Oxygen Tension and Vascular Density in Adenocarcinoma and Squamous Cell Carcinoma of the Uterine Cervix

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Acta Oncologica Vol. 37, No. 7/8, pp. 665–670, 1998

The prognosis of patients with carcinoma of the uterine cervix has been shown to depend on the oxygenation and vascularization status of the tumors. The purpose of the study reported here was to search for possible differences in oxygen tension and vascular density between adenocarcinomas and squamous cell carcinomas. Ten patients with adenocarcinoma and forty patients with squamous cell carcinoma were included in the study. Oxygen tension was measured polarographically using the Eppendorf pO_2 Histograph 6650. Vascular density was determined by histological examination of tumor biopsies. The adenocarcinomas were significantly better oxygenated than the squamous cell carcinomas. The squamous cell carcinomas and the adenocarcinomas did not differ significantly in vascular density. The difference in prognosis between patients with adenocarcinoma and patients with squamous cell carcinoma is probably not attributable to differences in tumor oxygenation or vascularization.

Received 31 March 1998
Accepted 28 September 1998

The prognosis of patients with carcinoma of the uterine cervix has been shown to depend on the tumor oxygenation status (1). The oxygen tension of cervical carcinomas is generally low compared with that of the normal cervix (2, 3) and differs substantially within and among individual tumors (4–6). The outcome of treatment is influenced by the oxygen tension of the primary tumor; patients with poorly oxygenated tumors show lower recurrence-free and overall survival probabilities than patients with well-oxygenated tumors (7, 8). Tumor hypoxia may also promote the malignant progression of cervical carcinomas (9), including lymph-vascular space involvement and parametrial infiltration (10). Moreover, patients with primary tumors with high lactate concentrations show a higher incidence of para-aortal and abdominal lymph node metastases than those with low-lactate primary tumors (11). High lactate concentration is indicative of extensive anaerobic metabolism and hence poor oxygenation in tumor tissue (12).

Tumor angiogenesis, possibly governed by the vascular endothelial growth factor (VEGF), has also been shown to influence the prognosis of patients with carcinoma of the uterine cervix (13–16). Locoregional recurrence following radiation therapy is related to long intercapillary distances in the tumor tissue (17, 18). A positive correlation between tumor vascular density and survival time after radiation therapy has also been reported for cervical carcinoma (19–21). Other studies have suggested that high vascular density is correlated with vascular space invasion, lymphatic involvement, and pelvic lymph node metastasis (22–24) and may predict short disease-free and overall survival times (25).

Adenocarcinoma and squamous cell carcinoma are the two most common tumor types of the uterine cervix (26). The stage-for-stage prognosis has been reported to be better for patients with squamous cell carcinoma than for patients with adenocarcinoma (27–29), squamous cell carcinomas generally showing a higher response rate to radiation therapy than adenocarcinomas (30, 31). The frequency of lymph node metastases at the time of diagnosis is higher for patients with adenocarcinoma than for patients with squamous cell carcinoma (32, 33). The biological differences between adenocarcinomas and squamous cell carcinomas that cause the difference in prognosis have not been recognized, but may be related to the tumor microenvironment. The purpose of the study

 ISSN 0284-186X

Table 1

Clinical stage and histological grade of adenocarcinomas and squamous cell carcinomas

	Adenocarcinoma		Squamous cell carcinoma	
	Patients	Frequency (%)	Patients	Frequency (%)
FIGO				
I b	3/10	30.0	7/40	17.5
II a, b	4/10	40.0	24/40	60.0
III a, b	1/10	10.0	7/40	17.5
IV a, b	2/10	20.0	2/40	5.0
Grade				
1, 2	5/10	50.0	23/38	60.5
3	5/10	50.0	15/38	39.5

reported here was to search for possible differences in oxygen tension and vascular density between adenocarcinomas and squamous cell carcinomas of the uterine cervix.

MATERIAL AND METHODS

Patients

Ten patients with adenocarcinoma and forty patients with squamous cell carcinoma of the uterine cervix were included in the study. Tumor stage according to FIGO and tumor grade according to the modified Broders' system are listed in Table 1. The largest tumor diameter, determined from pretreatment magnetic resonance images, was 2 cm or more. The tumor volumes were in the range of 4–126 cm^3 (adenocarcinoma) and 7–212 cm^3 (squamous cell carcinoma). Informed consent was obtained from all patients. The study was approved by the regional Ethics Committee.

Oxygen tension

Tumor oxygen tension (pO_2) was measured prior to treatment using a polarographic needle electrode (Eppendorf pO_2 Histograph 6650) (34). The electrode was moved automatically through the tissue in preset steps of 0.7 or 1.0 mm. Each forward step was followed by a backward step of 0.3 mm, leading to a distance of 0.4 or 0.7 mm between each pO_2 reading. A total of 57–252 readings in 2–6 tracks was performed in each tumor. The tracks were located peripherally (clock positions 3, 6, 9, and 12) or centrally and were directed perpendicularly to the tumor surface. The track lengths were determined by the tumor size, measured by analysing magnetic resonance images. A pO_2 frequency distribution was generated for each tumor by pooling the data from the individual tracks.

Vascular density

A needle biopsy, 1×18 mm in size, was taken from each electrode track, leading to two to six biopsies per tumor. 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 hematoxylin and eosin and subjected to analysis using a projecting light microscope (35). Blood vessels were identified as a lumen encircled by either a thick vessel wall or a lining of endothelial cells, using a magnification of $\times 410$. Vascular density, i.e. the number of vessel profiles per mm^2 of tumor tissue, was recorded.

Statistical analysis

The Mann–Whitney rank sum test was used to compare two pO_2 histograms and to investigate whether adenocarcinomas and squamous cell carcinomas differed in any pO_2 parameter, vascular density, or hemoglobin concentration. A significance level of $p = 0.05$ was used.

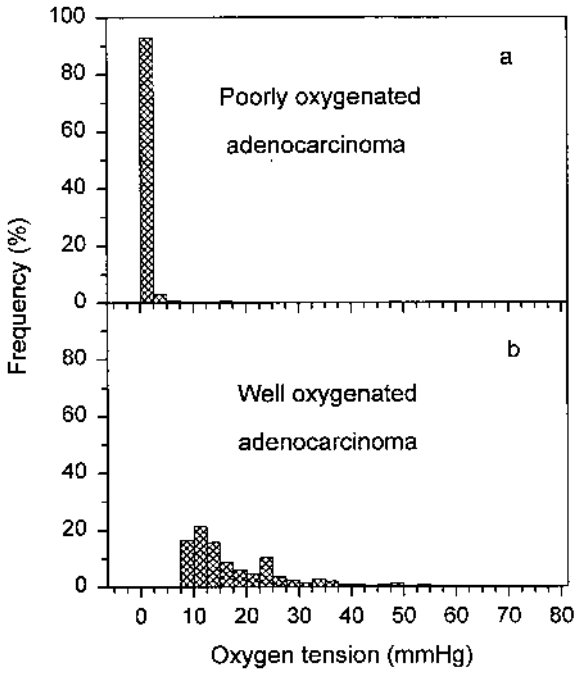

Fig. 1. Frequency distributions of pO_2 of (a) a poorly oxygenated and (b) a well-oxygenated adenocarcinoma of the uterine cervix.

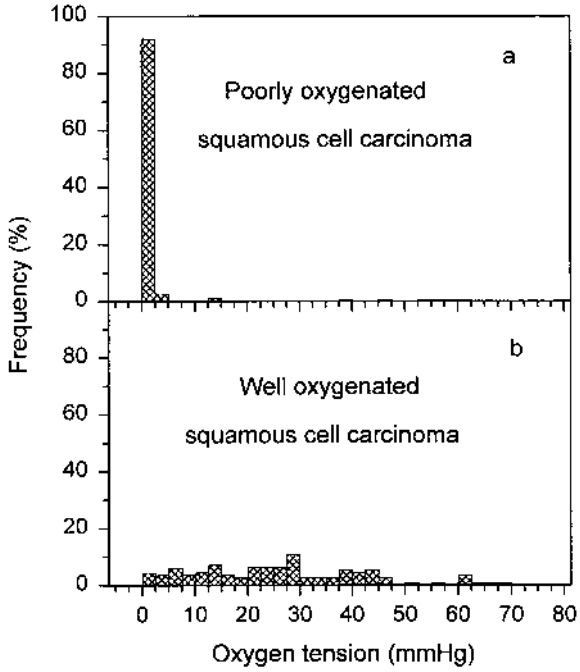

Fig. 2. Frequency distributions of pO_2 of (a) a poorly oxygenated and (b) a well-oxygenated squamous cell carcinoma of the uterine cervix.

RESULTS

Individual tumors showed highly heterogeneous pO_2 frequency distributions. The pO_2 frequency distributions differed substantially among individual tumors of the same histological type. This is illustrated in Fig. 1, which shows the pO_2 frequency distributions of a poorly oxygenated and a well-oxygenated adenocarcinoma, and Fig. 2, which shows the pO_2 frequency distributions of a poorly oxygenated and a well-oxygenated squamous cell carcinoma. Tumor oxygenation pattern did not correlate with clinical stage or histological grade in either of the two tumor types. Significant differences in oxygenation pattern between small and large tumors, between endophytic and exophytic tumors, or between tumors in premenopausal and postmenopausal women were not found, either in the adenocarcinomas or in the squamous cell carcinomas.

The adenocarcinomas were generally better oxygenated than the squamous cell carcinomas. The difference between the pO_2 frequency distributions of the adenocarcinomas and the squamous cell carcinomas was most pronounced at low pO_2-values, i.e. pO_2-values compatible with radiation resistance. The pO_2 frequency distributions of an adenocarcinoma and a squamous cell carcinoma of average oxygenation are presented in Fig. 3. The fraction of readings below a pO_2 of 2.5 mmHg can be seen to be substantially higher in the squamous cell carcinoma than in the adenocarcinoma. Statistical analysis showed that the two pO_2 frequency distributions are significantly different ($p < 0.001$).

Six parameters were derived from the pO_2 frequency distribution of each tumor: median pO_2, the 10th and 90th percentiles, and the fractions of readings below a pO_2 of 2.5 mmHg, 5 mmHg, and 10 mmHg. The median pO_2, the 10th percentile, and the 90th percentile tended to be higher in the adenocarcinomas than in the squamous cell carcinomas. The differences were on the borderline of being statistically significant ($p = 0.02–0.06$). As an example, median pO_2 was higher than 5 mmHg in 7 out of 10 adenocarcinomas, whereas only 10 out of 40 squamous cell carcinomas showed a median pO_2 above 5 mmHg. The fractions of readings below a pO_2 of 2.5 mmHg, 5 mmHg, and 10 mmHg were significantly higher in the squamous cell carcinomas than in the adenocarcinomas ($p = 0.003–0.04$). Cumulative frequency diagrams of these pO_2 parameters are presented in Fig. 4. The frequency diagrams illustrate that the adenocarcinomas were better oxygenated than the squamous cell carcinomas.

The vascular density differed substantially among individual tumors of the same histological type. The adenocarcinomas and the squamous cell carcinomas showed vascular densities ($\#/mm^2$) within the ranges of 5–28 and 6–35, respectively. The vascular density was not significantly different for the two tumor types ($p = 0.53$). The hemoglobin concentration of peripheral blood was not significantly different for the patients with adenocarcinoma and those with squamous cell carcinoma ($p = 0.58$).

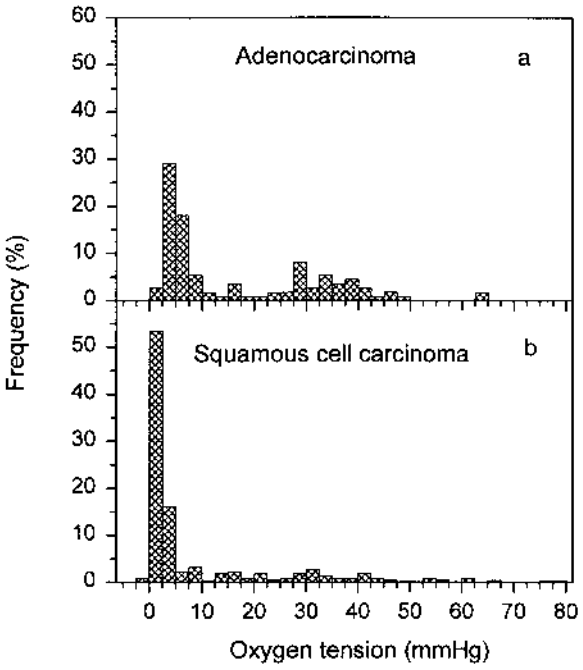

Fig. 3. Frequency distributions of pO_2 of (a) an adenocarcinoma and (b) a squamous cell carcinoma of the uterine cervix of average oxygenation.

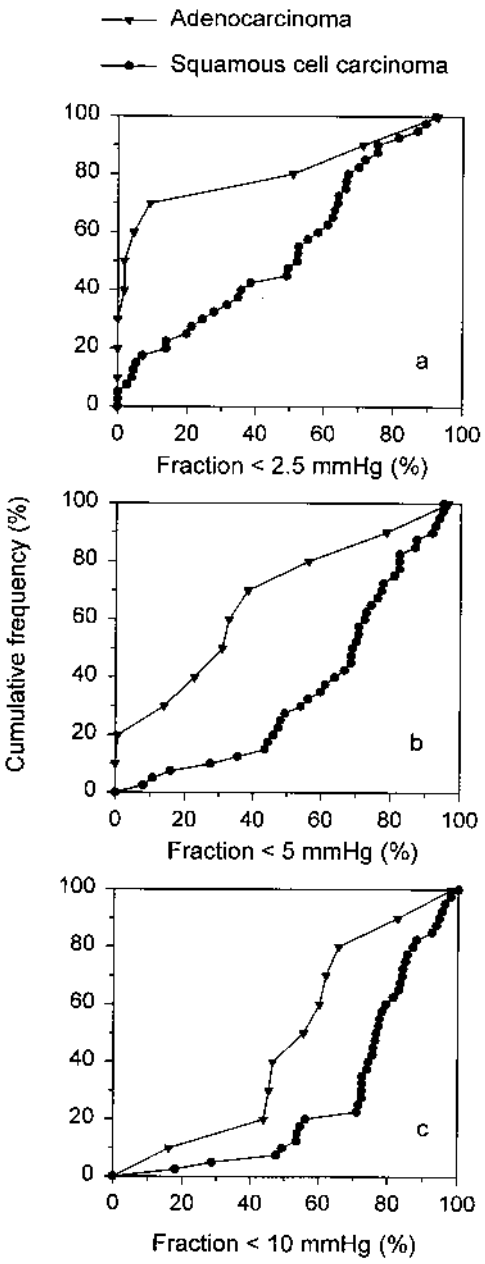

Fig. 4. Cumulative frequency diagrams of the fraction of readings below a pO_2 of (a) 2.5 mmHg, (b) 5 mmHg, and (c) 10 mmHg in adenocarcinomas and squamous cell carcinomas of the uterine cervix.

DISCUSSION

The prognosis of patients with carcinoma of the uterine cervix depends on the tumor histology; squamous cell carcinoma has a better stage-for-stage prognosis than adenocarcinoma (27–29). The biological properties of adenocarcinomas and squamous cell carcinomas causing the difference in prognosis have not been identified so far.

In this study we looked for differences in oxygen tension and vascular density between adenocarcinomas and squamous cell carcinomas. Tumor oxygen tension was measured polarographically using the Eppendorf pO_2 Histograph 6650. Feasibility and reproducibility studies performed in our laboratory (34–36) and in other laboratories (2, 3) suggest that reliable measurements of the oxygenation of cervical carcinomas can be obtained with Eppendorf polarographic needle electrodes.

Tumor vascular density was determined by counting vessels identified as a lumen encircled by either a thick vessel wall or a lining of endothelial cells. Specific endothelial stains have been used in many studies of tumor vascularization to highlight the vessels before the vascular density is scored at low magnifications ($\times$ 100–200). Preliminary studies performed in our laboratory showed that many tumor vessels were inadequately stained by using antibodies against factor VIII or CD31 antigens. Similar observations have been reported by others studying the vascularization of cervix carcinoma (15, 23, 37). The problem of varied staining was avoided in the present work by assessing the vascular density at a high magnification ($\times$ 410) without the use of specific endothelial stains. Repeated analyses of the same sections showed that the reproducibility of our method was at least as good as that of methods based on the use of specific endothelial stains. The main disadvantage of the method used here is that the vessel counting is time-consuming.

The oxygen tension was found to be significantly higher in the adenocarcinomas than in the squamous cell carcinomas included in the study. The difference in oxygen tension cannot be attributed to a difference in the extent of necrosis, as none of the tumor types showed significant necrotic fractions. The better oxygenation of the adenocarcinomas was not a consequence of a difference in tumor stage or grade either, as none of the oxygenation parameters showed significant correlations with these parameters in any of the tumor types. The latter conclusion is consistent with previous observations, which have indicated that the oxygenation of cervical carcinomas is independent of clinical stage and histological grade (3).

The oxygenation of tumors is determined by the rate of oxygen supply and the rate of oxygen consumption (12). The tumor vascular density and the hemoglobin concentration of peripheral blood were found to be similar in the patients with adenocarcinoma and the patients with squamous cell carcinoma. Although the rate of oxygen supply to tumors is not determined by these two parameters only, this observation suggests that the difference in oxygenation between the adenocarcinomas and the squamous cell carcinomas was caused by a difference in the rate of oxygen consumption rather than by a difference in the rate of oxygen supply.

The radioresponsiveness of tumors depends on the intrinsic radiation sensitivity of the tumor cells, the rate of cell proliferation during the treatment period, and the fraction of radiobiologically hypoxic cells (38). The rate of response to radiation therapy is generally higher for

squamous cell carcinomas than for adenocarcinomas (30, 31). The study reported here suggests that adenocarcinomas are better oxygenated than squamous cell carcinomas. Consequently, the difference in the outcome of radiation therapy between adenocarcinomas and squamous cell carcinomas may be a result of a difference in the intrinsic radiation sensitivity of the tumor cells and/or a difference in the rate of cell proliferation during the treatment period rather than a difference in the fraction of radiobiologically hypoxic cells.

The progression of cervix carcinomas is governed by the genetic properties of the tumor cells and the characteristics of the tumor microenvironment, including the rate of angiogenesis and oxygenation status (10, 16, 22). Patients with adenocarcinoma show a higher frequency of lymph node metastases at the time of diagnosis than patients with squamous cell carcinoma (32, 33). Hypoxia can cause increased expression of several selected genes, including the genes encoding metastasis promoting proteins, and hence enhance the metastatic frequency of tumors (39, 40). High vascular density in the primary tumor may promote metastasis by increasing the opportunity of the tumor cells to gain access to blood and lymph vessels (16, 41). The present study suggests that adenocarcinomas and squamous cell carcinomas show similar vascular densities and that squamous cell carcinomas generally are more hypoxic than adenocarcinomas. Consequently, the difference in metastatic propensity between adenocarcinomas and squamous cell carcinomas may be caused by differences in the genetic properties of the tumor cells rather than by differences in the characteristics of the tumor microenvironment.

We conclude that the study reported here suggests that adenocarcinomas and squamous cell carcinomas of the uterine cervix do not differ significantly in vascular density and that squamous cell carcinomas show lower oxygen tensions than adenocarcinomas. The difference in prognosis between patients with adenocarcinoma and patients with squamous cell carcinoma can probably not be attributed to differences in tumor vascularization or oxygenation. The genetic properties of the tumor cells may be more important than the tumor microenvironment for the difference in prognosis.

ACKNOWLEDGEMENTS

This study was supported by The Bothner Foundation for Cancer Research and The Norwegian Cancer Society.

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