The effect of combined nicotinamide and carbogen treatments in human tumour xenografts: oxygenation and tumour control studies

Georg Stüben[a],*, Martin Stuschke[a], Kai Knühmann[a], Michael R. Horsman[b], Horst Sack[a]

[a]*Department of Radiotherapy, Strahlenklinik im Universitätsklinikum Essen, Hufelandstr. 55, D 45122 Essen, Germany*
[b]*Danish Cancer Society, Department of Experimental Clinical Oncology, Aarhus University Hospital, Norrebrogade 44, DK-8000 Aarhus C, Denmark*

Abstract

Background and purpose: This was an investigation to study the effect of giving carbogen and nicotinamide (CON) on pO_2 and the radiation response of human xenografted tumours.

Materials and methods: The human xenografts were two sarcomas (ENE2 and ES3) and a glioblastoma (HTZ17). Nicotinamide (500 mg/kg, i.p.) was administered 60 min before pO_2 measurements and irradiation, while carbogen was given for 5 min before and during these treatments. Tumour pO_2 was measured with an Eppendorf electrode and radiation response was assessed by local tumour control following irradiation with 10 daily fractions.

Results: All three xenografts were found to be poorly oxygenated (about 80% of all pO_2 values were ≤2.5 mmHg). CON treatment improved the oxygenation status in all three tumours such that 65, 52 and 71% of the pO_2 values were ≤2.5 mmHg in ENE2, ES3 and HTZ17, respectively. However, only in ES3 was this decrease significant. The TCD_{50} doses for all tumours were around 52–54 Gy. No significant improvement was seen with CON in ENE2 (TCD_{50} = 48 Gy) and HTZ17 (TCD_{50} = 56 Gy), but for the ES3 xenograft a significant decrease to 42 Gy was found.

Conclusions: The three tumours used in this study appeared to show the same level of hypoxia as measured both by pO_2 and radiation response. However, only one tumour showed a significant improvement after CON treatment, suggesting that not all hypoxic human tumours might benefit from this type of therapy.

Keywords: Nicotinamide; Carbogen; Hypoxia; Breathing modalities; Xenografts; Nude mice

1. Introduction

A major factor contributing to tumour radioresistance is tumour hypoxia. It is now clear that experimental tumours contain a significant proportion of hypoxic cells [27,38] and there is increasing evidence that hypoxia is relevant in the clinical situation [30,32]. Recently, more insight was gained into the complex mechanism of tumour hypoxia by identifying at least two distinct types, i.e. diffusion limited chronic hypoxia, as first described by Thomlinson and Gray [46], and perfusion limited acute hypoxia, which is thought to result from intermittent impairment of blood flow [5,8]. A possible approach to reduce both types of hypoxia is the combination of carbogen and nicotinamide (CON). The rationale for this combination is that while carbogen breathing increases the distance that oxygen can diffuse from the vessels into the tumour [14,41], nicotinamide on the other hand appears to reduce the temporally impaired blood flow and thus affects the proportion of acutely hypoxic tumour cells [17]. Using this combination, significant enhancements of radiation response both in single doses [17] and fractionated regimes [23,39] have been observed.

Although clinical phase I/II trials are currently being carried out, pre-clinical data for human tumours radiosensitized with carbogen and nicotinamide are limited. We have now evaluated the combined use of carbogen and nico-

* Corresponding author.

tinamide (CON) treatments on the fractionated radiation response of human tumours grown as xenografts in nude mice. These tumours probably have growth characteristics more relevant to the clinical situation than rodent tumours. Furthermore, there is evidence that the vascular characteristics of the original tumours are largely maintained in the nude mouse model [2,48].

In addition, measurements of tumour oxygen partial pressure (pO_2) distributions were made with the Eppendorf polarographic electrode to both identify the changes in pO_2 induced by the CON therapy and to see how these measurements correlated with the response to radiation.

2. Materials and methods

2.1. Animals and tumour models

Three human tumour xenografts were used, i.e. ENE2, a human neurogenic sarcoma established from a biopsy of a local recurrence of the primary with an average (±1 SE) tumour volume doubling time of 2.9 (±0.2) days, ES3, a spindle cell sarcoma established from a biopsy of the primary with a tumour volume doubling time of 2.7 (±0.4) days, and HTZ17, a glioblastoma established from a biopsy of the primary with a tumour volume doubling time of 5.3 (±0.4) days. Details of their derivation and maintenance have been previously given [6,10]. The tumours were xenografted by transplanting 2–3 mm chunks into the subcutaneous tissue of the right hind legs of the nude mice (nu/nu of NMRI inbred background). These mice were derived from the central laboratory at Essen University where the breeding was performed under pathogen-free conditions. For experiments, animals were housed in the laboratory of the Department of Radiation Oncology in laminar air flow units and had unlimited access to water (supplemented with chlortetracycline (10 g/l) and K-sorbate (1.35 g/l) acidified to a pH of 3.0) and high calorie lab food. The animals were used for experiments when they had reached an age of 6–9 weeks. All experimentation had previously been approved by the regional animal ethics committee. All three tumour lines were repeatedly characterized by means of DNA content, volume doubling time and isoenzyme pattern of LDH and GPD [6]. During the experimental period no changes of these parameters were observed, confirming the human origin of these tumours.

2.2. Breathing conditions

All pO_2 measurements and irradiations were performed under gas breathing conditions. These were either air or carbogen (95% O_2 plus 5% CO_2). Gas breathing was started for 5 min prior to pO_2 measurements or irradiation and continued during treatment, which for the radiation experiments ranged from 8 to 15 min, depending on the dose delivered. The flow rate was 6 l/min.

2.3. Nicotinamide

Nicotinamide was freshly prepared before each experiment by dissolving in sterile saline. It was injected i.p. at a dose of 500 mg/kg mouse body weight 60 min prior to each radiation fraction or pO_2 measurement. Control animals received sham treatments.

2.4. Anaesthesia

Details of the experimental set-up used for both the irradiations and the invasive pO_2 measurements were published previously [42]. Briefly, the mice were positioned concentrically to the mid-point of the experimental set-up, breathing an anaesthetic gas mixture through openings in the distributor. Enflurane (Ethrane) was circulated by a membrane pump and was mixed with air or carbogen. Further details of the narcotic procedure have been reported [1]. A decrease in body temperature during narcosis was avoided by gently surrounding the animal body with a perspex tube. In addition, two thermostat controlled fan heaters were positioned at a distance of 40 cm from the set-up during irradiation. Rectal probe measurements revealed the efficacy of these means.

2.5. pO_2 measurements

The invasive pO_2 measurements were performed with a computerized system (KIMOC 6650, Eppendorf, Hamburg). The details of this technique have been previously described [21]. Briefly, the intratumoural pO_2 was registered with a polarographic needle with a diameter of 300 μm. The sensitive membrane-covered cathode has a diameter of 12 μm. Forward movements of the needle (500 μm) were immediately followed by a backward step of 200 μm, in order to minimize tissue compression artefacts [21]. Tumour oxygenation measurements were performed along at least three tracks within one individual tumour. Data of the same histology, tumour size and identical treatments were pooled for analysis. At least 250 individual pO_2 data points were obtained for statistical evaluations. Negative values ranging 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 due to 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 analyzed with standard statistical software. The significance of differences between the various treatments was assessed with the Kolmogorov–Smirnov two-sample test [31].

2.6. Radiation treatments

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 by a 12 cm thick Lipowitz's metal shielding block, such that the whole body dose to mice was 8% of the total tumour absorbed dose. Field homogeneity was repeatedly checked with LiF (lithium fluoride) thermoluminescent 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 the time of treatment. The fractionated irradiation consisted of 10 fractions given on 10 consecutive days without gaps for weekends. The first irradiation fraction was given when the tumour reached a volume of 110 mm^3. The fraction size ranged from 2.8 to 8 Gy.

After irradiation the tumours were observed two to three times weekly up to 180 days after treatment. Eight to 16 mice were used for each radiation dose point. The percentage of animals showing local tumour control was then calculated and full radiation dose–response curves were constructed. The dose yielding 50% local control (TCD$_{50}$) with correction for censored data was then estimated by fitting a logistic regression to the entire dose–response data [51]. Values in brackets represent 95% confidence intervals.

3. Results

3.1. pO$_2$ measurements

Table 1 summarizes the results obtained with the electrode measurements of pO$_2$ in the three tumour types. All three human xenografts had a relatively low oxygenation status, with some 80% of all the pO$_2$ values being in the range of 0–2.5 mmHg. Treating the mice with a combination of carbogen and nicotinamide resulted in a shift in pO$_2$ towards improved oxygenation status in all tumours. For ENE2 and HTZ17 tumours the percentage of low values

($\leq$2.5 mmHg) decreased from 79 to 65% and from 82 to 71%, respectively. However, neither of these changes were statistically significant. On the other hand, the decrease from a value of 79% observed in the ES3 tumour under ambient conditions to 52% following carbogen plus nicotinamide was significant ($P = 0.04$).

3.2. Fractionated irradiation

The influence of carbogen and nicotinamide on the radiation response of these tumour xenografts when the radiation was administered in a 10 fraction regime is shown in Figs. 1–3. For the ENE2 neurogenic sarcoma (Fig. 1) the TCD$_{50}$ ($\pm$95% confidence interval) derived under ambient conditions was 52.3 Gy (49.2–55.7 Gy). A slight but not statistically significant radiosensitization was observed following combined treatment with carbogen and nicotinamide, with the TCD$_{50}$ being 48.5 Gy (45.4–51.9 Gy). With the ES3 spindle cell sarcoma (Fig. 2) the TCD$_{50}$ for 10 fraction irradiations was 54.1 Gy (50.6–57.8 Gy) under ambient conditions and a significant improvement in radiation sensitivity occurred with the combined treatment, as seen by the left shift of the dose–response curve, with the TCD$_{50}$ decreasing to 41.8 Gy (39.5–44.2 Gy). This difference between the radiation response of control and carbogen plus nicotinamide-treated tumours at the TCD$_{50}$ level corresponds to a dose modifying factor of 1.3. In the HTZ17 glioblastoma (Fig. 3) the TCD$_{50}$ value derived under ambient conditions was 52.3 Gy (48.5–56.4 Gy). No trend towards improved radiosensitivity was observed following treatment with carbogen and nicotinamide. In fact the TCD$_{50}$ value was slightly higher at 55.8 Gy (50.4–61.8 Gy), but the difference between this value and the one seen under ambient conditions was not significant.

4. Discussion

Despite the disappointing clinical studies with hyperbaric oxygen and chemical sensitizers, a recent meta-analysis

Table 1

Summary of the effect of carbogen and nicotinamide (CON) on the oxygenation status of three human tumour xenografts

Tumour	Condition[a]	$N/n/t$[b]	pO$_2$ (mmHg)[c]			pO$_2$ values (%)[c]	
			Mean	Median	IPR[d]	$\leq$2.5 mmHg	$\leq$5 mmHg
ENE2	Ambient	10/360/30	2.7 ± 2.2	0.7 ± 0.5	11.4	79 ± 11.8	85 ± 12.0
	CON	10/360/30	9.5 ± 7.8	1.5 ± 4.9	22.1	65 ± 21.6	72 ± 22.8
ES3	Ambient	9/324/27	3.1 ± 2.1	1.3 ± 1.2	9.2	79 ± 6.2	87 ± 14.2
	CON	7/252/21	13.2 ± 6.8	2.4 ± 5.0	39.7	52 ± 18.2	62 ± 21.2
HTZ17	Ambient	8/288/24	3.1 ± 2.2	1.3 ± 1.0	9.8	82 ± 10.8	89 ± 12.4
	CON	9/324/27	7.4 ± 4.9	1.6 ± 4.4	11.3	71 ± 18.4	78 ± 19.9

[a]Measurements of tumour pO$_2$ were made in sham treatment conditions (ambient), or 60 min after injecting nicotinamide (500 mg/kg, i.p.) and after breathing carbogen for 5 min before and during the pO$_2$ measurements (CON).
[b]Values indicate the total number of tumours (N) used and the total number of measurements (n) and tracks (t) made.
[c]All results are means ($\pm$ 1 SE) based on the total number of animals in each group.
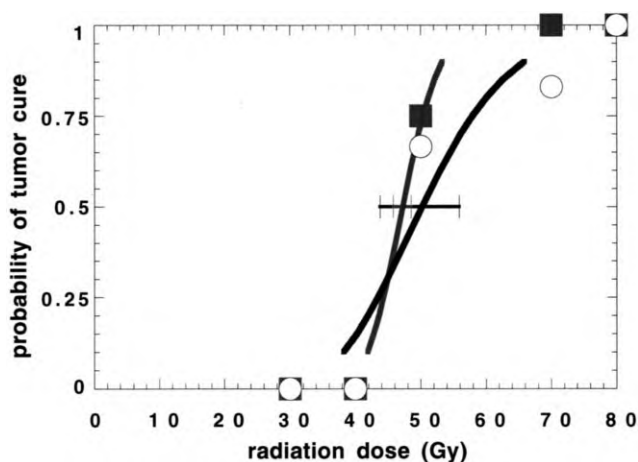[d]Indicates the 10–90% interpercentile range.

Fig. 1. The effect of carbogen and nicotinamide on the radiation response of ENE2 neurogenic sarcoma. Tumours were locally irradiated with 10 daily fractions either under ambient conditions (○) or after injecting the mice with nicotinamide (500 mg/kg, i.p.) 60 min before irradiating and gassing with carbogen for 5 min before and during the irradiation treatment (■). Results show local tumour control as a function of the total radiation dose for an average of eight mice per group. Lines were fitted by logit analysis and the errors show 95% confidence intervals on the TCD_{50} values.

involving more than 10 000 patients showed that strategies designed to reduce tumour hypoxia can improve survival and local tumour control following radiotherapy [33]. A more recent approach to overcoming hypoxia-related radio-resistance is the use of carbogen and nicotinamide (CON), especially when combined with accelerated radiotherapy (ARCON) [9,40]. In murine tumours CON has been shown to be a very effective radiosensitizing treatment both in single [17] and fractionated regimes [23,39]. However, limited data are available on the oxygenation and radiosensitivity of human tumour xenografts with CON.

The computerized pO_2 histograph (KIMOC 6650, Eppendorf, Hamburg) is increasingly used in the clinic to invasively measure tissue oxygenation. The available data show a huge variability of pO_2 even in identical histological types, with a trend towards low pO_2 values in most patients [12,15,50]. Since the polarographic system only investigates a small tumour volume and is unable to distinguish between viable hypoxic (stem cells) and necrotic areas irrelevant for the risk of recurrence, the relevance of the system remains controversial [19,22]. However, several clinical studies do indicate a correlation between initial pO_2 values and local control or survival [13,15,30].

The experimental tumours investigated in this study were characterized by a large proportion of low pO_2 readings; about 80% of all readings were ≤2.5 mmHg, where the sensitizing effect of oxygen is less than half maximal [49].

In our system the animals were anaesthetized during treatment, which may have been a factor that could have contributed to the low values, since data from both normal tissues and tumours show that anaesthetics may affect the radiation response by modification of oxygenation [16,27,

34,47]. This effect is also different with different times and durations of anaesthetics [29]. However, we used enflurane and two studies have specifically investigated the effect of this narcotic on oxygenation. Neely et al. [28] measured inner retinal oxygen tension in cats under general enflurane narcosis under normoxic conditions and found that enflurane anaesthesia did not cause hypoxia of the inner retina in cats breathing 21% inspired oxygen, indicating a limited effect of the drug on the oxygenation status of tissues. Also, in murine lip mucosa no significant effect of enflurane on radiation response could be observed for fractionated irradiation [43].

Furthermore, our values are not inconsistent with those found in other murine and xenografted human tumours [3,7,45,52]. Tumours with low pO_2 values are also often seen in the clinical situation [4,12,15,25,30].

In the present study, we also investigated the effect of CON on the oxygenation status of the human tumour xenografts. An improvement was seen in all three tumours, but it was only significant in ES3. This was also the only tumour in which we found a significant improvement in radiation sensitivity following a 10 fraction irradiation schedule. The enhancement ratio at the TCD_{50} level was 1.3. Similar values have been seen in other experimental systems for both murine tumours [17,23,39] and even human xenografts [20,44]. In the study by Kaanders et al. [20] an enhancement ratio of 1.3 was found for a human larynx carcinoma xenograft following single dose irradiation and CON. In a multifractionated regime (five and 20 fractions), Sun et al. [44] also reported an enhancement ratio of 1.3 for a human glioblastoma xenograft after combined treatment with carbogen and nicotinamide. What is not known is whether the 1.3 enhancement we obtained in the ES3 tumour was the result
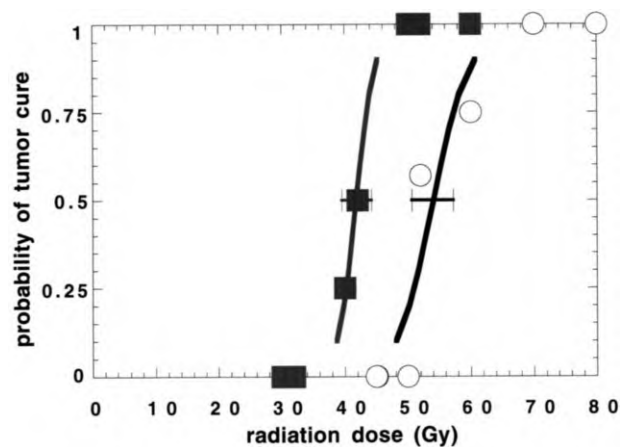

Fig. 2. The effect of carbogen and nicotinamide on the radiation response of ES3 spindle cell sarcoma. Tumours were locally irradiated with 10 daily fractions either under ambient conditions (○) or after injecting the mice with nicotinamide (500 mg/kg, i.p.) 60 min before irradiating and gassing with carbogen for 5 min before and during the irradiation treatment (■). Results show local tumour control as a function of the total radiation dose for an average of eight mice per group. Lines were fitted by logit analysis and the errors show 95% confidence intervals on the TCD_{50} values.
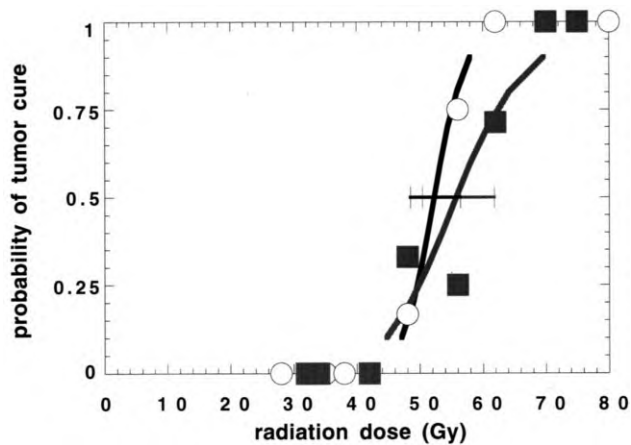
Fig. 3. The effect of carbogen and nicotinamide on the radiation response of HTZ glioblastoma. Tumours were locally irradiated with 10 daily fractions either under ambient conditions (○) or after injecting the mice with nicotinamide (500 mg/kg, i.p.) 60 min before irradiating and gassing with carbogen for 5 min before and during the irradiation treatment (■). Results show local tumour control as a function of the total radiation dose for an average of eight mice per group. Lines were fitted by logit analysis and the errors show 95% confidence intervals on the TCD_{50} values.

of the combination of carbogen and nicotinamide, or due to the action of just one of the treatment modalities. For ENE2 and HTZ17 xenografts we found no enhancement of fractionated radiation damage following treatment with carbogen and nicotinamide, despite the fact that both the pO_2 and radiation response data obtained under ambient conditions suggested that they were as hypoxic as the ES3 tumour. A similar lack of radiosensitization by carbogen and nicotinamide has been reported in an R1H tumour [36], again despite this tumour containing radiobiological hypoxia. Such results suggest that not all hypoxic tumours will necessarily respond to treatment with carbogen and nicotinamide.

Clinically, there are little data as yet available on the ability of CON to influence radiation damage. Several studies on brain tumours have suggested little or no benefit of this combination [11,24,35]. However, although low pO_2 values have been measured with the Eppendorf system in brain tumours [37], there is no evidence to suggest that radiobiological hypoxia influences the outcome of this site [18]. On the other hand, there is clear evidence of such a role for radiobiological hypoxia in head and neck tumours [13,30,33] and that the oxygenation status can be modified by carbogen breathing at least [26]. Thus, it is not surprising that preliminary studies in this site are beginning to indicate an improvement in radiation response following treatment with carbogen and nicotinamide [20]. The results from our current study clearly support the continued use of this type of treatment in specific human tumours.

Acknowledgements

References

[1] Ang, K.K., van der Kogel, A.J. and 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. 8: 145–148, 1982.

[2] Bernsen, H.J., Rijken, P.F., Oostendorp, T. and van der Kogel, A.J. Vascularity and perfusion of human gliomas xenografted in the athymic nude mouse. Br. J. Cancer 71: 721–726, 1995.

[3] Brizel, D.M., Lin, S., Johnson, J.L., Brooks, J., Dewhirst, M.W. and Piantadosi, C.A. The mechanisms by which hyperbaric oxygen and carbogen improve tumour oxygenation. Br. J. Cancer 72: 1120–1124, 1995.

[4] Brizel, D.M., Rosner, G.L., Harrelson, J., Prosnitz, L.R. and Dewhirst, M.W. Pretreatment oxygenation profiles of human soft tissue sarcomas. Int. J. Radiat. Oncol. Biol. Phys. 30: 635–642, 1994.

[5] Brown, J.M. Evidence for acutely hypoxic cells in mouse tumours and a possible mechanism of reoxygenation. Br. J. Radiol. 52: 650–656, 1979.

[6] Budach, V., Bamberg, M., Streffer, C., Budach, W., Stuschke, M. and Fabry, W. Establishment and characterization of human tumours in nu/nu-mice. Strahlenther. Onkol. 165: 500–501, 1989.

[7] Busse, M. and Vaupel, P.W. The role of tumor volume in 'reoxygenation' upon cyclophosphamide treatment. Acta Oncol. 34: 405–408, 1995.

[8] Chaplin, D.J., Olive, P.L. and Durand, R.E. Intermittent blood flow in a murine tumor: radiobiological effects. Cancer Res. 47: 597–601, 1987.

[9] Denekamp, J. and Fowler, J. ARCON – current status: summary of a workshop on preclinical and clinical studies. Acta Oncol. 36: 517–525, 1997.

[10] Dinges, S., Budach, V., Budach, W., Stuschke, M., Stüben, G. and Bamberg, M. Characterization of human soft tissue sarcoma and malignant glioma xenografts with particular reference to spontaneous changes during serial passages. Contrib. Oncol. 42: 60–65, 1992.

[11] Fatigante, L., Ducci, F., Cartei, F., Colosimo, S., Marini, C., Prediletto, R., Danesi, R., Laddaga, M., Del, T.M. and Caciagli, P. Carbogen and nicotinamide combined with unconventional radiotherapy in glioblastoma multiforme: a new modality treatment. Int. J. Radiat. Oncol. Biol. Phys. 37: 499–504, 1997.

[12] Füller, J., Feldmann, H.J., Molls, M. and Sack, H. Untersuchungen zum Sauerstoffpartialdruck im Tumorgewebe unter Radio- und Thermoradiotherapie. Strahlenther. Onkol. 170: 453–460, 1994.

[13] Gatenby, R.A., Kessler, H.B., Rosenblum, J.S., Coia, L.R., Moldofsky, P.J., Hartz, W.H. and Broder, G.J. Oxygen distribution in squamous cell carcinoma metastases and its relationship to outcome of radiation therapy. Int. J. Radiat. Oncol. Biol. Phys. 14: 831–838, 1988.

[14] Grau, C., Horsman, M.R. and Overgaard, J. Improving the radiation response in a C3H mouse mammary carcinoma by normobaric oxygen or carbogen breathing. Int. J. Radiat. Oncol. Biol. Phys. 22: 415–419, 1992.

[15] Höckel, M., Schlenger, K., Aral, B., Mitze, M., Schaffer, U. and Vaupel, P. Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. Cancer Res. 56: 4509–4515, 1996.

[16] Hornsey, S., Myers, R. and Andreozzi, U. Differences in the effects of anaesthesia on hypoxia in normal tissues. Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. 32: 609–612, 1977.

[17] Horsman, M.R. Nicotinamide and other benzamide analogs as agents for overcoming hypoxic cell radiation resistance in tumours. A review. Acta Oncol. 34: 571–587, 1995.

[18] Horsman, M.R. Tolerance to nicotinamide and carbogen with radiation therapy for glioblastoma. Radiother. Oncol. 43: 109–110, 1997.

[19] Horsman, M.R., Khalil, A.A., Siemann, D.W., Grau, C., Hill, S.A., Lynch, E.M., Chaplin, D.J. and Overgaard, J. Relationship between radiobiological hypoxia in tumors and electrode measurements of

148

tumor oxygenation. Int. J. Radiat. Oncol. Biol. Phys. 29: 439–442, 1994.

[20] Kaanders, J.H.A.M., Bussink, J. and van der Kogel, A.J. Radiotherapy with carbogen and nicotinamide for squamous cell carcinoma of the head and neck: tumor effects in mice and man. Third ARCON workshop, 1996.

[21] Kallinowski, F., Zander, R., Hoeckel, M. and Vaupel, P. Tumor tissue oxygenation as evaluated by computerized-pO2-histography. Int. J. Radiat. Oncol. Biol. Phys. 19: 953–961, 1990.

[22] Kavanagh, M.C., Sun, A., Hu, Q. and Hill, R.P. Comparing techniques of measuring tumor hypoxia in different murine tumors: Eppendorf pO2 histograph, [3H] misonidazole binding and paired survival assay. Radiat. Res. 145: 491–500, 1996.

[23] Kjellen, E., Joiner, M.C., Collier, J.M., Johns, H. and Rojas, A. A therapeutic benefit from combining normobaric carbogen or oxygen with nicotinamide in fractionated X-ray treatments. Radiother. Oncol. 22: 81–91, 1991.

[24] Lambin, P., Poortmans, M., Menten, J. and Hamers, H.P. Accelerated radiotherapy with carbogen and nicotinamide (ARCON) in high grade malignant gliomas. Radiother. Oncol. 43: 324, 1997.

[25] Laurence, V.M., Ward, R., Dennis, I.F. and Bleehen, N.M. Carbogen breathing with nicotinamide improves the oxygen status of tumours in patients. Br. J. Cancer 72: 198–205, 1995.

[26] Martin, L., Lartigau, E., Weeger, P., Lambin, P., Le, R.A., Lusinchi, A., Wibault, P., Eschwege, F., Luboinski, B. and Guichard, M. Changes in the oxygenation of head and neck tumors during carbogen breathing. Radiother. Oncol. 27: 123–130, 1993.

[27] Moulder, J.E. and Rockwell, S. Hypoxic fractions of solid tumors: experimental techniques, methods of analysis, and a survey of existing data. Int. J. Radiat. Oncol. Biol. Phys. 10: 695–712, 1984.

[28] Neely, K.A., Ernest, J.T. and Goldstick, T.K. Retinal tissue oxygen tension in normoxic cats under enflurane anesthesia. Invest. Ophthalmol. Vis. Sci. 36: 1943–1946, 1995.

[29] Nias, A.H. and Perry, P.M. Variation of tumour radiosensitivity with time after anaesthetic. Br. J. Radiol. 62: 932–935, 1989.

[30] Nordsmark, M., Overgaard, M. and Overgaard, J. Pretreatment oxygenation predicts radiation response in advanced squamous cell carcinoma of the head and neck. Radiother. Oncol. 41: 31–39, 1996.

[31] Ong, L.D. and LeClare, P.C. The Kolmogorov–Smirnov test for the log-normality of sample cumulative frequency distributions. Health Phys. 14: 376, 1968.

[32] Overgaard, J. Sensitization of hypoxic tumour cells – clinical experience. Int. J. Radiat. Biol. 56: 801–811, 1989.

[33] Overgaard, J. and Horsman, M.R. Modification of hypoxia-induced radioresistance in tumours by the use of oxygen and sensitizers. Semin. Radiat. Oncol. 6: 10–21, 1996.

[34] Pallavicini, M.G. and Hill, R.P. Effect of tumor blood flow manipulations on radiation response. Int. J. Radiat. Oncol. Biol. Phys. 9: 1321–1325, 1983.

[35] Pickles, T., Graham, P., Syndikus, I., Rheaume, D.E., Duncan, G.G., Green, A. and Marlow, C. Tolerance of nicotinamide and carbogen with radiation therapy for glioblastoma. Radiother. Oncol. 40: 245–247, 1996.

[36] Raabe, A., Rett, M., Krull, A. and Beck-Bornholdt, H.P. Combination of fractionated irradiation with nicotinamide and carbogen in R1H-tumours of the rat and its pulmonary metastases. Radiother. Oncol. 45: 183–189, 1997.

[37] Rampling, R., Cruickshank, G., Lewis, A.D., Fitzsimmons, S.A. and Workman, P. Direct measurement of pO2 distribution and bioreductive enzymes in human malignant brain tumors. Int. J. Radiat. Oncol. Biol. Phys. 29: 427–431, 1994.

[38] Rockwell, S. and Moulder, J.E. Hypoxic fractions of human tumors xenografted into mice: a review. Int. J. Radiat. Oncol. Biol. Phys. 19: 197–202, 1990.

[39] Rojas, A., Hirst, V.K., Calvert, A.S. and Johns, H. Carbogen and nicotinamide as radiosensitizers in a murine mammary carcinoma using conventional and accelerated radiotherapy. Int. J. Radiat. Oncol. Biol. Phys. 34: 357–365, 1996.

[40] Saunders, M.I., Hoskin, P.J., Pigott, K., Powell, M.E.B., Goodchild, K., Dische, S., Denekamp, J., Stratford, M.R.L., Dennis, M.F. and Rojas, A.M. Accelerated radiotherapy, carbogen and nicotinamide (ARCON) in locally advanced head and neck cancer: a feasibility study. Radiother. Oncol. 45: 159–166, 1997.

[41] Siemann, D.W. Tissue oxygen manipulation and tumor blood flow. Int. J. Radiat. Oncol. Biol. Phys. 22: 393–395, 1992.

[42] Stüben, G., Budach, W., Schick, K.H., Stuschke, M., Stapper, N., Müller, S. and Feldmann, H.J. A time-saving system for irradiation of experimental tumors. Strahlenther. Onkol. 170: 36–41, 1994.

[43] Stüben, G., Landuyt, W., van der Schueren, E. and van der Kogel, A.J. Different immobilization procedures during irradiation influence the estimation of alpha/beta ratios in mouse lip mucosa. Strahlenther. Onkol. 169: 678–683, 1993.

[44] Sun, L.Q., Buchegger, F., Coucke, P. and Mirimanoff, R.O. Fractionated radiotherapy combined with carbogen breathing and nicotinamide for human glioblastoma xenografts. Third ARCON workshop, 1996.

[45] Thomas, C.D., Prade, M. and Guichard, M. Tumour oxygenation, radiosensitivity, and necrosis before and/or after nicotinamide, carbogen and perflubron emulsion administration. Int. J. Radiat. Biol. 67: 597–605, 1995.

[46] Thomlinson, R.H. and Gray, L.H. The histological structure of some human lung cancers and the possible implications for radiotherapy. Br. J. Cancer 9: 539–549, 1955.

[47] Tozer, G.M., Penhalgon, M. and Nias, A.H. The use of ketamine plus diazepam anaesthesia to increase the radiosensitivity of a C3H mouse mammary adenocarcinoma in hyperbaric oxygen. Br. J. Radiol. 57: 75–80, 1984.

[48] Vaupel, P. and Gabbert, H. Evidence for and against a tumor type-specific vascularity. Strahlenther. Onkol. 162: 633–638, 1986.

[49] Vaupel, P., Kallinowski, F. and Okunieff, P. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. Cancer Res. 49: 6449–6465, 1989.

[50] Vaupel, P., Schlenger, K., Knoop, C. and Höckel, M. Oxygenation of human tumors: evaluation of tissue oxygen distribution in breast cancers by computerized O2 tension measurements. Cancer Res. 51: 3316–3322, 1991.

[51] Walker, A.M. and Suit, H.D. Assessment of local tumor control using censored tumor response data. Int. J. Radiat. Oncol. Biol. Phys. 9: 383–386, 1983.

[52] Yeh, K.A., Biade, S., Lanciano, R.M., Brown, D.Q., Fenning, M.C., Babb, J.S., Hanks, G.E. and Chapman, D.C. Polarographic needle electrode measurements of oxygen in rat prostate carcinomas: accuracy and reproducibility. Int. J. Radiat. Oncol. Biol. Phys. 33: 111–118, 1995.