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Feasibility of Measuring Oxygen Tension in Uterine Cervix Carcinoma

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Cellular hypoxia is a cause of radioresistance. The oxygen tension (pO_2) in normal tissues and in tumours can be measured by polarography. In this feasibility study we have measured the tissue pO_2 of 10 patients suffering from uterine cervix carcinoma, using the Eppendorf histograph. The measurements were performed at the time of the brachytherapy after external radiotherapy. The machine was found to be reliable and no adverse effect was noted. The mean pO_2 values in tumours were lower than those of normal tissues.
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Introduction

EXPERIMENTAL SOLID tumours often have hypoxic regions, and laboratory studies indicate that cellular hypoxia is one of the parameters which may influence the response to chemo- or radiotherapy [1,2]. Aerobic cells are about three times more sensitive to low LET radiations than are hypoxic cells and the regrowth of these hypoxic cells after fractionated radiotherapy could explain some local treatment failures [3, 4]. Considerable effort has been devoted to developing techniques able to detect such cells, and to overcoming their radioresistance [1]; non-invasive methods with radioactively-labelled radiosensitisers or magnetic resonance spectroscopy have been used to detect hypoxia [1]. Until the 1980s there had been few direct measurements of oxygen tension (pO_2) in human tumours by polarography [5–8]. Gatenby founded a correlation between tumour pO_2 levels and the response to radiotherapy in 1988 [7]. However, none of these polarographic techniques was easily reproducible.

A computerised polarographic histograph (Kimoc 6650-

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Eppendorf) has recently become available. Microcirculation is inevitably disturbed by insertion of a needle probe into tissue, and this can lead to a change in local tissue pO_2 at the site of the measurement. However, using this device, the local pO_2 readings are minimally influenced by those effects; the measurements are taken with fast responding electrodes, and the needle movements are programmed to advance and withdraw in stepwise fashion in order to avoid compression effects as much as possible [9]. This electrode responds much faster than the electrodes previously used in patients [5–8]. Experiments in animals have demonstrated that pO_2 can be measured *in vivo* without any adverse effects on tumour growth or on the development of metastases [10]. A correlation between pO_2 measurements and the hypoxic cell fraction in a murine tumour has been demonstrated [11]. The accuracy and reproducibility of pO_2 measurements in humans were tested by comparing two different sets of successive measurements in normal tissues [12]. The results obtained in normal and tumoral breast and uterine cervix tissues have been recently published [13–14]. The purpose of this study was to determine whether in our hands, the Eppendorf equipment performed as well as has been reported by the teams who primarily used this machine [13,15]. For this work we performed pO_2 measurements on 10 patients with carcinoma of the uterine cervix.

PATIENTS AND METHODS

Patients

10 patients (mean age: 57 years) with carcinoma of the uterine cervix were studied. Oxygen tension measurements were performed with the approval of the local committee for protection of individuals in biomedical research (GREBB), and after obtaining informed consent. The characteristics of the patients and their tumours are summarised in Table 1. As this was a feasibility study, all the patients but 1 were on treatment. 9 of the 10 had already received radiotherapy, and pO_2 measurements were performed at the time of the brachytherapy under general anaesthesia, brachytherapy was considered to be a boost at the end of the treatment protocol. pO_2 measurements were performed before initial brachytherapy on 1 patient. All measurements were carried out in the operating theatre of the Brachytherapy Department. Patients were ventilated with a FiO_2 of about 21%. Central body temperature was recorded. The mean central body temperature (36.1°C, range 35.3–36.8) was below physiological values. The temperature of the tumours were recorded with a thermocouple; mean tumour temperature was 35.2°C (34.5–36°).

Table 1. Patients and characteristics of tumours

Patient	Age	Stage	Size of tumour (cm)	Histology	Treatment*
A	59	III Left wall	5 × 6	Squamous carc.	50 Gy
B	57	IV Bladder	—	Squamous carc.	Chemotherapy + 45 Gy
1	45	IV Para aortic	6 × 6	Adenocarc.	20 Gy
2	70	IV Bladder	—	Squamous carc.	Chemotherapy + 50 Gy
3	66	III Right wall + vagina	—	Squamous carc.	45 Gy
4	59	IIB	—	Squamous carc.	30 Gy Brachytherapy
5	67	III Right + left walls	—	Squamous carc.	50 Gy
6	33	IIB	4 × 4	Squamous carc.	20 Gy
7	51	IIB	5 × 5	Squamous carc.	20 Gy
8	64	IIB	2.5 × 2.5	Squamous carc.	—

*External radiotherapy = 2–2.5 Gy per fraction, 4–5 fractions per week, Chemotherapy = cisplatin-5-fluorouracil, carc. = carcinoma.

Oxygen tension measurements

The pO_2 histograph was developed by Eppendorf (Hamburg). Oxygen tension was measured using polarographic needle O_2 electrodes. The gold electrode (12 μm diameter cathode) was mounted within an unbreakable stainless steel needle probe (300 μm diameter). The end of the hypodermic needle bevelled at an angle of 30°. The cathode was polarised against an Ag/AgCl anode placed on the skin of the patient (polarisation voltage: −700 mV, constant d.c. voltage). The resulting current was proportional in its strength to the oxygen partial pressure in the tissue. Electrode O_2 sensitivity (0.9% saline, 37°C, −700 mV) was 0.53–0.66 pA/kPa, with a response time (T_{90}) under 500 ms. Calibration was performed, before and after measurements (oxygen drift) in sterile phosphate-buffered saline solution (pH 8) equilibrated with air or 100% nitrogen (gas bubbling) at room temperature. All electrical connections to the patient were insulated at a level of 8 kV in such a way that the patient was in no danger from line voltage (German safety class I, type BF guidelines). For all measurements, the probe was placed through a trocar within the first millimetres of tissues. Measurements in normal tissues, i.e. paravaginal soft tissues (1–2 tracks with the same entrance points) were followed by measurements in tumours (tracks with different entrance points). The electrode was moved automatically into the tissues; each forward movement (0.3–0.5 mm) was immediately followed by a reverse one (0.1–0.3 mm) to minimise tissue compression. Tissue pO_2 was measured 1.4 s after the reverse step and in 0.1 s. All movements were programmed according to the estimated extent of the tumour in the area (length of tissues analysed). The length of the probe progression was programmed to stay within macroscopically clinical tumour determined by clinical examination under general anaesthesia, stage of the disease and radiological examens (ultrasound, CT scanner). Nevertheless, we cannot be sure that the full length of the probe progression was always within the tumoral tissues. Measurements were visualised on the computer screen during the electrode progression, indicating how far the probe has progressed and displaying pO_2 readings at each point (the probe can be stopped and restarted). The needle was automatically removed at the end of a measurement. The results and all parameters were printed and stored on a diskette. Results were given by the machine in mmHg and were converted to kPa (760 mmHg = 101.3 kPa). Electrodes were cleaned, dried and sterilised in formalin to prevent transmission of infectious diseases.

RESULTS

The histograph was easily moved in the operating theatre and its small size did not interfere with the usual anaesthetic procedures. The machine performed reliably throughout the measurements on the 10 patients. There were no breakdowns of the electrical and electronical devices; all computerised parameters were easy to use and the electrodes were particularly robust for use in hard cervix tumours. However, the procedure

Table 2. Mean (median) pO_2 in normal tissues and tumours

Patients	1	2	3	4	5	6	7	8
Normal tissue	0.79 (0.66)	5.05 (4.92)	7.31 (7.71)	6.51 (6.65)	5.71 (6.51)	4.52 (4.52)	6.78 (6.78)	7.58 (7.71)
	7.18 (7.71)	6.91 (6.78)	8.11 (8.11)	8.24 (8.24)	2.79 (1.19)	6.65 (7.18)	5.71 (6.38)	4.92 (4.52)
					4.38 (4.25)			3.19 (2.26)
Tumours	0.53 (0.53)	5.58 (5.45)	3.45 (2.66)	8.91 (8.24)	1.06 (0.26)	0.93 (0.26)	1.33 (0.93)	5.32 (3.72)
	0.53 (0.53)	0.53 (0.53)	2.66 (2.52)	3.59 (3.19)	0.79 (0.13)	0.26 (0.26)	1.86 (0.66)	2.12 (2.12)
			3.45 (3.19)	4.92 (5.45)		7.18 (7.31)	0.53 (0.53)	1.72 (1.72)
						0.13 (0.13)	5.98 (5.05)	1.19 (1.19)
						0.13 (0.13)		1.33 (1.33)
						1.46 (0.13)		

Each value = mean (or median) of pO_2 values recorded along one track. Results in kPa (1 kPa = 7.5 mmHg)

could not be completed for the first 2 patients (A and B). In the first case, a special long 10.5 cm electrode was too flexible and no pO_2 was recorded. In the second, a short circuit was induced by some moisture on the upper part (adaptor) of the probe after the measurement in normal tissues. pO_2 was measured in the normal tissues and tumours of the 8 remaining patients (Table 2).

The mean number of all pO_2 values recorded in normal tissues was 54 (range 38–90) and 118 (47–166) in tumour. The mean and median of pO_2 values along a single track in normal tissues and/or tumours varied within a single patient (Table 2) and from one patient to the other. The distribution of the values was non-Gaussian with a difference between mean and median values smaller in normal tissues than in tumours. The mean pO_2 in normal tissues of the 8 patients was 5.85 kPa (44 mmHg). In tumours, the differences between different tracks were negligible for patients 1, 3 and 5, and large for patients 2, 7 and 8. The mean pO_2 of a single track provided only partial information on the wide distribution of the individual pO_2 values recorded along the track. Two examples for 1 patient (patient 7) are presented in Fig. 1 (a) and (b). In Fig. 1(a), all the values were very similar, but in Fig. 1(b) there were large variations within the same tumour along the electrode track. In one patient (patient 6), pO_2 was recorded in different areas of the clinical tumour: macroscopically tumorous, necrotic or macroscopically non-tumorous area. The mean and median pO_2 within the macroscopically tumoral region varied greatly from less than 0.13 to over 7.31 kPa. The median pO_2 in necrotic areas was low (0.26 kPa in two tracks). A median value below 1.33 kPa (10 mmHg) was recorded in normal tissues in only 1 patient, but was recorded in tumours in 6 patients.

The whole procedure took 45–75 min, in part depending on the number of tumour measurements made (mean oxygen drift

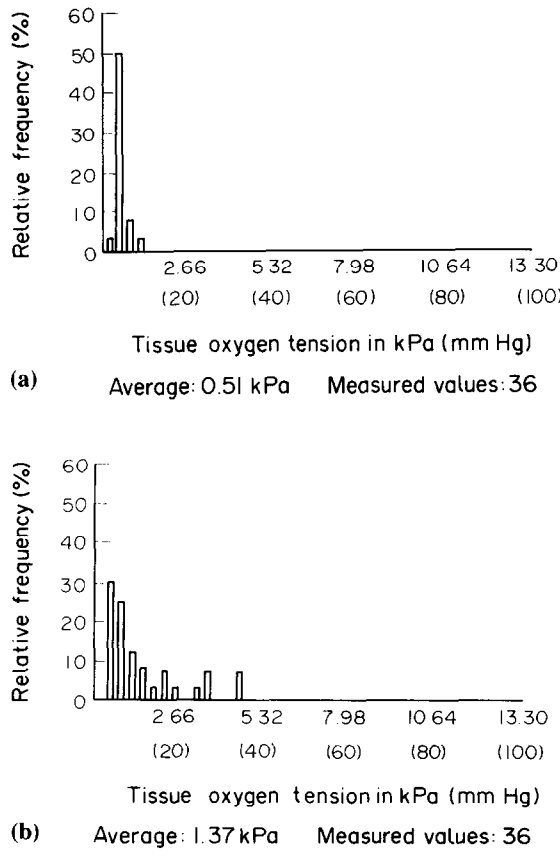

Fig. 1. pO_2 histograms obtained from tumour of patient number 7: (a) track number 1 (small variations), (b) track number 2 (large variations). "Measured values" represent the total number of measurements obtained along one track.

0.2%/min in 8 patients and 0.3%/min in 2 patients). The shortest time was obtained for the last patients, perhaps because of increasing operator skill. No patient showed any adverse effect, and measurements were always followed by brachytherapy. However, the procedure could be potentially hazardous in patients with cardiovascular or pulmonary deficiency as the duration of the general anaesthesia was notably increased. Two to four successive probe-sterilisations did not impair the normal function of the electrodes.

DISCUSSION

Polarographic methods have been used to measure pO_2 in tissues since the 1950s, and significant hypoxia has been reported in tumours [5–8]. However, the techniques were critised because of changes in pO_2 due to damage to the tissues by the large needle probe (18 gauge) and O_2 consumption by the large sensitive part (200 μm radius) of the electrode. These potential causes of error seem to be minimised in the Eppendorf histograph as the 12 μm electrode is placed within a 28 gauge needle (300 μm radius). Tissue injury due to the electrode progression is rather small, with no effect on the pO_2 values recorded [9]. The good reproducibility of pO_2 measurements in patients has been tested [12].

This study has shown that pO_2 measurements can be made in human tumours with this equipment without any adverse effects. The machine is reliable and can be used in an operating theatre. The relatively large oxygen drift between pre- and postcalibration (in our study superior to 10%/h) emphasises the need to shorten the complete procedure time, or to perform an intermediate recalibration between measurements. This total time could be shortened to around 30 min in which case measurements could be made in unanaesthetised patients without any pain [13]. This will allow central body temperature to be maintained at a physiological level without any potential change in the value of the recorded pO_2. The aim of this feasibility study was not to assess the validity of the pO_2 levels recorded. However, pO_2 values found in normal surrounding tissues were always higher than in tumours. This is an agreement with the results of one other study performed before treatment [6]. We recorded intratumour and intertumour variations; for some tumours the intratumour variations after radiotherapy were high as shown by Fleckenstein *et al.* in a metastasis of a squamous cell carcinoma [15]. Most of the patients were treated before this study and the impact of radiotherapy on tumour oxygenation is difficult to assess (% of tumour cells, viability, reoxygenation). Furthermore, pO_2 values measured in normal tissues were relatively high compared with oxygen values measured in mammalian tissues [16]. This could be due to leakage of oxygen from red cells around the electrode in the track, giving some systematic overestimation of the oxygen partial pressure in the tissues when compared with measurements by other types of electrodes [17].

The final goal of the technique is to select patients with tumours considered to be radioresistant due to hypoxia who may benefit from treatment with, for instance, hypoxic cell radiosensitisers. The best parameter which should be used to describe intratumoral hypoxia remains to be defined. For tumours with a wide range of pO_2, the non-Gaussian distribution would give the mean pO_2 little meaning; parameters such as the percentage of values below a certain level of oxygenation may be preferable. The effects of a modified treatment based on hypoxia will also depend on the "K curve" of the specific tumour cells. This has only been defined for a small number of rodent cell lines and there is a need for such studies in human tumour cell lines.

We are now beginning a prospective study on untreated patients with head and neck or uterine cervix carcinomas to assess the relevance of pO_2 measurements for local tumour control after radiotherapy.

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