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Radiotherapy and Oncology 39 (1996) 53–64

RADIOTHERAPY & ONCOLOGY

Radiosensitisation in normal tissues with oxygen, carbogen or nicotinamide: therapeutic gain comparisons for fractionated x-ray schedules

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Received 8 August 1995; revision received 2 November 1995; accepted 7 November 1995

Abstract

Methods: Radiosensitisation with oxygen, carbogen or nicotinamide alone and oxygen or carbogen combined with nicotinamide was compared in early and late responding normal tissues in rodents. X-ray treatments were delivered as single doses or fractionated schedules of 2 fractions in 1 day, 2, 12 and 36 fractions in an overall time of 12 days and 10 fractions in 5 or 12 days. Acute skin reactions, survival of intestinal crypts, breathing rate, reduction in the packed red-cell volume and clearance of ^{51}Cr-EDTA were used as assays of epidermal, gut, lung and renal damage. *Results*: Relative to air-breathing mice, carbogen or oxygen produced a small, and not always significant, increase in sensitivity (enhancement ratios ≤ 1.15) in gut, lung and kidneys; however, in skin a dose enhancement of 1.2–1.3 was observed. The effect of nicotinamide in air, carbogen or oxygen was studied only in lung and gut. The drug produced variable but generally significant increases in radiosensitisation (≤ 1.26) in all three gases. Relative to treatments in air, enhancement ratios for nicotinamide alone were usually slightly higher than those observed when either carbogen or oxygen were administered without the drug. With all three modifiers (i.e. oxygen, carbogen, nicotinamide alone or for the drug-gas combinations) there was no significant change in the enhancement ratios observed as the number of radiation dose fractions was varied. *Conclusions*: Comparisons with fractionated X-ray studies done previously in rodent tumours indicate that a therapeutic benefit, relative to lung, gut and renal damage, would be observed with oxygen or carbogen alone but not with nicotinamide alone. The greatest gain would be achieved with the combination of carbogen and nicotinamide, with which a benefit was observed even relative to epidermal damage. These results indicate that some decrease in normal tissue tolerance could be observed when using these modifiers in clinical radiotherapy and, although small, the appropriate dose reductions should be considered; caution should be exercised especially when carbogen and nicotinamide are used in conjunction with the more radical accelerated schedules.

Keywords: Oxygen; Carbogen; Nicotinamide; ARCON; Skin; Lung; Gut; Kidney; Fractionation; Therapeutic benefit

1. Introduction

Increasing the total radiation dose to large tumours in head and neck and cervix, does not markedly improve local tumour control. This is consistent with an expected increase in clonogen number and/or with a heterogeneous oxygenation of tumour clonogens. The improved response seen in some of the hyperbaric oxygen studies, especially with large doses per fraction, supports the existence of clonogenic hypoxic cells in late stage cancers [7,21]. Further evidence for this is the significant effect of blood transfusion on the radiosensitivity of tumours in anaemic patients [2,11], the finding that haemoglobin is an important prognostic parameter in treatment outcome [2,34], the positive correlation shown between the degree of response to radiotherapy and the oxygen content of lymph node metastases [17] and the increased local tumour control with misonidazole and nimorazole in head and neck cancer [34]. In a meta-analysis of the data from randomised clinical trials,

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0167-8140/96/$15.00
SSDI 0167-8140(95)01678-A

Overgaard has shown that hypoxic modification can increase both local tumour control and patient survival [33].

In fractionated radiotherapy of rodent tumours, normobaric oxygen and carbogen are more effective and less toxic radiosensitisers than misonidazole (for review see [37]). The gases improve tumour oxygenation by increasing the amount of dissolved oxygen in plasma and by raising the systemic blood pressure. Carbogen also enhances tumour blood flow and shifts the haemoglobin dissociation curve to the right, depressing the affinity of haemoglobin for oxygen. This latter mechanism may play an important role in areas of low oxygen concentration within tumours. Using a fluorescent immunologically labelled 2-nitroimidazole to stain hypoxic cells from a mouse mammary tumour, carbogen has been shown to decrease the proportion of labelled cells to a greater extent and enhance tumour radiosensitivity more effectively than oxygen [42]. The efficacy of both gases can also be enhanced by the addition of nicotinamide [5,6,27,39,40] which has been shown to sensitise mouse tumours by preferentially reducing the protective effect of cyclic hypoxia [5].

In most rodent normal tissues a dose enhancement of not more than 10% is seen with oxygen or carbogen with single dose radiation schedules; although much higher enhancements are observed in mouse epidermis and tail [37]. However, as in tumours, sensitisation can be increased further by the addition of nicotinamide [19,27]. Until now the majority of the studies on rodent normal tissues have been done with single dose X-ray treatments given usually in conjunction with only one of the sensitisers. To evaluate more fully the effect of these radiomodifiers when used alone or in combination, we have tested single dose and fractionated X-ray schedules in four different normal tissues. Both early and late reacting normal tissues were studied, namely skin, intestinal epithelium, kidney and lung. The sensitising effect of oxygen or carbogen, administered either as single agents (all four tissues) or combined with nicotinamide (lung and gut) to mice treated with 1 to 36 X-ray fractions, was compared with the response seen in mice treated in air.

2. Materials and methods

The studies were carried out according to the regulations stipulated by the UK Animals (Scientific Procedures) Act 1986. Albino WHT/GyfBSVS mice were used in the skin experiment and CBA/HtGyfTo mice were used in the kidney, lung and gut experiments. The mice were fed and watered ad lib for the duration of an experiment. Nicotinamide (NAM) treated mice received a dose of 500 $mg \cdot kg^{-1}$, injected intraperitoneally 1 h before each irradiation. The drug was dissolved in saline at a concentration of 50 $mg \cdot ml^{-1}$, and a volume of 0.01 $ml \cdot g^{-1}$ was injected. Air was delivered 2 min and oxygen or carbogen 2 to 5 min before and during each radiation fraction, at a flow-rate of 5 $l \cdot min^{-1}$. Table 1 summarises the different experimental protocols used with each tissue.

2.1. Assays

2.1.1. Skin

The gross skin reaction on the left-hind foot was measured [9]. Adult albino female mice aged 13–15 weeks were irradiated in dose groups of four. Single dose X-rays, 12 or 36 fractions in 12 days were given to mice breathing air or 100% oxygen (Exp. 1 in Table 1). The 36F/12 days regime was administered as 3 fractions per day with a 6-h interval between the 3 fractions; at this time virtually complete repair has occurred in mouse skin [43]. Acute skin reactions were scored three times a week, from 6 to 38 days after single doses and from 10 to 44 days after the first dose in the fractionated regimes, using an arbitrary scale for oedema, dry and moist desquamation [13]. An average skin reaction was calculated for each animal over a 22-day period, (typically 10–32 days after the first dose, which covers the appearance and disappearance of the reactions in the lower dose groups), from the sum of the interpolated daily scores divided by the total number of days [8].

2.1.2. Lung

Whole thorax irradiation was given to unanaesthetised adult male mice [50]. In Exp. 2 (Table 1) single doses or 10 fractions in 5 days were delivered in air or 100% oxygen, the latter given 2 or 5 min before and during irradiation. In Exp. 3, the 10F/12 days regime was given in air, oxygen or carbogen alone or combined with NAM. Breathing rates were measured at various times after X-ray treatment. Repeated measurements were made, usually every 4 weeks, with the first assessment done 16 weeks after the first radiation dose. Data from Exp. 2 were obtained by direct frequency measurement utilising a rate-meter and chart recorder in conjunction with a plethysmographic chamber, as described by Travis et al. [50]; with this arrangement only one animal at a time can be assessed. Data from Exp. 3 were acquired using a novel technique which enabled up to four mice to be assessed simultaneously. This procedure is automated and relies on the extraction of breathing-rate frequency using Fourier transformation of pressure variations within the plethysmographic chamber. Briefly, pressure transducer outputs from eight chambers, four of which are acquiring data at any one time, are connected to a multichannel data acquisition card in an Apple Macintosh computer. The cyclic pressure variation signals, due to the animal's breathing, are digitised and a fast-Fourier transform (FFT) is calculated on a 10-s frame of acquired data. This gives a spectrum of

Table 1
Experimental protocols

Exp. no.	Tissue No.	X-ray fractions	Interfraction interval (h)	X-ray overall time (days)	No. mice/dose group	Gas alone	Gas plus NAM[a] (500 mg·kg^{-1})
1	Skin	1	—	0	4	Air, oxygen	Not done
		12	24	12			
		36	6 and 12	12			
2	Lung	1	—	0	4–8	Air, oxygen	Not done
		10	6 and 18	5			
3	Lung	10	24 and 72	12	8	Air, oxygen, carbogen	Air, oxygen, carbogen
4	Kidney	2	264	12	6	Air, oxygen, carbogen	Not done
		12	24	12			
		36[b]	6 and 12	30			
5	Gut	2	6	0	4	Air	Carbogen
6	Gut	10	6 and 18	5	4–8	Air, oxygen, carbogen	Air, oxygen, carbogen

[a]Nicotinamide.
[b]The planned schedule was 36F/12 days but, due to gut damage, the irradiations were suspended after 31 fractions. The remaining 5 fractions were given 18 days after as 5 fractions in 2 days.

frequencies present in the transducer's signal, over the range 0.125–50 Hz. The process is then repeated and the resulting FFTs are averaged over a period of up to 2 min; typically 8–16 spectra are averaged. The result is a spectrum with distinct peak at the breathing frequency and this peak can be found by the software in the range 3–10 Hz (i.e. 180–600 breaths per min). This process is performed simultaneously on the four 'active' chambers; during the acquisition phase, a new group of mice are being acclimatised in the other four chambers. The software for this procedure has been developed in-house using commercially available libraries and is based on a graphical user interface. A degree of automation is designed in this process and the technique introduces considerably less experimenter bias. It has allowed us to speed up the acquisition of data by a factor of at least four. The procedure is described in full elsewhere [51].

2.1.3. Kidney

Localised bilateral kidney irradiations were given as 2F/12 days, 12F/12 days or 36F/30 days in air, oxygen or carbogen to female mice aged 14–16 weeks (Exp. 4 in Table 1), using the arrangement first described by Williams and Denekamp [52]. The intention was to deliver all three regimes in 12 days. However, with the 36 fraction regime the dose per day to the surrounding gut was too high and the animals presented signs of severe gut damage on day 10. To allow the mice to recover, the irradiations were interrupted after 31 fractions and resumed 18 days later. The remaining 5 fractions were then given as 3F/day over 2 days. Renal function was assayed at 25, 29 and 33 weeks after the first X-ray fraction using clearance of ^{51}Cr-EDTA and reduction in the packed red-cell blood volume [1,52].

2.1.3.1. Isotope clearance. The clearance of ^{51}Cr-EDTA was used to assess glomerular filtration. The retention of the isotope was measured by determining its plasma concentration 1 h after an i.p. injection of 0.37 MBq (~200 μg EDTA), given as 0.1 ml of a solution with an activity of 3.7 MBq·ml^{-1}. A 70-μl sample was taken from the orbital sinus, centrifuged for 2 min at 1200 *g* and the plasma separated and counted for residual activity in an LKB 1282 auto gamma counter. The measurements were expressed as the percentage of injected activity per ml of plasma at 1 h after injection, and this increased with both total radiation dose and time post-irradiation [52].

2.1.3.2. Haematocrit. The percentage of packed red cells was measured, after centrifugation of the blood sample taken for the clearance assay, using a standard microhaematocrit reader. The progressive reduction in red cells observed after bilateral kidney irradiation possibly reflects the loss of the erythropoietin-producing juxta-glomerular cells [1].

2.1.4. Gut

The microcolony assay for crypt survival was used to assess intestinal damage after whole abdominal irradiation to unanaesthetised mice [53]. A schedule of 2F/1 day or 10F/5 days was given, with an interfraction interval of 6 h between pairs of fractions, to female mice (19 weeks old in Exp. 5 and 14 to 15 weeks old in Exp. 6;. Table 1). In Exp. 5, the intestinal sensitivity of mice breathing air was compared to that of animals treated with carbogen plus NAM. In Exp. 6, the effect of air, carbogen or oxygen alone was compared to that when each gas was combined with NAM. Mice were sacrificed 96 h after the second dose in Exp. 5 and 48 h after the last fraction in Exp. 6. After sacrifice, the intestine was removed and gently flushed through with neutral buf-

fered formalin and stored in the solution until ready for histological processing. At that time five consecutive specimens, 5 mm in length each, were sectioned from the jejunum starting 5 cm away from the pylorus and working distally. The five sections from each intestine were bundled together and bound with surgical Micropore tape. The bundles were tied with surgical suture to prevent unwinding of the tape during histological processing. Two transverse 5 μ sections, 1–3 mm apart, were cut from each bundle and the sections were then stained with haematoxylin and eosin. The surviving crypts per circumference (10 circumferences per mouse) were counted under light microscopy [46].

2.2. X-ray treatments

X-rays were generated with a Pantak X-ray set operating at 240 kVp and filtered with 0.25 mm Cu and 1 mm Al to give an HVL of 1.3 mm Cu. Unanaesthetised mice were restrained in specially-designed perspex and lead jigs enabling precise collimation of X-rays to both kidneys or to the left-hind leg; lung and gut-irradiated mice received either whole thorax or whole abdominal irradiation. The jigs were in turn enclosed in a 23 × 21 × 5.5 cm perspex box through which air, pure oxygen or carbogen was flushed at a rate of 5 $l \cdot min^{-1}$. In skin, the dose rate was 2.4 $Gy \cdot min^{-1}$ with a dose fall-off from the dorsal to ventral aspects of the foot of $< 1.5\%$. Since the dose variation between the four feet in each group was less than 0.3%, the animals were arbitrarily positioned at each fraction. For kidney irradiation, the animals were treated through lateral 20 × 13 mm tangential fields to include both kidneys with a dose fall-off across this field of $\approx 10\%$ and the dose rate was 1.7 $Gy \cdot min^{-1}$. To minimise dose non-uniformity, the mice were rotated through 180° at successive treatments. Whole thorax irradiations were given through sagittal 22 × 20 mm fields at a dose rate of 2.5 $Gy \cdot min^{-1}$ and the mice positioned, at each following fraction, in the field which gave the lowest dose variation between the four animals in the group. The dose rate for gut irradiations was 1.5 $Gy \cdot min^{-1}$ and whole abdominal irradiations were delivered through sagittal 60 × 20 mm fields. The animals were turned from a prone to a supine position at alternate treatments.

2.3. Data Analysis

The response of skin, lung (Exp. 2 in Table 1) and kidney was plotted against dose as the mean and standard error for the mice in each dose group (4, 4–8, and 6 animals per dose group, respectively). The curves drawn through the data (±95% CL) were obtained by fitting a generalised logistic equation to the average daily skin scores, breathing rate, percent of injected activity per ml of plasma or percent packed cell volume *for each* mouse against dose, using non-linear least-squares regression [25]. Isoeffect doses were obtained from each curve by back-substitution in the equation fitted to each set of data points by the regression analysis and these values were used to calculate enhancement ratios (ER)[1] relative to each gas and relative to treatments given in air alone.

In the second lung experiment (Exp. 3 in Table 1), the percentage of mice whose breathing rate was ≥ 1.15 times that of sham-irradiated controls was plotted. The dose-response curves were obtained by fitting the data using logit analysis and the ED_{50} values, (dose required to increase the breathing rate in 50% of the animals by a factor of 1.15), obtained from the fits were used to calculate enhancement ratios and confidence intervals. This analysis could not be done with the data from Exp. 2 since the response with most of the schedules was either below (all single dose data at 24 weeks) or above this threshold (10F/5 days in oxygen at 35 weeks).

Dose-response curves from the two separate experiments on gut (Exps. 5 and 6 in Table 1) were obtained by fitting the surviving crypts per circumference against dose using direct analysis, with the criterion of minimising negative LogLikelihood. The data were fitted to the expression

$$k[1 - (1 - e^{-\alpha RD})^n]$$

where kn is the back-extrapolate on the y-axis, α the slope parameter, D dose and R the dose-modifying factor. The statistical package JMP (SAS Institute Inc., Cary, NC, USA) was used for the computation.

For each tissue, the significance of differences between ERs was estimated by Student's t-test using a standard computer package and these results, along with the corresponding enhancement ratios, are summarised in Tables 2–5.

3. Results

Fig. 1 shows dose-response curves for acute skin reactions in albino mice after a single X-ray dose, 12 or 36 fractions in 12 days administered in air or 100% oxygen. For all three regimes in oxygen, the dose-effect curves were steeper and displaced to the left of those irradiated in air with an increase in effective dose of up to 30% ($P < 0.001$). In the 36F/12 day regime, the dose modification varied with level of effect from 1.19 to 1.31; in the other two schedules the variation was far less. Table 2 summarises the ERs calculated for a skin reaction level of 1.5, equivalent to moist desquamation in one small area of the foot.

[1] $ER = \frac{\text{X-ray dose without modifier}}{\text{X-ray dose with modifier}}$ at the same level of effect

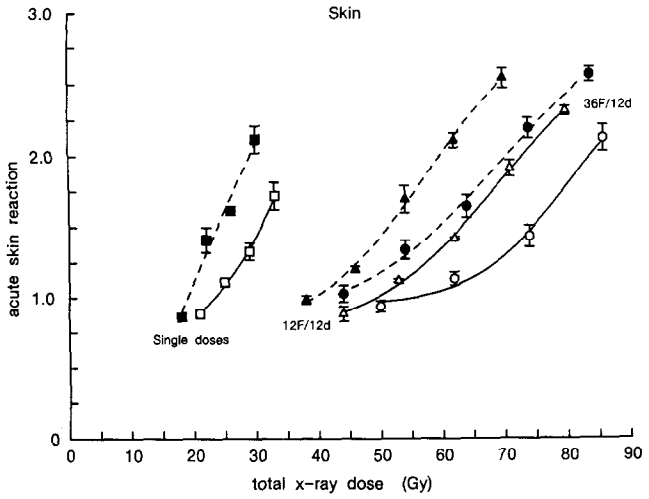

Fig. 1. Dose-response curves for acute skin reactions in mice treated with single X-ray doses (□, ■), 12 fractions in 12 days (Δ, ▲) or 36 fractions in 12 days (○, ●). Treatments were given in air (empty symbols) or 100% oxygen (filled symbols) administered 2 min before and during each fraction. Errors on the data points are ±1 S.E.M.

Dose-response curves for renal clearance and reduction in packed red cell volume 29 weeks after 2, 12 or 36 fractions in air, oxygen or carbogen, are shown in Fig. 2. With both gases the increases in radiosensitivity observed relative to air were small and not always significant. Table 3 summarises isoeffective ERs for both oxygen and carbogen with each schedule, 29 and 33 weeks after irradiation; there was no significant difference in the ERs as a function of fraction number, gas, testing time or assay. The gases did not behave in a truly dose modifying manner. In the 2F/12 day schedule, sensitiser enhancement ratios, obtained at different isoeffective levels, varied from 0.9 to 1.07 for oxygen and from 0.9 to 1.13 for carbogen. With both 2 and 12 fraction schedules, the increase, relative to air-breathing mice, was not more than 9% for oxygen and 13% for carbogen, and these dose enhancements were not always statistically significant. In the 36 fraction regime, given as 3 fractions per day with a 6-h interfraction interval, the dose received by the surrounding gut was too high and the majority of the animals died, from acute gut damage, during the second week of the irradiation schedule. The mortality was similar for all three gases and comparable numbers of mice were assessable for renal damage at later times. Due to the paucity of data, the fitting procedure was done only for the control air alone data points to allow an estimate of renal sensitisation, at the effect levels measured for carbogen or oxygen. No confidence limits could be obtained from the fit and errors are therefore indeterminate. As shown in Fig. 2 (panels c and f), the level of response with carbogen and oxygen was only slightly higher than in air-breathing mice, and corresponds to an enhancement ratio of at most 1.03 in oxygen and 1.06 in carbogen (Table 3).

Fig. 3 shows the breathing rate at 35 weeks after whole-thorax irradiation given as single or fractionated X-ray doses to mice breathing either air or oxygen given 2 or 5 min before and during each irradiation (Exp. 2). At this time, a small but generally significant increase in sensitisation was seen (ER 1.05–1.11) with both pre-irradiation breathing times. With the fractionated regime, sensitisation was slightly lower than that observed with single doses. Table 2 shows ERs at isoeffective levels of damage at two different times of assay (24 and 35 weeks). At the earlier time of 24 weeks (data not shown) in the single dose schedules, the response in most dose groups in oxygen was similar to that in air. The data for 2 min pre-irradiation breathing time (PIBT) could not be fitted by the regression procedure although an ER of 1.07 was calculated at the level of effect achieved with 11.5 Gy; a lower and non-significant enhancement was observed for 5 min PIBT. With the 10 fraction regime, ERs were similar to those observed at 35 weeks (Table 2).

Table 2
Enhancement ratios (±95% CL) and P values for sensitisation by oxygen relative to air

Exp. no.	Tissue/time of assay	PIBT	Isoeffect level	Single doses	10 fractions	12 fractions	36 fractions
1	Skin (5 weeks)	2 min	Moist desquamation (SR = 1.5)	1.29 ± 0.08 ($P < 0.001$)		1.23 ± 0.05 ($P < 0.001$)	1.26 ± 0.08 ($P < 0.001$)
2	Lung (24 weeks)	2 min	SD @ 360 bpm, 10F @ 400 bpm	1.07	1.11 ± 0.04 ($P < 0.001$)		
		5 min		1.03 ± 0.02 ($P = 0.07$)	1.10 ± 0.03 ($P < 0.001$)		
2	Lung (35 weeks)	2 min	SD @ 400 bpm. 10F @ 450 bpm	1.11 ± 0.07 ($P = 0.003$)	1.05 ± 0.04 ($P = 0.07$)		
		5 min		1.11 ± 0.07 ($P = 0.003$)	1.06 ± 0.04 ($P = 0.05$)		

PIBT, pre-irradiation breathing time; bpm, breath per minute.

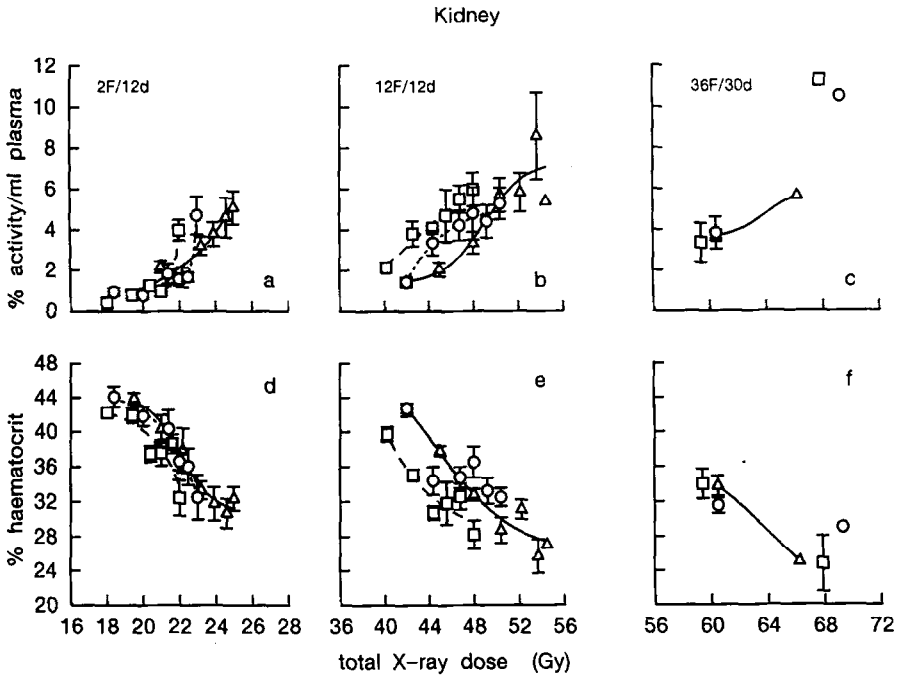

Fig. 2. Dose-response curves for increased retention of ^{51}Cr-EDTA (upper panels) and reduction in haematocrit (lower panels) 29 weeks after 2 fractions (panels a, d) and 12 fractions (panels b, e) in an overall time of 12 days, and 36 fractions in 30 days of X rays (panels c, f), given to mice breathing air (Δ), oxygen (O) or carbogen (□). The curves drawn through the data were obtained by fitting a generalized logistic equation to the percent of injected activity per ml of plasma or percent packed cell volume for each mouse against dose, using non-linear least-squares regression [25]. Error bars represent ±1 S.E.M.

In the second experiment in lung (Exp. 3), air, oxygen or carbogen were administered either alone or in conjunction with 500 mg·kg^{-1} of NAM given 1 h before each fraction. Dose-response curves at 46 and 70 weeks after irradiation are shown in Fig. 4 for the fraction of mice whose breathing rate was ≥1.15 times that of sham-irradiated controls. These animals were jigged for a comparable period of time as irradiated mice using the same schedule of 10 fractions in 12 days, in air or carbogen with or without NAM (4 mice/group). At the earlier assay time of 46 weeks, NAM significantly enhanced radiosensitisation under all three gases, but only that of carbogen and oxygen at 70 weeks. At both assay times, there was a negligible increase in response in mice treated with oxygen or carbogen alone compared with animals irradiated in air. Finally, a dose enhancement of 8–14% was seen for the combination of carbogen and NAM relative to air alone. Table 4 summarises the ERs calculated at the ED_{50} level and the significance of difference between the schedules used.

Table 3
Enhancement ratios (±95% CL) and *P* values for sensitisation by oxygen or carbogen relative to isoeffective renal damage in air

Assay	Assay week	2 fractions		12 fractions		36 fractions[a,b]	
		Oxygen	Carbogen	Oxygen	Carbogen	Oxygen	Carbogen
Clearance @ 3.5% level	29	1.04 ± 0.04 ($P = 0.1$)	1.08 ± 0.04 ($P = 0.01$)	1.07 ± 0.07 ($P = 0.05$)	1.12 ± 0.07 ($P = 0.003$)	1.03	1.05
	33	1.01 ± 0.05 $P = 0.4$	1.01 ± 0.04 $P = 0.4$	1.09[a]	1.15[a]	1.02	1.04
Haematocrit @ 37% level	29	1.00 ± 0.04 ($P = 0.5$)	1.04 ± 0.04 ($P = 0.1$)	1.07 ± 0.05 ($P = 0.01$)	1.10 ± 0.05 ($P = 0.001$)	1.03	1.01
	33	1.04 ± 0.07 ($P = 0.2$)	1.05 ± 0.07 ($P = 0.1$)	1.04 ± 0.05 ($P = 0.1$)	1.09 ± 0.04 ($P = 0.001$)	1.01	1.06

[a]No confidence limits available.
[b]Week 29: ERs were calculated at 4.5% clearance and 31.5% haematocrit; Week 33: ERs were calculated at 6.5% and 6.2% clearance level and 29% and 31.5% haematocrit for oxygen and carbogen, respectively.

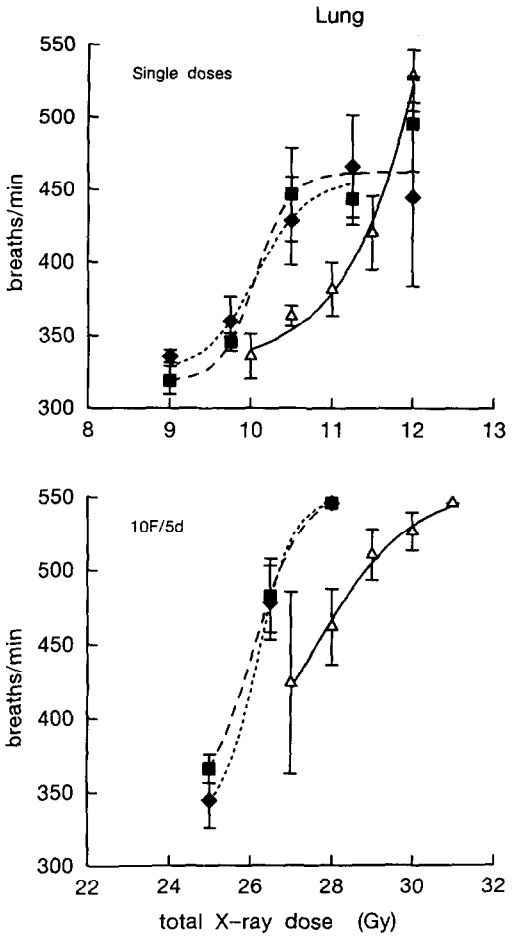

Fig. 3. Breathing rate in mice 35 weeks following whole-thorax irradiation with single X-ray doses (top) or with 10 fractions in 5 days (bottom). Mice were treated in air (Δ) or 100% normobaric oxygen. the latter given either 2 min (◆) or 5 min (■) before and during irradiation. Error bars represent ±1 S.E.M.

Fig. 5 illustrates intestinal crypt survival after 2 and 10 fractions of X rays in an overall time of 1 or 5 days, both given with a 6-h interval between fractions. With the 10F/5 days regime, air, oxygen or carbogen were used with and without NAM. There was a small non-significant increase in radiosensitisation with carbogen and no effect of oxygen on gut radiosensitivity. NAM significantly enhanced the effect of all three gases, giving a dose modifying factor of 1.2 to 1.3. For the comparison between carbogen plus NAM with air alone an ER of 1.24 ± 0.01 (S.E.M) was obtained. With the 2 fraction schedule, the response to air alone was compared with the response to giving carbogen plus NAM, which achieved a dose enhancement of 1.15 ($P < 0.0001$). Table 5 shows ERs obtained from the fitting procedure and P values for each comparison.

4. Discussion

The renewed interest in identifying alternatives to nitroimidazoles has led to a re-evaluation of oxygen, carbogen and NAM as tumour hypoxic-cell radiosensitisers. Table 6 summarises published ERs for each modifier given alone or for the combined gas-drug administration, in a variety of rodent tumours. Oxygen and carbogen are usually effective tumour sensitisers both in single dose and, unlike misonidazole, also in fractionated X-ray regimes. NAM alone achieves significant tumour sensitisation with single radiation doses, but, in the only fractionated study so far reported, the ER of 1.17 in CaNT tumours did not translate into a significant therapeutic ratio relative to renal damage in mice [27]. A similar small sensitising effect of NAM alone can be extrapolated from a more recent study where both 30 and 40 X-ray fraction regimes were administered in air alone, carbogen alone or combined with NAM (see Table 6). It is therefore unlikely that the drug can be used in humans as a sole agent, but since it enhances the action of both carbogen and oxygen, the combined use of carbogen and NAM has been proposed for use in radiotherapy [36]. At 1–2 Gy per fraction, carbogen combined with a clinically-relevant dose of NAM, achieves an ER of 1.6 and 1.7 in 30 and 40-fraction schedules in a mouse mammary carcinoma [39].

Table 4
Enhancement ratios at the ED_{50} level for lung damage after a 10-fraction in 12-days X-ray schedule, and P values for the indicated comparisons

Comparison	Week 46			Week 70		
	ER ± S.E.M.	ν[a]	P value	ER ± S.E.M.	ν	P value
Air vs. oxygen	1.03 ± 0.03	119	0.3	1.02 ± 0.05	110	0.8
Air vs. carbogen	1.05 ± 0.03	113	0.2	0.97 ± 0.05	110	0.5
Air vs. air+NAM	1.20 ± 0.05	115	0.0003	1.01 ± 0.06	106	0.9
Air vs. oxygen+NAM	1.16 ± 0.04	118	0.0003	1.16 ± 0.06	113	0.01
Air vs. carbogen+NAM	1.14 ± 0.04	116	0.0001	1.08 ± 0.06	114	0.2
Oxygen vs. oxygen+NAM	1.12 ± 0.03	125	0.0002	1.14 ± 0.04	117	0.0001
Carbogen vs. carbogen+NAM	1.09 ± 0.03	117	0.0003	1.11 ± 0.03	118	0.0001

[a]Degrees of freedom.

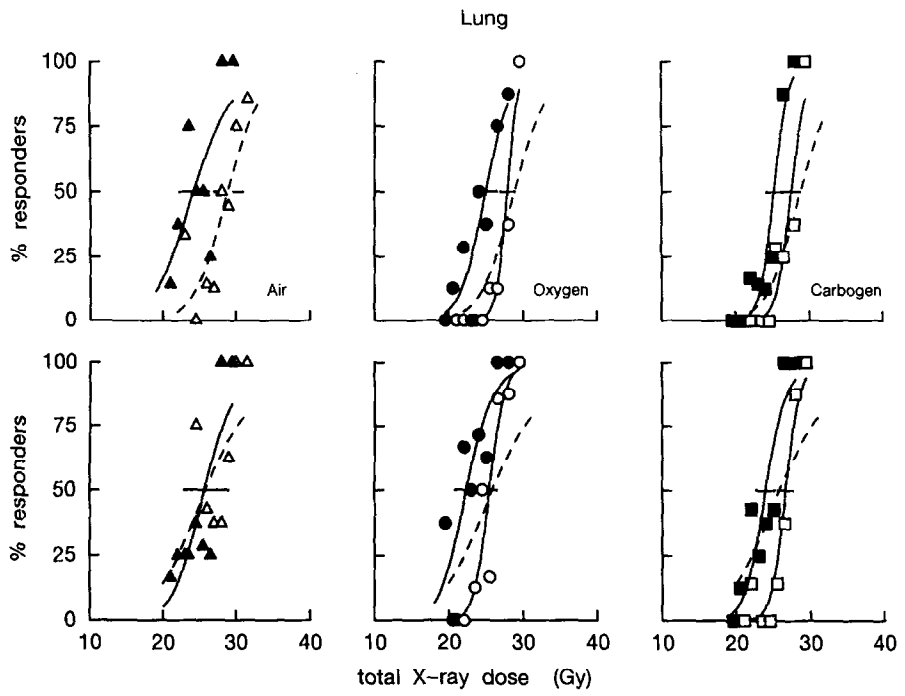

Fig. 4. Percentage of mice whose breathing rate was ≥1.15 times that of sham-irradiated controls measured 46 weeks (top panels) or 70 weeks (bottom panels) after an X-ray schedule of 10F/12 days. Air (Δ, ▲), oxygen (○, ●) or carbogen (□, ■) were administered alone (empty symbols) or combined with 500 mg·kg^{-1} of nicotinamide (filled symbols), injected 1 h before each fraction. Errors at the ED_{50} level are ±95% confidence limits. The dashed line in each panel represents the response to air alone at the appropriate time of assay.

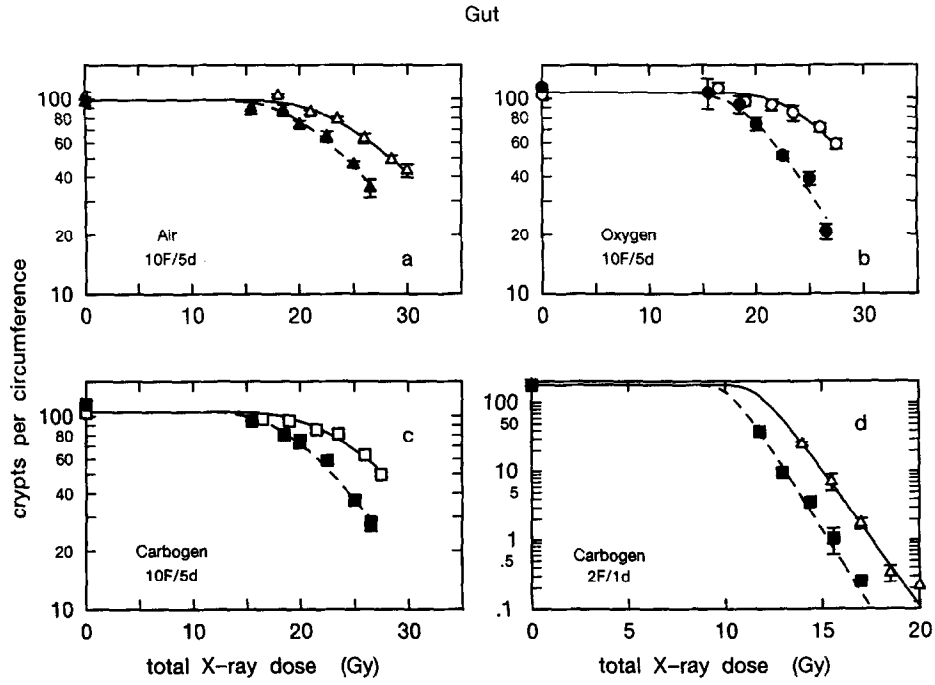

Fig. 5. Dose-response curves for survival of intestinal crypts after whole-abdominal irradiation with a schedule of either 10F/5 days (panels a–c) or 2F/1 day (panel d). Air (Δ, ▲), oxygen (○, ●) or carbogen (□, ■) were administered alone (empty symbols) or combined with 500 mg·kg^{-1} of nicotinamide (filled symbols), injected 1 h before each fraction. Error bars represent ±1 S.E.M.

Table 5
Enhancement ratios for intestinal crypt survival and *P* values for the indicated comparisons

Comparison	No. X-ray fractions	ER ± S.E.M.	*P* value	ν^a
Air vs. oxygen	10	0.96 ± 0.01	0.01	66
Air vs. carbogen	10	1.02 ± 0.01	0.1	62
Air vs. air+NAM	10	1.17 ± 0.01	<0.0001	65
Air vs. oxygen+NAM	10	1.24 ± 0.01	<0.0001	60
Air vs. carbogen+NAM	10	1.24 ± 0.01	<0.0001	63
	2	1.15 ± 0.01	<0.0001	36
Oxygen vs. oxygen + NAM	10	1.26 ± 0.02	<0.0001	62
Carbogen vs. carbogen+NAM	10	1.21 ± 0.01	<0.0001	61

[a]Degrees of freedom.

Although the fractionated protocols have been done in only two tumour models, it is of interest that in CaNT, the ERs with carbogen plus NAM do not decrease with increasing number of fractions. Using a dose of 500 $mg \cdot kg^{-1}$ of the drug, ERs of 1.8–1.9 were obtained with 10 fraction regimes compared with 1.9–2.1 using 20 fraction radiation schedules (Table 6).

The therapeutic benefit with these agents will of course depend on the degree of normal tissue sensitisation. Table 7 summarises the published data and the data presented in this paper. With the exception of mouse skin and tail, ERs are similar in both early and late responding normal tissues and independent of the number of X-ray fractions. Sensitisation is no more than 10% for carbogen or oxygen alone, with a similar additional increase when NAM is administered alone or in combination with the gases. Mouse skin and tail are known to be under a considerable degree of hypoxia and over-estimate the sensitisation likely to be seen in other normal tissues in rodents and in humans [20,47]. From the ERs observed with fractionated regimes in the six more clinically-relevant tissues compared with the two rodent tumour models done so far, a therapeutic benefit would generally be observed with carbogen or oxygen alone, but not with NAM alone. The greatest gain would be obtained with the combined use of carbogen and NAM (Table 6); with this combination a benefit was observed even relative to the somewhat enhanced epidermal damage in mice [27]. Although the normal tissue experiments summarised in Table 6 have been done with high doses of NAM (500–1000 $mg \cdot kg^{-1}$) it is unlikely that the therapeutic gain would increase when

Table 6
Sensitiser enhancement ratios, relative to treatments in air alone, in rodent tumours irradiated in oxygen, carbogen or nicotinamide (NAM) alone or with the drug-gas combination

Tumour	No. X-ray fractions	Oxygen	Carbogen	Air + NAM	Oxygen + NAM	Carbogen + NAM	Reference
KHT	8					1.3	[12]
SCCVII	8					1.6	[12]
RIF-1	8					1.5	[12]
SCCVII	1					1.4	[6]
C3H	1 and 5	1.3 and 1.4	1.2				[18]
KHT	2–15		1.1–1.3				[22]
EMT6	1			1.4–1.7			[23]
Lewis lung	1			1.5–1.6			[23]
RIF-1	1			1.2–1.6			[23]
C3H/Tif	1			1.0–1.5			[23]
SCCVII	1			1.2–1.6			[23]
C3H	1			1.2			[26]
CaNT	10	1.3	1.2	1.2	1.7	1.8	[27]
CaRH	10		1.7			1.8	[27]
SCCVII	1		1.2–1.3	1.5–1.8		1.8–2.6	[30]
EMT6	1		1.2–1.4	1.1–1.2		1.5–1.9	[30]
SCCVII	1		1.5			1.8–1.9	[32]
CaNT	6–36	1.2 to 1.5					[38]
SaF	1		1.3			1.5	—[a]
CaNT[b]	10	1.3	1.6				[42]
CaNT	10		1.5			1.6–1.9	[40]
CaNT	20					1.9 and 2.1	[41]
CaNT	30 and 40		1.4 and 1.5			1.6 and 1.7	[39]
KHT[b]	2–7		1.4				[44]
KHT	1		1.9	1.9		1.9	[45]
C3H[b]	10	1.4	1.3				[49]

[a]Rojas et al., unpublished data.
[b]ERs with the optimum pre-irradiation breathing time.

Table 7
Sensitiser enhancement ratios, relative to treatments in air alone, in rodent normal tissues irradiated in oxygen, carbogen or nicotinamide (NAM) alone or with the drug-gas combination

Tissue	No. X-ray fractions	Oxygen	Carbogen	Air + NAM	Oxygen + NAM	Carbogen + NAM	Reference
Bone marrow	1			1.1			[3]
	1		1.0				[31]
	1					1.2	[32]
	1		1.1				—[b]
Gut	1		1.0				[46]
	1			1.2			[23]
	1					1.1	[32]
	2					1.1	—[a]
	10	1.0	1.0	1.2	1.2	1.2	—[a]
Lung	1	1.1					[14]
	1	1.1					[35]
	1	1.1					—[a]
	10	1.1					—[a]
	10	1.0	1.1	1.2	1.2	1.1	—[a]
Kidney	1	1.1					[46]
	10	1.1	1.1	1.1	1.1	1.2	[27]
	2	1.0	1.1				—[a]
	12	1.1	1.1				—[a]
	36	1.0	1.1				—[a]
Cord	10					1.2	[19]
Testis	1			1.2			[23]
Skin	1	1.1	1.1				—[c]
	1	1.3	1.4				[46]
	1			1.2			[23]
	10	1.2	1.3	1.2	1.5	1.5	[27]
	1	1.3					—[a]
	12	1.2					—[a]
	36	1.3					—[a]
Tail	1			1.5			[26]

[a]This paper.
[b]Rojas et al., unpublished data.
[c]Denekamp et al., unpublished data.

smaller doses of NAM are used. Recent studies indicate that sensitisation in mouse skin does not decrease as the dose of NAM is reduced from 500 to 100 $mg \cdot kg^{-1}$ (Rojas et al., unpublished data) but a small reduction in tumours is seen using the latter, clinically relevant dose of NAM [39].

The use of carbogen and NAM is currently undergoing clinical evaluation [24,54]. Although rodent haemoglobin has a lower affinity for oxygen than that of humans, significant and rapid increases in tumour oxygen partial pressure are obtained by administering either oxygen or carbogen to patients [4,15,16,28,29]. Pharmacokinetic studies of NAM indicate that 80 $mg \cdot kg^{-1} \cdot day$ is close to the maximum tolerated dose that can be given repeatedly to patients [24,48]. This dose achieves a mean plasma concentration of 0.99 $\mu mol \cdot ml^{-1}$ in humans, and plasma concentrations of 0.69–1.02 $\mu mol \cdot ml^{-1}$ in mice, at the time of irradiation, can yield enhancement ratios of up to 1.7 [39,40]. The benefit of carbogen plus NAM in clinical radiotherapy will depend on the extent of tumour sensitisation and on the degree of normal tissue sensitisation. In patients, hyperbaric oxygen produced an increase of about 3% in the sensitivity of gut, cartilage, spinal cord and skin [10] and, therefore, some enhancement of normal tissue damage may occur when administering any of these modifiers to humans. Great care must be taken when devising radiotherapy schedules especially if the sensitisers are to be used in conjunction with the more radical accelerated regimes, since acceleration itself can exacerbate the acute normal tissue reaction. Animal studies do indicate, however, that a substantial benefit could be obtained in human tumours in which hypoxia limits the outcome of radiotherapy.

Acknowledgements

We are grateful to Mr. P. Russell and his staff for care of the animals and for the acquisition of the breathing-rate data in the second lung experiment. This work was funded by the Cancer Research Campaign, UK.

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