Experimental studies on the possible influence of invasive oxygen measurements on tumour radiosensitivity

Georg Stüben, Martin Stuschke, Kai Knühmann, Christoph Pöttgen, Horst Sack

Angaben zur Veröffentlichung / Publication details:

Stüben, Georg, Martin Stuschke, Kai Knühmann, Christoph Pöttgen, and Horst Sack. 1998. "Experimental studies on the possible influence of invasive oxygen measurements on tumour radiosensitivity." *Acta Oncologica* 37 (4): 369–73. https://doi.org/10.1080/028418698430593.

Experimental Studies on the Possible Influence of Invasive Oxygen Measurements on Tumour Radiosensitivity

Georg Stüben, Martin Stuschke, Kai Knühmann, Christoph Pöttgen and Horst Sack

From the Department of Radiotherapy, Universitätsklinikum Essen, Germany

Correspondence to: Dr Georg Stüben, Strahlenklinik im Universitätsklinikum, Hufelandstr. 55, D-45122 Essen, Germany. Tel: +49 201 7232818. Fax: +49 201 7235960. E-mail: georg.stueben@uni-essen.de

The effects of tissue damage associated with invasive pO_2 measurements on radiation sensitivity were investigated using a xenografted squamous cell carcinoma model. For the tumour cure experiments, single dose irradiations were given following different regimens of polarographic pO_2 measurements associated with different degrees of mechanical tissue damage. With a dose of 32 Gy, 57% of animals were cured. Following 3 tracks of needle measurements, 73% of tumours were locally controlled, and 75% were cured after 8 needle tracks. The polarographic measurements gave virtually identical oxygenation data for recurrent or cured tumours (both median pO_2 1.0 mmHg), respectively. There was thus no evidence of decreased radiosensitivity associated with tissue damage after invasive pO_2 measurements. The pre-therapeutic oxygenation status gave no evidence for a prediction of radiation response on an individual basis.

Invasive needle measurements of intratumoral pO_2 are increasingly used in the clinical situation, as rapid in situ measurements of oxygenation are possible. For clinical practice, the measurements have been reported to be reliable and some studies have reported that oxygen electrode measurements can predict the outcome of radiotherapy (1–3).

However, the considerable concern over possible adverse effects of the measurements has been discussed (4–6). The present investigation was initiated to evaluate the effects of the tissue damage associated with invasive polarographic oxygen tension measurements on tumour radiosensitivity. The background of the investigation was the possible impact of hypoxia induced by tissue and vessel damage as a consequence of the needle insertions. The cure probability of tumours was assessed following single dose radiotherapy. Therefore, the significance of pre-therapeutic O_2 partial pressure, as measured polarographically, could also be analysed by comparison with the radiation response.

MATERIAL AND METHODS

Animals

Nude mice (nu/nu of NMRI inbred background) were used for this study. The mice were derived from the central animal laboratory of Essen University, where the breeding was performed under pathogen-free conditions. For the experiments, the animals were housed in laminar air flow units in the laboratory of the Department of Radiation Oncology. Animals had unlimited access to water (supplemented with chlortetracyclin (10 g/l) and K-sorbate (1.35 g/l) acidified to a pH of 3.0) and high caloric laboratory food. The animals were entered in the experiment at an age of 6 to 9 weeks. All experimentation had previously been approved by the regional animal ethics committee.

Tumours and transplantation

A human squamous cell carcinoma established from a biopsy of a head and neck primary tumour (WAN) with a tumour volume doubling time of 3.5 ($\pm$0.2) days was used for this investigation.

Tumour chunks of 2 to 3 mm were transplanted into the s.c. tissue of the right hind leg of the nude mice. The tumour line was xenografted into nude mice and repeatedly characterized by means of DNA content, volume doubling time, and isoenzyme pattern of LDH and GPD (7). During the experimental period, no changes in these parameters were observed, confirming the human origin of the tumour.

Assessment

The animals were assigned to treatment when the tumours reached a volume of 110 mm^3. After irradiation the tumours were scored 2 to 3 times weekly until a recurrence was observed, or the experiments were terminated 80 days after the observation of the last recurrence at day 180 after the end of the treatment. Eight to 16 mice were used for each radiation dose point. Six radiation dose levels were given in order to construct a full dose response relationship. The dose yielding 50% local control rate (TCD_{50}) with correction for censored data was estimated by fitting a logistic regression to the entire dose/response data (8). Values in parentheses represent 95% confidence intervals. For the irradiation with a fixed single dose (32 Gy about TCD_{50}), 99 animals were irradiated after measuring 3 tracks and 40 animals after 8 tracks invasive measurements, respectively.

Anaesthesia

Details of the experimental set-up used for both the irradiations and the invasive pO_2 measurements have been published previously (9). Briefly, the mice were positioned concentrically to the midpoint of the experimental set-up, breathing an anaesthetic gas mixture through openings in the distributor. Enflurane (Ethrane®) was circulated using a membrane pump and was mixed with air as carrier. Further details of the narcotic procedure can be found in Ang et al. (10). A decrease in body temperature during narcosis was avoided by surrounding the animal body gently with a perspex tube. In addition, two thermostatically controlled fan heaters were positioned at a distance of 40 cm to the set-up during irradiation.

Polarographic measurements of pO_2

Polarographic intratumoral oxygen partial pressure (pO_2) measurements and irradiations were performed under identical conditions (tumour size, positioning of animals, depth and duration of anaesthesia, avoidance of body temperature drop). The invasive pO_2-measurements were performed using a computerized system (KIMOC 6650, Eppendorf, Hamburg). The details of this technique have been previously described (11). Briefly, the intratumoral pO_2 was registered with a polarographic needle, 300 μm in diameter. Forward movements of the needle (500 μm) were immediately followed by a backward step of 200 μm, in order to minimize tissue compression artefacts (12). Tumour oxygenation measurements were performed either along 3 tracks within one individual tumour or along 8 tracks, respectively (see Fig. 1). Data of the same histology, tumour size, and number of tracks were pooled for analysis. More than 900 individual pO_2 data points (924–3790) have been obtained for statistical evaluations. Negative values in the range from –1 to 0 mmHg, which are thought to appear due to the accuracy of the system, were treated as belonging to the lowest class reading (0–2.5 mmHg). Values below that range (i.e. < -1 mmHg), which usually appear because of bending of the needle, were not observed in our investigations. The drift was $< 0.4\%$/min. The raw data of the pO_2 measurements were exported from the KIMOC device into a personal computer and analysed using standard statistical software. The significance of differences between the various regimens of measuring pO_2 (3 vs. 8 tracks) was assessed by means of the Kolmogorov-Smirnov 2-sample test (13).

Irradiations

Tumour-bearing legs of mice were irradiated with 15 MeV photons generated by a linear accelerator (Mevatron) at a dose rate of 2.5 Gy/min. The focus isocentre distance was 100 cm with field sizes of 3×2 cm^2 at the isocentre. The remaining body of the animals was shielded effectively by a 12 cm thick Lipowitz's metal shielding block from the direct beam and mainly exposed to scattered radiation. The whole body dose of mice was 8% of the total tumour absorbed dose.

The field homogeneity was repeatedly checked using LiF (Lithium Fluoride) thermolumiscent dosimeters. The dose variation was found to be less than 4% over a length of 6 mm in the centre of the collimated fields, which corresponds to the size and position of the tumours at time of treatment.

RESULTS

Single-dose irradiation

The radiation dose-response curve for the single-dose irradiation of tumours, which were not pO_2 measured, is presented in Fig. 2. The TCD_{50} derived under unclamped air breathing conditions was 32.0 Gy (28.3–36.2 Gy).

With a dose of 32 Gy given to tumours, which were not measured invasively before irradiation, 8 out of 14 animals (57%) were cured. Following 3 tracks of needle measurements, 73 out of 99 animals (73%) were locally controlled with 32 Gy, while 30 out of 40 animals (75%) were cured after 8 needle tracks, respectively.

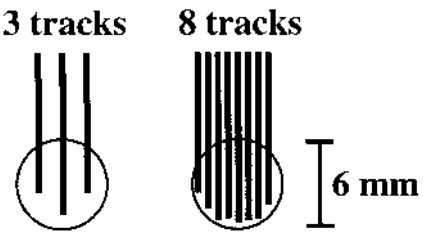

Fig. 1. Different regimens of polarographic oxygen tension measurements for tumours transplanted on the legs of animals.

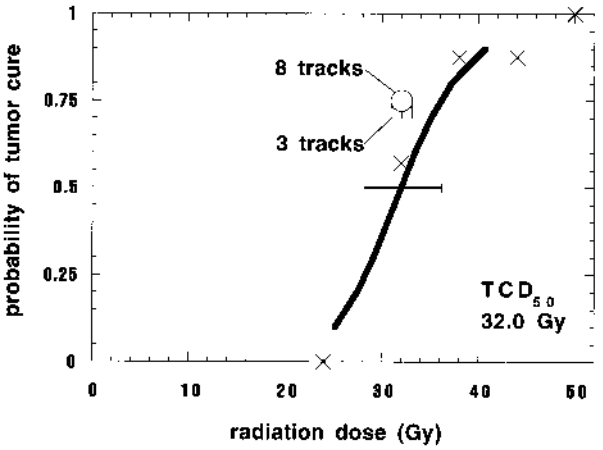

Fig. 2. Dose/response curve of the WAN tumour for single-dose irradiation.

pO_2 measurements

The xenografted squamous cell carcinoma (WAN) investigated revealed a significant amount of hypoxic readings under ambient conditions, as assessed polarographically. In tumour volumes of 110 mm^3, more than 80% low-class pO_2 values in the range from 0 to 2.5 mmHg occurred. Details of the results of pO_2 measurements are presented in Table 1.

A stratification of pre-irradiation pO_2 readings by response to 32 Gy single dose is summarized in Table 1.

Briefly, no significant differences in the oxygenation status of the recurrent or cured tumours could be detected. After 3 tracks the cured tumours showed a median pO_2 of 1 mmHg, while the recurrent tumours also had a median pO_2 of 1 mmHg.

Comparing the oxygenation data derived with 3 tracks or 8 tracks, respectively, virtually identical pO_2 values were observed. No increase in resolution or statistical power of the data was seen with more tracks and consequently more data points.

DISCUSSION

The computerized pO_2 histograph (KIMOC 6650; Eppendorf, Germany) is increasingly used in the clinic to measure tissue oxygenation invasively. The available data show a wide range of pO_2 values even in identical histological types, with a trend towards low pO_2 values in most patients (2, 14) . Since the polarographic system investigates a small volume of the tumour and is unable to distinguish between viable hypoxic (stem cells) and necrotic areas irrelevant for the risk of recurrence, there remains controversy on the relevance of the system (15, 16). However, some clinical studies indicate a correlation between initial pO_2 values with local control or survival (1, 3, 17).

The present investigation was initiated to evaluate the effect of tissue damage associated with invasive intratumoral needle measurements on radiation response. As a high resolution of the experiment was necessary, a large group of animals (n = 139) was irradiated with a radiation dose, which corresponded to the TCD_{50} level of unmeasured animals. At this dose level the dose/response curve is steep, which should be associated in principle with an increased resolution of the assay. The TCD_{50} for unmeasured tumours was 32 Gy; in other words, this radiation dose led to a 50% probability of local control. With the identical radiation dose, 73% of animals were cured after 3 tracks of needle measurements and 75% were locally controlled after 8 needle tracks, respectively. Clearly, no decreased radiation sensitivity was observed. However, the situation might be different in a tumour which is better oxygenated. In that situation the impact of induced hypoxia as consequence of vessel damage on radiation sensitivity might be more pronounced. In our study a slightly enhanced radiation response was noticed for the measured tumours, regardless of the measurement regimen. The reason for this is most likely the tumour cell kill associated with the measuring procedure.

Very few studies have investigated adverse effects of probe tracks in tumours (5, 6). In transplantable tumours neither effect of probe progression on tumour growth nor incidence of metastases was observed (5) . Furthermore, the survival of mice was not changed after pO_2 measurements compared with controls (5).

In a histological study on xenografts Steinberg et al. (6) described aggregates of erythrocytes from microvessel rupture within the needle track. In some tumours the tumour vessel perforation resulted in large extravasations with a diameter of up to ten times that of the needle itself. However, the implantation site at the lateral thorax might have lead to an overestimation of the needle-induced damage, as breathing movements might have increased the damage of the inserted needle.

In contrast to the present paper, the number of measurements have in some studies been variable within groups of animals or patients and are subject to criticism (4). Oedema and haemorrhage associated with other modes of invasive measurements might lead to a modification of tumour hypoxia. Our data give no evidence that the numbers of tracks (3 vs. 8) affect the validity or resolution of the pO_2 readings concerning the radiation response.

The experimental tumour investigated in this study was characterized by a large proportion of low pO_2 readings. About 80% of all readings were observed to be ≤ 2.5 mmHg where the sensitizing effects of oxygen are less than half maximal (19). This finding is at the low end of reported values for both murine and xenografted human tumours (18–20).

The applied anaesthetic might be a factor participating toward the low oxygen tensions. Data on both normal

Table 1

Results of pO_2 measurements of the WAN-tumour stratified by response to 32 Gy single-dose radiation treatment

Status of animals	Mean pO_2 ($\pm$S.D.)	Median pO_2 ($\pm$S.D.)	%$\leq$25 mmHg ($\pm$S.D.)	%$\leq$5 mmHg ($\pm$S.D.)	No. of readings
3 tracks					
All (99)	2.5 ± 5.3	1.0 ± 6.3	84 ± 12.2	88 ± 12.9	3540
Cured (73/99)	2.7 ± 4.9	1.0 ± 6.5	83 ± 11.2	87 ± 11.8	2616
Recurrent (26/99)	1.8 ± 6.5	1.0 ± 5.9	87 ± 14.8	90 ± 15.7	924
8 tracks					
All (40)	3.2 ± 2.7	1.0 ± 0.3	82 ± 11.5	86 ± 10.3	3790
Cured (30/40)	3.4 ± 2.9	1.1 ± 0.3	82 ± 11.7	85 ± 10.4	2821
Recurrent (10/40)	2.6 ± 2.1	0.9 ± 0.3	85 ± 11.1	87 ± 10.3	969

Data were derived with 3 or 8 tracks, respectively. Values in parentheses represent the number of animals.
S.D. = Standard deviation.

tissues and tumours show that anaesthetics may affect the radiation response by modification of oxygenation (21–23). The data on the effect of the commonly used enflurane narcosis on radiation response are limited. Neely et al. (24) measured inner retinal oxygen tension in cats in general enflurane narcosis under normoxic conditions. Enflurane anaesthesia did not cause hypoxia of the inner retina in cats breathing 21% inspired oxygen, indicating a limited effect of the drug on oxygenation status of tissues. Furthermore, in murine lip mucosa, no significant effect of enflurane on the radiation response could be observed for fractionated irradiation (25). However, low pO_2 values have also been described in the clinical situation (26–28).

In view of the patient numbers needed for clinical trials, a major challenge is the selection of patients who might actually benefit from sensitizing methods. Our experimental design allows an approximation of the predictive potency of pO_2 readings on an individual basis. As the observed dose response curves are frequently not as steep as could be expected with inbred strains and identical tumour size, differences in the oxygenation could be a reason for the differences in radiation response, which determines the incidence of cure following radiation.

In our study we also attempted to correlate results of radiotherapy with the oxygenation status as assessed polarographically. Within the limited range of pO_2 values observed, the pO_2 readings did not predict the cure probability of a single-dose irradiation on an individual basis.

ACKNOWLEDGEMENT

This work was supported by the Deutsche Forschungsgemeinschaft (DFG, grant STU 151-4/4).

REFERENCES

1. Gatenby RA, Kessler HB, Rosenblum JS, et al. Oxygen distribution in squamous cell carcinoma metastases and its relationship to outcome of radiation therapy. Int J Radiat Oncol Biol Phys 1988; 14: 831–8.
2. Höckel M, Schlenger K, Aral B, Mitze M, Schaffer U, Vaupel P. Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. Cancer Res 1996; 56: 4509–15.
3. Nordsmark M, Overgaard M, Overgaard J. Pretreatment oxygenation predicts radiation response in advanced squamous cell carcinoma of the head and neck. Radiother Oncol 1996; 41: 31–9.
4. Brizel DM, Lin S, Johnson JL, Brooks J, Dewhirst MW, Piantadosi CA. The mechanisms by which hyperbaric oxygen and carbogen improve tumour oxygenation. Br J Cancer 1995; 72: 1120–4.
5. Lartigau E, Lespinasse F, Vitu L, Guichard M. Does the direct measurement of oxygen tension in tumors have any adverse effects? Int J Radiat Oncol Biol Phys 1992; 22: 949–51.
6. Steinberg F, Hildenhagen-Brüggemann E, Konerding MA. Oxygen electrode injury in tumour tissue. In: Vaupel PW, Kelleher DK, Günderoth M, eds. Funktionsanalyse biologischer Systeme. Mainz: Akademie der Wissenschaften und der Literatur, 1995: 185–93.
7. Budach V, Bamberg M, Streffer C, Budach W, Stuschke M, Fabry W. Establishment and characterization of human tumours in nu/nu-mice. Strahlenther Onkol 1989; 165: 500–1.
8. Walker AM, Suit HD. Assessment of local tumor control using censored tumor response data. Int J Radiat Oncol Biol Phys 1983; 9: 383–6.
9. Stüben G, Budach W, Schick KH, et al. A time-saving system for irradiation of experimental tumors. Strahlenther Onkol 1994; 170: 36–41.
10. Ang KK, van der Kogel AJ, van der Schueren E. Inhalation anesthesia in experimental radiotherapy: a reliable and time-saving system for multifractionation studies in a clinical department. Int J Radiat Oncol Biol Phys 1982; 8: 145–8.
11. Vaupel P, Okunieff P, Kallinowski F, Neuringer LJ. Correlations between 31P-NMR spectroscopy and tissue O_2 tension measurements in a murine fibrosarcoma. Radiat Res 1989; 120: 477–93.
12. Kallinowski F, Zander R, Höckel M, Vaupel P. Tumor tissue oxygenation as evaluated by computerized-pO_2-histography. Int J Radiat Oncol Biol Phys 1990; 19: 953–61.
13. Ong LD, LeClare PC. The Kolmogorov–Smirnov test for the log-normality of sample cumulative frequency distributions. Health Phys 1968; 14: 376.
14. Vaupel P, Schlenger K, Knoop C, Höckel M. Oxygenation of human tumors: evaluation of tissue oxygen distribution in breast cancers by computerized O_2 tension measurements. Cancer Res 1991; 51: 3316–22.

15. Horsman MR, Khalil AA, Siemann DW, et al. Relationship between radiobiological hypoxia in tumors and electrode measurements of tumor oxygenation. Int J Radiat Oncol Biol Phys 1994; 29: 439–42.
16. Kavanagh MC, Sun A, Hu Q, Hill RP. Comparing techniques of measuring tumor hypoxia in different murine tumors: Eppendorf pO_2 Histograph, [3H]misonidazole binding and paired survival assay. Radiat Res 1996; 145: 491–500.
17. Höckel M, Knoop C, Schlenger K, et al. Intratumoral pO_2 predicts survival in advanced cancer of the uterine cervix. Radiother Oncol 1993; 26: 45–50.
18. Busse M, Vaupel PW. The role of tumor volume in 'reoxygenation' upon cyclophosphamide treatment. Acta Oncol 1995; 34: 405–8.
19. Yeh KA, Biade S, Lanciano RM, et al. Polarographic needle electrode measurements of oxygen in rat prostate carcinomas: accuracy and reproducibility. Int J Radiat Oncol Biol Phys 1995; 33: 111–8.
20. Teicher BA, Schwartz GN, Dupuis NP, et al. Oxygenation of human tumor xenografts in nude mice by a perfluorochemical emulsion and carbogen breathing. Artif Cells Blood Substit Immobil Biotechnol 1994; 22: 1369–75.
21. Hornsey S, Myers R, Andreozzi U. Differences in the effects of anaesthesia on hypoxia in normal tissues. Int J Radiat Biol Relat Stud Phys Chem Med 1977; 32: 609–12.
22. Sheldon PW, Chu AM. The effect of anesthetics on the radiosensitivity of a murine tumor. Radiat Res 1979; 79: 568–78.
23. Tozer GM, Penhaligon M, Nias AH. The use of ketamine plus diazepam anaesthesia to increase the radiosensitivity of a C3H mouse mammary adenocarcinoma in hyperbaric oxygen. Br J Radiol 1984; 57: 75–80.
24. Neely KA, Ernest JT, Goldstick TK. Retinal tissue oxygen tension in normoxic cats under enflurane anesthesia. Invest Ophthalmol Vis Sci 1995; 36: 1943–6.
25. Stüben G, Landuyt W, van der Schueren E, van der Kogel AJ. Different immobilization procedures during irradiation influence the estimation of alpha/beta ratios in mouse lip mucosa. Strahlenther Onkol 1993; 169: 678–83.
26. Brizel DM, Rosner GL, Harrelson J, Prosnitz LR, Dewhirst MW. Pretreatment oxygenation profiles of human soft tissue sarcomas. Int J Radiat Oncol Biol Phys 1994; 30: 635–42.
27. Höckel M, Vorndran B, Schlenger K, Baussmann E, Knapstein PG. Tumor oxygenation: a new predictive parameter in locally advanced cancer of the uterine cervix. Gynecol Oncol 1993; 51: 141–9.
28. Laurence VM, Ward R, Dennis IF, Bleehen NM. Carbogen breathing with nicotinamide improves the oxygen status of tumours in patients. Br J Cancer 1995; 72: 198–205.