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Oxygen tension and vascular density in human cervix carcinoma

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Summary Hypoxia-induced radiation resistance has been proposed to be a consequence of low vascular density in tumours. The purpose of the study reported here was to investigate possible relationships between pretreatment oxygen tension (pO_2) and vascular density in patients with cervix carcinoma. Tumour pO_2 was measured by the use of polarographic needle electrodes. Biopsies were taken from the electrode tracks and vascular density and tissue composition, i.e. volume fraction of carcinoma tissue, stroma and necrosis, were determined by stereological analysis. The vascular density of individual biopsies was related to the median pO_2 of the corresponding electrode track. Tumour regions with vascular density below 24 mm mm^{-3} always showed low pO_2, whereas tumour areas with vascular density above 24 mm mm^{-3} could show a high or a low pO_2. This indicates the existence of a threshold value of about 24 mm mm^{-3} for vascular density in cervix carcinoma; a vascular density above this value is probably needed before high pO_2 can occur. Low vascular density might, therefore, be a useful predictor of hypoxia-induced radiation resistance in cervix carcinoma. High vascular density, on the other hand, can probably not be used to exclude radiation resistance. The differences in pO_2 among tumour regions with high vascular density were not a consequence of differences in the amount of necrosis or stroma or in the haemoglobin concentration in peripheral blood of the patients. Model calculations indicated that these differences in pO_2 could be explained by differences in the oxygen delivery alone and by differences in the oxygen consumption rate alone.

Keywords: cervix carcinoma; oxygen tension; vascular density; radiotherapy; predictive assay

Clinical studies have indicated that oxygen concentration is of major importance for the response to radiotherapy of some histological types of tumours, e.g. cervix carcinoma (Bergsjø and Kolstad, 1968; Dische *et al*., 1983; Révész and Balmukhanov, 1987; Höckel *et al*., 1993), head and neck carcinoma (Glassburn *et al*., 1977; Henk *et al*., 1977; Gatenby *et al*., 1988; Okunieff *et al*., 1995) and breast carcinoma (Okunieff *et al*., 1993). Streffer *et al*. (1989) have suggested that hypoxia-induced radiation resistance can be attributed to low vascular density in tumours. Thus, studies of the radiation response of cervix carcinoma have shown that high local recurrence rate is related to large intercapillary distances (Kolstad, 1968; Awwad *et al*., 1986). Moreover, a positive correlation between vascular density and survival time after radiotherapy has been reported for cervix carcinoma (Siracká *et al*., 1982, 1988; Révész *et al*., 1989) and nasopharyngeal carcinoma (Delides *et al*., 1988). A similar study on oral squamous cell carcinoma, however, showed no such relationship (Lauk *et al*., 1989). Studies of experimental tumours have indicated that factors other than vascular density are also important for hypoxia-induced radiation resistance, e.g. blood flow, haemoglobin concentration, oxyhaemoglobin (HbO_2) saturation and rate of oxygen consumption (for review, see Coleman, 1988; Stone *et al*., 1993; Horsman, 1995). The importance of vascular density for the development of hypoxia in tumours is, therefore, unsettled.

Studies are performed to develop clinically useful methods for (1) prediction of radiation resistance caused by hypoxia and (2) improving tumour oxygenation (for review, see Hirst, 1986; Moonen *et al*., 1990; Stone *et al*., 1993). Several strategies based on different principles are used for these purposes. Identification of the most important biological factors leading to the development of hypoxia in tumours would be of considerable help in choosing the most appropriate strategies. Tumour oxygen tension (pO_2) is currently measured in patients with cancer of the uterine cervix at The Norwegian Radium Hospital. One aim of the project is to identify biological factors influencing tumour pO_2. In the present work, relationships between pO_2 and vascular density measured before the start of treatment are reported. The pO_2 in individual electrode tracks was related to the vascular density in biopsies taken from the tracks immediately after the pO_2 measurement.

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Materials and methods

Patients

Patients with carcinoma of the uterine cervix (stage Ib, IIb or IIIb, according to the FIGO) and a histological diagnosis of squamous cell carcinoma were included in the study. The largest tumour diameter was 2 cm or more. The study was approved by the local ethical committee, and informed consent was obtained from all patients.

Measurement of pO_2

Measurement of pO_2 was performed before the start of radiotherapy using polarographic needle electrodes with a shaft diameter of 300 μm (Eppendorf pO_2 histograph 6650) (Lyng *et al*., 1995). A venflon (20G) was used whenever the tumour was surrounded by connective tissue to guide the oxygen electrode into the tumour tissue. The electrode was moved automatically through the tissue in preset steps of 1 mm. Each forward step was followed by a backward step of 0.3 mm, leading to a distance of 0.7 mm between each pO_2 reading. Measurements were performed in four to six different tracks in each tumour. The tracks were located in tumour periphery and centre and were directed perpendicular to the tumour surface (Lyng *et al*., 1995). The length of each track was determined from the size of the tumour, measured from pretreatment magnetic resonance (MR) images. A total of 100-220 measurements was performed in each tumour.

Heart rate, arterial blood pressure, arterial HbO_2 saturation and rectal temperature were recorded throughout all measurements, which were performed under general anaesthesia (Propofol, i.v.). This anaesthetic does not modify body temperature or tumour pO_2 in cervix cancer patients significantly. The haemoglobin concentration in peripheral blood was determined the day before or the same day as the pO_2 measurements were performed.

Histological examination

A needle biopsy, 1 × 18 mm in size, was taken from each measurement track, leading to four to six biopsies per tumour. The biopsies were fixed in phosphate-buffered 4% paraformaldehyde, embedded in paraffin casts and cut in the length direction to 5-μm-thick sections. The sections were stained with haematoxylin and eosin and subjected to stereological analysis using a projecting light microscope and a counting frame, 20 × 20 cm in size. Tissue composition and vascular density were determined for each biopsy. Three different types of tissue were found in the biopsies, i.e. carcinoma tissue, stroma and necrosis. The volume fraction of each tissue type was determined by point counting, using a magnification of 160× (Weibel, 1979). Blood vessels were identified as a lumen encircled by either a thick vessel wall or a lining of endothelial cells. The vessels were classified according to their diameter and the type of tissue in which they were found, using a magnification of 410×. Vascular density, i.e. total vessel length, total vessel surface and total vessel volume per unit tissue volume, was quantified as an average value for each biopsy by stereological calculations as described previously (Lyng *et al.*, 1991).

Analysis of pO_2 *vs vascular density*

The pO_2 values measured in an electrode track were analysed *vs* the vascular density in the biopsy taken from that track. To compare pO_2 and vascular density at the microregional level using this approach, the biopsies should be taken exactly on the electrode track. This was aimed at by taking the biopsy immediately after the oxygen electrode was withdrawn from a track, i.e. the biopsy was taken before the subsequent pO_2 track in the tumour was recorded. This procedure facilitated the positioning of the biopsy needle in the electrode track.

Measurement tracks that were homogeneous in pO_2, i.e. tracks in which none of the pO_2 readings deviated from the median pO_2 by more than 50%, were selected for the analysis. The experimental procedure used here is not suitable for analysis of pO_2 *vs* vascular density at the microregional level in heterogeneous tumour regions, because minor differences in location between electrode track and biopsy might introduce large errors.

Statistical analysis

Statistically significant correlation between median pO_2 and vascular density was searched for by linear regression analysis. An analysis of variance was applied to investigate whether tissue composition and haemoglobin concentration differed significantly among groups of tumours, and a Student–Newman-Keuls test was applied to identify the groups that differed from each other. Ratios of intratumour to intertumour heterogeneity in pO_2 and vascular density were calculated using the exploratory method described by Brizel *et al.* (1995), making no assumptions regarding relationships between median values and variances. A significance level of $P = 0.05$ was used throughout.

Results

The tumours showed heterogeneous pO_2 distributions. Heterogeneity in pO_2 was also observed within most individual tracks. A coarse mapping of the vascular density along electrode tracks revealing clear pO_2 gradients, suggested that pO_2 was related to vascular density along a track.

Twenty-four tracks in eight tumours were homogeneous in pO_2, according to the criterion described above, and these pO_2 tracks and the corresponding biopsies were subjected to detailed quantitative analysis. Median pO_2 is plotted *vs* vascular density (total vessel length per unit tissue volume) in Figure 1. Tumour regions with a low vascular density (total vessel length per unit tissue volume < 24 mm mm^{-3}) always showed a low median pO_2, whereas tumour areas with a high vascular density (total vessel length per unit tissue volume > 24 mm mm^{-3}) could show a low or a high median pO_2 (Figure 1). There was no correlation between median pO_2 and total vessel length per unit tissue volume for tumour regions with high vascular density ($P = 0.17$). The ratio of intratumour to intertumour heterogeneity was 1.02 for pO_2 and 1.33 for vascular density, i.e. the intratumour heterogeneity was larger than the intertumour heterogeneity, justifying the analysis in Figure 1. Qualitatively similar data

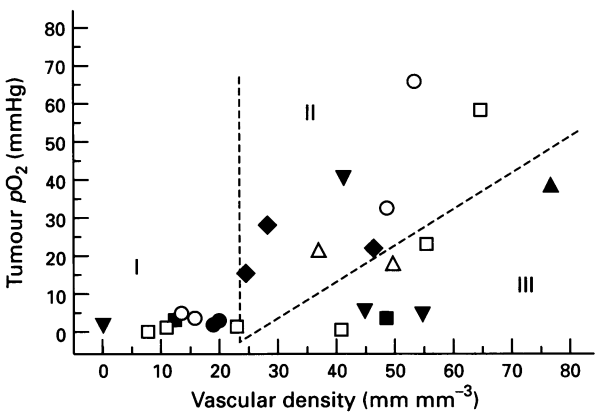

Figure 1 Tumour pO_2 *vs* vascular density for human cervix carcinoma ($P = 0.17$ for vascular density > 24 mm mm^{-3}). The pO_2 values represent median values of pO_2 distributions measured in single electrode tracks. The values for vascular density represent average values for single biopsies and refer to total vessel lengths per unit tissue volume in biopsies taken from the pO_2 tracks. Each point thus represents data from individual pO_2 measurement tracks and the corresponding biopsy. Points of similar symbols refer to the same tumour.

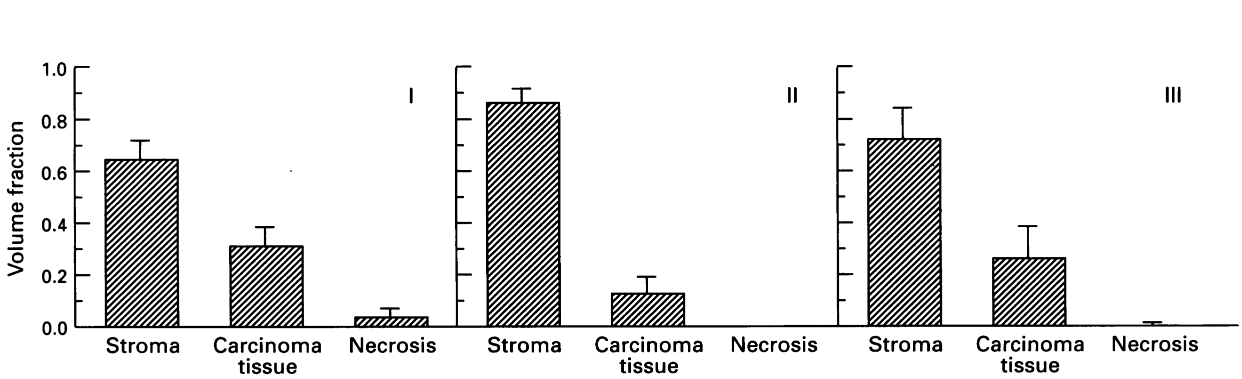

Figure 2 Volume fraction of stroma, carcinoma tissue and necrosis in biopsies from human cervix carcinoma. Each column represents the mean value for the tumour regions depicted in Figure 1 (groups I, II and III). Standard errors are marked.

were achieved when mean or mode pO_2 was considered and when total vessel surface or total vessel volume was considered (data not shown).

Possible explanations of the data in Figure 1 were searched for by dividing the data into three groups, i.e. one group with low vascular density and low pO_2 (I), one group with high vascular density and relatively high pO_2 (II) and one group with high vascular density and relatively low pO_2 (III). The tissue composition of the biopsies in the three groups is shown in Figure 2. All groups had a considerable amount of stroma and carcinoma tissue. Only a few biopsies contained necrosis and all except one were in group I. The volume fraction of stroma was lower for group I than for group II ($P<0.05$). Other significant differences in the tissue composition were not found. There was no significant difference in haemoglobin concentration in peripheral blood among the three groups (data not shown).

Similar analysis was also performed after having divided the data in Figure 1 into groups in two other ways: (1) two groups, one for tumour regions with total vessel length per unit tissue volume <24 mm mm^{-3} and the other for tumour regions with total vessel length per unit tissue volume >24 mm mm^{-3}; and (2) three groups, one for tumour regions with total vessel length per unit tissue volume <24 mm mm^{-3}, a second for tumour regions with total vessel length per unit tissue volume >24 mm mm^{-3} and median pO_2 >10 mmHg and a third for tumour regions with total vessel length per unit tissue volume >24 mm mm^{-3} and median pO_2 <10 mmHg. The latter grouping was justified by the observation of Höckel *et al.* (1993) that the survival rate differs between patients with tumours showing a median pO_2 below and above 10 mmHg. Significant differences in tissue composition or haemoglobin concentration among groups were not found, irrespective of the way of grouping.

Discussion

The tumour regions in group I had low vascular density and low pO_2. Moreover, these regions had more necrosis and less stroma than the regions in group II. The pO_2 is generally low in necrosis and poorly vascularised stroma of cervix carcinoma, whereas well-vascularised stroma generally has high pO_2 (Lyng *et al.*, 1995). The low pO_2 in group I was, therefore, consistent with the histological observations. The observation that all poorly vascularised tumour regions had low pO_2 indicates the existence of a threshold value of about 24 mm mm^{-3} for vascular density in cervix carcinoma; a vascular density above this value is probably needed before high pO_2 can occur.

The tumour regions in group II had high pO_2 compared with the tumour regions in group III, although the vascular density was similar for the two groups. The two groups showed no difference in tissue composition. The difference in pO_2 between the two groups was, therefore, not caused by the presence of more necrosis or less stroma in group III than in group II. Moreover, the difference in pO_2 was not a consequence of a difference in haemoglobin concentration in peripheral blood either. This conclusion is consistent with studies which have shown that large differences in haemoglobin concentration may exist between individual capillaries in tumours, independent of the haemoglobin concentration in the supplying vessels (Brizel *et al.*, 1993). Differences in factors other than tissue composition and haemoglobin concentration in peripheral blood may, therefore, have caused the difference in pO_2 between groups II and III. The oxygen tension in tumours is determined by the balance between oxygen delivery and oxygen consumption.

In addition to the vascular density, oxygen delivery depends on the erythrocyte flux, i.e. the number of erythrocytes passing through the vessels during a defined time interval, and the HbO_2 saturation of the erythrocytes (Vaupel, 1990). Both erythrocyte flux and HbO_2 saturation may differ considerably among tumour regions with similar vascular density. Brizel *et al.* (1993) found that the erythrocyte flux in neighbouring tumour capillaries could differ by more than a factor of four. Temporal fluctuations and cessation of the erythrocyte flux have also been reported (Chaplin and Hill, 1995). The HbO_2 saturation of the erythrocytes decreases as the cells move from the arterial to the venous side of the capillary network, leading to significant differences in HbO_2 saturation within a single capillary. The large differences in erythrocyte flux and HbO_2 saturation that exist within tumours, independent of the vascular density, may lead to large differences in intracapillary pO_2, i.e. intracapillary pO_2 may range from zero mmHg to the pO_2 of arterial blood, which is about 90 mmHg (Vaupel, 1993). In the present work, tumour pO_2 ranged from 0.5 mmHg in group III to 41 mmHg in group II at a vascular density of 40 mm mm^{-3}, and from 5 mmHg in group III to 66 mmHg in group II at a vascular density of 55 mm mm^{-3}. These pO_2 values are within the range of variation for intracapillary pO_2 in tumours. The difference in pO_2 between groups II and III observed at similar vascular density can, therefore, be explained by a difference in the oxygen delivery alone. This observation suggests that vascular density is not a representative measure of the functional efficiency of oxygen and nutritive supply.

The oxygen consumption rate of the tumour cells may have a significant influence on the oxygen tension in tumour tissue (Secomb *et al.*, 1995). Considerable differences in oxygen consumption rate exist among tumours; oxygen consumption rates in the range from 2.0 μl oxygen g^{-1} tissue min^{-1} to 40 μl oxygen g^{-1} tissue min^{-1} have been reported for tumours in humans (Vaupel *et al.*, 1989). The influence of the oxygen consumption rate on oxygen tension in tumours can be estimated by the use of a simple one-dimensional model describing the transport of oxygen from a single capillary into the tissue (Appendix). Calculations based on this model show that the differences in pO_2 observed at similar vascular densities in the present work can occur among tumour regions differing only in oxygen consumption rate, i.e. among tumour regions located at the same distance from capillaries with similar intracapillary pO_2. For example, oxygen tensions ranging from 0.5 mmHg to 41 mmHg, which were observed at a vascular density of 40 mm mm^{-3}, can occur halfway between two capillaries with an intracapillary

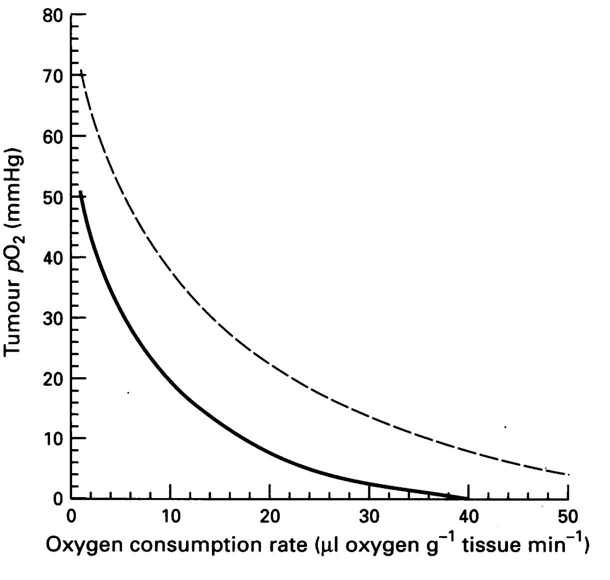

Figure 3 Tumour pO_2 halfway between two vessels *vs* oxygen consumption rate. The pO_2 values were calculated from equation (2) in the Appendix.——, vascular density (VD) is 40 mm mm^{-3} and intracapillary pO_2 (P_{cap}) is 70 mmHG. - - -, vascular density is 55 mm mm^{-3} and intracapillary pO_2 is 90 mmHg.

pO_2 of 70 mmHg, if the oxygen consumption rate ranges from 2.0 μl oxygen g^{-1} tissue min^{-1} to 37 μl oxygen g^{-1} tissue min^{-1} (Figure 3). Moreover, oxygen tensions ranging from 5 mmHg to 66 mmHg, which were observed at a vascular density of 55 mm mm^{-3}, can occur halfway between two capillaries with an intracapillary pO_2 of 90 mmHg for approximately the same range of the oxygen consumption rate (Figure 3). The estimated values for oxygen consumption rate are within the range of those reported elsewhere (Vaupel *et al.*, 1989). Although a simple model was used here, the calculations make it plausible that the difference in pO_2 between groups II and III might also be explained by a difference in the oxygen consumption rate alone.

The present results may have some implications for the use of vascular density to predict hypoxia-induced treatment resistance of cervix tumours. First, it was found that tumour regions with vascular densities below a threshold value of about 24 mm mm^{-3} always had low oxygen tensions, indicating that low vascular density might be a good predictor of tumour resistance to radiotherapy. Second, tumour regions with vascular densities above 24 mm mm^{-3} could show low or high oxygen tensions, indicating that vascular density is not useful for predictive purposes in well-vascularised tumours. The apparent discrepancy between our results and those from earlier studies on cervix carinoma (Kolstad, 1968; Awwad *et al.*, 1986) can probably be explained by differences in the data analysis. In the present work, a direct comparison of oxygen tension and vascular density measured in the same tumour regions was performed. In the other studies, however, mean values for vascular density were related to mean values for local recurrence rate for groups of patients. An analysis of our data, based on mean pO_2 values for tumour regions with low, median and high vascular density, showed a correlation between pO_2 and vascular density in agreement with the results reported earlier (data not shown). However, the use of vascular density as a predictive parameter necessitates a correlation between vascular density and oxygen tension based on individual tumours.

The present work may also have some implications for the choice of strategy for improving the oxygenation of cervix tumours. It was found that the low oxygen tension in some of the well-vascularised tumour regions could be explained by inadequate oxygen delivery alone and also by high oxygen consumption rates alone. Low oxygen tension can, therefore, be a consequence of low oxygen delivery in some tumours and high oxygen consumption rates in others. A method to distinguish tumours with low oxygen delivery from tumours with high oxygen consumption rates would, therefore, be useful for choosing strategy for improving tumour oxygenation. However, because a method for this purpose is not available yet, a combined strategy including both an increase in the oxygen delivery and a decrease in the oxygen consumption rate is probably needed to achieve satisfactory improvement of oxygenation in cervix carcinoma.

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Appendix

The oxygen transport from a capillary into tumour tissue can be described in one dimension by:

$$P(x) = \frac{M}{2D\alpha}x^2 + \mathrm{A}x + \mathrm{B} \tag{1}$$

where P is tissue $p\mathrm{O}_2$, x is the distance from the capillary, M is the oxygen consumption rate, D is the diffusion coefficient and α is the solubility coefficient of oxygen in tissue, and A and B are constants depending on the boundary conditions (Dewhirst *et al.*, 1994). Equation (1) assumes that the oxygen diffusion occurs only in a plane through the capillaries and in the direction perpendicular to the vessels. Oxygen diffusion into tissue above and below the plane of the capillaries or diffusion resulting from gradients in $p\mathrm{O}_2$ in the direction parallel to the vessels is not included in the equation. However, $p\mathrm{O}_2$ values estimated from equation (1) correlate well with measured values (Dewhirst *et al.*, 1994).

Intracapillary $p\mathrm{O}_2$ may be used as a measure of tumour $p\mathrm{O}_2$ close to capillaries (Dewhirst *et al.*, 1992). The $p\mathrm{O}_2$ profile can be calculated by assuming that the derivative of $P(x)$ is zero when $P(x)$ is zero (Tannock, 1972) and that the oxygen consumption rate is constant:

$$P(x) = \frac{M}{2D\alpha}x^2 - \sqrt{\frac{2MP_{\mathrm{cap}}}{D\alpha}}x + P_{\mathrm{cap}} \tag{2}$$

where P_{cap} is intracapillary $p\mathrm{O}_2$.

A value of 2.0 10^{-5} $\mathrm{cm}^2\ \mathrm{s}^{-1}$ and 3.3 10^{-5} cm^3 oxygen cm^{-3} tissue mmHg^{-1} was used for D and α respectively (Degner and Sutherland, 1988). $P(x)$ halfway between two vessels was calculated for x values of 95 μm and 112 μm, corresponding to a vascular density of approximately 55 mm mm^{-3} and 40 mm mm^{-3} respectively.

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