

Carbogen breathing with nicotinamide improves the oxygen status of tumours in patients

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Summary Nicotinamide and carbogen breathing are both effective radiosensitisers in experimental tumour models and are even more effective in combination. This study was to investigate the feasibility of using the agents in combination in patients and to measure their effect on tumour oxygenation. Twelve patients with advanced malignant disease were treated with 4–6 g of oral nicotinamide (NCT) in tablet formulation. Ten of these 12 patients breathed carbogen (95% oxygen, 5% carbon dioxide) for up to 20 min at presumed peak plasma NCT concentration (C_{peak}) and had tumour oxygen partial pressure (pO_2) measured using the Eppendorf pO_2 histogram. The mean C_{peak} values were 82, 115 and 150 $\mu\text{g ml}^{-1}$ for NCT doses of 4, 5 and 6 g respectively and were dose dependent. The time of C_{peak} was independent of dose with an overall mean of 2.4 h (range 0.7–4 h). NCT toxicity occurred in 9 out of 12 patients and was mild in all but one; carbogen was well tolerated in all patients. Following NCT only two patients had significant rises ($P < 0.05$) in tumour median pO_2 . During carbogen breathing, eight out of ten patients had early highly significant rises in pO_2 ($P < 0.0001$), of which six continued to rise or remained in plateau until completion of gas breathing. Six patients had hypoxic pretreatment values less than 5 mmHg, which were completely abolished in three and reduced in two during carbogen breathing. In conclusion, the combination of NCT and carbogen breathing was generally well tolerated and gave rise to substantial rises in tumour pO_2 which were maintained throughout gas breathing. These results should encourage further study of this potentially useful combination of agents as radiosensitisers in the clinic.

Keywords: nicotinamide; carbogen; pharmacokinetics; tumour oxygen partial pressure; radiosensitiser

Strategies to improve tumour oxygenation during radiation treatment remain an important area of laboratory and clinical research. Despite positive findings in some trials of hyperbaric oxygen and radiation sensitisers, these agents have failed to become part of routine clinical practice to date (Overgaard, 1992). This has been partly because of inadequate patient numbers in many of the trials, but also to do with problems of compliance in the case of hyperbaric oxygen and unacceptable toxicity in the case of sensitisers (Henk, 1981; Dische, 1985). Current approaches should now address the issue of tumour repopulation (Dische, 1991) as well as the existence in tumours of both acute and chronic hypoxia (Coleman, 1988).

Combining nicotinamide with carbogen breathing (95% oxygen, 5% carbon dioxide) and accelerated hyperfractionated radiation regimens (ARCON) is one such approach currently being evaluated in Europe. Both nicotinamide and carbogen breathing (aimed at overcoming acute and chronic hypoxia respectively) can enhance radiosensitivity and improve the therapeutic ratio in murine tumours when used alone (Horsman *et al.*, 1987; Horsman *et al.*, 1989; Rojas, 1991). Maximum enhancement is achieved when the agents are used in combination and has been demonstrated with both single and multifraction radiation treatments (Kjellen *et al.*, 1991; Chaplin *et al.*, 1993; Simon *et al.*, 1993). By delivering the radiation in an accelerated regimen, in an attempt to prevent tumour repopulation, even further enhancement can be achieved (Rojas, 1992). Clinical studies using combinations of these agents are now under way in patients, but their exact modes of action and optimum ways of delivery remain under investigation.

The B vitamin derivative nicotinamide appears to improve radiosensitivity primarily by overcoming acute hypoxia which may occur as a result of the transient closure of oxygen and nutrient supplying vessels within the tumour microcirculation (Horsman *et al.*, 1988, 1989; Chaplin *et al.*, 1990). Although it has been previously used with little toxicity in daily doses

of up to 12 g in the treatment of benign disease (Zackheim, 1981), its use in patients with neoplastic disease is novel. Its pharmacokinetics has been measured in man at doses up to 6 g (Stratford *et al.*, 1992; Horsman *et al.*, 1993a), but its optimum dose, formulation and timing of administration in relation to radiation treatment are not yet fully established. Early studies suggested that radiosensitisation in murine tumour models occurs only at doses which would be toxic in man (Brown, 1992), but a recent study has suggested that enhancement is independent of dose provided that radiation is given when nicotinamide plasma concentration is at its peak (Horsman *et al.*, 1993a) and that doses of 6 g in man should be enough to produce tumour sensitisation.

Normobaric oxygen and carbogen breathing are well established in animal models as radiation sensitisers (Rojas, 1991). They are thought to overcome hypoxia by increasing the dissolved oxygen partial pressure (pO_2) and increasing the intracapillary haemoglobin saturation, thus making more oxygen available to chronically deprived hypoxic tumour cells located beyond the maximum diffusion distance of oxygen (Kruuv *et al.*, 1967). The preirradiation breathing time (PIBT) appears to be a critical factor in radiosensitisation, with optimum enhancement occurring within short defined time periods in murine tumours (Siemann *et al.*, 1977). However, its importance is diminished by the concurrent use of nicotinamide (Chaplin *et al.*, 1993). Some of the previous studies of carbogen and oxygen breathing in man may have failed owing to the use of extended periods of PIBT (Keresteci and Rider, 1973; Rubin *et al.*, 1979). However, in one randomised controlled study in patients with carcinoma of the cervix breathing normobaric oxygen for 5–10 min before irradiation, both local recurrence and overall survival were improved by the addition of oxygen (Bergsjö and Kolstad, 1968).

On the assumption that both nicotinamide and carbogen breathing may improve radiosensitisation by their action on tumour hypoxia, there has been some attempt to date to measure the effects of the agents on tumour pO_2 distribution. Measurement of tumour pO_2 distribution was previously hampered by the lack of a reliable direct method. The computerised Eppendorf pO_2 histogram oxygen microelectrode system has now become established as a safe, reproducible

and relatively non-invasive method for measuring pO_2 in accessible tumours and normal tissues (Kallinowski *et al.*, 1990; Vaupel *et al.*, 1991). This method has been used to study tumour pO_2 distribution changes in animal models following treatment with either nicotinamide or carbogen. Rises in pO_2 following nicotinamide treatment have been shown in only one study to date (Lee and Song, 1992), whereas others have shown no change (Kelleher and Vaupel, 1993). Significant rises have been shown following treatment with carbogen breathing (Horsman *et al.*, 1993b; Vitu-Loas *et al.*, 1993). In man, tumour pO_2 rises have been demonstrated during carbogen breathing in two studies to date (Falk *et al.*, 1992; Martin *et al.*, 1993) but pO_2 has not been measured following treatment with nicotinamide or the combination.

In the current study, we have measured changes in tumour pO_2 distribution in patients with advanced malignant disease following nicotinamide administration and during subsequent carbogen breathing. In addition, we have studied nicotinamide pharmacokinetics and the toxicity of the combined treatment.

Materials and methods

Patient characteristics

Twelve patients were entered into the study, of whom ten received the combination of nicotinamide and carbogen breathing before irradiation and had measurements of tumour pO_2 distribution performed. Two patients received nicotinamide alone and were eligible for toxicity and pharmacokinetic assessment. Table I shows the characteristics and tumour details of the treated patients. All had advanced recurrent or metastatic histologically proven malignancy requiring palliative irradiation, with accessible tumours of an adequate size to obtain a satisfactory number of pO_2 readings without tissue trauma. Tumours were not cystic, haemorrhagic or too firm for pO_2 measurements to be performed. Only one had been previously irradiated in the site used for pO_2 measurements (no. 5). All patients were of WHO performance status ≤ 2 and had no serious cardiac or respiratory illness. All had haemoglobin values above 11 g dl^{-1} . Patients were defined as smokers if they had smoked at any time during the past 5 years. The protocol was approved by the local ethical committee and written informed consent was obtained from all patients.

Nicotinamide administration

All patients received oral nicotinamide at a dose of 4–6 g as 500 mg tablets (Larkhall Natural Health, UK). It had been our intention to treat all patients with a standard dose of 6 g, but following unexpected acute toxicity in the second patient we modified our dose to 4 g until further experience had been gained. Tablets were taken 3–4 h after a light breakfast. No cigarettes, food or drink other than water were allowed after breakfast until completion of carbogen breathing.

Carbogen breathing

Carbogen breathing was commenced as close to the estimated time of peak plasma nicotinamide concentration as possible. Published data suggested that this would be at around 90 min (Stratford *et al.*, 1992) and this time was used for the initial patients. However, as large individual variation was found, approximate nicotinamide concentrations were measured directly in the clinic in six patients (see Pharmacokinetic assessment below) and carbogen breathing was commenced at the time of individually estimated peak plasma nicotinamide concentrations. During carbogen breathing patients wore an air-tight scuba-diving face mask covering the nose and mouth (SOS Products) and were in control of holding the mask in place. The inhaled carbogen (95% oxygen, 5% carbon dioxide; from British Oxygen) was delivered to the face mask using a closed system with regulated flow rate of 4–5 l min^{-1} through an anaesthetic ambubag and two-way valve (supplied by Intersurgical). Exhaled gas was removed using elephant tubing connected between the valve and a window to the outside. Patients were asked to breathe carbogen for as long as they were able to do so for up to 20 min. On completion of breathing carbogen, all patients were treated with palliative irradiation.

Pharmacokinetic assessment

Serial 5 ml venous blood samples were collected from a heparinised 22 G cannula (Venflon, Viggo, UK) at regular time points after nicotinamide administration. Urine was also collected for 24 h. Plasma and urine nicotinamide concentrations were determined using a method of high-performance liquid chromatography (HPLC) analysis based on the procedure described by De Vries and Stratford (De Vries *et al.*, 1980; Stratford *et al.*, 1992). Briefly, 200 μl of plasma was added to 400 μl of acetonitrile containing 25 $\mu\text{g ml}^{-1}$ Ro-31-602 as an internal standard, and after vortexing the sample was centrifuged (9500 g, 5 min). A 50 μl aliquot of the supernatant was diluted with 150 μl of mobile phase and injected onto the HPLC system. Separations were achieved on a Nova-Pak C_{18} (8 mm \times 10 cm Radial Pak cartridge) with a mobile phase of 25% acetonitrile, and 75% of an aqueous solution of 2 mM sulphosuccinate 1 mM trimethylamine, 20 mM orthophosphoric acid and 5 mM sodium dihydrogen orthophosphate at a flow rate of 2 ml min^{-1} . Nicotinamide was detected by UV absorbance at 254 nm and quantified by peak area with reference to calibration standards using Waters Expert-Ease software V2.1.

Having found wide variability in the time of peak plasma nicotinamide concentration in initial patients, we subsequently used a rapid detection method in the clinic. Plasma nicotinamide concentration was estimated using an LKB UV spectrophotometer. This is an approximate method which does not distinguish between nicotinamide and its metabolites but nonetheless gives an indication of increasing and decreasing blood concentrations of detectable compounds. Briefly, plasma was obtained by centrifugation of 1 ml blood samples at 7500 g for 3 min. A 1 ml aliquot of

Table I Patient and tumour characteristics

Patient no.	Sex	Age (years)	Height (m)	Weight (kg)	Hb (g dl^{-1})	Smoker in last 5 years	Tumour site	Tumour histology	Tumour volume (cm^3)
1	M	57	-	74	11.7	Y	Skin	met AC caecum	4
2	M	72	1.70	77	14.9	N	Neck node	met SCC bladder	8
3	M	68	1.77	79	14.4	N	Chest wall	met SCC kidney	11
4	F	58	1.58	73	12.1	Y	Neck node	met SCLC	23
5	F	64	1.58	75	13.9	N	Groin node	met SCC cervix	29
6	M	57	1.70	81	12.3	Y	Suprasternal mass	met SCLC	6
7	F	66	1.58	59	11.0	N	Skin	met AC stomach	57
8	M	71	1.75	78	12.5	N	Groin node	met SCC bronchus	28
9	F	68	1.60	52	11.1	N	Breast	rec AC breast	10
10	M	42	1.80	60	11.5	Y	Neck node	met SCC bronchus	64
11	M	86	1.71	56	na	Y	Chest wall	met AC bronchus	104
12	F	57	1.62	84	14.8	N	Neck node	met AC breast	113

met, metastatic; rec, recurrent; SCC, squamous cell carcinoma; SCLC, small cell lung cancer, AC, adenocarcinoma.

dried acetonitrile was added to 200 μ l of plasma and the mixture was vortexed and then centrifuged for a further 3 min at 7500 g. The supernatant was transferred to a clean glass cuvette and measured at 260 nm. When sample absorbance levels showed a plateau or decline it was assumed that peak plasma nicotinamide concentration had been reached. The remainder of the initial 5 ml blood samples from these patients were stored on ice for subsequent HPLC analysis and comparison with spectrophotometer results.

Assessment of toxicity

Patients all had an initial full history and physical examination performed with particular attention to measurement of the tumour in which pO_2 measurements were to be performed. Resting pulse rate, blood pressure and arterial oxygen saturation (measured by pulse oximeter supplied by Omeda) were performed before and at 15 min intervals after nicotinamide administration and until completion of carbogen breathing. Any unexpected symptoms or change in vital signs were noted. On completion of the radiation treatment pulse rate and blood pressure were monitored every 4 h for 24 h. Patients were assessed for early toxicity by history and examination before discharge at 24 h and reassessed in the clinic 3–4 weeks after the procedure.

Tumour oxygen measurements

Tumour oxygen partial pressure pO_2 was measured using the computerised KIMOC 6650 Eppendorf pO_2 histogram as previously described in detail (Vaupel *et al.*, 1991). In brief, the polarographic electrode system was calibrated for 15 min in buffered sterile physiological saline with air and 100% nitrogen before and after each series of measurements. After initial calibration a 22 G intravenous cannula (Venflon) was inserted into or near the tumour following the subcutaneous injection of 2 ml of local anaesthetic (lignocaine 2% without adrenaline). Tumour temperature was measured using a tissue-implantable thermocouple microprobe (Physitemp) with BAT 10 data display system and was programmed into the histogram so that all pO_2 readings were corrected for temperature. The microelectrode probe was then inserted into the tumour through the cannula in order to protect the membrane of the cathode. The electrode was allowed to stabilise and the set of measurements then taken. The probe was programmed to move forwards in steps of 1 mm immediately followed by a step of 0.3 mm backwards in order to minimise tissue compression artefacts.

The track length and number of tracks taken were individually selected according to tumour size, and sets of readings were obtained from across the maximum diameter of the tumour. Patients remained supine in a warm and comfortable position during the procedure. Sets of measurements were performed before nicotinamide administration, at estimated peak plasma nicotinamide concentration (immediately before carbogen breathing) and then at 3–4 time points during carbogen breathing to obtain a time course. Each individual measurement was displayed on the histogram as it was taken and sets were then presented as frequency histograms.

Evaluation of data and statistical analysis

Sets of pO_2 measurements from each patient were transferred from the Eppendorf histogram to a Macintosh SE/30 and analysed using Statview software (Abacus Concepts) to obtain median values, inter-percentile ranges and percentages of values less than 10, 5 and 2.5 mmHg for individual patients. Statistical analysis was by the non-parametric Mann–Whitney *U*-test to assess the significance of any changes in pO_2 distribution after treatment with nicotinamide and during carbogen breathing. Intra- and inter-tumour heterogeneity were compared using analysis of variance and using the median pO_2 of each individual track as the parameter of oxygenation. Three time intervals were chosen based on data availability and compared using Snedecor's

variance ratio test (Moroney, 1990). Pharmacokinetic data were analysed using Kaleidagraph software (Albeck) for the calculations of means, standard errors and correlation coefficients.

Results

Nicotinamide pharmacokinetics

Pharmacokinetic data were analysed from 12 patients and are shown in Table II; four patients received a dose of 4 g, three received 5 g and five received 6 g. There was wide variation between patients in both peak plasma nicotinamide concentration and time to reach it. The mean peak plasma nicotinamide concentrations were 82, 115 and 150 μ g ml⁻¹ for doses of 4, 5 and 6 g respectively, the overall mean \pm (s.e.) being 115 (\pm 10) μ g ml⁻¹. There was a linear relationship between peak plasma nicotinamide concentration and nicotinamide dose expressed either in g, mg kg⁻¹ or g m⁻² (correlation coefficients, *R* = 0.73, 0.68 and 0.73 respectively). Figure 1 shows the relationship between administered nicotinamide dose in grams and peak plasma nicotinamide concentration. In contrast, no relationship was found between administered nicotinamide dose and the time of peak plasma nicotinamide concentration, which had an inter-patient range of 0.7–4 h with a mean (\pm s.e.) of 2.4 (\pm 0.3) h.

We were particularly concerned to begin carbogen breathing as near to the time of peak plasma nicotinamide concentration as possible. Having found marked variation in this time in the initial patients we then used the spectrophotometric assay in six subsequent patients. We assumed that peak nicotinamide concentration had been reached when a plateau or fall in UV absorption was detected. At this stage a set of oxygen measurements was performed and then carbogen breathing commenced. A time lag between the true time of the peak and commencement of carbogen thus inevitably occurred. Figure 2 shows the comparative profiles of the percentages of peak plasma nicotinamide concentrations in the six patients as measured by the two different assays and their relationship to the time of carbogen breathing. It demonstrates that the rapid spectrophotometric assay gave a reasonable indication of the peak in all except one patient (no. 9). Of the five patients who breathed carbogen four (80%) had mean nicotinamide plasma concentrations (as measured by subsequent HPLC analysis) during gas breathing which were within 10% of the peak. In contrast, if a standard 90 min time to reach the peak had been assumed throughout the study, only 33% of the patients would have had nicotinamide plasma concentrations within 10% of the peak at the commencement of carbogen breathing (data not shown). Overall, the mean plasma concentration of nicotinamide during carbogen breathing was within 20% of peak plasma nicotinamide concentration in eight out of ten patients.

Toxicity associated with nicotinamide

After nicotinamide administration 9 out of 12 patients suffered from some toxicity (Table II). Diastolic blood pressure fell in three patients (> 15 mmHg diastolic), of whom two were asymptomatic and one had accompanying mild dizziness. Five patients noticed flushing, one accompanied by mild nausea, and three had mild headaches. One patient with a long-standing history of asymptomatic hypertension, concurrently receiving bendrofluzide medication, suffered significant toxicity at the time of peak nicotinamide concentration, with profound hypotension resulting in a transient cardiorespiratory arrest. He quickly regained consciousness but remained relatively bradycardic and hypotensive for several hours. He recovered completely with no further sequelae.

Table II Summary of nicotinamide pharmacokinetics

Patient no.	Surface ^a area (m ²)	Dose (g)	Dose (mg kg ⁻¹)	Dose (g m ⁻²)	C _{peak} ^b (µg ml ⁻¹)	T _{peak} ^c (h)	t _{1/2β} ^d (h)	AUC _{0-∞} ^e (µg ml ⁻¹ h)	NCT concentration during carbogen breathing (µg ml ⁻¹)	Side-effects
1	—	6	81	—	138	0.7	6.5	1400	118	Vertigo, hypotension
2	1.9	6	78	3.2	136	1.7	9.5	2400	—	Nausea, vomiting, hypotension, cardiorespiratory arrest
3	1.9	4	51	2.1	55	3.6	3.5	380	35	—
4	1.7	4	55	2.3	78	1.5	1.9	760	63	—
5	1.8	4	53	2.3	102	1.0	6.6	930	91	Flushing, headache
6	1.9	5	62	2.6	151	2.0	6.1	1900	145	—
7	1.6	4	68	2.5	94	3.6	2.7	990	91	—
8	1.9	5	65	2.6	89	3.1	2.5	1200	82	Flushing, headache, hypotension
9	1.5	5	96	3.3	105	4.0	3.6	1200	81	Headache, hypotension
10	1.8	6	100	3.4	177	2.0	7.1	2300	165	Flushing
11	1.6	6	108	3.6	146	1.5	10.7	2400	—	Flushing
12	1.8	6	71	3.2	103	4.0	7.4	1800	97	Flushing, nausea

^aSurface area, estimated from a normogram; ^bC_{peak}, peak nicotinamide plasma concentration; ^cT_{peak}, time of peak nicotinamide plasma concentration; ^dt_{1/2β}, nicotinamide plasma elimination half-life; ^eAUC_{0-∞}, area under curve.

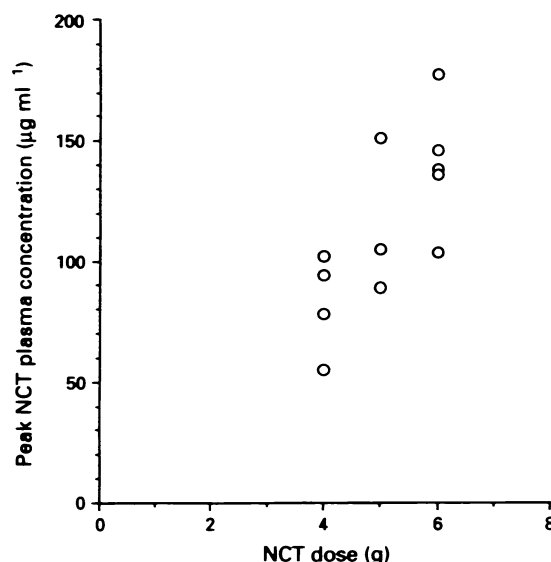


Figure 1 Peak plasma concentration of nicotinamide (NCT) as a function of dose in g.

Carbogen breathing

Of the 12 patients in the study, two did not breathe carbogen. The patient in whom major toxicity occurred was obviously unable to complete the study, and patient no. 11 was able to tolerate nicotinamide but judged to be too frail to breathe carbogen. Carbogen breathing was well tolerated by all remaining patients. It was performed for between 15 and 20 min in all except one (no 1), who was only able to complete 10 min of gas breathing. One patient removed his mask for 4 min after 13 min (no. 6) and then recommenced breathing again for a further 3 min. During carbogen breathing the mean time to reach 100% arterial oxygen saturation was 2.35 min (range 0.5–6 min).

Toxicity associated with combined treatment

Following radiation treatment all patients were assessed at 24 h and at 3–4 weeks. No obvious increase in normal tissue toxicity was noted.

Tumour oxygenation

Sets of tumour pO_2 measurements were obtained in ten patients. Between 30 and 150 measurements were performed per set of readings using typically 3–4 tracks. Table III shows the number of readings per set and the parameters of tumour oxygenation in terms of the median pO_2 value in mmHg and the percentage of values < 5 mmHg obtained during each set of readings. We have chosen to present the value of % < 5 mmHg to represent hypoxia as only one patient had > 1% of readings of < 2.5 mmHg before treatment (Hall, 1987). Table IV shows the estimates of variance, using median pO_2 as the parameter of oxygenation, before treatment, at presumed peak nicotinamide concentration and during carbogen breathing. At all three time points there was greater variation of oxygenation between tumours than within tumours, confirming that despite wide inter-percentile ranges per set an adequate number of readings had been taken to exclude any bias (Nordsmark *et al.*, 1994).

Figure 3 shows the changes in median pO_2 and the interpercentile ranges (10–90%) for each set of measurements performed in all patients. Following nicotinamide only two patients had significant rises in median tumour pO_2 ($P < 0.05$) compared with pretreatment. During carbogen breathing, and comparing pO_2 distributions with those obtained after nicotinamide, nine out of ten patients had highly significant rises in median tumour pO_2 ($P < 0.0001$). These rises occurred within 10 min of commencing carbogen in all except one patient (no. 4) and continued

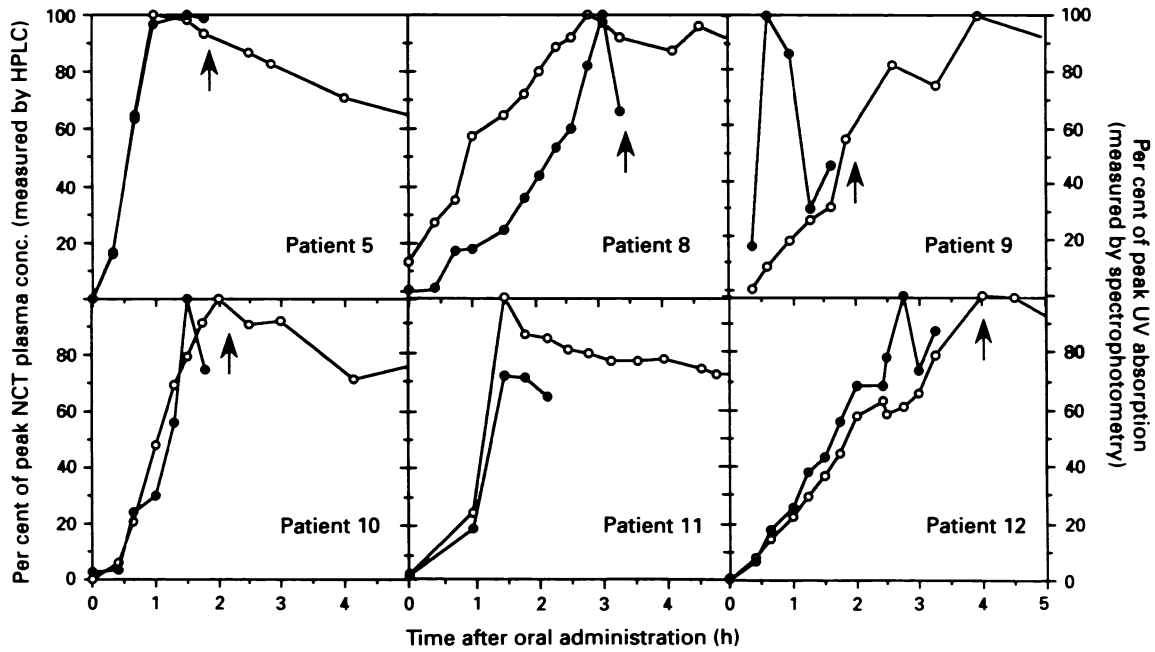


Figure 2 Percentages of peak plasma concentration of nicotinamide (NCT), as measured by (○) HPLC and (●) spectrophotometer assay system, as a function of time after oral administration of NCT in six patients and their relationship to the commencement of carbogen breathing (↑).

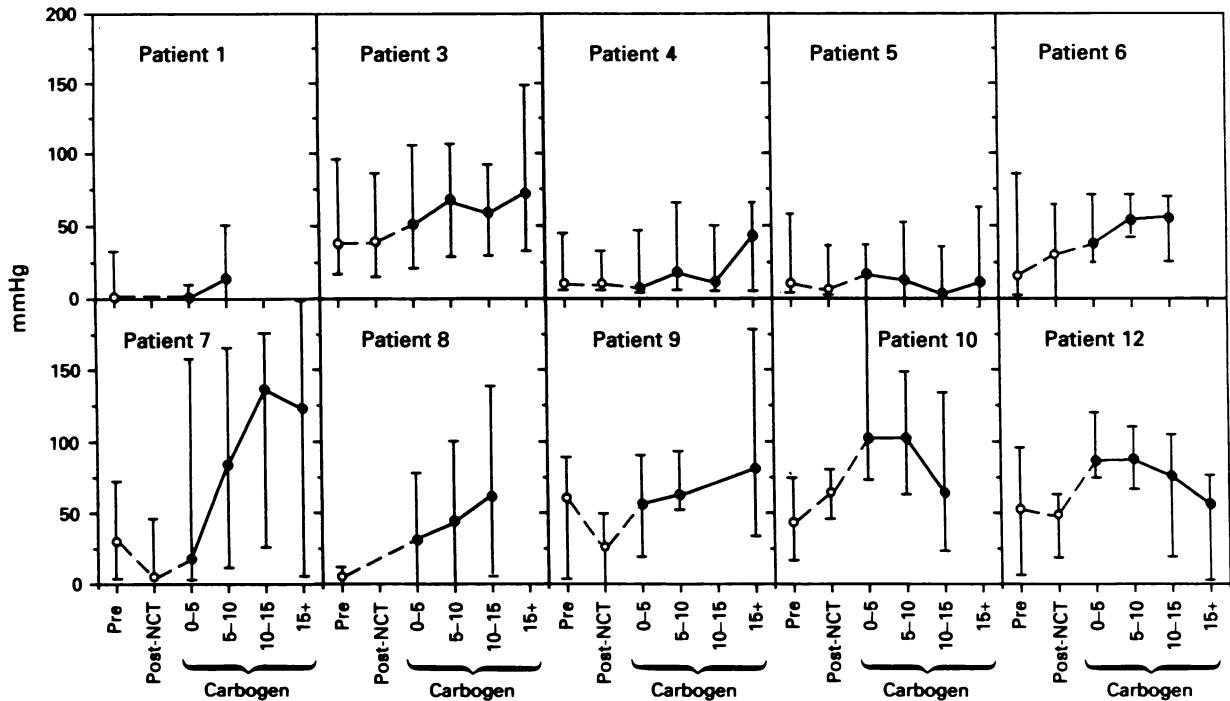


Figure 3 Median pO_2 values and 10–90% inter-percentile ranges in ten patients before ingestion of nicotinamide (pre), at presumed peak plasma nicotinamide concentration (post-NCT) and at times 0–5, 5–10, 10–15 and > 15 min after commencement of carbogen breathing.

to rise significantly or remain in plateau in all except two (nos. 10 and 12). Of the six patients with pretreatment values of less than 5 mmHg, these had been completely abolished in three and were reduced in two during carbogen breathing. Patient no. 5 was the only patient who had previously been irradiated in the measured site and was also the only patient to show no significant rise in tumour median pO_2 .

Discussion

The aim of this study was to investigate whether combining treatment with nicotinamide and carbogen breathing altered

pO_2 distribution in accessible tumours of patients with advanced malignant disease. In addition, data were obtained on changes in pO_2 after nicotinamide alone, nicotinamide pharmacokinetics and toxicity of the combined treatment.

The nicotinamide pharmacokinetic data confirmed and extended those for man already in the literature. Following doses of 4–6 g in tablet formation (51–108 mg kg⁻¹) the magnitude of peak concentration and plasma clearance were consistent with previous findings on normal volunteers (Stratford *et al.*, 1992; Horsman *et al.*, 1993a) and were both dose dependent. The time to reach peak plasma concentration was widely variable and was independent of dose, as in Stratford's study using the same formulation, but unlike

Table IV Estimates of variance, using median PO_2 as oxygenation parameter

	Sum of squares	Degrees of freedom	Variance estimate	F	P-value
Pretreatment ($n = 10$)					
Between patients	11385	9	1265		
Between tracks in a patient	14828	29	511	2.47	$0.01 < P < 0.05$
Post-NCT ($n = 8$)					
Between patients	7585	7	1084		
Between tracks in a patient	3478	15	232	4.7	$0.005 < P < 0.01$
During carbogen breathing at 5–10 min ($n = 10$)					
Between patients	26071	9	2897	9.3	$P < 0.001$
Between tracks in a patient	5625	18	313		

F = Snedecor's variance ratio. P-value obtained from F-distribution tables, using the tabulated degrees of freedom.

et al., 1994). These observations are consistent with the findings of maximum enhancement of radiosensitisation occurring after combining the agents (Kjellen *et al.*, 1991).

In man there have been no other published studies of the measurement of tumour pO_2 distribution following combined treatment with nicotinamide and carbogen breathing. The findings of greater between- than within-tumour heterogeneity at three time points support the impression of wide individual variation between tumours and make pooling of individual patient data irrelevant. Each individual was therefore assessed separately, and nine out of ten showed significant rises in tumour oxygen distribution. As in our previous study of carbogen breathing alone (Falk *et al.*, 1992), we found a wide range of change in median pO_2 with elimination of some but not all values of pO_2 below 5 mmHg. The rises in median pO_2 tended to be of greater magnitude than in the previous study and were sustained until 15 min and over in the majority of our patients, whereas in the previous study a fall in median tumour pO_2 occurred in all patients between 12 and 18 min.

Our current results suggest that the combination of nicotinamide and carbogen breathing may give rise to greater improvements in tumour hypoxia than carbogen breathing alone, and in particular may result in a sustained rise rather than a rise followed by a progressive fall. These results are supported by experimental animal data. Thus Chaplin *et al.* (1993) have shown that the influence of the length of the pre-irradiation breathing time of carbogen on radiosensitivity was diminished by the concurrent use of nicotinamide, following which hypoxia was apparently abolished. Our

findings, overall, support the results in experimental tumour models of a greater reduction in tumour hypoxia after the use of the combined agents compared with either agent alone. In addition, the combination appears to be effective over a longer time period than carbogen alone, which in the clinic would ensure less risk of missing the time of presumed tumour radiosensitisation.

In conclusion, we have treated ten patients with advanced malignant disease with the combination of oral nicotinamide and carbogen breathing before palliative radiotherapy and a further two patients received nicotinamide alone. Side-effects were generally mild except for one patient who suffered from a major toxic event. Despite being unable to demonstrate marked changes in tumour oxygen distribution close to peak plasma nicotinamide concentration and therefore at assumed optimum time for radiosensitisation, large and highly significant rises in median tumour pO_2 were demonstrated during carbogen breathing which were sustained for at least 15 min. Although these changes in pO_2 cannot be shown to be occurring in the milieu of the radioresistant hypoxic cells, our results are encouraging and should stimulate further clinical studies of this potentially highly effective combination of agents in order to establish whether the impressive therapeutic gains seen in animal models will be repeatable in man.

Acknowledgements

We would like to thank Dr Davina Honess for helpful discussions and criticisms of this work.

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