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INTERACTIONS OF RADIATION, CYCLOPHOSPHAMIDE AND NIMORAZOLE IN A C3H MAMMARY CARCINOMA *IN VIVO*

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The combined effect of adjuvant Cyclophosphamide (CTX) and the hypoxic radiosensitizer, Nimorazole (NIM), on the radiation response was studied in a C3H mammary carcinoma in CDF1 mice. The effect of NIM and CTX alone or in combination without radiation was assessed by tumor growth delay measured by tumor growth time (TGT). Administration of CTX (100 mg/kg) increased the TGT from 5.2 days in untreated controls to 18.8 days. NIM (1000 mg/kg) had no effect on the TGT. The combined treatment with NIM given 4 hrs before CTX did not increase the TGT compared with CTX alone, which suggests that NIM does not potentiate CTX. The possible effect of an interaction between the therapeutic parameters was determined by administration of NIM, CTX, and radiation in different sequences to C3H mammary tumor bearing mice. The drugs were administered as single doses before or after graded single doses of irradiation. The end point was the radiation dose required to achieve local tumor control in 50% of the mice (TCD50). The enhancement ratio (ER)—defined as TCD50 for radiation alone relative to TCD50 for radiation combined with drug—was 1.2 for CTX given either 15 min before or 4 hrs after radiation. NIM given 30 min before radiation showed an ER of 1.6, but no enhancement was obtained when NIM was given after radiation. When NIM was given immediately after radiation, followed 4 hrs later by CTX, the ER was 1.2. However, applying NIM 30 min before radiation and CTX 3.5 hrs after radiation, the ER increased to 1.6. NIM given 30 min before, together with CTX given 15 min before radiation, showed an ER of 1.8. Our data suggest that: (a) an improved tumor response may be expected when CTX is added to a radiation and hypoxic radiosensitizer treatment; (b) this improvement is attributable to an additive effect based on the chemotherapy response alone rather than to chemopotentiation by the hypoxic radiosensitizer.

Nimorazole, Cyclophosphamide, Radiation, Tumor *in vivo*.

INTRODUCTION

Hypoxic cells are crucial in determining local control in tumors treated with radiation and, possibly, also with chemotherapy.[2] Hypoxic radiosensitizers like Nimorazole (NIM) and Misonidazole (MISO) can reduce the resistance of hypoxic cells to radiation.[7] Some hypoxic radiosensitizers increase the effect of certain chemotherapeutic agents on hypoxic cells, which has been exemplified by MISO and cyclophosphamide (CTX) *in vitro*,[1] but it has been more difficult to prove it *in vivo*.[3,4,10,11] Further, CTX has been shown to interact with radiation, but whether it is caused by potentiation or if it is only an additive effect remains unknown.[15] Based on these facts, the combination of a chemotherapeutic agent, a hypoxic radiosensitizer, and radiation could therefore be of considerable interest.

Because of the apparently low toxicity of NIM and its hypoxic radiosensitizing potential,[7,8,14] we have chosen this compound for a combined study of a hypoxic radiosensitizer in combination with a chemotherapeutic agent (CTX) and radiation.

METHODS AND MATERIALS

All experiments were carried out with a C3H/Tif mammary carcinoma transplanted to the right hind limb of male CDF1/Bom mice, as previously described.[15] Treatment was given to tumors with a volume of approximately 200 mm^3, determined by the formula: $\pi/6 \times D1 \times D2 \times D3$.

NIM was dissolved in isotonic saline to a concentration of 25 mg/ml immediately before administration. CTX was dissolved in sterile distilled water to a concentration of 5 mg/ml.

Both drugs were administered intraperitoneally as single doses, according to body weight. CTX was given in a volume of 0.02 ml/g and NIM in a volume of 0.04 ml/g.

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Radiation was given as single doses to unanaesthetized mice with a 250 kV Müller X ray unit (15 mA, filtration 2 mm Al, HVL 1.1 Cu, dose rate 3.25 Gy/min). The mice were placed in a lucite jig, with the tumor bearing leg immersed in a water bath to secure homogeneity of the radiation dose.[6]

In the evaluation of data, tumor response to drug therapy alone was assessed by tumor growth time (TGT), that is, the time required for a tumor to reach a volume 5 times that of the treatment day. The exponential regrowth phase was evaluated as the tumor doubling time. The calculations were based on curves for each individual animal.

To determine the possible effect of an interaction between NIM, CTX, and radiation, graded doses of radiation alone and in combination with drugs were evaluated by calculation of the radiation dose required to achieve local tumor control in 50% of the mice (TCD50).

Tumor control was defined as absence of macroscopically detectable tumor after 120 days. Animals who died prior to this day without evidence of tumor were excluded.

The TCD50 values, based on pooled data, were calculated by logit analysis[13] from assays containing 50–70 mice divided into 7–8 dose groups.

The results were estimated by the enhancement ratio (ER): ER = TCD50 for radiation alone/TCD50 for radiation + drug.

RESULTS

Tumor growth time after treatment with NIM and CTX alone or in combination in various schedules is seen in Table 1. This end point was only used because it was impossible to achieve tumor control with drug treatment alone. CTX alone produced a marked increase in TGT compared with the control values (19.4 days vs 5.2 days). Addition of NIM 15 min prior to CTX reduced TGT, but not significantly compared to CTX alone. Other studies[1,4] have shown that protracted injection may prove more efficient than administration in one dose. Therefore, NIM was given as three injections followed by CTX, but no effect on the TGT was recorded (Table 1). Compared with the controls, there was no difference in tumor doubling time for any of the treatments. Thus, it is likely to expect that the increase in TGT is because of a kill of a certain amount of cells, rather than a consequence of a delayed proliferation.

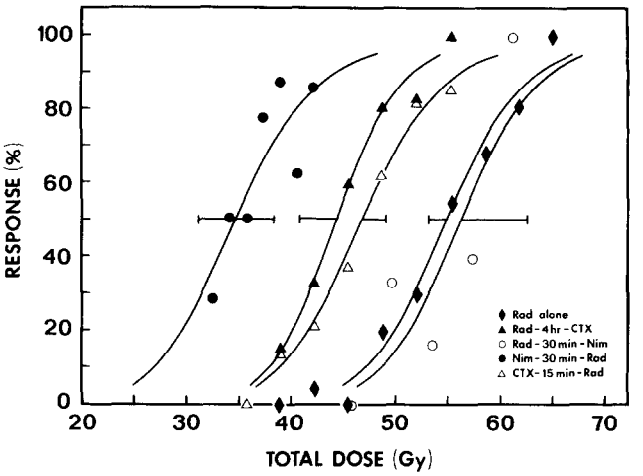

Fig. 1. Dose-response curves for radiation alone and radiation combined with either nimorazole or CTX.

In the study, where drugs and radiation were combined (Figs. 1 and 2), NIM was given either 30 min before or after radiation, whereas CTX was given either 15 min before or 4 hrs after radiation, to avoid interference with repair of sublethal radiation damage.

As seen in Figure 1, there was no difference in TCD50 when CTX was given either before or after radiation, but as expected, the ER for NIM given before radiation was significantly different from the ER when NIM was given after radiation. TCD50 values and enhancement ratios are given in Table 2.

The ER for CTX given 15 min before radiation was 1.17. Supposing that an additive effect is obtained by the interaction of CTX with NIM, NIM given 30 min before radiation should yield approximately the same value as the ER obtained by a combined schedule with NIM–15 min–CTX–15 min–radiation, a value which, in this case,

Table 1. Tumor growth time and doubling time after treatment with drugs alone and in combination

Treatment	No. of mice	Tumor growth time (days)	Tumor doubling time (days)
Untreated control	40	5.2 (4.8–5.7)*	2.5 (2.3–2.7)
NIM 1 mg/g	18	5.8 (5.3–6.2)	2.6 (2.3–3.0)
CTX 100 mg/kg	38	19.4 (17.6–21.1)	2.6 (2.3–2.9)
NIM 1 mg/g 15 min before CTX 100 mg/kg	9	14.0 (12.6–15.4)	2.9 (2.5–3.2)
NIM 1 mg/g 4 hrs before CTX 100 mg/kg	15	17.0 (14.8–19.2)	2.5 (2.1–2.9)
NIM 0.4–2 hrs–NIM 0.3–2 hrs–NIM 0.3–15 min–CTX 100 mg/kg†	11	16.1 (13.6–18.5)	3.2 (2.4–4.0)

* Numbers in parentheses are 95% confidence interval or mean.
† NIM 0.4 mg/g–2 hrs–0.3 mg/g NIM–2 hrs–0.3 mg/g NIM–15 min–CTX 100 mg/kg.
All calculations were based on curves for each individual mouse.

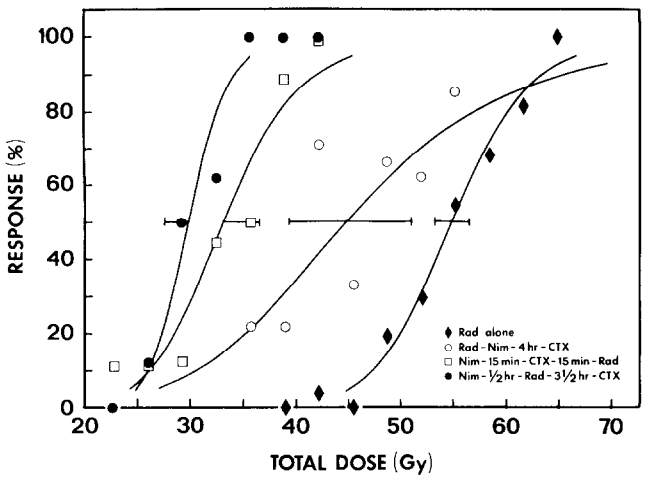

Fig. 2. Dose-response curves for radiation alone and radiation combined with schedules of nimorazole and CTX.

was 1.16 (1.84/1.58). The same could apply when CTX was added in a treatment where NIM was given after radiation. Thus, compared with radiation plus NIM, an enhancement of 1.24 (1.22/0.98) was obtained by a combined treatment schedule with radiation–NIM–4 hrs–CTX. As seen in Table 2, the ER for CTX given 4 hrs after radiation was also 1.24.

Table 2. Dose effect factors for different sequences of NIM, CTX, and radiation

Treatment	No. of mice	TCD50 (Gy)	ER*
Radiation alone	222	54.88 (53.32–56.49)†	
NIM 30 min before radiation	55	34.69 (31.24–38.52)	1.58 (1.30–1.91)
Radiation 30 min before NIM	27	56.14 (50.42–62.52)	0.98 (0.83–1.15)
CTX 15 min before radiation	112	46.76 (44.54–49.09)	1.17 (1.06–1.29)
Radiation 4 hrs before CTX	51	44.27 (40.92–47.88)	1.24 (1.08–1.42)
NIM–15 min–CTX 15 min–radiation	54	29.85 (27.68–32.20)	1.84 (1.33–2.12)
NIM–30 min–radiation–3½ hrs–CTX	60	33.18 (30.16–36.50)	1.65 (1.38–1.97)
Radiation–NIM–4 hrs–CTX	55	44.84 (39.37–51.07)	1.22 (0.96–1.54)

* ER = enhancement ratio = TCD50 for radiation alone/TCD50 for radiation + drug.

† Numbers in parentheses are 95% confidence interval.

DISCUSSION

The present study was performed with the aim of uncovering a possible effect of the combined treatment with CTX, NIM, and radiation. The expected enhancement of the therapeutic effect did occur, and based on the present data it can only be described as additive. Assuming an independent effect of NIM and CTX, respectively, with radiation, the expected enhancement ratio is the same as the ER for NIM given 30 min before radiation multiplied with the ER for CTX given 15 min before radiation, that is 1.85 (1.17 × 1.58).

Pre-radiation treatment with both NIM and CTX gave an ER of 1.84, suggesting that the observed enhancement is caused by an independent additive effect.

The lack of chemosensitization may be ascribed to the tumor size, since sensitization is a function of tumor size, and for very small tumors, the differential effect is markedly decreased.[4,12] However, this is not the case, since NIM was found to sensitize radiation, which indicates a significant hypoxic fraction in our tumor system.

Previous studies in different tumor systems have given various results from a relatively high extent of chemosensitization to a very little effect.[4,5,9] The presumption is that the results depend on the tumor system applied, therefore, it cannot be ascertained whether the observed lack of a NIM-induced chemosensitization in the present study is because NIM does not have sensitizing properties, or if such sensitization cannot be detected in the system employed. However, ongoing experiments with a combination of Misonidazole and CTX in the same tumour system indicate that Misonidazole has a pronounced chemopotentiating effect (Zachariae, C. and Overgaard, J., unpublished data, 1985). Thus, it is likely that NIM has no significant effect as chemosensitizer.

We can conclude from these experiments that the improvement by CTX is an additive effect based on chemotherapy response alone, rather than on chemopotentiation by the hypoxic radiosensitizer. The combination of a hypoxic sensitizer, chemotherapy, and radiation may have a potential clinical value which needs to be explored further, especially with regard to normal tissue complications.

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