Clinical Outcome and Tumour Microenvironmental Effects of Accelerated Radiotherapy with Carbogen and Nicotinamide

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Experimental studies have shown an almost 2-fold increase in effectiveness if accelerated radiotherapy combined with carbogen and nicotinamide (ARCON) was compared with standard radiotherapy. This combination was chosen in order to overcome repopulation of clonogens during radiotherapy and to minimize tumour hypoxia. Analysis of microenvironmental parameters is required to identify tumours that can benefit from these new treatment approaches. In this study 124 patients with stage III or IV head and neck squamous cell carcinomas received ARCON treatment. Vascular architecture, perfusion, proliferation and oxygenation were studied in two human laryngeal squamous cell carcinoma xenograft lines and the effects of carbogen and nicotinamide were analysed. Loco-regional control for stage III–IV larynx carcinomas was 85%, for hypopharynx carcinomas 50% and for oral cavity and oropharynx carcinomas 65%. In the experimental studies, carbogen treatment resulted in one tumour line in a decrease of blood perfusion, which was reversed if nicotinamide was added. The other tumour line showed no perfusion changes after carbogen or nicotinamide treatment. Both tumour lines showed a drastic reduction of hypoxia after carbogen breathing only or carbogen breathing plus nicotinamide. The ARCON schedule results in high loco-regional tumour control rates. Analysis of tumour microenvironmental parameters showed differences in response to carbogen and nicotinamide between different tumour lines of similar histology and site of origin. This indicates that it may be advantageous to base the selection of patients for oxygenation modifying treatment on microenvironmental tumour characteristics.

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Radiotherapy is often preferred to surgery for the treatment of head and neck cancer, because of the good results in loco-regional control combined with preservation of organ function. The treatment of early laryngeal cancer with radiotherapy alone, for example, can achieve cure rates of 66–93% (1). However, there has also been on-going work aiming to improve the results of radiotherapy for more advanced local disease.

Advanced local disease can only be treated successfully if radiotherapy is given in combination with a strategy to overcome the effect of radiation-resistance mechanisms. The three radio-resistance mechanisms that are considered to be most important for radiation treatment failure are: intrinsic radio-resistance; tumour cell repopulation during treatment; and tumour hypoxia. Overcoming these resistance mechanisms almost always results also in an increase in side effects. It is therefore important to aim at treatment intensification in combination with careful selection of patients for new treatment modalities.

To overcome treatment failure due to intrinsic radio-resistance the tumour dose has to be increased. This can be achieved by dose escalation with hyperfractionation or with 3-D planned conformal radiotherapy (2, 3).

The second mechanism that is known to be important for failure of loco-regional tumour control is cellular repopulation during treatment. It has now been recognized as an important cause for radiation treatment failure in various cancers, particularly squamous cell carcinomas (4, 6–8). One week of prolongation of the overall treatment time during a course of fractionated radiotherapy can result in a 3–25% loss of local control (5). Randomized clinical studies have shown that patients with head and neck and bronchus carcinomas benefit from reducing the overall treatment time to counteract repopulation of clonogenic tumour cells during treatment (4, 6–8).

The third mechanism that can be a cause of treatment failure is tumour hypoxia. A meta-analysis of 72 randomized clinical trials showed that the combination of radiotherapy with a treatment to modify tumour oxygenation resulted in an improvement of loco-regional control and survival compared with radiotherapy alone (9). This meta-analysis included, for example, randomized studies on the

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use of hyperbaric oxygen in head and neck carcinoma and in carcinoma of the uterine cervix which demonstrated an improvement in loco-regional control and survival if irradiation took place under hyperbaric conditions relative to treatment in air (10, 11). In a recently published randomized study, an increase in tumour control and survival was found in patients with supraglottic and pharynx carcinomas with the hypoxic radiosensitizer nimorazole (12).

Often in the above-mentioned studies there were groups of patients showing a better response to the modified treatment than other groups. For example in the meta-analysis by Overgaard and Horsman the improvement was dominated by head and neck tumours (9).

Because of the difference in response between categories of patients to certain treatment modifications it is important to select patients before treatment is started. This selection needs to be based on the factors that the treatment modification is aiming at. Predictive assays for the various resistance mechanisms have been developed and the potential value of some of these assays has been shown in clinical radiotherapy studies.

INTRINSIC RADIO-RESISTANCE

Assessment of the radiosensitivity of tumour cells by a clonogenic survival assay gives a direct measure of the fraction of cells capable of forming colonies after a given dose of irradiation. However, clonogenic assays are not feasible if cells fail to proliferate in a tissue culture system or, as is the case in some primary and early passage cultures, if the tumour consists of several sublines with differences in growth rate and/or intrinsic radiosensitivity. Mechanism based assays of intrinsic radiosensitivity are being proposed and tested to use instead. The relative degrees of chromosome and DNA break induction and repair, and the subsequent clonogenic cell survival does not only depend on the cell type, but also on changes throughout the cell cycle. This means that non-clonogenic endpoints of radiation sensitivity must be used with caution (13).

The intrinsic radiosensitivity of squamous cell carcinomas analysed by clonogenic assays is intermediate relative to tumours of other histology (14). Differences in parameters derived from clonogenic assays are correlated with the probability of loco-regional control in head and neck cancer (15). However, it is not possible to select individual patients based on intrinsic radiosensitivity testing alone, because the discriminating power is not sufficient and often a clonogenic assay is not fast enough for clinical application.

TUMOUR CELL PROLIFERATION KINETICS

The fraction of cells in the S-phase can be analysed after labelling with thymidine analogues, such as bromodeoxyuridine (BrdUrd). The fraction of labelled cells is the Labelling Index (LI) which is a measure of the proliferative activity of a cell population. The LI can be determined either by counting under the microscope (16) or by flow cytometry (FCM) (17). Data collected from 10 different European trials showed that the pre-treatment LI, analysed by flow cytometry, is correlated with local control after conventional radiotherapy (18). The tissue architecture is lost because of the processing for flow cytometry, which is a disadvantage because it precludes analysis of histological patterns of proliferation and relations with other structures such as blood vessels. Analysis of whole tissue sections shows various differences in distribution patterns of proliferating cells, even though the overall LI is the same (19). The spatial information of proliferation patterns can give additional information which can help in selecting patients for accelerated treatment in certain tumours more than overall LI based on flow cytometry alone (19). Recently, we presented a semi-automatic computer-controlled method for the analysis of tumour cell proliferation and tumour blood perfusion in relation to tissue architecture in whole tissue sections (20).

TUMOUR OXYGENATION

Well-oxygenated cells are more radiosensitive than hypoxic cells (21). In the classical model of Thomlinson and Gray, hypoxic cells are located at a relatively constant distance from blood vessels, which is dependent on the diffusion capacity of oxygen in tissues ('chronic' or 'diffusion-limited' hypoxia) (22). Another form of hypoxia is induced by local and temporary fluctuations of tumour blood perfusion, this has become known as 'acute' or 'transient' hypoxia (23). It has been demonstrated that hypoxia is present to various degrees in most rodent and xenografted tumours (24). Estimates of the amount of hypoxia in tumours range from 1% to 98%. These estimates depend on several factors, including the method used for measuring hypoxia, tumour size, transplantation site and characteristics of the host. In clinical studies measurement of tumour hypoxia has been shown to predict for the treatment outcome of patients receiving radiotherapy for carcinomas of the uterine cervix, head and neck carcinomas and for soft tissue sarcomas (25–28). The most commonly used methods for measuring the oxygenation status of tumours and tissues are oxygen electrodes and, more recently, time resolved luminescence measurements for direct measurement of pO_2. Another way to quantitate hypoxia is by using immunohistochemical staining of bioreductive chemical probes, such as the 2-nitroimidazoles (29, 30). After injection and staining they can be analysed by flow cytometry (31) or visualized in tissue sections (32). In situ detection of hypoxic probes has been used to analyse the distribution of tumour hypoxia in whole tissue sections (33).

The improvement of loco-regional tumour control is in the order of 5–20% if the treatment is modified to counteract one of the above mentioned radio-resistance mechanisms (4, 6–8, 10–12, 34). Even though these are important improvements, many individual patients will still remain uncontrolled. It is likely that a combination of these treatment modifications will lead to an additive effect. The DAHANCA trials studied the combination of shortening the overall treatment time of radiotherapy together with a hypoxic cell sensitizer. Significant increases in local control and survival were found after first adding the hypoxic cell sensitizer nimorazole and, second, reducing the overall treatment time by giving 6 instead of 5 treatments per week (4, 12).

The combination of Accelerated Radiotherapy with CarbOgen breathing and Nicotinamide (ARCON) that was first proposed by Rojas et al. is applied in the present study (35, 36). Carbogen (95% O_2 and 5% CO_2) breathing causes a rise of the oxygen partial pressure in blood and tissues and reduces chronic hypoxia. In patients with head and neck cancer this effect was shown by direct measurements with oxygen electrodes in metastatic lymph nodes (37). Nicotinamide, the amide derivative of vitamin B_3, can reduce the intermittent closure of blood vessels in experimental rodent tumours and consequently decreases transient hypoxia (38, 39). With carbogen alone, a TCD-50 enhancement ratio of 1.5 was obtained in mouse tumours with irradiation doses close to those used clinically (36). Addition of nicotinamide gave further radiosensitization to an enhancement ratio of 1.7 (36). Finally, when also accelerated fractionation was incorporated the complete ARCON treatment resulted in an enhancement ratio of 1.9, indicating an almost 2-fold increase in the effectiveness of this treatment relative to conventional radiotherapy alone (36). These promising results justified testing of this treatment in the clinic.

It is clear that it is necessary to combine treatment modalities in order to increase tumour control. Often this will lead to an increase of side effects. Therefore, in parallel with treatment modifications, predictive tests are being developed to select patients or categories of patients that are more likely to benefit from these new treatment approaches. In our microenvironmental study we aim to combine tumour architecture, vascularization, tumour cell proliferation and hypoxia into one predictive profile. This profile will enable us in the future to identify patients or categories of patients for these new treatment combinations.

In this paper the results of the ARCON treatment for head and neck cancer and the recent advances in the development of predictive profiles to select patients for this treatment are presented.

MATERIAL AND METHODS

Clinical study

Study design and patients. Patients with squamous cell carcinomas of four major head and neck sites: oral cavity, oropharynx, larynx and hypopharynx, are included in this study. All patients have advanced disease, i.e. stage III or IV. Also voluminous stage II hypopharyngeal lesions are eligible.

Between October 1993 and May 1998 124 patients received the ARCON treatment. The study started with larynx carcinoma patients only, but from November 1995 patients with hypopharynx, oral cavity and oropharynx carcinomas were also eligible. The distribution by tumour site and TNM-stage (UICC 1992) is shown in Table 1.

The study was approved by the local ethics committee.

Accelerated radiotherapy. The treatment started with giving one fraction of 2 Gy per day, five times a week. During the last 1.5 weeks, treatments were given twice daily (2 Gy per fraction) with an interval between the fractions of at least 6 h. The overall treatment time was 36–38 days. Total dose was 68 Gy for gross disease and 44 Gy for the nodal areas treated electively. Sensitization of the laryngeal cartilage has been reported after radiotherapy in hyperbaric oxygen. A 10% reduction of the total dose in a subsequent study of hyperbaric oxygen reduced the laryngeal complications to a level seen with treatment in air (10). Since a similar effect might be expected from normobaric carbogen with nicotinamide, the maximal permissible dose to the larynx was reduced from 70 Gy to 64 Gy and, as a consequence, the dose for primary tumours of the larynx and hypopharynx did not exceed this limit. Involved nodes received 68 Gy. Because a decrease in the

Table 1

Patients treated with the ARCON regimen. Distribution by tumour site and TNM-stage (UICC 1992)

	N0	N1	N2	N3	Total
Larynx					
T1	–	–	1	1	2
T2	–	4	6	–	10
T3	22	9	7	–	38
T4	11	–	2	–	13
Total	33	13	16	1	63
Hypopharynx					
T1	–	1	–	–	1
T2	3	4	7	1	15
T3	–	2	3	–	5
T4	1	3	7	1	12
Total	4	10	17	2	33
Oral cavity/oropharynx					
T1	–	–	1	–	1
T2	–	–	–	–	–
T3	1	4	7	–	12
T4	2	4	7	2	15
Total	3	8	15	2	28

tolerance of the rat spinal cord of ~20% was observed when radiation was combined with carbogen and nicotinamide (40), total dose to the spinal cord was not higher than 40 Gy.

Carbogen breathing. Scuba-diving equipment was used for carbogen delivery. Details of this breathing system have been described earlier (41). Carbogen breathing commenced 4 min before start of irradiation and was continued throughout the treatment.

Nicotinamide. Nicotinamide was administered orally as a liquid formulation, 1.5 h before irradiation. On days when two fractions were given, only one dose of nicotinamide was taken before the first treatment. Initially, the daily dose was 6 g. Based on pharmacokinetic studies (42), this was changed to a weight-adjusted dose of 80 mg/kg with a maximum of 6 g from April 1994 and the interval between intake and irradiation was reduced to 1 h from November 1995. Also from November 1995, a dose reduction to 60 mg/kg was introduced for those patients who experienced severe side effects.

Chemotherapy. A total of 23 patients received chemotherapy prior to radiation treatment. This consisted of cisplatin weekly for 1–6 courses. All these patients had far advanced unresectable tumours. None of the patients with laryngeal tumours received chemotherapy.

Tumour vasculature and microenvironment

Two tumour lines (SCCNij3 and SCCNij19) derived from different primary human squamous cell carcinomas of the larynx were studied. Tumour vasculature (endothelium marker 9F1), blood perfusion (Hoechst 33342), hypoxia (NITP) and proliferation (BrdUrd) were studied in whole tissue sections. For details of the staining procedure see Bussink et al. (20, 33). The effects of carbogen breathing and nicotinamide on these parameters and the differences in response between the two tumour lines were analysed.

The tissue sections were analysed with an image processing system after immunohistochemical staining of NITP, BrdUrd and vasculature. A high-resolution intensified solid-state camera on a fluorescence microscope (Zeiss Axioskop) with a computer-controlled motorized stepping stage was used. A detailed description of the scanning method has been given by Rijken et al. (43). Tumour cross sections were sequentially scanned three to four times, using different filters for the different fluorescent signals. After each scan of complete tissue sections, one composite image was reconstructed of the different fluorescent images. As a final step, the tumour area was delineated by drawing a contour line. This area was used as a mask in further image analysis excluding non-tumour tissue and necrotic areas from the analysis.

Quantitative data of perfusion and vascularity were derived from the Hoechst and 9F1 images. The area of perfused vascular structures divided by the total vascular area of the tissue section yielded the perfusion fraction (43).

The labelling index (LI) was determined from the ratio of the BrdUrd positive surface (FITC) and the total nuclear surface (Fast Blue) (20).

For analysis of the relationship between proliferation and vascularity, functional units, so called 'vascular domains' were calculated. Delineation of these domains is based on imaginary lines which are equidistant from adjacent vessels and which are determined by computerized image processing (20).

In order to describe the distribution of hypoxic regions in relation to the vasculature, the relative amount of hypoxia was calculated as the fraction of NITP-stained area in different zones around a perfused vessel (= relative hypoxic area) (Rijken et al. personal communication).

RESULTS

Clinical study

Carbogen and nicotinamide, compliance. Full compliance with carbogen breathing was 76%. Some patients experienced sensations of suffocation and/or hyperventilation and were not able to cope with the breathing procedure.

The most common side effects of nicotinamide were nausea and vomiting, which occurred in 61% and 30% of the patients, respectively. Thirty percent of the patients discontinued the drug intake and 13% required a dose reduction because of severe symptoms.

Early reactions of mucosa and skin. With the ARCON treatment 69% of the patients developed moist desquamation of the skin with a mean duration of 2.5 ± 2 weeks (SD). Healing was always complete.

Confluent mucositis occurred in 95% of the patients with larynx and hypopharynx tumours and in 90% of the patients with oral cavity and oropharynx tumours. Mean duration of confluent mucositis was 6.0 ± 3.2 weeks (SD) in larynx and hypopharynx tumours and 6.7 ± 4.0 weeks (SD) for oral cavity and oropharynx tumours. Healing was always complete.

Loco-regional tumour control. Median follow-up at the time of analysis was 22 months for larynx carcinomas, 11 months for hypopharynx carcinomas and 11 months for oral cavity and oropharynx carcinomas. No patients have been lost to follow-up. The actuarial loco-regional control rate at 3 years for larynx carcinomas was 85%, for hypopharynx carcinomas 50% and for oral cavity and oropharynx carcinomas 65% (at 2 years). Loco-regional tumour control for the various sites is shown in Fig. 1.

Tumour vasculature and microenvironment

The semi-automatic method for computer-controlled quantitative analysis of whole tissue sections allows study of architectural patterns of proliferation, vascularization and oxygenation of tumours. Perfused and non-perfused

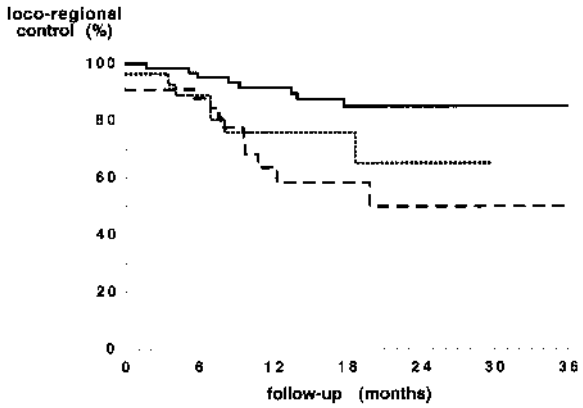

Fig. 1. Actuarial loco-regional tumour control after treatment according to the ARCON schedule for larynx —, hypopharynx –– and oral cavity and oropharynx carcinoma ---.

vessels can be distinguished and specific regions of interest can be analysed separately (20, 33).

Effects of carbogen and nicotinamide

Perfusion. SCCNij19 showed no changes in tumour blood perfusion after carbogen or nicotinamide treatment. For SCCNij3 a decrease of tumour blood perfusion of 11% was found after carbogen breathing only (from 66% to 55%). The combination of carbogen and nicotinamide resulted in a 12% increase of the perfusion fraction to 78%.

Proliferation. Nicotinamide treatment resulted in an increase in LI. SCCNij3 showed an increase in LI from 10.7% to 14.1% and for SCCNij19 an increase from 12.6% to 14.5% was found.

Carbogen treatment resulted in both tumour lines in a decrease of the LI: for SCCNij3 from 10.7% to 6.8% and for SCCNij19 from 12.6% to 5.2%.

The combination of carbogen and nicotinamide resulted in a different effect on the LI in the two tumour lines: for SCCNij3 the LI decreased to a level close the carbogen only group (5.7%), but for SCCNij19 the LI stayed close to the level of the control animals (10.8%).

Hypoxia. The NITP stained area was drastically reduced if tumours were treated with carbogen or carbogen in combination with nicotinamide. At an increasing distance from 150 μm to 300 μm from the nearest perfused vessel the hypoxic fraction of untreated tumours increased from 3.5% to 11% in SCCNij3 and from 3.5% to 6% in SCCNij19.

After carbogen breathing the hypoxic fraction was reduced to 1.5% or less from 150 μm to 300 μm from the nearest perfused vessel in both tumour lines. Treatment with nicotinamide alone did not significantly change the NITP-staining pattern in either tumour line.

As an example, Fig. 2 shows the binary images of untreated and treated tumours (SCCNij3). There is an almost complete disappearance of hypoxia after treatment with carbogen alone (not shown) or carbogen in combination with nicotinamide (top right). The bottom images show the decrease in proliferation after treatment with carbogen and nicotinamide (right) relative to the untreated tumour (left).

DISCUSSION

Failure of a tumour to respond to radiotherapy has been attributed mainly to three radio-resistance mechanisms: intrinsic radiosensitivity; accelerated repopulation during radiotherapy; and tumour hypoxia. This paper focuses on treatment modification to overcome the latter two. Both the clinical response of the ARCON treatment and the development of pre-treatment testing to select patients that can benefit from this new treatment approach are presented. The changes in the tumour microenvironment after carbogen breathing and nicotinamide are described.

Fig. 2. Binary images of tumour sections of a human laryngeal squamous cell carcinoma xenograft tumour line (SCCNij3), showing microenvironmental changes after oxygenation modifying treatment. Left: control; right: combination of carbogen and nicotinamide. Top images; scanned at 100 × magnification for analysis of tumour blood perfusion (Hoechst, blue), vascular structures (9F1, red) and tumour hypoxia (NITP, green). Bottom images: scanned at 200 × magnification for analysis of proliferation characteristics (BrdUrd, green) in relation to vasculature (9F1, red) and tumour blood perfusion (Hoechst, blue). The boxes in the top images show the area that is shown at 200 × magnification in the bottom image (consecutive tissue section).

Clinical study

Patients with squamous cell carcinomas of the head and neck were selected because there was already evidence that this category of patients can benefit both from acceleration and from hypoxic modification. Additional arguments were that the loco-regional control rate for the more advanced cases is not yet satisfactory and that organ preservation in the head and neck region is of great value. This region is easily accessible for assessment of normal tissue reactions and tumour response. Finally, hypoxic modification and treatment acceleration increases loco-regional control (10, 12).

In the present study patients were treated with a combination of accelerated radiotherapy, nicotinamide and carbogen. This approach has led to a loco-regional control rate for stage III–IV larynx carcinomas of 85% at 3 years, which is considerably higher than the control rates of 23–53% that are obtained with conventional irradiation schedules (44–46). In fact, better results have not previously been reported. The results of the ARCON treatment for hypopharynx and oral cavity and oropharynx tumours are not as high as for the larynx carcinomas. However, these patients had more voluminous tumours, with 70% of the hypopharynx tumours and 82% of the oral cavity and oropharynx tumours being stage IV (see Table 1). Most of the patients with oral cavity and oropharynx tumours had unresectable disease and were referred for palliative treatment. A 65% 2-year loco-regional control rate for this category of patients is certainly very encouraging.

Although these results are promising, an increase in mainly acute toxicity is observed. Both early skin and mucosal reactions were increased, with acceleration and hypoxic modification. A gradual increase of the severity and duration of these reactions occurred with successive addition of the three components: acceleration, carbogen and nicotinamide (47–49). This emphasizes the need for predictive testing, in order to select patients who are likely to benefit from the intensified treatments. Patients with sensitive tumours who are likely to do well with conventional treatment can thus be spared the enhanced toxicity.

Tumour vasculature and microenvironment

Multiple staining of the same tissue section is a difficult procedure. For instance antibodies used for visualizing one antigen can be damaged if visualization of the next antigen needs aggressive treatment, such as acid to expose BrdUrd-labelled DNA. Image analysis with the semi-automatic method for quantitative analysis as described, enabled us to perform triple and quadruple staining of sections. In order to preserve optimal staining quality and signal to noise ratio, tissue sections were scanned between the staining sessions, i.e. each fluorescent signal was captured before the staining of the next marker (antigen) was commenced.

Proliferation and hypoxia can be analysed in whole tissue sections, but also as a distribution pattern throughout the tissue section or in relation to vasculature (20, 33).

The two tumour lines that were used in these studies, SCCNij3 and SCCNij19, were both derived from male patients with advanced laryngeal carcinoma (both clinical stage T4N2cM0). The differentiation grades of both squamous cell carcinomas were similar (SCCNij3 moderately to well differentiated, SCCNij19 moderately differentiated). However, the vascular pattern, proliferation characteristics and the effect of carbogen and nicotinamide on the tumour microenvironment were different.

Hypoxia and perfusion. Carbogen breathing gave a different response in the two tumour lines. In SCCNij3 there was a decrease in perfusion, whereas SCCNij19 showed no change in perfusion after carbogen treatment. Tumour-dependent changes in blood perfusion during carbogen breathing were also observed by other investigators (50). A difference in response to carbogen or nicotinamide may be related to endogenous vasoconstrictors (e.g. endothelin-1) and vasodilators (e.g. NO) that are oxygen dependent and tumour dependent (51).

The absence of a significant increase of tumour blood perfusion after nicotinamide treatment may be attributed to a high baseline perfusion fraction in these tumours that can not be much further increased by nicotinamide. If, however, tumour blood perfusion is not at the maximum level due to carbogen treatment, nicotinamide can compensate and cause an increase in the perfusion fraction, which was shown for SCCNij3.

Proliferation. The decrease in LI after carbogen treatment may be caused by a cell cycle delay in S-phase as a result of glucose and other nutrient deprivation (both lines showed a reduced perfusion after carbogen), or a G2 delay caused by reperfusion injury to previously poorly oxygenated cells by the overall increased pO_2. The decrease of the extracellular pH after carbogen breathing does not result in a change of intra-cellular pH (52), the consequences of this for the proliferative activity of tumour cells is unclear at the moment. The observed anti-proliferative effect of carbogen could complement the radiation modifying effects on the hypoxic compartment of tumours by also reducing the proliferative activity of tumour cells during a course of fractionated radiotherapy. However, the anti-proliferative effect in SCCNij19 was reversed by nicotinamide, which suggests that the anti-proliferative effect of carbogen may not be obtained in all tumours with the full ARCON treatment.

The differences in response to nicotinamide and carbogen between the two tumour lines that were analysed show that even in tumours of the same site and histology a pre-treatment analysis may in the future help to select patients for treatments that reduce tumour hypoxia. The goal of the microenvironmental study is to develop an assay that can aid the selection of patients or patient

categories that may benefit from radiotherapy treatment modification. Recently, we have started a study that applies most of the described methodology directly to primary human tumour material. Patients are injected with a hypoxic marker (pimonidazole) and a proliferation marker (IdUrd or BrdUrd), after which diagnostic biopsies are taken and analysed. Initial results indeed show large differences of microenvironmental parameters between individual patients.

CONCLUSION

The clinical outcome of the treatment of head and neck cancer patients with advanced disease according to the ARCON schedule results in high loco-regional tumour control rates. The microenvironmental study shows that the analysis of multiple parameters related to the tumour tissue architecture can reveal different responses to carbogen and nicotinamide, even in tumour lines of similar histology. This indicates that a pre-treatment analysis of tumour characteristics may aid the selection of patients who can benefit from accelerated fractionated radiotherapy and oxygenation modification.

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