Int. J. Radiation Oncology Biol. Phys., Vol. 42, No. 4, pp. 807–810, 1998

0360-3016/98/$–see front matter

PII S0360-3016(98)00309-5

● *Chemical Sensitizers and Protectors*

PILOT STUDY OF NIMORAZOLE AS A HYPOXIC-CELL SENSITIZER WITH THE "CHART" REGIMEN IN HEAD AND NECK CANCER

CHRISTOPHER P. COTTRILL, PH.D., M.R.C.P., F.R.C.R.,* KATE BISHOP, B.SC., R.G.N.,* MICHAEL I. WALTON, PH.D.,† AND J. MICHAEL HENK, F.R.C.R.*

*Department of Radiotherapy, The Royal Marsden Hospital, London, U.K.; †Cancer Research Campaign Centre for Cancer Therapeutics, The Institute of Cancer Research, Sutton, Surrey, U.K.

Purpose: A potential disadvantage of accelerated fractionation in radiotherapy is the lack of time for reoxygenation, so that hypoxia becomes a more potent cause of failure. Accordingly, we have combined nimorazole, the only hypoxic radiosensitizer shown to significantly improve local control in head and neck cancer, with continuous hyperfractionated accelerated radiation therapy (CHART).
Methods and Materials: Twenty-two patients with locally advanced (stage IV) squamous cell carcinoma of the head and neck were treated with escalating doses of nimorazole given concomitantly with CHART (three fractions of 1.5 Gy per day, spaced 5½ hours apart, on 12 consecutive days). All patients received 1.2 g/m^2 nimorazole 90 minutes before each first daily fraction. Seventeen patients received a further 0.6 g/m^2 before each second daily fraction and six of these patients received an additional dose of 0.6 g/m^2 before each third fraction.
Results: The three times daily schedule yielded mean plasma drug concentrations at the time of irradiation of 37.7 μg/ml with the morning fractions, 31.2 μg/ml with the afternoon fractions, and 30.4 μg/ml with the evening fractions. In view of these results the midday dose was increased to 0.9 μg/m^2 in an ongoing Phase II study. Drug toxicity was limited to nausea and vomiting apart from two cases of mild paraesthesia at the highest dose level.
Conclusions: Comparison with a historical group of patients, treated with the CHART regimen alone and matched for irradiation volume and technique, showed that nimorazole did not increase the severity of acute normal tissue radiation effects. Encouraging tumor responses have been seen in the patients receiving nimorazole with every radiotherapy fraction. **© 1998 Elsevier Science Inc.**

INTRODUCTION

The principal causes of radiotherapy failure are intrinsic radioresistance, repopulation during treatment, and hypoxia. Of these, the last two are amenable to therapeutic intervention.

The results of the U.K. Medical Research Council (MRC) continuous hyperfractionated accelerated radiation therapy (CHART) trials (1) clearly demonstrate the significance of tumor cell repopulation and underline the importance of limiting the overall treatment time. Despite a 12 Gy reduction in total dose, the 12-day CHART regimen gave equivalent local control of head and neck tumors with reduced late morbidity compared with the conventional 6½ weeks treatment.

Various methods of overcoming tumor hypoxia have been tested in clinical trials, including hyperbaric oxygen, carbogen breathing, and electron-affinic radiosensitizers. The results have often been inconclusive but an overview analysis of over 80 randomized studies has suggested that the local control of head and neck cancers in particular could be improved by hypoxic modification (2).

The 5-nitroimidazole nimorazole (Naxogen, Pharmacia–Upjohn) has been widely used as an antimicrobial agent with little reported toxicity. It is also one of the simplest and most reliable hypoxic cell sensitizers with demonstrated efficacy in supraglottic and pharyngeal tumors (3).

A potential disadvantage of accelerated fractionation in radiotherapy is the lack of time for reoxygenation, so that hypoxia becomes a more potent cause of failure. As a consequence, a hypoxic cell radiosensitizer may have a greater effect with accelerated rather than conventional fractionation. Accordingly, we are investigating the combination of accelerated hyperfractionation with nimorazole and report here the results of a Phase I dose-escalation study in which the sensitizer has been given concomitantly with CHART.

METHODS AND MATERIALS

Between January 1995 and May 1996, 22 patients with stage IV squamous cell carcinoma of the head and neck were treated with the CHART regimen (1) in combination

Presented at the 10th International Conference on Chemical Modifiers of Cancer Treatment, Clearwater, Fl, Jan 28-31, 1998.

Reprint requests to: Dr. J. M. Henk, Department of Radiotherapy, Royal Marsden Hospital, Fulham Road, London SW3 6JJ, UK.

Acknowledgments—We are grateful to Pharmacia–Upjohn for supplies of nimorazole, and to the Scott of Yews Trust for financial support. This study was made possible by the enthusiastic support of the radiographers (technologists), nursing staff, and physicists of the Royal Marsden Hospital.

Accepted for publication 6 July 1998.

with escalating doses of nimorazole in a Phase I study. The study had approval from the Royal Marsden Hospital Research Ethics Committee and all patients gave written informed consent.

Radiotherapy planning and treatment

The CHART regimen was identical to that used in the MRC randomized trial (1). The schedule consisted of 3 fractions of 1.5 Gy per day, spaced 5½ hours apart, on 12 consecutive days to a total dose of 54 Gy in 36 fractions. A larger volume, including primary tumor and regional lymphatics (according to the MRC protocol guidelines), was treated 3 times on day 1 and thereafter twice daily (morning and evening) on days 2–12 to a dose of 37.5 Gy in 25 fractions. A reduced volume, comprising primary tumor plus involved nodes excluding the spinal cord, was treated once daily, in the afternoon of days 2–12, to a dose of 16.5 Gy in 11 fractions. Doses were prescribed at the beam intersection point. All patients were treated with 5 or 6 MV photons, using either lateral opposed fields or oblique wedged fields as appropriate. Lateral electron beams were used as part of the reduced volume where necessary to treat nodes overlying the spinal cord. In all cases the total dose to the spinal cord dose was limited to 40Gy.

Nimorazole dosage

All 22 patients received 1.2 g/m^2 of nimorazole as a single oral dose each day, 90 minutes before the first radiotherapy fraction. The first 5 patients were dosed once daily only. The next 11 patients received a second dose of 0.6 g/m^2 each day before the second radiotherapy fraction and the final six patients received a further 0.6 g/m^2 each day before the third fraction. Where possible, samples for plasma nimorazole concentrations were taken immediately after each fraction of radiotherapy at the beginning, middle, and end of the 12-day course.

As part of an ongoing Phase II study, a further 19 patients have received 1.2 g/m^2, 0.9 g/m^2, and 0.6 g/m^2 nimorazole 90 minutes before the first, second, and third daily fractions, respectively. Drug toxicity data from these patients are included in the present report.

Plasma nimorazole concentrations

Plasma nimorazole concentrations were analyzed by reverse phase high-performance liquid chromatography (HPLC), using Ro 07-1051 [benznidazole, N-benzyl-(2-nitroimidazoyl) acetamide] as an internal standard, in a similar manner to that previously described (4). The authentic standard nimorazole (4-[2-(5-nitroimidazole-1-ly) ethyl]-morpholine) was supplied by Pharmacia–Upjohn and the internal standard by Dr. M. Stratford (CRC Gray Laboratory, Northwood, Middlesex, UK).

Plasma samples (100 μl) were extracted on ice with 4 volumes of methanol containing internal standard (Ro 07-1051, 3 μg/ml), mixed, centrifuged (14,000 rpm, 5 minutes) and the supernatant recovered for injection onto the HPLC. Chromatography was carried out using Kontron 325 pumps, Perkin Elmer 1SS 100 automatic sample injector, and Waters model 440 fixed wavelength UV detectors, and the system was controlled via a Kontron 450 MT2 data system. Separations were performed on a stainless steel column (150 × 4.6 mm) containing Spherisorb C18 ODS2 (5 μm bead size, Sigma-Aldrich) fitted with a C18 guard column. Chromatography was carried out under isocratic conditions using 35% acetonitrile in 20 mM phosphate buffer (pH 6.5) as a mobile phase, at a flow rate of 1 ml/min with UV detection at 313 nm. Nimorazole was identified by co-chromatography with authentic material and UV absorbance characteristics. Retention times for nimorazole and Ro 07-1051 were 2.64 and 4.21 minutes respectively. Drug quantification was by peak area with reference to linear calibration curves over the range 1–200 μg/ml. Drug recovery was greater than 95% and the same day coefficient of variation for 7 replicate samples was 2.65% (10 μg/ml in plasma).

Morbidity assessment and follow-up

Drug toxicity was assessed using the National Cancer Institute Common Toxicity Criteria (NCI CTC) grading. Acute radiation toxicity was recorded weekly, from the start of treatment until fully healed, using the same morbidity scales as in the MRC CHART study (1).

Nineteen of the CHART + nimorazole (CHART-N) Phase I study patients could be matched for irradiation volume and technique with patients we had previously treated with CHART alone as part of the MRC study. Acute morbidity scores for the 38 matched patients, weighted for severity and duration, were ranked and compared by the Wilcoxon rank-sum test.

Table 1. Numbers of patients (percentage in parentheses) experiencing each Common Toxicity Criteria grade of nausea and vomiting at the four nimorazole dose levels

Nimorazole dose g/m^2/day	CTC grade: Nausea 0	Nausea 1	Nausea 2	Nausea 3	Vomiting 0	Vomiting 1	Vomiting 2	Vomiting 3
1.2 (n = 5)*	2 (50)	1 (25)	1 (25)	0	2 (50)	2 (50)	0	0
1.8 (n = 11)	3 (27)	3 (27)	4 (36)	1 (9)	7 (64)	2 (18)	2 (18)	0
2.4 (n = 6)	3 (50)	0	1 (17)	2 (33)	3 (50)	0	2 (33)	1 (5)
2.7 (n = 19)	6 (32)	3 (16)	4 (21)	6 (32)	7 (27)	3 (16)	8 (42)	1 (5)

*Toxicity data available for four patients only.

RESULTS

Nimorazole toxicity

Toxicity data are available for 21 of the 22 Phase I study patients and 19 of the ongoing Phase II study patients. At doses of nimorazole below 2.7 g/m^2/day, nausea and vomiting were the only observed side effects. Table 1 lists the frequency of these according to the NCI CTC grading. Although the numbers are small there is no clear relationship to dose. In most cases the symptoms were successfully

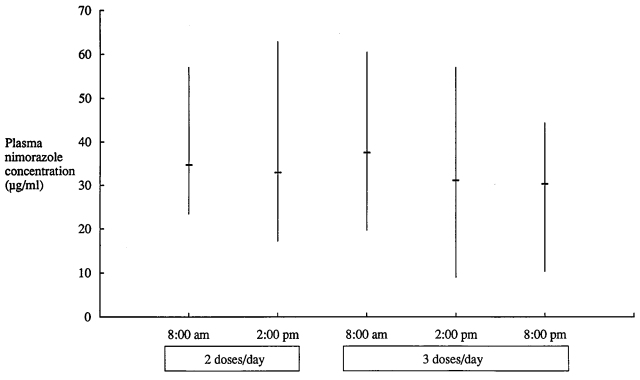

Fig. 1. Mean plasma nimorazole concentrations and ranges measured at the time of radiotherapy in patients receiving two and three doses per day.

controlled with simple anti-emetic medication and they did not persist beyond the treatment period. Only one patient refused further drug as a result of these side effects (after receiving 2.7 g/m^2/day for 1 week).

Two patients treated at 2.7 g/m^2/day developed mild paraesthesia of the extremities not associated with any neurological deficit but which persisted for several months. In view of this, no further dose escalation has been attempted.

Plasma nimorazole concentrations

Plasma nimorazole concentrations were measured immediately after irradiation on 36 separate occasions in the 11 patients dosed twice daily, and on 51 separate occasions in the six patients dosed thrice daily. The mean concentrations are shown in Fig. 1.

In the latter group the mean values were 37.6 μg/ml, 31.2 μg/ml, and 30.4 μg/ml respectively.

Acute radiation reactions

There were no unforeseen radiation reactions. The frequencies of maximum skin reaction (moist desquamation, dry desquamation, or erythema), and the time course of skin reactions were very similar in the CHART-N and the matched CHART-alone groups. No significant difference emerged when the scores in the two groups ranked for severity and duration of skin reaction were compared using the Wilcoxon rank-sum test. Similarly there was no difference between the two groups in the maximum grade or duration of mucositis, pain on swallowing, or analgesia usage. Almost twice as many CHART-N patients required feeding by nasogastric tube or gastrostomy at some point, but this was attributed to their more extensive tumors rather than to radiation reaction per se.

Patient outcome

Of the 22 patients in the Phase I study, 8 are alive and disease-free at a median follow-up of 23.5 months, including 4 of the 6 who received nimorazole with every fraction. Four have died without evidence of loco-regional failure, and 10 from progressive disease.

DISCUSSION

The 5-nitroimidazole nimorazole has been widely used as an antibiotic with acceptable toxicity at cumulative doses of up to 25 g over 10 days (5). The only side effects reported have been nausea and vomiting, which appeared to be dose-independent and responsive to simple anti-emetics. An oral dose of 1.2 g/m^2 has been shown to achieve a median peak plasma concentration of 33 μg/ml 90 minutes after ingestion (4). Preclinical studies suggested that this should achieve a therapeutic tumor concentration giving a sensitizing enhancement ratio of the order of 1.3–1.4 (6). In the Danish Head and Neck Cancer Study (DAHANCA 5), this dosage was given before every fraction of a conventionally fractionated 6-week course of radiotherapy, without evidence of neurotoxicity; a significant improvement in local control compared with placebo was observed (5).

Nimorazole is a less potent radiosensitizer *in vivo* than the 2-nitroimidazoles such as misonidazole. It has however proved more clinically effective, probably because the cumulative neurotoxicity of the latter precludes their use with every fraction of radiotherapy.

In our Phase I study we were able to give nimorazole before every fraction of CHART to a total dose of 28.8 g/m^2 in 12 days, and achieve plasma concentrations at the time of each fraction of radiotherapy that were in the range of effective sensitization. All 22 patients completed the full prescribed dose of nimorazole; this compares with only 57% of patients in the DAHANCA 5 study, where radiation-induced dysphagia was reported to be the major cause of noncompliance. In the case of CHART, mucosal reaction sufficient to cause dysphagia does not appear until after completion of treatment, which may in part explain the better compliance. In the further dose-escalation to 2.7 g/m^2/day, 13 of the first 19 patients experienced nausea with or without vomiting, but in only one patient did these symptoms result in refusal to continue taking the drug.

The two cases of persistent paraesthesia were perhaps surprising, as once daily dosage to a total of 36 g/m^2 had caused no neurological problems. However, it is possible that the neurotoxicity of nitroimidazoles is related to the intensity of exposure (area under the curve) as well as the peak plasma concentration (6), so no further dose-escalation was attempted.

CHART, perhaps unexpectedly, has been shown to produce less severe acute skin reactions and late radiation morbidity than conventional fractionation (1). So far we have found no evidence that nimorazole exacerbates the normal tissue effects of CHART, although longer follow-up is of course necessary to assess late effects. This study is an attempt to combine the potential therapeutic benefits of acceleration and hypoxic-cell sensitization. We have succeeded in achieving therapeutically relevant plasma drug concentrations at the time of each fraction of CHART with promising results, so a Phase II efficacy study is in progress.

REFERENCES

1. Dische S, Saunders M, Barrett A, Harvey A, Gibson D, Parmar M. A randomized multicenter trial of CHART versus conventional radiotherapy in head and neck cancer. *Radiother Oncol* 1997;44:123–136.
2. Overgaard J, Horsman MR. Modification of hypoxia-induced radioresistance in tumors by the use of oxygen and sensitizers. *Semin Radiat Oncol* 1996;6:10–21.
3. Overgaard J, Sand Hansen H, Lindelov B, Overgaard M, Jorgensen K, Rasmussen B, Berthesen A. Nimorazole as a hypoxic radiosensitizer in the treatment of supraglottic larynx and pharynx carcinoma. First report from the Danish Head and Neck Cancer Study (DAHANCA) protocol 5-85. *Radiother Oncol Suppl* 1991;20:143–149.
4. Overgaard J, Overgaard M, Timothy A. Studies of the pharmacokinetic properties of nimorazole. *Br J Cancer* 1983;48:27–34.
5. Timothy AR, Overgaard J, Overgaard M. A phase I clinical study of nimorazole as a hypoxic radiosensitizer. *Int J Radiat Oncol Biol Phys* 1984;10:1765–1768.
6. Overgaard J, Overgaard M, Nielson OS, Pederson KA, Timothy AR. A comparative investigation of nimorazole and misonidazole as hypoxic radiosensitizers in a C3H mammary carcinoma in vivo. *Br J Cancer* 1982;46:904–911.