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BROAD-SHOULDERED SURVIVAL CURVES OF A HUMAN MELANOMA XENOGRAFT

Implications for radiation therapy in the absence and presence of misonidazole

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Broad-shouldered survival curves with relatively low D_0-values have been reported for melanoma cells irradiated under aerobic conditions in vitro (DEWEY 1971, BARRANCO et coll. 1971). Theoretic surviving fractions for various fractionation regimes calculated on the basis of these survival curves indicated that treatment with few fractions and relatively high doses per fraction may be superior to conventional treatment (30 fractions of 2.0 Gy over 6 weeks; ELLIS 1974, WHELDON & KIRK 1975, HABERMALZ & FISCHER 1976). However, WHELDON (1979) and MCNALLY (1980) showed that conclusions drawn from such calculations may depend on whether the radiation response of a tumour is determined by the aerobic or the hypoxic cells, i.e. whether the tumour is reoxygenated efficiently or inadequately between successive fractions. Recently, DENEKAMP et coll. (1980) have calculated theoretic surviving fractions for irradiation in the absence and in the presence of the hypoxic cell radiation sensitizer misonidazole, using several fractionation regimes and a variety of survival curve parameters and reoxygenation patterns. They concluded that usually, whether misonidazole is present or not, many small radiation fractions may give lower survival levels than few, relatively high radiation fractions for the same NSD values. However, they also concluded that in clinical situations where experience has shown no marked disadvantage of fewer high fractions of radiation alone, this would be the recommended mode of treatment in the presence of misonidazole.

The calculations referred to were either based on aerobic survival curves of cells cultured in vitro or on theoretic survival curves. Cells from a human melanoma (E.E.), irradiated as solid tumours in athymic nude mice and assayed in vitro in soft agar, show survival curves with broad shoulders and relatively low D_0-values (ROFSTAD 1981). Sensitization by misonidazole, repair of potentially lethal damage in the absence and in the presence of misonidazole, as well as reoxygenation after clinically relevant radiation doses have been reported in detail for this melanoma (ROFSTAD & BRUSTAD 1980, ROFSTAD). The purpose of the present work was to calculate theoretic surviving fractions for several fractionation regimes in the absence and in the presence of misonidazole. The model used for the calculations is similar to that described by DENEKAMP et coll., but the para-

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meters used are determined from experimental data on E.E. melanoma irradiated in vivo.

Methods

Theoretic surviving fractions for the fractionation regimes listed in the Table were calculated and compared. The overall treatment time for these regimes was 6 weeks, and they all gave NSD = 1 765 ret.

As discussed by DENEKAMP et coll., the choice of fractionation regime for irradiation in the presence of misonidazole may be influenced by the neurotoxic side effects of the sensitizer. The maximum tolerated dose of misonidazole in patients is approximately 12 g/m^2, whether the sensitizer is administered in few or many fractions (DISCHE et coll. 1977, 1979). Thus, a higher concentration of misonidazole will be obtained with each fraction when fractionation regimes with few fractions are used as compared with fractionation regimes with many fractions. According to the survival data of ADAMS et coll. (1976) and MCNALLY et coll. (1978), the sensitizing effect of misonidazole is highly dependent on the concentration of the sensitizer. The concentration of misonidazole in the tumour, which can be obtained for each of the fractionation regimes in the Table, was calculated on the basis of the clinical data of DISCHE et coll. (1977, 1979). The concentration in the tumour was assumed to be 50 per cent of that in the serum, as previously measured for E.E. melanoma (ROFSTAD & BRUSTAD 1979). The enhancement ratio for the hypoxic tumour cells for each concentration of misonidazole was calculated on the basis of the experimental data of ADAMS et coll. and MCNALLY et coll. The sensitizing effect of misonidazole (500 mg/kg body weight) on E.E. melanoma (ROFSTAD & BRUSTAD 1978, ROFSTAD) has been shown to be comparable with that on the murine tumours used by MCNALLY et coll. The calculated values for the drug concentrations in the tumour and the corresponding enhancement ratios are presented in the Table. It must be emphasized that these enhancement ratios relate only to the hypoxic cells present in the tumour during irradiation and not to the whole tumour. The enhancement ratios for the whole tumour will depend on the hypoxic fraction and the reoxygenation pattern of the tumour.

The inactivation data for E.E. melanoma, on which the calculations of the surviving fractions were based, have been published previously (ROFSTAD) and are redrawn in Fig. 1. The following assumptions were made for the calculations:

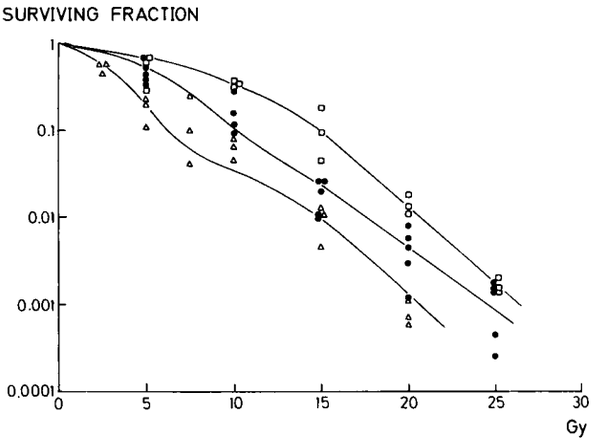

Fig. 1. Theoretic survival curves and experimental data for cells from E.E. melanoma irradiated as solid tumours in nude mice. Each point represents the surviving fraction of cells from one tumour. Dead mice; assay immediately after irradiation (□). Air-breathing mice; assay immediately after irradiation (△). Air-breathing mice; assay 14 hours after irradiation (●).

Table

Tumour concentrations and enhancement ratios of misonidazole for various fractionation regimes

Fractionation regime (NSD = 1 765 ret)	Fractions per fortnight	Concentration of misonidazole (μg/g)	Enhancement ratio
30×2.00 Gy/6 weeks	10	9	1.15
18×2.95 Gy/6 weeks	6	16	1.28
12×4.00 Gy/6 weeks	4	24	1.35
6×6.80 Gy/6 weeks	2	47	1.52
3×11.50 Gy/6 weeks	1	95	1.72

(1) The survival curves for the hypoxic and for the aerobic cells in the tumour can independently be described by the 'multitarget with a one-hit component' equation:

$$S = \exp\left(-\frac{D}{D_1}\right) \times \left(1 - \left[1 - \exp\left(-\frac{D}{D_2}\right)\right]^n\right),$$

$$\frac{1}{D_2} = \frac{1}{D_0} - \frac{1}{D_1}$$

where S = surviving fraction, D = dose, D_0 = final slope, D_1 = initial slope, and n = extrapolation number. The literature contains a large number of

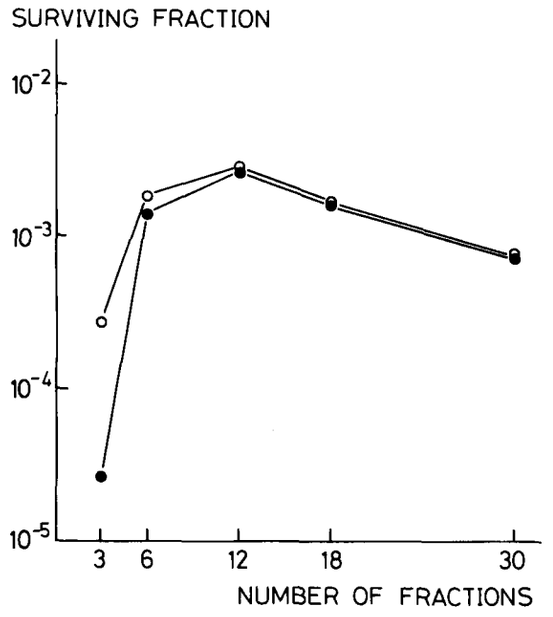

Fig. 2. Theoretic surviving fractions of cells from E.E. melanoma for the fractionation regimes in the Table. Without misonidazole (○). With misonidazole (●).

examples of curves which become exponential for large radiation doses and are well fitted by this equation (ELKIND 1977).

(2) The data for tumours irradiated in dead mice are representative for the naturally occurring hypoxic cells in the tumour. The best fit to the data was obtained with n=42, D_0=2.47 Gy, and D_1 = 12.35 Gy, indicating that the initial slope is five times larger than the final one. The theoretic survival curve and the experimental data are presented in Fig. 1.

(3) Oxygen acts as a dose modifying agent with enhancement ratio 2.8. This assumption is in close agreement with experimental results from in vitro irradiations of the same cells (ROFSTAD).

(4) The fraction of hypoxic cells in the tumour is 10 per cent (ROFSTAD). A theoretic survival curve for cells from tumours irradiated in air-breathing mice was calculated on the basis of the assumptions mentioned in the paragraphs 3 and 4. The theoretic survival curve gives a good fit to the corresponding experimental data (Fig. 1).

(5) The repair of potentially lethal damage is dose modifying both for the aerobic and the hypoxic cells, and is not affected by the presence of misonidazole (ROFSTAD). The best fit to the experimental data (cells from tumours irradiated in air-breathing mice, left in situ for 14 hours and then assayed in vitro) was obtained with repair factors (dose modifying factors) 1.8 (aerobic cells) and 1.2 (hypoxic cells; Fig. 1). These repair factors are within the range reported by others for cells irradiated in vitro and as solid tumours (WEICHSELBAUM et coll. 1977, MCNALLY & SHELDON 1977).

(6) The tumour is reoxygenated back to a hypoxic fraction of 10 per cent during each interval between successive fractions, i.e. the fraction of hypoxic cells in the tumour is 10 per cent at the time of each exposure. This assumption is based on previous investigations (ROFSTAD & BRUSTAD 1980), which indicated that the tumour is reoxygenated fast and efficiently after clinically relevant doses of gamma rays.

(7) Misonidazole acts on the hypoxic cells in the tumour as a dose modifying agent (ASQUITH et coll. 1974) with enhancement ratios as indicated in the Table.

(8) The shape of the survival curves as well as the ability of misonidazole to penetrate into (ROFSTAD & BRUSTAD 1979) and sensitize the hypoxic cells in the tumour do not change during a course of fractionated radiation therapy.

Results and Discussion

The theoretic surviving fractions calculated for the fractionation regimes in the Table appear in Fig. 2. The following conclusions can be drawn from the figure:

(1) In the absence of misonidazole surviving fractions below 1×10^{-4} were not obtained. As tumours consist of about 10^9 cells/g tumour weight, the present tumour can probably not be cured with any of the fractionation regimes in the Table. This is in agreement with results reported previously (ROFSTAD et coll. 1977a, b), which showed that E.E. melanoma was not cured when irradiated to the tolerance level of human connective tissue.

(2) The lowest surviving fraction in the absence of misonidazole was obtained with 3 fractions of 11.50 Gy. However, the variation in surviving fraction among the various fractionation regimes was relatively small (about one decade).

(3) The sensitizing effect of misonidazole, which is indicated by the vertical distance between the open and closed symbols in Fig. 2, was small for all fractionation regimes except for that consisting

of 3 fractions of 11.50 Gy. This result is in agreement with experimental results presented previously (ROFSTAD & BRUSTAD 1980).

(4) The surviving fraction for 3 fractions of 11.50 Gy in the presence of misonidazole was considerably lower than those calculated for the other treatment regimes. Thus, if the present model is applicable to radiation therapy, Fig. 2 indicates that the treatment of malignant melanomas which behave like E.E. melanoma, may be improved by the use of misonidazole in combination with fractionation regimes with high individual doses. However, since the lowest surviving fraction obtained was higher than 1×10^{-5}, the probability for tumour cure may be extremely low also for the most favourable fractionation regime in the presence of misonidazole.

Clinical investigations have also indicated that radiation therapy of malignant melanomas may be improved by the use of few fractions and relatively high doses per fraction (HABERMALZ & FISCHER, HORNSEY 1978, OVERGAARD 1980). Thus, the results from the present calculations are in agreement with these clinical investigations. However, it should be noted that not all human melanoma xenografts show as broad-shouldered survival curves as E.E. melanoma (FLATEN et coll: 1981).

The surviving fractions shown in Fig. 2 are highly dependent on the initial shape of the survival curves in Fig. 1. Since the exact shape of the shoulder region of the survival curves is difficult to determine experimentally, it must be emphasized that experimental uncertainties may be involved in the calculated surviving fractions.

It should also be noted that the present calculations are based on a relatively simple model. Thus, regrowth of the tumour as well as changes in the distribution of the cells in the cell cycle in the intervals between the fractions are not considered in the model. Investigations of the proliferation kinetics in E.E. melanoma have indicated that the median cell cycle time as well as the growth fraction and the cell loss factor are changed after exposure to single doses of radiation (ROFSTAD et coll. 1980). Since the sensitivity of mammalian cells varies through the cell cycle (SINCLAIR 1968), the response of the tumour to the last exposures is not necessarily equal to that to the first ones in a fractionation regime. This may limit the predictive value of the present model. However, since the present surviving fractions were calculated on the basis of experimental data for tumours irradiated in vivo, they may be more relevant for clinical practice than those calculated by ZEITZ & MCDONALD (1978) and WHELDON, which were based on melanoma cells irradiated in vitro.

SUMMARY

A human malignant melanoma (E.E.) was irradiated in vivo in athymic nude mice, with subsequent assay of single cell survival in vitro. By assuming the survival curves for the hypoxic as well as for the aerobic cells in the tumour to be of the form $S=\exp(-D/D_1)\times(1-[1-\exp(-D/D_2)]^n)$, theoretic survival curves were fitted to the experimental data for tumours irradiated in air-breathing mice. Assumptions were made about hypoxic fraction, oxygen enhancement ratio, sensitization by misonidazole, repair of potentially lethal damage, and reoxygenation, all based upon own experimental data on E.E. melanoma. Theoretic surviving fractions were calculated for several clinically relevant fractionation regimes, both for irradiation in the absence and in the presence of misonidazole. The results indicate that tumours with biologic parameters like those of E.E. melanoma are best treated with fractionation regimes with few fractions and high doses per fraction, whether misonidazole is present or not.

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