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The Relationship Between the Unmodified Initial Tissue pH of Human Tumours and the Response to Combined Radiotherapy and Local Hyperthermia Treatment*

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Abstract—*The relationship between unmodified tumour pH before treatment and tumour response was investigated in patients receiving combined radiotherapy and local hyperthermia treatment. Tumour pH showed a statistically significant positive correlation with the response rate (Spearman correlation coefficient* 0.31, n = 50, $2P < 0.05$). *The mean pH of tumours showing a complete response (CR) was significantly higher than that of tumours showing a partial response (PR) as well as those showing no change (NC). As the means of the PR and NC groups were not significantly different from each other, these groups were combined for further analysis. The pH of the CR group was also significantly different from that of the NC + PR group (CR:* 7.36 ± 0.05, *median* 7.36, n = 18, NC + PR: 7.16 ± 0.06, *median* 7.21, n = 32); *Mann–Whitney test*: $2P < 0.05$).

Stratification of the data with respect to radiotherapy dose, hyperthermia dose or tumour volume showed that these factors were not associated with tumour pH to such a degree that they might have seriously biased the results.

The results suggest that enhancement of the cytotoxic effects of hyperthermia by low pH known from experiments with cell cultures is not observed in tumours which are treated with radiotherapy and hyperthermia, and that even the converse may occur. The reasons for this are discussed at length and it is suggested that sudden modification of the tumour pH directly prior to or during treatment is imperative to obtain any sensitizing effect.

INTRODUCTION

IT HAS BEEN KNOWN for a number of years that cells incubated in media at low pH values are sensitized to the cytotoxic effects of hyperthermia. This effect is particularly pronounced at moderate temperatures, i.e. below 43°C, which is the temperature range most frequently applied in clinical hyperthermia (this field has been extensively reviewed by several authors [1–4]).

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It is commonly assumed that the therapeutic effects of hyperthermic treatment may be attributed to the occurrence of a low tissue pH in tumours. It can now be taken as established that the pH of human tumours is low with respect to that of normal tissues or blood [2]. In previous studies we have demonstrated that the average pH of human mammary carcinoma is approx. 0.4 pH units lower than that of the subcutis [5], and furthermore that there was no significant difference between different types of tumours in this respect [6]. Interestingly, the tumour pH appeared to increase very consistently after treatment with a combination of localized hyperthermia and radiotherapy [7]. This indicates that low pH is a characteristic of tumours which may be normalized by therapy. The pH of tumours might thus be an indicator of the success of treatment, and possibly serve as a prognostic parameter. It was the aim of this study to analyse the relation-

ship between tumour pH (as measured directly before the start of a series of combined localized hyperthermia and radiotherapy treatments) and tumour response in a group of patients who were evaluable for response according to WHO criteria.

MATERIALS AND METHODS

pH determinations

Tumour pH determinations were performed using the Philips C902S tissue pH electrode as previously described [5]. Briefly, following sterilization in Cidex solution (Johnson and Johnson, Benelux B.V.) the electrode was calibrated in sterile NBS buffers at pHs 6.841 and 7.385. As this type of electrode has a relatively fragile glass tip it cannot be used to puncture the skin. It was therefore necessary to make a small incision in the skin into which the electrode could be carefully inserted. In some cases the skin was numbed prior to incision using a chloroethylene spray, which we have found does not affect the tissue pH. The electrode was then secured to the skin using adhesive tape. pH was monitored continuously until a stable reading was obtained. The electrodes were recalibrated immediately after removal from the tumour. All measurements were performed prior to the first treatment session.

Patients and tumours

The patient group consisted of 49 patients who were referred for treatment with local hyperthermia and who gave consent after having been fully informed about the experimental nature of the measurements. Selection was based on the technical feasibility of positioning the patient as well as the electrode such that the electrode could be kept in place undisturbed for at least 1 h. Only those patients who were evaluable for response according to WHO criteria (complete response, partial response or no change) were included in this report. The tumours were mostly recurrences of mammary carcinoma (41/50). In one patient, two different lesions were measured, which occurred with an interval of 6 months at different sites.

Local hyperthermia and radiotherapy treatment

The combined treatment consisted of 2–5 weekly fractions of radiation up to a total dose ranging from 18 to 60 Gy (26 of the tumours receiving 24 Gy), and 3–13 hyperthermia treatments given twice weekly following radiotherapy with a mean time interval of 40 min. Hyperthermia was induced using 27, 433 or 2450 MHz radiation or a combination of two of these frequencies, depending on tumour dimensions and depth. Technical details have been published in a previous paper [8]. A typical hyperthermia treatment consisted of 60 min (generator time) up to the maximum tolerated temperature, which was either indicated by the patient or by a temperature measurement within normal tissue exceeding 43°C. The calculation of heat dose has been previously described in great detail [8]. In this paper two parameters were used, *viz.* EQT43min and EQT43mean, being the sums of the minimum and the mean equivalent times at 43°C observed in the tumour during the whole treatment series. The concept of equivalent time is based on an empirical relationship between temperature and exposure time at a certain temperature to an exposure time at a standard temperature, usually 43°C. In formula: $EQT43 = \Sigma \triangle t.b^{(43-T)}$, in which T = treatment temperature, t = time interval during which this (constant) temperature was measured and b = an experimentally determined constant which is 0.17 at temperatures below 43°C and 0.5 above 43°C (means of values taken from a review by Field and Morris [9]).

RESULTS

A descriptive summary of the pH data observed is presented in Table 1. The pH values of tumours which showed a complete response (CR) were significantly higher than those showing a partial response (PR) or no change (NC) ($2P < 0.05$, Mann–Whitney test). Since the PR group did not differ significantly from the NC group it was logical to combine these groups. The difference between the means was mainly caused by a single low value observed in the NC group. The pH values in the combined NC + PR group were significantly lower than those in the CR group ($2P > 0.05$).

The cumulative distribution of pH values in these two groups clearly show a shift towards higher pH values with increasing response (Fig. 1). However, this on its own does not prove that tumours with a high pH will show a better response. Since factors such as tumour volume, radiotherapy dose and hyperthermia dose were not kept constant in this study, their possible influence on the tumour response should also be considered.

The influence of hyperthermia dose is difficult to assess, since a generally accepted definition of heat dose is not available. In a previous study we have demonstrated that definitions of heat dose (equivalent time, degree-minutes, maximum or minimum temperature) relating to the coldest spot in the tumour showed the best correlation with the response rate. In this study the equivalent time at 43°C was taken as basic dose definition. The mean tumour dose (EQT43mean), as well as the dose at the coldest spot (EQT43min), was calculated, since EQT43min may be sensitive to a wide variation in the number of measurement sites. Due to recent improvements of the temperature measurement system temperatures were measured at a considerably larger number of sites in the last few patients. This

Table 1. *Summary description of tumour pH values grouped according to clinical tumour response*

	N	Mean	S.D.	S.E.M.	Min	Median	Max
No change (NC)	8	7.06	0.48	0.17	5.98	7.20	7.46
Partial response (PR)	24	7.19	0.28	0.06	6.48	7.22	7.65
Subtotal (NC + PR)	32	7.16	0.33	0.06	5.98	7.21	7.65
Complete response (CR)	18	7.36	0.22	0.05	6.90	7.36	7.87
Total (NC + PR + CR)	50	7.23	0.31	0.04	5.98	7.21	7.65

CR was significantly different from both NC and PR as well as NC + PR (Mann–Whitney test, $2P < 0.05$).

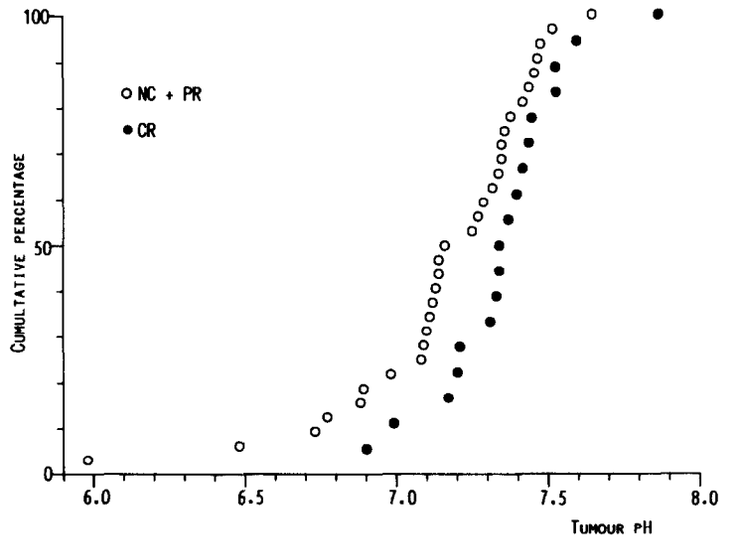

Fig. 1. *Cumulative distributions of initial pH values in tumours showing complete response (CR) vs. partial response (PR) or no change (NC) after treatment with combined radiotherapy and local hypothermia.*

increase in number of sites raises the probability of measuring a low temperature at some spot in the tumour.

The correlations between the tumour response and each separate factor are shown in Table 2. A significant correlation between tumour pH and response is apparent ($2P > 0.05$). The correlations of the hyperthermia dose parameters with the response were not much different, and in neither case statistically significant (Table 2), although they pointed in the expected direction. Tumour volume also did not correlate significantly with response. Furthermore this table shows a significant effect of the radiotherapy total dose ($P < 0.05$) on the response. If the radiotherapy dose was, by chance, positively associated with the observed pH values, then this could account for the observed effect of tumour pH upon response. A similar reasoning applies to the other factors, even if the correlations of these factors with response are not statistically significant on their own.

Table 2. *Spearman rank correlation coefficients of tumour response with tumour and treatment parameters* (n = 50)

Tumour pH	0.31	($2P < 0.05$)
Tumour volume	−0.10	
Radiotherapy dose	0.26	($P < 0.05$)
EQT43min	0.14	
EQT43mean	0.17	

In order to assess the influence of these factors simultaneously one should ideally use a multivariate technique, e.g. a logistic regression analysis. In this case, however, the number of data is rather small with respect to the number of factors, so that a more qualitative approach is more appropriate. The pH values were, therefore, divided into three arbitrary classes (low pH: pH $<$ 7.20, medium pH: 7.20 $\leq$ pH $<$ 7.40 and high pH: pH $\geq$ 7.40), whereas response and the other factors were each divided into two classes: high and low response (CR and NC + PR), small and large tumour volume

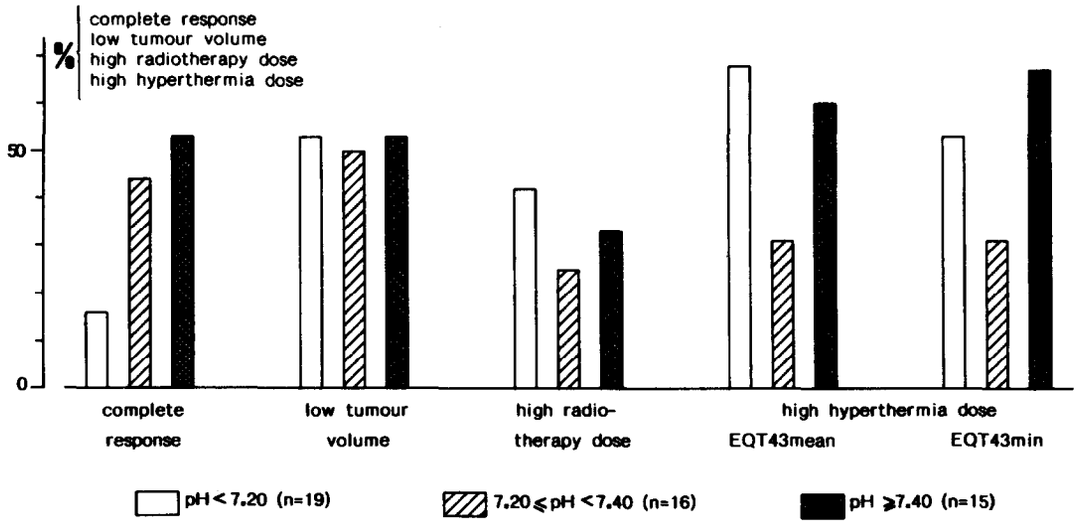

Fig. 2. Comparison of the complete response rate with other tumour or treatment parameters grouped according to initial tumour pH. Parameters were each divided into two categories, high and low. The percentages of the categories which may be expected to positively influence the complete response rate are depicted, i.e. low tumour volume (<20 cm^3, n = 24), *high radiotherapy dose* (>24 Gy, n = 17), *and high hyperthermia dose* (*EQT43mean* > 120 min, n = 27 *or EQT43min* > 10 min, n = 25). *A matching pattern of the CR data with one of the parameters shown would indicate that a correlation between tumour response and tumour pH may be due to a correlation between tumour pH and one of the parameters.*

(cutpoint 20 cm^3), low and high radiotherapy dose (cutpoint 24 Gy) and low and high hyperthermia dose (EQT43min, cutpoint 10 min; EQT43mean, cutpoint 120 min). It would be logical to assume that the complete response rate would be greater with low tumour volume, high radiotherapy dose or high hyperthermia dose. Therefore the percentages of complete responses were compared with those of low tumour volume, high radiotherapy dose and high hyperthermia dose for each of the classes of pH values and presented in a bar diagram (Fig. 2). The diagram shows a 16% complete response rate in the low pH group, whereas this was 53% in the high pH group. If the percentages for any of the response modifying factors (low volume or high radiotherapy or hyperthermia dose) were to show a similar trend, then this factor could be responsible for the observed increase in response rate. On the other hand, a decreasing trend would indicate that the true pH effect may be even stronger.

The percentages of small tumours in the low and high pH groups were equal (53%), so that the effect of pH cannot be explained by differing tumour size. The percentage of high radiotherapy doses was 33% in the high pH group, whereas it was 42% in the low pH group. This indicates that a difference in radiotherapy dose cannot account for the different response rates in the high and low pH range. The proportion of high hyperthermia doses (EQT43min) was higher in the high pH group (67%) as compared with the low pH group (53%), but this difference was so small that it cannot account for the observed difference in complete response rates (53% vs. 16%, respectively). For EQT43mean these values were 60% and 68%, respectively. It thus appears that none of the factors can be associated with tumour pH to such a degree that it might introduce a spurious relationship or mask a true relationship between pH and response rate.

DISCUSSION

The generally made assumption that an increase in acidity of tumour tissue enhances the therapeutic effect of hyperthermic treatment is based on the results of *in vitro* investigations using cell cultures [1–4]. To our knowledge there have been no such studies involving experimental or human tumours *in situ*. We have been investigating the pH of human tumours for several years in order to assess whether this characteristic could be useful as a prognostic parameter in the clinic. The average tissue pH of human tumours was found to be significantly lower than that of normal tissue [5], and to be normalized after treatment [7]. On the other hand, the wide scattering of individual pH values and the apparent lack of correlation with other tumour characteristics seemed to diminish the usefulness of tumour pH as a prognostic factor [6]. However, its relationship with tumour response had yet to be established. In this study we retrospectively analysed the available data of those patients who received a full course of combined hyperthermia and radiotherapy treatments and who were evaluable for response according to the WHO criteria.

The results do not indicate that acidic tumours show a better response to treatment, but rather that

the converse seems to be true. In the first place it should be emphasized that the distributions of tumour pH in the response categories CR and PR + NC show a considerable overlap (Fig. 1). Nevertheless the difference between the means appeared to be statistically significant (Table 1). This is an unexpected result, which requires close examination. The inherent heterogeneity of tumour tissue as well as several possible sources of bias in tumour and treatment might cause the expected positive correlation between low tumour pH and tumour response to be obscured or even reversed. Such bias would be induced by unequal distribution of factors which may be assumed to influence tumour response, such as tumour type, tumour volume or treatment dose.

Tumour tissue may be considered heterogeneous in several respects, one of these being the degree of oxygenation, which may vary due to local variations in perfusion. Since hypoxia leads to acid metabolite production via glycolytic energy metabolism, regional differences in tissue pH may be expected. Such heterogeneity has been demonstrated in experimental animals by measuring pH as well as oxygen tension using microelectrodes. Most of the published data on the pH distribution within tumours show cumulative data of more than one tumour, but some reports indicate that the range within a single tumour may be 0.3 [10] to 0.6 [11] pH units when measured by microelectrodes with tip diameters of 10 and 1 μm, respectively. The maximum ranges we have observed using a much more robust electrode were not higher than 0.4 pH units. Although our technique tends to average out variations at the microscale, the remaining heterogeneity will introduce some scattering in the data when single point pH values are compared with other tumour parameters. It should be noted, however, that we found the variance between tumours to be about twice that observed within tumours [6]. Thus, despite the heterogeneity, it should be possible to demonstrate certain relationships involving tumour pH, as we in fact have shown in previous studies.

The results of this study would be biased if factors which influence the response rate were unequally distributed with respect to tumour pH. Tumour type was not expected to be a confounding factor, since in previous work no differences in tumour pH or response with respect to tumour type have been observed [6, 8] (moreover, in this study the tumours investigated were mainly mammary carcinomas, 41/50). It has been reported that in experimental tumours the pH is negatively correlated with tumour volume [12]. Small tumours, which as such may be expected to show a favourable response to radiotherapy, might therefore have a relatively high pH. Furthermore, any fortuitous correlation between radiotherapy dose or hyperthermia dose with tumor pH might have influenced the result (it was not possible to study the effects of pH on hyperthermic treatment alone, since such treatment is not usually given as a monotherapy). Careful analysis, however, failed to show any sign of bias which could have been the cause of a reversal of the expected positive effect of low pH on the response rate (Table 2 and Fig. 2). The radiotherapy dose, which showed a significant effect upon the response rate, was even relatively high in the low pH group.

Despite the inevitable uncertainties in the interpretation of the results of this study it may, therefore, be concluded that it seems very unlikely that the tumour response is enhanced by low tumor pH, i.e. the unmodified pH in the tumour before combined treatment. Since many *in vitro* studies have suggested that such an enhancement occurs the question arises as to why this discrepancy was observed. The tumour pH values observed were generally higher than those shown to be most effective in *in vitro* studies. In fact, only about 40% of all reported pH values in human tumours are below 7.0, and only 10% are below 6.7 [2]. This would suggest that unmodified tumour pH is generally too high to detectably amplify the therapeutic effect of hyperthermia. The apparently increased response rate at high pH may reflect the influence of better tissue oxygenation on the cell killing effect of radiation, since low pH may be expected to be accompanied by low oxygen pressure to a certain extent [11] and the radiotherapy dose was not positively correlated with tumour pH.

A different and interesting explanation can be based on recent experiments by Hahn and Shiu [13], which indicated that tumour cells have a remarkable ability to adapt to low environmental pH *in vitro*. This results in a diminished susceptibility to hyperthermia, a maximum degree of adaptation being achieved after approx. 100 h of incubation at low pH prior to heat treatment. In the usual *in vitro* experiments incubation times are much shorter and are assumed to have no influence on the pH-dependent hyperthemia effects studied [14]. If tumour cells *in vivo* are adapted to their environment it may be necessary to lower the tumour pH before or during heat treatment in order to achieve maximum cell kill.

It has been shown in experimental tumours that hyperthermia itself can lower the tumour pH at sufficiently high temperatures and sufficiently long treatment times (more than 1 h at or above 42.5–43°C) [4]. Furthermore tumour pH may be lowered by administering glucose in order to intensify the production of acid metabolites. The clinical application of this well known concept was originally proposed by Von Ardenne [15]. Recently Thistlethwaite *et al.* [16] presented a few cases

showing that such a procedure may be feasible and successful in humans. A pH drop induced in this way might show a better correlation with tumour response than the unmodified pH before treatment. Preliminary data show that tumour pH decreases can be observed in humans during microwave induced hyperthermia by continuous monitoring using a fibre optic pH probe.*

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