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Original Paper

Variations in Tumour Oxygen Tension (pO_2) During Accelerated Radiotherapy of Head and Neck Carcinoma

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The study was performed to assess the effect of accelerated radiotherapy on oxygenation of primary tumours and metastatic nodes in patients with advanced head and neck tumours. In 14 patients with head and neck tumour, oxygen tension (pO_2) was evaluated in normal tissues and tumours (primary tumour or metastatic neck node) before (0 Gy) and after 2 weeks (32 Gy) of accelerated radiotherapy (70 Gy in 3.5 weeks, with three daily fractions). Radiotherapy was combined with carbogen breathing in 5 patients. pO_2 was measured using a polarographic technique. For pooled normal tissues, median pO_2 was 38 mmHg before treatment and 46 mmHg after 2 weeks. For tumours, very low values (< 2 mmHg) represented 20% of the recorded values before treatment and 10% after 2 weeks. The relative increase in tumour oxygenation was more pronounced for primary tumours (median pO_2 12 mmHg before treatment versus 26 mmHg after 2 weeks, $P < 0.05$) than for metastatic nodes (respectively, 20 and 27 mmHg $P = 0.1$). For the 5 patients who breathed carbogen during accelerated radiotherapy, the median pO_2 was 44 mmHg at 2 weeks, compared with 13.5 mmHg before treatment ($P = 0.05$). Very low pO_2 values, corresponding to tumour hypoxia, were found in the tumours (primary and metastatic neck nodes) prior to accelerated treatment. During the first 2 weeks of accelerated treatment, an increase in median pO_2 was found in nine of the 14 tumours, together with a decrease in the frequency of very low values.

Key words: head and neck carcinoma, pO_2, accelerated radiotherapy, carbogen breathing
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INTRODUCTION

SOLID TUMOURS in patients are known to contain hypoxic areas [1–3]. Results obtained for oxygen tension (pO_2) measurements in human tumours have already been published and very low pO_2 values (< 2 mmHg), at which cells are likely to be radioresistant, have been found [4–6]. The treatment outcome of cervical and head and neck tumours has been correlated with tumour pO_2 distribution [7–11].

Tumour oxygenation can be increased using different modalities. Previous clinical results with hyperbaric oxygen (HBO), which decreases the radioresistance linked to diffusion limited (chronic) hypoxia, have been encouraging [12, 13]. However, HBO has been abandoned due to an increase in observed toxicity, together with technical difficulties and poor patient tolerance [13]. Normobaric oxygen and carbogen (95% O_2, 5% CO_2) have been tested without conclusive clinical results, in part due to heterogeneity in patient selection and probably due to a preradiotherapy breathing time which was too long (> 1 h) [14–16].

Interruptions of treatment during radiotherapy of head and neck carcinomas have been shown to decrease the probability of local tumour control. This is commonly attributed to tumour cell proliferation which starts during the first 4 weeks of treatment [17–19]. Thus, accelerated radiotherapy, delivering a curative total dose in a shorter overall treatment time than conventional radiotherapy, has been extensively tested in various centres [20, 21]. However, only limited data have been published with a 'high total dose' (> 65 Gy) delivered by accelerated radiotherapy using a low dose per fraction (< 1.8 Gy) [21].

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Recently, new ways of treatment have been advocated to overcome both tissue hypoxia and tumour repopulation. One of these ways is to combine accelerated radiotherapy and oxygen manipulation [22, 23]. Such a combination is the ARCON protocol (accelerated radiotherapy with carbogen and nicotinamide), now undergoing phase II trials. However, one of the remaining questions concerns the optimal time for sensitising hypoxic cells, i.e. at the start or during a fractionated radiotherapy. The answer to this question could depend on the demonstration of variations in tumour oxygenation taking place during radiotherapy, especially if it is a very accelerated scheme (overall treatment time < 5 weeks). Only limited, historical, data are available on variations in tumour oxygenation during conventional radiation treatment (overall treatment time 7 weeks), all showing an increase in tissue oxygenation during the course of the treatment [24–27].

Our study was undertaken to determine the variations in tumour oxygenation during a very accelerated radiotherapy for advanced head and neck carcinoma, which was combined with carbogen breathing in 5 patients. The acute tolerance of normal tissues under carbogen breathing and accelerated radiotherapy was evaluated.

PATIENTS AND METHODS

Patients

The changes in tumour pO_2 distribution and the tolerance to accelerated radiotherapy combined with carbogen breathing was studied in 14 patients. All patients (12 men and 2 women) had T3–T4, N0–N2c, M0 tumours of the oropharynx and/or oral cavity and were previous smokers. The patients' characteristics are shown in Table 1. The accelerated radiotherapy delivered 70 Gy in 3.5 weeks to the primary tumour and clinically involved nodes. Three daily fractions were given from Monday to Friday (0.9 Gy, 0.9 Gy, 1.4 Gy at 9 a.m., 1 p.m. and 5 p.m.) and two fractions were given on Saturday mornings (0.9 Gy and 1.4 Gy at 9 a.m. and 1 p.m.) with ^{60}Co machines. The spinal cord dose was limited to 36.6 Gy. Cervical posterior nodes were boosted with one daily fraction with electrons up to 50 Gy total dose for N0 and 65–70 Gy in the case of palpable nodes. The inferior cervical areas were irradiated with conventional fractionation (anterior field, ^{60}Co, 50 Gy at 3 cm depth).

Acute tolerance and tissue reactions were recorded and graded according to the ROTG-EORTC grading system. Informed consent was obtained before pO_2 measurements were carried out and this work was approved by the local Ethical Committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale de Bicêtre).

Carbogen breathing

If the patients agreed (non-randomised study), they were given carbogen to breathe throughout every fraction delivered to the primary tumour site and involved nodes. The methodology has been previously described [28]. Briefly, carbogen was started 5 min prior to the beginning of each radiotherapy fraction and was stopped immediately at the end (1 atm, flow rate 15 l/min). It was breathed in via a rubber mouth piece connected to a plastic two-way valve; this system was connected to a 3 l breathing bag. A nose-clip prevented room air breathing. Only 5 of 14 patients breathed carbogen during this study (see Table 1).

pO_2 measurement

pO_2 was measured using a computerised polarographic system (KIMOC-6650, Eppendorf, Hamburg, Germany). A gold microcathode (12 μm in diameter) in an unbreakable stainless steel needle (300 μm in diameter) was polarised against an Ag/AgCl anode placed on the skin of the patient. The resulting current was proportional to the oxygen partial pressure in the tissue. Calibration was performed, before and after measurements, in phosphate buffered saline (pH 8) equilibrated with air or 100% nitrogen at room temperature. pO_2 values were corrected for barometric pressure and temperature of the tissues (recorded with a thermocouple).

pO_2 values were recorded in non-irradiated normal tissues (subcutaneous tissues of the homolateral shoulder), followed by independent tracks in the tumour. For normal tissues and metastatic neck nodes, the skin was anaesthetised (Xylocaine spray) and a 22 gauge plastic trocar was inserted; the sterilised probe was then introduced through the trocar into the first few millimetres of the tissues. For primary tumours (oral cavity and oropharynx), the probe was directly inserted in the tissues. The length of the track was clinically defined as being 1.5–2 cm for normal tissues, and the longest clinical axis for the tumours. The probe was automatically moved through the tissues as defined at the beginning of each experiment, with each forward movement followed by a backward step. A median number of 70 pO_2 values (range 51–100) was recorded

Table 1. *Patient characteristics*

Patient	Sex	Age (years)	Primary tumour site	TNM	Carbogen	Measurements in*
1	F	51	Oral cavity	T4 N1 M0	–	Tumour
2	F	52	Oropharynx	T4 N1 M0	+	Tumour
3	M	68	Oropharynx	T4 N0 M0	+	Tumour
4	M	54	Oropharynx	T4 N0 M0	+	Tumour
5	M	51	Oral cavity	T4 N0 M0	–	Tumour
6	M	76	Oral cavity	T4 N0 M0	+	Tumour
7	M	58	Oropharynx	T4 N2b M0	+	Node
8	M	48	Oropharynx	T3 N2c M0	–	Node
9	M	61	Oropharynx	T4 N2e M0	–	Node
10	M	65	Oropharynx	T4 N1 M0	–	Node
11	M	46	Oropharynx	T4 N2c M0	–	Node
12	M	60	Oropharynx	T3 N2a M0	–	Node
13	M	47	Oropharynx	T3 N2b M0	–	Node
14	M	74	Oropharynx	T4 N3 M0	–	Node

*Tumour, primary tumour; node, metastatic neck node.

in order to be representative of pO_2 distribution in the tissues. At the end of the measurement, the needle probe was immediately and automatically removed. A complete procedure (measurement in normal tissues and in tumour) took 30 min. One set of pO_2 measurements was performed in the 24 h before the start of the radiotherapy and a second one at the end of the second week in the 4 h following the first daily fraction (dose delivered 32 Gy). Tracks before treatment and after 2 weeks were made in the same tumour area. A comparison of the pO_2 measurements was made with a Wilcoxon rank test.

RESULTS

All patients had grade III acute skin reactions at the end of the treatment. Mucositis was always very pronounced, the patients had grade II mucositis at the end of the second week and grade III mucositis at the end of the treatment. The resolution of the mucositis was observed after 2–3 months. Due to mucositis and dysphagia, a naso-gastric tube was used in two-thirds of patients.

All pO_2 measurements were well tolerated. The overall time for measurements in normal tissues and in tumours did not exceed 30 min. For normal tissues outside the radiotherapy field, oxygen distribution was slightly increased during treatment, with a pooled median pO_2 of 38 mmHg before treatment and of 46 mmHg after 2 weeks of treatment. However, the difference was not statistically significant. The pooled 10th percentile was, respectively, 11 and 15 mmHg before and during treatment. Very low values (< 2 mmHg) represented less than 2.5% of the recorded values before and during radiotherapy. Results for normal tissues before treatment are shown in Figure 1 (a) and after 2 weeks in Figure 1 (b). The slight increase in normal tissue oxygenation could be due to smoking cessation for some of the patients. However, it was not always possible to obtain any information on the precise date at which the patients had stopped smoking.

For tumours, the pooled median pO_2 was 13 mmHg before treatment and 33 mmHg after 2 weeks of treatment. This difference was significant ($P = 0.05$). Very low values (< 2 mmHg) represented 20% of the recorded values before treatment and 10% after 2 weeks and low pO_2 values (< 10 mmHg) accounted for, respectively, 45 and 25% of the recorded values. The 10th percentile was, respectively, 1 and 2 mmHg. The relative increase in tumour oxygenation was more pronounced for the six evaluated primary tumours (median pO_2 12 mmHg before treatment versus 26 mmHg after 2 weeks, $P < 0.05$) than for the eight metastatic nodes (respectively, 20 and 27 mmHg, $P = 0.10$). The exact variations in tumour sizes due to 2 weeks of treatment were sometimes difficult to assess, mostly for the primary tumours. Results for all head and neck tumours (primary plus nodes) are shown in Figure 2 before treatment and at 2 weeks, and for primary tumours only in Figure 3.

When tumours were compared individually, large interpatient heterogeneity was found. An increase in median pO_2 was shown in nine of 14 tumours, a decrease in four of 14 and no change in one of 14. Individual variations are presented in Table 2. For the nine tumours with values recorded below 2 mmHg, a decrease in the percentage of very low values (increased oxygenation) was found in 7 cases after 2 weeks of treatment.

For the 5 patients who breathed carbogen during accelerated radiotherapy, a marked increase in tumour oxygenation was found in four of five of the tumours. After 2 weeks, the median pO_2 of the five tumours was 44 mmHg compared with 13.5 mmHg before treatment ($P = 0.05$). This limited number of patients makes any comparison between breathing and non-breathing carbogen patients difficult.

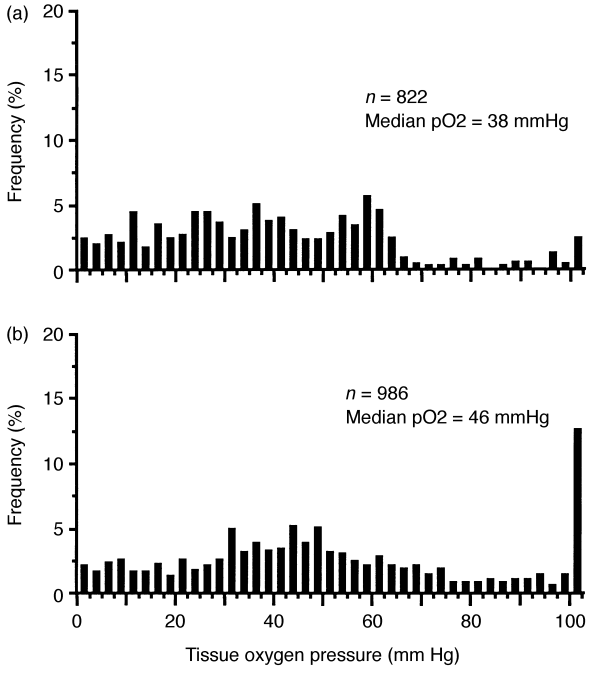

Figure 1. Histograms of oxygen tension distribution before (a) and during (b) accelerated radiotherapy (end of week 2) in non-irradiated normal tissues. (n = number of recorded values).

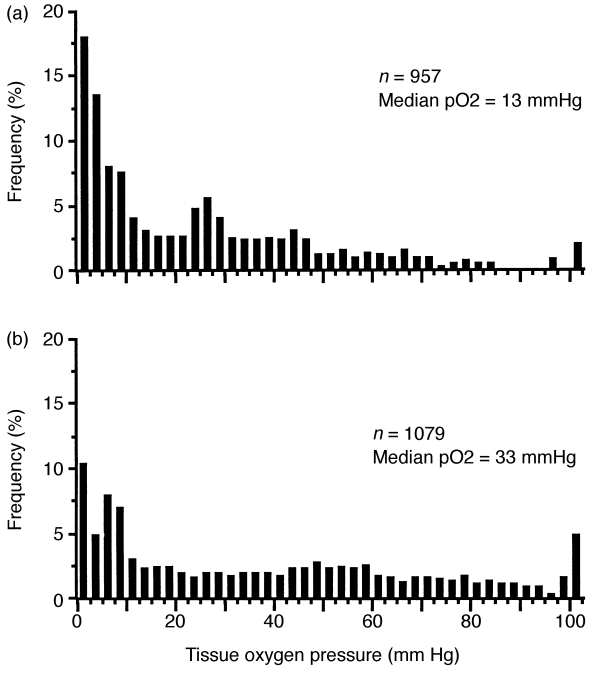

Figure 2. Histograms of oxygen tension distribution before (a) and during (b) accelerated radiotherapy (end of week 2) in all evaluated head and neck tumours. (n = number of recorded values).

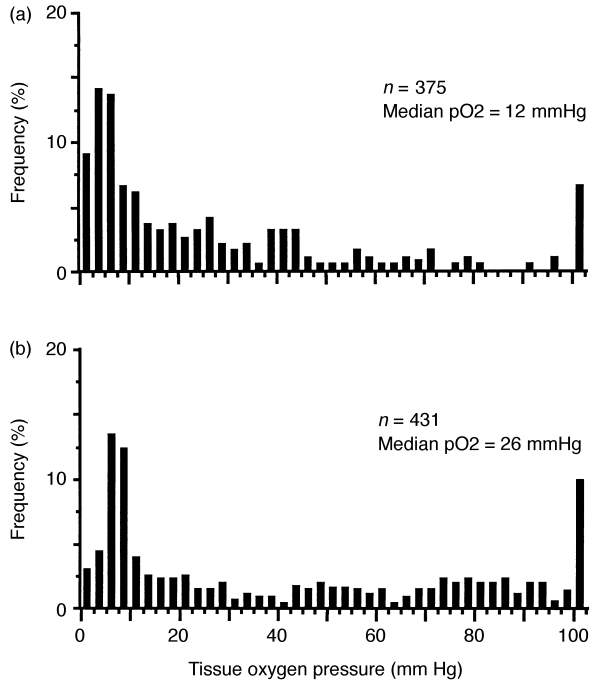

Figure 3. Histograms of oxygen tension distribution before (a) and during (b) accelerated radiotherapy (end of week 2) in primary tumours only. (*n* = number of recorded values).

DISCUSSION

One of the most important biological parameters of tumour response to radiotherapy is the dose-modifying role of oxygen (oxygen enhancement ratio) [29, 30]. To assess the oxygenation status of human tumours, needle probes were first used in the 1950s, despite technical limitations [24–26] and changes in pO_2 were monitored with patients breathing air or oxygen. These techniques were improved in the late 1980s through the KIMOC-6650 histograph [4, 5, 31]. The KIMOC-6650 histograph uses fast responding electrodes programmed to minimise the effects of tissue compression. Massive oxygen consumption by the electrode and tissue compression are largely overcome. However, there is some systematic overestimation of oxygen partial pressure by this equipment (which could be due to bleeding along the probe track) when compared with measurements made with micro-electrodes [4].

The use of the combination of carbogen breathing and accelerated radiotherapy could be limited by an increase in acute normal tissue reactions. Even though the number of patients is limited, data presented show that carbogen breathing appears to be safe for use in clinical situations and well tolerated with accelerated radiotherapy. The normal tissue reactions were not more pronounced than those seen in previous protocols, giving 70 Gy in 5.5 weeks, then in 4.5 weeks, or in our previous patients treated with the same protocol of 70 Gy in 3.5 weeks [21]. The appearance and resolution of the reactions occurred with the same timing as in a comparable accelerated regimen, such as CHART [32]. No bone or cartilage necrosis or skin or mucosa ulcerations were seen in our patients. The spinal cord dose was kept well below 40 Gy to avoid any myelitis.

It has been shown with the KIMOC 6650 that normal tissues are, in general, better oxygenated than solid tumours and this has been again confirmed in this study [4–6, 10, 11]. This finding probably reflects the better vascularisation of normal tissues than tumours and the higher interstitial pressure present in tumours. In our study, more than 90% of the values recorded in normal tissues were above 10 mmHg. At this pO_2 the relative radiosensitivity is close to a maximum and only a small increase in sensitivity can be expected with an increase in oxygen delivery. Our previously published study on 20 patients with head and neck carcinomas showed that tumour tissues often had low pO_2 and a high percentage of values < 10 mmHg [5], with median pO_2 values of 9 mmHg compared with 13 mmHg in the current study (38 mmHg for normal tissues). Due to the limited number of patients, it is difficult to compare oxygen distribution in primary tumours and in metastatic neck nodes. However, it can be said that the relative increase in tissue oxygenation was more pronounced for primary tumours than for neck nodes. Thus, re-oxygenation seems to occur during hyperfractionated radiotherapy such as the protocol used in our study, with which there is a marked increase in tumour oxygenation within 2 weeks of starting treatment (median pO_2 33 mmHg). In accordance with our previously published data [5], no systematic variations in tumour oxygenation were found from the periphery to the centre of the tumour, as

Table 2. Oxygen tension (pO_2) measurements before and during accelerated radiotherapy

	pO_2 before treatment			pO_2 at 2 weeks		
Patient	Median (mmHg)	10% (mmHg)	% < 2 mmHg	Median (mmHg)	10% (mmHg)	% < 2 mmHg
1	6.0	3.6	0	6.4	5.6	0
2*	6.8	3.7	0	20	15	0
3*	14.5	3.8	5	16	2	10
4*	10.4	1	40	59	13.5	0
5	8.2	4	0	4.4	3	0
6*	33.7	5	0	100	10	0
7*	1.5	1	60	25	2	17
8	37	7	3	63	32	0
9	38	12	5	3	1	30
10	31	2	20	19	3	0
11	13	2	12.5	13	5	0
12	27	23	0	4	14	0
13	27	1	17	62	27	0
14	3	1.5	35	10	3	10

*Patients with carbogen breathing.
10%, 10th percentile.

described by other authors [31]. However, like all techniques with pO_2 electrodes, the data presented do not allow the proportion of clonogenic hypoxic cells to be estimated nor to discriminate between 'chronic' and 'intermittent' hypoxia. However, two recently published studies in head and neck and uterine cervix carcinomas indicate that pretreatment pO_2 measurements by needle probe are of predictive value for the tumour response to radiotherapy [10, 11]. However, the best parameter to demonstrate 'predictive tumour hypoxia' is still not clearly defined. Two distinct parameters have been described: the 'hypoxic fraction' based on median pO_2 [10] and the percentage of pO_2 values below 2.5 mmHg [11].

One efficient way to decrease the percentage of low pO_2 values in tumours is to ask the patient to breathe carbogen or pure oxygen, but the effect on oxygen distribution in the tissues will vary from one patient to another. Results previously obtained in 13 patients suggested that carbogen breathing could improve the overall oxygenation of the tumour in only two-thirds of patients [28]. Based on these results, it could be worthwhile to select the patients who should benefit from carbogen breathing on the basis of pre-irradiation pO_2 measurements. However, as demonstrated in this study, an increase in tumour oxygenation is observed in 70–80% of cases during the course of accelerated treatment, This finding, observed for both air and carbogen breathing patients, means that the impact of carbogen breathing on tumour response to treatment could be less than predicted by pre-irradiation pO_2 investigations. With this limited number of patients, it can only be said that some changes in pO_2 distribution do occur during accelerated radiotherapy with a decrease in the frequency of low values after 2 weeks. The timing of measurements was respected for all patients (interval between radiotherapy session and pO_2 measurement) and, in our experience, it would be very difficult to assess tumour oxygenation at the end of the treatment. The skin and mucosal reactions made the measurements painful for the patients and the clinical evaluation of the residual disease was often extremely difficult.

The remaining question is to determine the best timing in order to sensitise hypoxic cells, i.e. at the start or during fractionated radiotherapy, knowing that variation in tumour oxygenation will take place during fractionated radiotherapy for most patients. One of the more logical ways could be to have the maximum increase in tumour oxygenation at the start of the treatment (erythropoietin, sensitisers, carbogen breathing) and to use bioreductive drugs over the course of the treatment, in order to be active on hypoxic clonogenic cells remaining in non-oxygenated tumours [33–35]. The clinical impact of such 'oxygen-based' radiotherapy remains to be demonstrated.

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