ARCON—Current Status: Summary of a Workshop on Preclinical and Clinical Studies

Juliana Denekamp and John F. Fowler

From the Department of Oncology, Umeå University, Sweden (J. Denekamp), and the Department of Oncology, University of Leuven, Belgium (J.F. Fowler)

Correspondence to: Dr Juliana Denekamp, Department of Oncology, Umeå University, S-901 85 Umeå, Sweden. Tel: +46 90 785 15 02. Fax: +46 90 77 54 03. E-mail: Juliana.Denekamp@Onkologi.umu.se

Acta Oncologica Vol 36, No. 5, pp. 517–525, 1997

The current status of the experimental and clinical studies of ARCON was presented. This is a new approach aimed at increasing the effectiveness of radiotherapy by acceleration in order to avoid tumour cell regeneration during therapy and using two forms of radiosensitizer of hypoxic cells. The background knowledge about human tumour proliferation and oxygenation was expertly reviewed. New experimental data were presented for various aspects of carbogen, Nicotinamide and a range of other potential sensitizers. These included mechanistic investigations, radiobiological studies of clinically relevant dose levels, tumour and normal tissue effects, pharmacology and blood flow measurements. Clinical data for both toxicity and initial estimates of the tumour response were presented. Extensive discussions about the side effects of Nicotinamide took place. The main conclusions from the preliminary clinical results were encouraging, especially in head and neck and bladder tumours. Two studies showed that local control is considerably increased compared with previous experience and with results from centres of excellence elsewhere. Several other clinicians reported optimism. So far, ARCON has not been taken into randomized clinical trials and the data are therefore still, of necessity, compared with historical controls or with general clinical experience.

Received 18 September 1996
Accepted 11 March 1997

INTRODUCTION

The third in this series of translational research workshops took place in Umeå, Sweden. This follows two previous ARCON workshops in 1991 and 1994 at the Gray Laboratory, England. In the intervening time many papers on ARCON have been published. Several clinical studies have been undertaken, but the results from most of these have not yet been published. The third workshop therefore provided the opportunity for an up-to-date presentation and discussion of both published and unpublished data. More than forty people were present, representing the work from eleven different countries. The meeting ran for three days, with approximately half the time allocated to clinical and half to experimental studies. The workshop format provided plenty of time to discuss each of the 41 presentations, and the discussions were intense and broad-ranging. These continued in the extended lunch breaks at local beauty spots and in the relaxed and informal evening events. It was a striking example of effective translational research activities, involving multiple disciplines focusing on a common question.

Data were presented from more than 400 patients who have been entered into phase I/II studies (see Table 1). More than half of these patients have received the full ARCON combination. These radiotherapy treatments have been backed up with further studies of tumour proliferation rates, tumour oxygenation, blood flow and pharmacokinetic studies on Nicotinamide, in animals and in the patients and human volunteers. The mix of clinical- and laboratory-based scientists active in this field provided a firm basis for fertile interaction and detailed discussions.

The clinical dose-limiting toxicity from Nicotinamide is mainly nausea and vomiting, which can be severe at 80–85 mg/kg per day, and seems to be refractory to anti-emetics. No other type of severe toxicity was reported. The enhancement of acute radiation reactions in normal tissues was absent or small in most centres. No increase in late reactions was reported. Consensus recommendations were reached for future clinical trials of ARCON.

ARCON BACKGROUND

The acronym ARCON stands for Accelerated Radiotherapy with Carbon dioxide, Oxygen, and Nicotinamide. This

Summary of presentations and conclusions from the Third International ARCON Workshop, Umeå, Sweden, 14–17 August 1996

© Scandinavian University Press 1997. ISSN 0284-186X

Table 1

Summary of clinical studies presented at third ARCON workshop

Centre	Reported by	No. of patients	Intolerance to 80 mg/kg NAM	Tumour site	Accelerated r/t	R/t plus carbogen	R/t plus NAM	R/t plus both	R/t schedule
Nijmegen	Kaanders	124	65%	H&N		32		92	32–34 × 2 Gy/5 weeks concomitant boost
Northwood	Rojas/Saunders	35	52%	H&N		8	12	15	36 × 1.5 TID/12 days
Northwood	Hoskin, Rojas	11	0%	H&N, rectum				11	ditto pharmacology
Northwood	Hoskin, Rojas	30	50%	bladder		10	10	10	20 × 2.5 Gy/4 weeks
EORTC	Bernier, Mirimanoff	40	17%	H&N		11	11	18	3 Fx/day with split 45 × 1.6 Gy/5.5 weeks
EORTC	Bernier, Mirimanoff	39	27%	lung		10	12	17	20 × 2.0 + 0.75 Gy/4 weeks concomitant boost
EORTC	Bernier, Mirimanoff	2	NA	bladder		2	0	0	20 × 2.5 Gy/4 weeks
EORTC	Mirimanoff	95	19%	brain		21	16	58	40 × 1.5 Gy BID/4 weeks
Pisa	Cartei/Fatigante	42	37%	brain	7		8	27	40 × 1.5 BID/4 weeks
Umeå	Karlsson	22	higher doses	H&N	13			9	30 × 2 BID/3 weeks or 30 × 2/6 weeks
Amsterdam	Hulshof	19	not known	brain		6	6	7	blood flow
Cambridge	Laurence, Honess	22	100% (6/6)	mixed			2	10&10 carb & PXF	Pharmacokinetics & pO_2

was first proposed as a multifactorial approach to over coming tumour resistance at the first of this series of workshops in Northwood in 1991, based on a large body of animal data for both single doses (Horsman et al.) and fractionated schedules (Rojas et al.).

The separate elements of ARCON require consideration of:

- normal tissue and tumour responses to fractionated schedules;
- proliferation rates in human tumours;
- compensatory regenerative response of tumours and normal tissues to injury;
- hypoxia in human tumours and normal tissues;
- alterations of oxygenation patterns between treatments;
- the relationship between vasculature, perfusion, hypoxia and proliferation parameters;
- the systemic and local responses in blood flow and oxygenation after administering carbogen and NAM.

The preclinical data leading to the idea of implementing ARCON in the clinic were obtained in murine tumour models, so it is also necessary to consider the relevance of these tumours to spontaneous human malignancies. Several groups presented data on human tumour xenografts, often considered a useful bridging model. Since the first workshop, many clinical studies have been initiated and several hundred patients have been treated with the different elements, accelerated regimes, with either carbogen or Nicotinamide, and, finally, with the whole combination. The radiotherapy schedules have varied widely, being based on the previous experience of the individual investigators.

The original rationale for using ARCON was that it would deal simultaneously with several potential causes of radioresistance and would spare late damage to normal tissues by using very small doses in each fraction. To obtain any gain by dealing with these causes of resistance requires, of course, that there should be a differential between tumour and normal tissues for these resistance factors.

Radioresistance can result from tumour cell multiplication during therapy increasing the tumour burden. In an untreated tumour very rapid cell division is balanced by extensive cell death as the blood vessels are incapable of providing an adequate nutrition. In many solid tumours 90–95% of the newly born cells usually die of starvation, giving an appearance of slow, overall tumour growth. However, as soon as some cells are killed by the radiation, the nutrient supply to the rest improves, the natural process of cell death is disturbed and the tumour can effectively proliferate much faster. This may occur within the time taken for a normal radiotherapy schedule and hence may contribute to treatment failure.

A threefold radioprotection is also acquired by any cell in the absence of oxygen or at unphysiologically low oxygen tensions. Hypoxia is rare in normal tissues, but exists in solid tumours for two reasons, either because tumour cells are pushed too far from their supplying blood vessel, (chronic and progressive hypoxia leading to death as described above) or because individual microvessels close for a short period when all the dependent cells become acutely but reversibly hypoxic.

The ARCON combination has been shown in mice to be extraordinarily effective in multifraction experiments,

using large numbers of doses, each of a few grays. The degree of sensitization can be expressed as an SER (sensitizer enhancement ratio) for comparison with other approaches to sensitize tumours. (SER is the radiation dose without sensitizer, divided by the dose with sensitizer that produces the same level of effect. An SER of 2 would be equivalent to being able to administer twice as much of the radiation in the standard schedule).

The SER for a hypoxic sensitizer results from sensitizing a subset of cells, i.e. only those that are hypoxic. Their presence is almost undetectable at low doses but totally dominates the response at high doses, when almost all the sensitive well-oxygenated cells have been eliminated. If a fractionated series of small doses is given, then the importance of the hypoxic subset will become greater as the oxic cells are killed, unless the oxygenation status changes between fractions. A natural process of reoxygenation of the hypoxic cells has been shown to occur after a small dose of radiation, so that the response to each small dose is again dominated by the well-oxygenated cells and the effect of a substitute for oxygen is then reduced with fractionation. This is what has been observed in practice with earlier attempts to overcome hypoxic resistance, e.g. with hyperbaric oxygen, neutrons and chemical sensitizers. However, in stark contrast to the other approaches, the SER for ARCON observed with clinically relevant schedules is *larger than* the effect with single doses and much greater than has been seen in animal experimental tumours with any of the earlier modifiers. (See discussion of this phenomenon below). There is a slight sensitization of some normal tissue in the mouse but the considerably greater effect in tumours than in normal tissues means that a therapeutic gain is predicted. Thus:

- Accelerated radiotherapy deals with resistance by avoiding the possibility of rapid tumour cell proliferation during treatment.
- Carbogen (95% O_2 + 5% CO_2) increases the amount of oxygen in the blood and oxygenates the chronically hypoxic tumour cells.
- Nicotinamide (NAM) sensitizes acutely hypoxic cells by preventing intermittent vascular shut-down in tumours.

The experimental data on which this approach is based came mainly from the Gray Lab and from the Stanford, Vancouver and Aarhus radiobiology groups. The animal experiments from Rojas and colleagues have demonstrated large SER values of 1.7–1.9 in fractionated studies on rodent tumours even with clinically relevant extended fractionation schedules. These are in sharp contrast with earlier studies from the same laboratory, using the same tumour model, of fractionated neutrons and of chemical sensitizers. In the previous studies a considerable benefit was seen with large single doses, but this was usually reduced or totally abolished when many small fractions were used in the more clinically relevant schedules.

The SER or 'effective enhancement of the dose' has to be considered in terms of the likely increase in local control before knowing how big an SER would be useful in clinical practice. This can be deduced from clinical data for each tumour site where a dose dependence has been shown. Often a 10% increase in dose (corresponding to an SER of 1.1) results in a 10–20% increase in the proportion of tumours controlled. Thus the observed large SER values with ARCON are very favourable and even a much smaller SER than that observed in mouse tumours would still be useful in the clinic and would be detectable in a clinical trial of reasonable size. Of course the possibility of enhancing normal tissue injury has also been considered. It has been shown to occur in some mouse tissues, but to be much less than in the tumour, leaving a considerable potential for therapeutic gain, but a need to go carefully into clinical studies with curative radiotherapy regimes, which are already close to the tolerance limit.

CLINICAL STUDIES PRESENTED AT THE WORKSHOP

Several groups presented an interim report of their clinical activities, as shown in Table 1. Pilot and Phase I/II clinical studies have been under way since the first workshop in a number of radiotherapy centres, alongside four multicentre EORTC Phase I/II randomized trials with patient intake since 1994. These pilot studies were usually designed as three sequential studies to treat, for example, 10 patients with accelerated radiotherapy plus carbogen, 10 with accelerated radiotherapy plus Nicotinamide, then 20 or more patients with both substances administered with the accelerated radiotherapy, which is the full ARCON treatment. In centres with no previous experience of an accelerated radiotherapy schedule, the introduction of an unconventional schedule required additional testing on its own. The accelerated schedules ranged widely, from shortening to 12 days overall time (as in CHART at Northwood), or reducing the treatment time by only a week or two from the conventional 6–7 week treatments.

INTERIM CLINICAL RESULTS

The largest number of patients treated in a single institute was from Nijmegen, where, since November 1992, Kaanders et al. have treated 124 advanced inoperable head and neck patients, with impressive improvements in actuarial local control at 18 months (n = 104 evaluable), compared with their historical controls or the best literature results. The result was particularly good for the larynx. The next ARCON workshop will be held in Nijmegen in June 1998, at which time it will be possible to assess whether these exceptionally good results hold up in the longer term.

Good improvements in tumour clearance were reported from Saunders et al. (Northwood) for 35 advanced head and neck patients treated with the 36 Fx/12 day regime

with the addition of carbogen and NAM. Hoskin et al. (Northwood) also reported a large increase in cystoscopic clearance at 6 months for T3 bladder tumours (17/19: 87%) treated with a 4-week regime even though these patients tolerated NAM poorly and did not all receive the total combination for the whole treatment. This compared favourably with historical data using the same radiotherapy regime at the London Hospital or literature results from Edinburgh or Toronto which ranged from 45 to 58% for the same tumour type and clearance criterion.

In the EORTC studies, 95 glioblastomas, 40 head and neck, 39 non-small-cell lung and 2 bladder cancer patients were assessed. Table 1 shows the distribution into the different steps of the ARCON evaluation. The main toxicity was from nausea and vomiting, resulting from the high dose Nicotinamide. There were some reports in this multicentre study of lung tumours of nausea associated with the carbogen inhalation, but this was not observed in any of the other studies presented at this workshop. Curiously, the full carbogen and Nicotinamide treatment was associated with fewer side effects than the individual components. The full ARCON treatment was only given after experience had been gained with the individual elements so perhaps this reflects a learning curve with this new approach. It was too early to assess local control, but response at 2 months was good. CR + PR was 72% for the advanced head and neck tumours (n = 40), and 89% for the unresectable lung tumours (n = 39) (Bernier & Mirimanoff). Results on glioblastoma treatments, with two treatments per day over 4 weeks, showed no particular improvements, but it was clear that when surgical removal was complete, the results were better than when incomplete (Mirimanoff et al.). Similar findings have been reported for glioblastomas by several other groups, both in the literature and at this workshop, and a non-significant improvement in survival was shown by the Pisa group (Fatigante et al.).

Although the follow up periods are of course too short for definitive conclusions about local control, even of head and neck tumours, the tumour responses in all sites were considered to be encouraging, and there was enthusiasm to continue with further clinical studies of the full ARCON combination.

Radiation morbidity

There was little, or no, increase in the acute radiation reactions in normal tissues as a result of adding the hypoxic cell sensitizers. There was, of course a considerable increase in mucosal reactions due to accelerated schedules of radiotherapy alone, as predicted by the preclinical studies and as has been found in most studies of acceleration. The healing of these tissues during protracted radiotherapy schedules is prolonged when the schedule is shortened. For some accelerated schedules a decrease in total dose has already been made to allow for the increase in acute reactions. (In CHART, for example, a 10–20% downward dose adjustment for lung and head and neck cancer has been made compared with 6–7-week schedules, but it is nevertheless showing significant benefits in the multicentre studies.) Some centres reported delayed healing in a small proportion of patients treated with full ARCON. In one centre (Umeå, Sweden), the total dose was reduced by 10% when accelerated or conventional therapy was combined with ARCON (n = 22) but this was an exception. In the bladder, a precautionary dose reduction from 52.5 Gy to 50 Gy in 20 fractions in 4 weeks at Northwood had been reversed after the first 6 patients and doses of 52.5 Gy were now being regularly used with ARCON (Hoskin et al.).

No increase in late complications was reported from the centres which had been using ARCON longest, although of course the time available for evaluation is short and further follow-up is required to test this observation.

Nicotinamide side effects

The toxicity that was of most concern was the nausea and vomiting associated with the large doses of Nicotinamide (NAM). This nausea occurs to some extent in many patients at the recommended dose of 80 mg/kg, usually occurring within the first week of treatment. In one study there was a sharp increase in toxicity between 80 and 90 mg/kg (Hoskin et al., Northwood). The nausea has not responded to any of the battery of sophisticated anti-emetics that are now available. In the more severe cases it led to unacceptable distress and even to vomiting. If the dose of NAM was reduced by 25% in those patients showing severe intolerance, the symptoms became more manageable (Kaanders et al., Nijmegen). In some studies this side effect was the main cause of non-compliance and the treatment in those cases was continued using the radiation and carbogen without Nicotinamide.

Addressing this problem occupied a large part of the workshop. In about 20% of the patients given both carbogen and NAM, administration of NAM had to be stopped, although the irradiation was always completed as planned. That proportion varied widely in different institutes (see Table), for reasons that were difficult to identify. Glioblastoma patients tolerated NAM better and there was speculation about the contribution of steroids to this better tolerance. At Northwood the earlier patients with large head and neck tumours and in poorer condition had tolerated NAM better than those entered in the most recent bladder studies. It was suggested (Pero, Lund) that prophylactic use of anti-emetics might be more helpful than treating the patients after the symptoms develop, and would perhaps avoid the conditioning of the patient to anticipate nausea with subsequent doses.

In most centres carbogen and radiotherapy were very well tolerated except in the EORTC lung tumour trials, where it was also reported to induce severe nausea in 4/10 patients, though treatment was never interrupted for this reason.

PHARMACOLOGICAL STUDIES

Comprehensive pharmacological measurements have been made in some human volunteers and in many of the patients. It is generally agreed that a peak of NAM in plasma is seen between 0.5–4 h, with a peak value that can vary by almost a factor of two for the same administered dose (mg/kg). 80 mg/kg was reported to give plasma levels above the 'target' of 0.7 μmol/ml in 70% of patients within 1–2 h at Northwood (Hoskin et al.) and in all patients between 0.5 and 3 h at Nijmegen (Kaanders et al.). In the latter study the mean value for peak levels was 1.15 μmol/ml and 82% of patients were above the target value at the chosen time of irradiation (2 h) when no adjustment had been made for individual differences in pharmokinetics.

The half-life for excretion is long (6–9 h) after these high doses, and therefore reasonable levels are still present at the time of the second irradiation, 6 h later. The plasma level usually falls close to background level in 24 h, but in some patients there was a dramatic accumulation, accompanied by severe side effects, for example in Umeå (Littbrand, Karlsson). This may relate to concomitant medication, e.g. for cardiac disorders, and cannot be predicted on the initial liver or kidney function tests. It would be desirable therefore to monitor the NAM levels in all patients, especially to determine the level and timing of the peak, and the level prior to the next day's administration as an indicator of accumulation. Since repeated blood sampling is undesirable Stratford et al. (Northwood) have developed a technique for assaying NAM levels in saliva. They found that it is rapidly converted to nicotinic acid by bacterial flora of the mouth, and that it is necessary to keep the sample on ice during processing and to measure both NAM and the metabolite. This was shown to correlate well in a series of patients with blood samples taken at the same time, and was therefore proposed as a substitute for plasma measurements.

The formulation and the route of administration have been investigated, using oral tablets, capsules or a solution, or rectal suppositories. The fastest and most reproducible peak is obtained with liquid given orally, and is reached somewhat faster in patients who have not had breakfast before receiving the drug. With 80 mg/kg most patients achieve peak plasma levels within 1–2 h above the target of 0.7 μmol/ml and still have substantial levels 6 h later at the time of the second radiation fraction each day. In Pisa (Cartei, Fatigante et al.) this target plasma level was achieved with 4 g doses (approx. 50 mg/kg), but an intercomparison of all the pharmacokinetic profiles (Horsman, Aarhus) showed the Pisa data to be generally higher than that from other centres.

PROLIFERATION STUDIES OF HUMAN TUMOURS

Begg, Wilson et al. (Amsterdam & Northwood) reported the updated analysis of pre-treatment potential doubling time (Tpot) and LI (labelling index of cells synthesizing DNA) from a meta-analysis of data supplied to them from many European clinical series in which these two laboratories have acted as reference centres in the assessment of proliferation parameters. All the patients have been given a small dose of IUdR or BUdR several hours before a biopsy. The tissue had been analysed by flow cytometry after disaggregation, to determine the fraction of labelled cells (LI) and the duration of the S phase from the relative movement of labelled cells towards mitosis in the interval between labelling and biopsy.

So far, 435 patients have been assessed from nine European centres in which they were treated with conventional 6–7-week radiotherapy for head and neck cancer. The relationship between the measures of proliferation on pretreament biopsies and the response to therapy is being studied for its prognostic value. Local control was indeed significantly correlated with LI, but with Tpot showed 'only' a strong trend. A possible explanation for this difference between LI and Tpot may be due to the difficulties in accurately determining the value of Ts, see below.

Another set of data that is in the pipeline, consists of a large series of sequential patients with many types of tumours, being treated with conventional radiotherapy (6–7 weeks). This study is being coordinated in Lausanne by Coucke, in Besancon by Pavy and in Northwood by Wilson. A total of 102 specimens from cervix, head and neck, rectum and lung tumours have been analysed for the LI and Tpot measurements in a quality control assessment of specimen and data handling in the three centres. This tripartite study has so far shown similar short Tpot values to those seen in other centres (median Tpot values of 3–5 days). A considerable variation has been seen between samples processed and/or evaluated in the three centres. A detailed analysis of the source of the variation was presented in an attempt to identify the best methods of achieving objectivity and agreement. This showed that Ts is much more difficult to derive from the histograms than LI and there is still a long way to go before patients can reliably and unequivocally be identified in multicentre studies as having a particular potential doubling time. This will have a negative impact on its use as a predictive factor for accelerated regimes in multicentre trials.

The observed difference in the prognostic value of LI and Tpot was therefore considered as probably due to the

variety of analytic and preparative techniques to obtain Ts (duration of DNA synthesis) with the relative movement assay. It was suggested that if better quality control for measuring Ts could be developed, the correlation with Tpot might be improved. Furthermore, the analysis has so far been based only on the flow cytometry assessment of the labelling index. It is recognized that the labelling index of the tumour cells (without stromal cell contamination) is often higher than the flow cytometry estimate if it is assessed visually in histological specimens, especially for diploid tumours. Some of the tumours have both measurements, so that further analyses should reveal the contribution this makes.

Neither Tpot, nor the BUdr or Ki-67 labelling index using the MIB-1 antibody (Wilson) should yet be rejected in comparisons with clinical outcome, even though it would be simpler to obtain the labelling or Ki-67 index in practice. Although it may not be possible to identify a cut-off line for Tpot above and below which the prognosis is different, it was shown again that cell doubling times within tumours are much shorter than volume doubling times, with most of the median Tpot values being only a few days. Thus, if natural cell loss is reduced when treatment starts, proliferation may be important in most tumours, rather than in an identifiable subset.

A kappa test of the agreement between LI, Tpot and Ki-67 in classifying individual tumours as rapidly or slowly proliferating, to determine which might correlate best with clinical response, was described by Fowler, Haustermans & Wilson (Leuven & Northwood). This showed that if the range of LI and Tpot is wide, as in 35 breast carcinomas, good agreement can be expected. However, if the range is small, as in 60 oesophageal tumours (and probably many other types of tumour), agreement is poor, in practical terms of predicting any individual value, despite a high correlation coefficient. Therefore all three parameters should continue to be investigated for their value as indicators of proliferation rate.

Wilson also described the four categories of histological *pattern* of proliferating cells (shown either by BUdr or Ki-67 labelling) with respect to their depth of labelling into the micro-islands of cells in tumours: marginal, intermediate, mixed, and random. This reflects the deviation from the normal pattern of epithelial proliferation where the label is confined to the layers close to the basement membrane. It appears to have prognostic significance, independent of the Tpot value, with increasing deviation from the normal pattern of proliferative organization having the worst prognosis. In the CHART treatment of head and neck tumours, this proliferative organization was a significant, prognostic parameter ($n = 95$) but, surprisingly, this was not the case in patients treated conventionally (overall time 6–7 weeks). Further investigations are planned at the Gray Lab to investigate the significance of these observations, using a series of human tumour xenografts in mice with different differentiation patterns (Rojas).

OXYGENATION AND BLOOD FLOW MEASUREMENTS IN HUMAN TUMOURS

Vaupel (Mainz) reviewed the convincing evidence from the use of Eppendorf probes that at least a third of human tumours of the breast, of Ca cervix and of head and neck contain sizeable regions sufficiently hypoxic to be radioresistant. He demonstrated that metastases and recurring tumours had lower pO_2 values than primaries. Tumours which reoxygenate respond better to treatment than tumours that do not, and reoxygenation can be seen after one week of radiotherapy. Mean pO_2 does not correlate with tumour size or stage, yet pre-therapeutic tumour oxygenation is the most powerful predictor of overall and disease-free survival in Ca cervix, followed by FIGO stage. Multivariate Cox regression also identifies tumour oxygenation as the most important independent risk factor for poor outcome. It was a remarkable finding that the same correlation was found for Ca cervix patients treated by surgery, not radiotherapy. Thus more than hypoxic radioresistance must be involved in this correlation. There was speculation about the induction by hypoxia of a more aggressive phenotype in some tumours. A further new and unexpected correlation was that tumours with high vascularity that were also hypoxic had a poor outcome no matter what the modality, surgery or radiotherapy.

Horsman reviewed the historical data, especially that from a little-read paper by Kolstad 1968, which showed that local control in Ca cervix was much worse for relatively poorly vascularized and hypoxic tumours. Kolstad showed a correlation of 5-year local control rates with vascularity (17% vs. 51%, $n = 105$), and with surface pO_2 values above or below 10 mm Hg (70% vs. 40%, $n = 31$). Horsman also reported the striking new results for nodal metastases of head and neck tumours from Nordsmark et al., Aarhus. She has found a highly significant difference (77% vs. 33%, $n = 35$) in the 2-year local recurrence-free rates for patients stratified as being below or above 15% (the median percentage) of pO_2 values below 2.5 mm Hg, assessed with the Eppendorf electrode.

Hill et al. (Northwood) showed the changes in blood flow that have been seen in 13 human tumours and 18 xenografts using Doppler flowmetry. She also demonstrated changed MRI images using the difference in MR characteristics of haemoglobin and oxyhaemoglobin in 28 patients. Fluctuations in blood flow in any microregion within a tumour occur in human tumours and xenografts, as they do in murine tumours. However, the extent and magnitude may differ. Using Doppler readings over a 1-h sample time to monitor changes in local perfusion, in murine tumours 50% of readings show a twofold and 16%

a fivefold variation. In xenografts the corresponding figures are 37% and 10%, and in human tumours they are 26% and 3%. There was no indication of a compensatory shut-down of blood flow when the patients breathed carbogen. Fluctuations in flow continue, but they were no greater than in normal air breathing conditions.

Hulshof (Amsterdam) presented data on 25 human glioblastomas imaged with 99mTc HMPAO in patients breathing air, i.e. normal conditions or after administration of NAM or carbogen. He was unable to demonstrate any significant improvements in blood perfusion with these treatments.

PRECLINICAL LABORATORY STUDIES

Rojas (Northwood) reviewed concisely her large series of fractionated experiments on the CaNT mouse tumour which, with carbogen and Nicotinamide, had previously shown SER values of 1.8–3.0 for short schedules using high-dose NAM vs. the control arm of conventional treatment in air over 6 weeks. The separate components of benefit from acceleration, carbogen and NAM have been dissected. She also showed recent data using lower, clinically relevant doses of NAM, giving a plasma level of 1.0 μmol/ml with 30 Fx/40 days (SER = 1.7) and 40 Fx/28 days (SER = 1.6). SER values for epidermal, gut, lung and renal damage had been measured at 1.0–1.3 with a high dose of NAM, the highest being in the skin of mice. Corresponding values for lower doses of NAM are not available. There is a large therapeutic gain, but a dose reduction may be necessary for some sites in the body. (Enhanced reactions in the skin of patients have *not* been a problem in the clinical studies.) Nicotinamide (NAM) abrogates the critical timing of carbogen breathing for tumour sensitization. A benefit of NAM in addition to the carbogen has been seen down to 100 mg/kg, giving in mice the same plasma levels as are seen in the patients at 80 mg/kg. Further experiments are now required to determine whether even lower concentrations of NAM would also be effective. This relates to the question of whether the clinical dose could be reduced below 80 mg/kg. It is also important to determine whether human tumour xenografts would yield the same high SER values as those seen in murine tumours (see below).

Denekamp and Waites presented a dynamic subpopulation computer modelling exercise of the sensitization achieved with the carbogen and Nicotinamide in the Ca NT tumour by Rojas and co-workers. They showed that the very large SER values with small doses of 2–4 Gy are not compatible with simple oxygen sensitization if they accept the measured values of 20% hypoxic cells and reoxygenation back to this value between fractions. Reoxygenation should dilute the effect of the hypoxic cells and should yield much smaller ER values of 1.05–1.10. They concluded that either the hypoxic fraction of the clonogenic cells must be grossly underestimated by the standard techniques, or there is an additional mode of action of the carbogen (± the Nicotinamide) which affects a larger and persistent subpopulation of cells. Even killing all the hypoxic cells present at the start of treatment (analogous to reperfusion injury) cannot explain these high SER values, unless there is a regeneration of hypoxic cells as treatment proceeds, i.e. rehypoxiation rather than reoxygenation. This modelling exercise should allow the identification of critical experiments that could test between these alternatives.

Horsman et al. (Aarhus) showed in three lines of mouse tumour, using large single x-ray doses, SER values of 1.3 to 1.4. He tested for the time dependence and the dose dependence using an excision assay to determine the surviving fraction, or a growth delay assay. The dose dependence was different in the three tumour types, producing maximal sensitization in C3H tumours with 137 mg/kg (growth delay), but only 80% and 40% of the maximum SER in the SCCVII and KHT respectively (survival assay). This dose was chosen to match the peak plasma levels in humans after a dose of 80 mg/kg. The maximum SER could only be achieved if the irradiation was carried out at the time of peak plasma concentration of NAM, but much better sensitization was observed with a standard 20 min interval than the previously published data with a 1–2 h interval.

Olsson et al. (Lund) presented data relating to the original hypothesis that Nicotinamide is a DNA damage repair inhibitor. This had originally been postulated to be the mechanism of sensitization, based on radiation data from cells in vitro. In vivo data now were presented for two types of tumour tissue and for the spleen. They showed that NAM itself induced DNA strand breaks and also caused inhibition of the rejoining of DNA breaks at doses that radiosensitize (100–1000 mg/kg), but no inhibition at the low doses that do not radiosensitize (10 mg/kg). Pero et al. (Lund) presented data on a range of N substituted benzamides and nicotinamides and showed that the N substitution alters the mode of action. Their studies include estimates of ADPRT activity and the induction of apoptosis. N substitution of Nicotinamide abolishes its ADPRT inhibition but selectively induces apoptosis.

Sun et al. (Lausanne) found SER values with NAM and carbogen of about 1.3 in human glioblastoma xenografts in mice, for 5F/5 days and 20F/2 weeks. Skin reaction SER values were lower, at about 1.2 showing only a small therapeutic gain. Stern et al. (Paris) also showed lower SER values for ARCON in xenografts but that was using single doses, not fractionated schedules, and is consistent with the Gray Lab data. At this time it is not clear whether xenografts in general will show lower ERs or similar high values to those seen in murine tumours.

Several groups had worked on sensitizing chemicals other than Nicotinamide and carbogen: Verovski et al.,

Brussels, with sodium nitroprusside; Pero et al., Lund, with various N-substituted benzamides and nicotinamides; Coucke et al., Lausanne, with(E)-2-deoxy-(fluoromethylene)cytosine) and Kaleta et al., Gliwice, with cis platinum (clinically).

Stern & Guichard (Paris) compared the radiosensitizing effect of NAM, carbogen, tirapazamine and perflubron emulsion (alone or in combination) in two human tumour xenografts and one animal tumour. They reported different results in the three types of tumour. Most of the treatments decreased the transient perfusion. The percentage of mismatched stained vessels (a measure of acute intermittent hypoxia) only correlated with change in radiosensitivity in one cell line. Their message was to use great caution in interpreting experimental tumour results, since their determination of the optimum combination of sensitizers reached a different conclusion in the different tumour types.

Honess reported excessive hypotensive toxicity in 4/12 patients with 4–6 g doses of NAM in Cambridge and had therefore sought, in the murine tumour RIF1, an alternative sensitizer which might also act by diminishing intermittent vessel closure. One with an attractive immediate clinical applicability was Pentoxyfilline (PXF), already used in the clinic for intermittent claudication and possibly less toxic than Nicotinamide. Using the Eppendorf microelectrode, she showed that NAM had a bell-shaped dose response curve, with 200 and 400 mg/kg resulting in higher median pO_2 values than either 100 or 700. All doses of PXF between 20 and 50 mg/kg gave a similar effect. The time dependence for NAM was less sharp, peaking at 1 h, than with PXF where the peak occurred at 15 minutes and had substantially diminished by 20 min. When the short half-life in mice was corrected for by a series of small injections totalling 40 mg/kg over 40 min, PXF was more effective at improving oxygenation than Nicotinamide. The half-life of PXF in man is sufficiently long (1 hour) so that repeated administrations should not be necessary. This approach seemed promising if PXF is less toxic than NAM. Doses of 400 and 800 mg PXF have already been given to patients and, when combined with carbogen breathing, have yielded reductions (1) or elimination (4) of hypoxic values within 10–20 min after ingestion in the 6 out of 10 patients who showed hypoxic regions (Laurence et al. Cambridge).

Siemann & Fenton (Gainesville, USA) showed that carbogen alone improved tumour cell-killing in the mouse KHT sarcoma, with an SER of 1.8 for single doses of radiation but only 1.2 or 1.4 after a week of fractionated irradiation (5F × 2Gy or 5 Gy). This loss of sensitization with fractionation is reminiscent of that seen in the earlier work with sensitizers and differs from Rojas's data with the pseudoclinical full ARCON fractionation regimes. Carbogen continued to produce higher levels of oxyhaemoglobin, measured with cryospectrophotometry, than air breathing even in the irradiated tumours. Siemann also reported that a clinical trial is planned at Gainesville, USA, using carbogen alone with their twice daily schedule of 1.2 Gy fractions for head and neck cancer. They plan to add the hypoxic stain EF5 to identify hypoxia in biopsies, when it is approved by the FDA.

Experiments by Hill et al. (Northwood) investigated a range of percentages of CO_2 in carbogen from 0 to 10%, and showed that 1 to 2.5% was better than either 5 or 10% for improving oxygenation and for radiosensitization. This could help reduce the few percent of patients unable to tolerate the 5% level, although the problems with carbogen clinically have been so slight that few clinicians wished to adjust the CO_2 level at this stage.

Thews (Mainz) illustrated the change in tumour oxygenation and in blood flow by electrode and Doppler techniques in rat tumours. He compared the improvement in oxygenation from pure oxygen, or with the addition of 2.5% or 5% carbon dioxide, at normal pressures or at two atmosphere pressures. In normobaric pressures all the gases improved the oxygenation but did not eliminate the hypoxia. There was a size-dependence effect, with carbogen being more efficacious in the larger tumours, and giving a better perfusion. High-pressure gas breathing improved the oxygenation further and almost eliminated the hypoxia, but there was still a benefit in larger tumours of adding CO_2, possibly due to a central effect on arterial blood pressure increasing perfusion.

DISCUSSION

During the discussion it was agreed that the clinical tumour responses in all the sites studied (with the possible exception of the brain) were encouraging, and that further clinical trials were warranted. A number of options for reducing the toxicity and non-compliance for Phase III clinical trials were discussed at length:

- Continue with the same level of 80 mg/kg of Nicotinamide, and accept the 20% average non-compliance.
- Reduce the NAM dose stepwise, in further pilot studies.
- Tailor the NAM dose to each patient, with plasma or saliva measurements of concentration at peak and/or at 24 h. This was felt to be ideal for centres with sufficient laboratory resources, but probably not practical for multicentre trials.
- Give NAM on alternate days.
- Give NAM on alternate weeks.
- Drop NAM and only use carbogen, which in some tumours gives most of the sensitizing effect anyway.
- Use an alternative to NAM such as Pentoxyfilline; an interesting possibility that justifies further exploratory work.
- Use other sensitizers with carbogen, such as Nimorazole or Tirapazamine; although this was held to be

confusing the rationales because these compounds are believed to deal with chronically hypoxic cells rather than the acutely hypoxic ones that NAM is intended to combat.

- Use with conventional schedules or hyperfractionation (such as 1.2 Gy fractions twice a day) instead of accelerated radiotherapy, to avoid the higher acute reactions that accompany accelerated regimes.
- Reduce the CO_2 to 2.5% instead of 5% to reduce the possible difficulties with administering carbogen.

During the discussion it was strongly suggested by Horsman, Rojas and van der Kogel that to reduce the dose of NAM below 0.7–1.0 μmol/ml in plasma might bring the sensitization by this mechanism (of acutely hypoxic cells) to a subthreshold and negligible level, and this should not be risked. This peak plasma concentration is achieved in some clinics, or with some patients, with 50–60 mg/kg but in most centres higher doses of 80 mg/kg were needed to achieve this level in all patients. However, a reduced dose could not be rejected because NAM also seems to make the time dependence of pre-treatment carbogen breathing less critical. More animal experiments were deemed necessary to determine how steep the NAM dose vs. response curve was in the relevant dose range for different types of tumour, including human xenografts. It was also necessary to check that the abolition of time dependence of the carbogen administration persisted at low NAM doses. Preclinical data are lacking on the effectiveness of NAM when it is used on alternate days instead of on a daily basis. In animals it has only been demonstrated to be better if used with all the fractions rather than with the first half or the last half of a 10-fraction schedule.

CONSENSUS RECOMMENDATIONS

A consensus view was reached about the future directions, based on both the laboratory and clinical experience.

It was recommended that clinical trials should continue into Phase III, using the same dose of NAM as presently recommended, 80 mg/kg by weight to a maximum of 6 g, once a day, and to adjust the dose only if it was considered intolerable.

Patients who still develop severe nausea and vomiting could be given NAM at 25% lower doses (60 mg/kg) thereafter. All patients should continue on carbogen, and there should be no interruption of the planned radiotherapy.

It was recommended that liquid NAM should be used, given 1 h before irradiation, (instead of 1.5 h as now), except after food, when there should be a 2-h interval. Either permitting or withholding a light breakfast could be acceptable, provided the appropriate interval was used.

The results from the work that is now in progress will be presented at the Fourth ARCON Workshop, which is planned to be held in Nijmegen in June 1998. Further information can be obtained from Professor A. van der Kogel and/or Dr J. Kaanders.

ACKNOWLEDGEMENTS

We are grateful to the participants for their assistance in checking the accuracy of this summary and for allowing the wider dissemination of these 'work in progress' results. The reader is advised to contact the named presenters directly if they require further details. We appreciate the financial support from Umeå Lions and Umeå Cancerforskningsfond, which made the workshop possible.