. 619–623, 1984.
Printed in Great Britain.

0277-5379/84$3.00+0.00

Human Tumour pH Changes Following Hyperthermia and Radiation Therapy*

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Abstract—*Measurement of human tumour pH was performed during treatment with whole-body hyperthermia (WBHT) at* 41.8°C, *and prior to and following a series of combined local hyperthermia (LHT) and radiotherapy treatments. During WBHT no changes were seen in the* 11 *tumour measurements performed during heating, 'plateau' and cooling phases of treatment. Tumour pH rose significantly in* 24 *tumours in which paired determinations were performed prior to and following local therapy (mean rise,* 0.23 *pH units), while the* 11 *subcutaneous controls, measured in a non-treated area, remained unchanged. The lack of change in tumour pH during WBHT is probably related to the moderate treatment temperature* (42°C *or less), whereas the rise seen following local therapy is probably the result of changes in tissue oxygenation and blood flow following therapy. These changes may have implications to the treatment of malignant disease.*

INTRODUCTION

HYPERTHERMIA has been used in our institute as a treatment modality for malignant disease for a period of about 4 yr. It is usually administered in combination with radiotherapy. The finding that environmental pH affects the survival of cells treated with hyperthermia [1–4] prompted us to investigate the interstitial pH in human tumours. Once we had confirmed that human tumours are indeed more acid than normal tissue [5] we became interested in the effects of hyperthermic treatment upon tumour pH. To this end we attempted the continuous measurement of tumour pH (and subcutaneous control) in patients during treatment with whole-body hyperthermia (WBHT) and both prior to and following treatment with combined local hyperthermia (LHT) and radiotherapy. The determination of tumour pH during local hyperthermia treatment was unfortunately not possible as the high-frequency electromagnetic radiation that is used to induce hyperthermia interferes with electrical monitoring equipment. As a single pH determination requires about an hour to stabilize, measurements before and immediately after a single LHT treatment (which also lasts an hour) would have been impracticable. For this reason determinations were performed prior to and following the treatment series.

MATERIAL AND METHODS

Whole-body hyperthermia

Tissue pH was measured in 8 consenting patients undergoing WBHT. Treatments were given in combination with radiotherapy (19.5–65 Gy) and in one case also with the anti-neoplastic agent 5-fluorouracil (10 mg/kg body wt). WBHT was given within a conventional radiotherapy schedule of 5 fractions per week, fraction size 1.5–2.5 Gy. Hyperthermia was induced in the Pomp–Siemens cabin, the use of which has been described elsewhere [6]. Briefly, the anaesthetized patient was heated to a rectal temperature of 41.8°C by hot air and a hot water circulating mattress on which the patient lay. The 'plateau' temperature was maintained for a period of 2 hr, after which the patient was rapidly cooled. Further details of WBHT treatment are reported by Van der Zee *et al.* [6]. Following induction of anaesthesia one or more pH electrodes were introduced s.c. or into the tumour when this was

Accepted 3 November 1983.
*Supported by the Koningin Wilhelmina Fonds, grant Nos. EUR 77-4, RRTI 83-4.
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accessible, or both. Tissue pH was monitored continuously throughout the course of the treatment.

Local hyperthermia

Determinations of tumour and subcutaneous pH were performed in consenting patients prior to and following a series of LHT and radiotherapy treatment (10–36 Gy). LHT was administered once or twice weekly immediately following radiotherapy using either microwaves (2450 or 433 MHz) or radiofrequency (27 mHz). Hyperthermia treatment typically consisted of 6 sessions of 60 min at the maximum temperature tolerated by the patient (average tumour temperature 42.3°C ± 2.1 S.D.). In as far as it was possible, the pH electrodes were placed in the same positions for pre- and post-treatment determinations in each patient. Patients with no detectable tumour following therapy were excluded from this study. The post-treatment determinations were performed at least 24 hr after the last hyperthermia session.

pH determination

The pH determinations were performed using the specially developed Philips C902 S tissue pH electrode, based on an original design by Stamm *et al.* [7]. The technique used was the same as that described by Van den Berg *et al.* [5]. Briefly, after calibration of the electrode in sterile N.B.S. buffers (pH 6.841 and 7.385, Ingold) the skin was cleaned with 70% alcohol and a small incision made with a sterile scalpel. This was not always necessary in ulcerating tumours. In some patients the skin was numbed prior to incision by the use of a chloroethylene spray, which we have found does not affect the tissue pH. One or more electrodes were carefully inserted into the tissue and then secured to the skin using adhesive tape. In patients undergoing WBHT subcutaneous determinations were performed in the thigh. In those treated with LHT and radiotherapy determinations were performed in the upper arm—in patients with uni-lateral mastectomy in the contra-lateral arm, to avoid possible effects from operatively induced disturbances in blood or lymph circulation. Stabilization required on average about 50 min. After each determination the electrodes were removed and re-calibrated.

RESULTS

Whole-body hyperthermia

A total of 11 tumour and 11 subcutaneous determinations were performed on 12 occasions during treatment with WBHT. In 2 cases tumour and subcutaneous pH were measured simultaneously. On the other occasions one or two determinations of either tumour or subcutaneous pH were performed per patient. A typical trace is shown in Fig. 1. Tumour pH did not change during WBHT treatment (see Table 1). As we were interested in the *paired* differences in tissue pH during the course of treatment, the data have been tabulated as median pH change over the given intervals. This prevents skewing of the data at the points with fewer determinations (tumour pH can vary to a great extent). During heating, plateau or cooling phases of the treatment pH values did not change significantly (Wilcoxon signed rank test), with the exception of subcutaneous pH values, which rose slightly during heating from a central temperature of 39°C to 40°C ($2P < 0.05$). The values over the whole treatment (from 39°C before plateau phase to 40°C after plateau) were also not significantly different. For tumour the median pH values at these two points in time were 7.32 (range 6-71–7.60) and 7.23 (range 6.89–7.51), and the subcutaneous values for

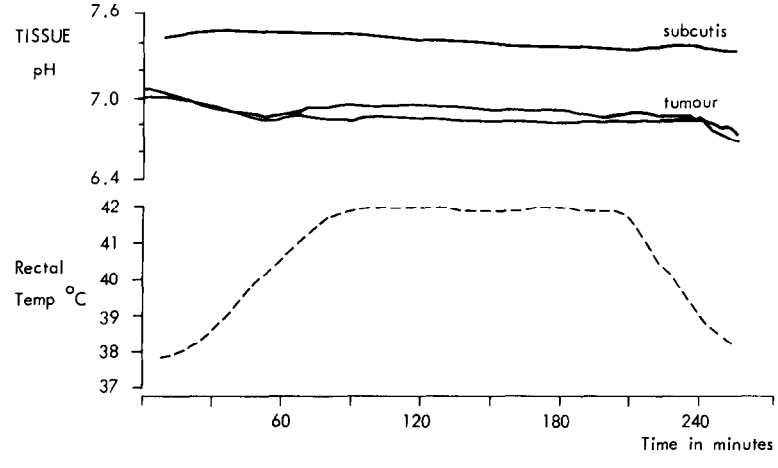

Fig. 1. The course of tumour and subcutis pH as determined in one patient during treatment with whole-body hyperthermia. The lower trace represents the rectal temperature.

Table 1. The median pH changes for all treatments over various treatment intervals for tumour (left) and subcutis (right)

Treatment interval (°C)	Tumour n	Tumour Δ pH	Tumour Range	Subcutis n	Subcutis Δ pH	Subcutis Range
38–39	3 (4)	0.02	−0.05–0.07	3(6)	−0.03	−0.05–0.01
39–40	7(11)	−0.01	−0.07–0.05	7(11)	0.03*	0.00–0.10
40–SP	7(11)	−0.02	−0.15–0.05	7(11)	0.02	−0.09–0.11
SP–MP	7(11)	0	−0.07–0.14	7(11)	−0.03	−0.13–0.05
MP–EP	7(11)	0	−0.06–0.09	7(11)	−0.04	−0.06–0.02
EP–40	5(6)	0	−0.01–0.02	6(9)	0.03	0.00–0.08
40–39	3(4)	−0.01	−0.07–0.03	4(6)	0.02	−0.01–0.05

In cases where 2 simultaneous determinations were performed during one treatment the mean change was used in the calculations. The total number of determinations is given in parentheses. SP = start of plateau phase (41.8°C), MP = mid-plateau (i.e. after 1 hr at 41.8°C), EP = end of plateau phase (i.e. after 2 hr at 41.8°C).
*Significant (Wilcoxon signed rank test, $2P < 0.05$).

the same times were 7.55 (range 7.49–7.63) and 7.53 (range 7.39–7.63) respectively.

Local hyperthermia

Twenty-six paired determinations of tumour pH in 24 tumours (before and after therapy) and 11 paired subcutaneous determinations (in a non-treated area) were performed. A highly significant increase in tumour pH was seen following combined LHT and radiotherapy treatment ($2P < 0.001$, Wilcoxon signed rank test). The pH increased in all but one tumour (see Fig. 2), the mean increase in all 24 tumours being 0.23 (median 0.21) pH units. There was a significant correlation between initial pH values and the pH changes (Spearman rank correlation test, $2P = 0.042$). The changes in subcutaneous values were smaller and showed no significant trend.

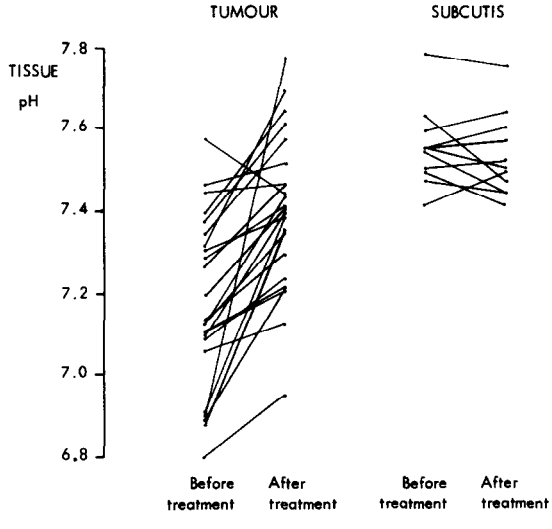

Fig. 2. Tumour pH as measured before and after a series of treatments with combined local hyperthermia and radiotherapy (left) and subcutis controls, in untreated area (right). In cases where more than one determination was performed in a tumour, the average values are shown.

DISCUSSION

Several reports have appeared in the literature indicating that treatment with hyperthermia lowers tumour pH [8–10]. The authors account for these changes by changes in blood flow and tissue oxygenation during the hyperthermic treatment. Indeed, Bicher *et al.* [8] have demonstrated that blood flow in murine tumours increases as tumour temperature increases up to about 41°C and that tissue oxygen tension in tumours increases up to about 41.5°C. Both decline at higher temperatures. It has also been shown that the lactic acid content of tumours is elevated immediately after hyperthermia, and remains so for many hours [11]. During whole-body hyperthermia we saw no changes in tumour pH. The small rise in subcutaneous pH during heating was probably the result of treatment-induced physiological changes induced by raising the body temperature. Tissue pH is a sensitive indicator of the oxygenation status of tissues [12] and the subcutaneous pH followed the same trends during treatment as those followed by the mean pulmonary artery pressure and oxygen consumption values (for further details see Faithfull *et al.* [13]), although the small number of observations precludes statistical analysis.

The lack of change in tumour pH during WBHT could be a question of temperature. Vaupel *et al.* [14] suggest that hyperthermia-induced changes in tumour pH occur only at temperatures of 43°C or higher, and, indeed, Song *et al.* [9], Bicher *et al.* [8] and Vaupel [10] all used temperatures of 43°C or more. Our treatment temperature was 41.8°C. Experiments on Yoshida sarcoma at 42°C showed no change in pH [15], although changes were seen in this tumour following hyperthermia at 44° for 60 min [14]. The fact that many of the patients in this study had very large, necrotic tumours may also have

contributed to the lack of hyperthermia-induced effect on tumour pH. Vaupel *et al.* [14] found that the pH of small tumours was reduced immediately after LHT whilst that of large, necrotic ones remained unchanged.

Interestingly, a significant rise in tumour pH was seen following a treatment series of LHT combined with radiotherapy. Previous experimental work in this field has mostly been directed towards the effect of one hyperthermic treatment session on tumour pH during or directly following treatment, when a pH drop is usually seen [8, 10]. Our investigation was aimed at the long-term effects of hyperthermia treatment following a complete treatment series. Up to 3 weeks may elapse between the first and last LHT sessions. In addition, the post-treatment determinations were performed at least 24 hr after the last LHT treatment. Tumours may well have recovered from direct LHT-induced pH changes by this time. Indeed, Vaupel *et al.* [14] found that 24 hr after LHT the average tumour pH in Yoshida sarcoma was not significantly different from the pre-treatment value. The rise in pH following therapy seen by us is probably a result of improved oxygenation and blood circulation in the tumour as a result of therapy. Such changes have been reported following radiotherapy. Mantyla *et al.* [16] found an average increase in flow from 20.1 to 31.3 ml/100 g/min during the first week of radiation treatment, although this is followed by a gradual decrease in flow. Oxygen tension, however, continues to rise, as demonstrated by Badib and Webster [17], who found increases ranging from 29 to 46% in different tumour groups. Pappova *et al.* [18] found that oxygen tension in human mammary carcinoma rose by more than 50% following radiation therapy. These investigations support our findings as one would expect tumour regression following therapy—with radiotherapy destroying the well-oxygenated, actively metabolizing cells and hyperthermia attacking the hypoxic cells. This presumably leads to reduced lactic acid production and more effective removal of metabolites. Logically, then, the interstitial pH should rise, as does in fact occur. Moreover, tumour regression of varying degrees was seen following treatment in all but one patient (in whom tumour pH also increased after therapy). The observed pH changes did not, however, correlate with the regression rate, radiation dose or heat dose as it is presently derived, but with the initial pH measured in the tumor. The change was thus determined by the tumour rather than by the treatment. This is in accordance with the work of Thomlinson [19]. In studies performed over many years he has observed that tumour regression following therapy is not dependent upon either the dose or nature of the treatment, but is rather a characteristic of the tumour.

The tumours in which the lowest initial pH values were determined were thus the ones showing the greatest changes. There appears to be a trend towards tissue pH 'normalization' following therapy that is most pronounced in tumours with low pH values. Whether this is due to the fact that cells in more acid environments are more sensitive to hyperthermia is not known. The lack of a trend in the subcutaneous pH changes demonstrates that the increase in tumour pH was specific for the treatment area.

It would be interesting to investigate the effects of hyperthermia treatment given alone upon tumour pH. At out clinic, however, this treatment is no longer given alone as the response, when obtained, was only of very short duration. Studies are now in progress to determine the effect of radiotherapy alone upon tissue pH, although this is difficult as radiotherapy at the low doses used in the present study is not often given (in this clinic) without supplementary hyperthermia.

Knowledge of tumour pH, and the fact that it rises following local hyperthermia and radiation therapy, may well have applications in the clinical setting. The action of several antineoplastic agents has been shown to be influenced by pH. Born and Eicholtz-Wirth [20] have shown that both adriamycin and bleomycin are less effective against cells at a low environmental pH. Conversely, thiotepa, 5-fluorouracil and *N*-oxide mustard have been shown to be more effective in acid environments [21–23]. Determination of the tumour pH enables selection of an agent with a pH optimum in that range or, alternatively, the tumour pH could be modified by the application of LHT and radiotherapy to obtain a pH more favourable for the action of the desired drug.

In conclusion, the results of this study indicate that *during* hyperthermic treatment at temperatures below 42°C the pH of human tumours does not change. No additional tumour-sensitizing effect from hyperthermically induced pH reduction can thus be expected during WBHT, which is usually given at 42°C or less. The application of local hyperthermia combined with radiotherapy causes, in the long term, a significant rise in human tumour pH. This may have applications in the treatment of malignant disease.

Acknowledgements—The authors are grateful to Mr R. de Jong for help in designing and constructing the 4 channel pH meter used in this study and to Ms J. van de Gein for typing the manuscript.

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