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BIOLOGY CONTRIBUTION

NEUTRON AND PHOTON CLONOGENIC SURVIVAL CURVES OF TWO CHEMOTHERAPY RESISTANT HUMAN INTERMEDIATE-GRADE NON-HODGKIN LYMPHOMA CELL LINES

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Background: The potential role of neutron therapy in the management of intermediate-grade non-Hodgkin lymphoma (IGNHL) has not been examined because of the belief that the anticipated radiobiological effectiveness (RBE) would be uniformly very low.
Purpose: To determine the fast neutron RBE for two chemotherapy-resistant IGNHL cell lines.
Methods and Materials: Conventional soft agar clonogenic survival curves following irradiation by ^{60}Co and fast neutron were established for two IGNHL cell lines. These cell lines, WSU-DLCL2 and SK-DHL2B, were found in previous studies to be able to repair sublethal damage, and were also resistant to L-Pam and doxorubicin chemotherapy.
Results: When the surviving fraction after 2 Gy photon was chosen as the biological endpoint, the RBE for WSU-DLCL2 and SK-DHL2B measured 3.34 and 3.06. Similarly, when 10% survival was considered, the RBE for these two cell lines measured 2.54 and 2.59. The RBE, as measured by the ratios α neutron/α photon, for WSU-DLCL2, SK-DHL2B cell lines are 6.67 and 5.65, respectively. These results indicate that the RBE for these IGNHL cell lines is higher than the average RBE for cell lines of other histological types.
Conclusion: Fast neutron irradiation may be of potential value in treating selected cases of IGNHL. © 1999 Elsevier Science Inc.

Non-Hodgkin lymphoma, Lymphoma cell lines, Neutron radiotherapy.

INTRODUCTION

Fast neutron external irradiation was shown in randomized trials to be superior to photon irradiation in the management of salivary gland tumors and prostate cancer (1, 2). Encouraging results from Phase II studies of soft tissue sarcoma have also been reported (3).

Intermediate-grade non-Hodgkin lymphoma (IGNHL) is generally considered a radiosensitive tumor that can be controlled with moderate radiation doses (4–6). Several *in vitro* experiments demonstrated that lymphoma cells are radiosensitive and that their clonogenic survival curves are characterized by a small or absent shoulder, implying their inability to accumulate or repair sublethal damage (7–9). In view of these characteristics, it is generally believed that the relative biological effectiveness (RBE) of fast neutron for lymphoma cells would be very low (10) and therefore, the potential role of fast neutron therapy in the management of IGNHL was examined adequately neither in the laboratory nor in the clinic.

We have recently reported the radiobiological characteristics of two human IGNHL cell lines (WSU-DLCL2 and SK-DHL2B) and noticed that these cells are only moderately radiosensitive and able to repair sublethal damage efficiently (11). This observation prompted us to establish the *in vitro* survival curves of these cells following neutron irradiation and to measure the resulting RBE.

METHODS AND MATERIALS

Cell lines

Two human IGNHL cell lines (WSU-DLCL2 and SK-DHL2B) were used for this experiment. Both cell lines were established from patients who demonstrated clinical chemoresistance, and the established cell lines have also shown chemoresistance in laboratory experiments (11). A description of the establishment of these lines and their characterization have been published (12, 13).

Maintenance and cell lines

Cell lines were maintained in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum (Hyclone,

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Inc., Logan, UT), 1% L-Glutamine, 100 μg/ml penicillin G, and 100 μg/ml streptomycin. Cells were fed by complete change of culture medium three times/week and incubated in a humidified 5% CO_2 incubator at 37°C.

Photon irradiation

Survival curves were obtained by the *in vitro* clonogenic assay method after single-dose irradiation. A ^{60}Co beam was used to irradiate the cells in their exponential growth phase. The flasks containing the cells were irradiated in a lucite phantom at a dose rate of 85–100 cGy/min. One flask was not irradiated and served as the control. WSU-DLCL2 cells were irradiated to six different dose points, while SK-DHL2B cells were irradiated to seven different dose points.

Neutron irradiation

The Wayne State d(48.5) +Be fast neutron beam was used. The flasks containing lymphoma cells were placed in a tissue equivalent plastic phantom (TEP-A150) at the isocenter of the machine. The calibration of the experiment setup was performed according to the international protocol for neutron dosimetry (14). The cells were irradiated at a dose rate of 20–30 cGy/m. Each cell line was irradiated to seven different doses of 0.25, 0.5, 1, 1.5, 2, 2.5, and 4 Gy.

Culture

Immediately after irradiation, the cells were cultured in soft agar as previously described (15). Briefly, 1 ml of 1% low-melting agarose (prepared in RPMI-1640 medium, cooled at 35°C) was added to each petri dish and gently mixed. The dishes were solidified and incubated at 37°C in a 3% CO_2 humidified incubator. The number of irradiated cells seeded into each dish increased with radiation dose. Three petri-dishes were seeded/dose point. The irradiated cells were allowed to grow until the surviving cells produced macroscopic colonies that could be readily counted, usually after 10–14 days following irradiation. Only colonies containing 50 or more cells were counted and the surviving fraction was calculated as follows: Surviving fraction = colonies counted/cell seeded × PE/100, where PE is the plating efficiency.

Statistical analysis

Surviving fractions were plotted following each dose point against the radiation dose on a semi-logarithmic scale and the best fit to the equation $S = e^{-\alpha D - \beta D2}$ was obtained (S = surviving fraction and D = radiation doses).

RESULTS

Figure 1 demonstrates the clonogenic photon and neutron survival curves of the examined cell lines. Table 1 depicts the values of α, β, α/β values for the cell lines following both photon and neutron irradiation. As anticipated, both photon survival curves, particularly the WSU-DLCL2 curve, are curved more than the corresponding neutron

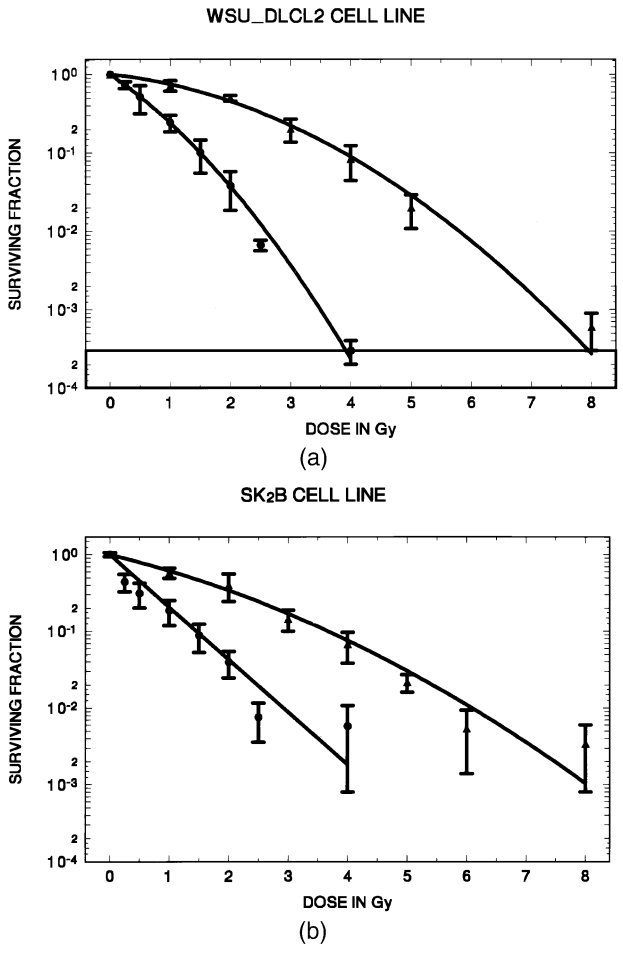

Fig. 1. Clonogenic photon and neutron survival curves for WSU-DLCL2 and SK-DHL2B.

curves. The photon α/β value of WSU-DLCL2 of 1.64 is much lower than what is expected for a typical lymphoma cell line and is consistent with the observed ability of this cell line to repair sublethal damage efficiently. The photon α/β value for MSK-DHL2 is also lower than the anticipated value for lymphoma cell lines. The ratio α neutron/α photon (α_n/α_p) estimated the relative radiosensitivity to neutron irradiation as described by Warenius *et al.* (16). We have also calculated the RBE for these cell lines using two different biological endpoints: the surviving fraction following 2 Gy photon irradiation (RBE 2 G_{yp}) and 10% survival ($RBE_{10\%}$). The mean inactivation dose following photon and neutron irradiation was calculated using the method of Fertil *et al.* (17) and the resulting RBE (RBE_{mid}) was noticed. These results are shown in Table 2.

DISCUSSION

Few studies have compared the radiosensitivity of tumor cell lines to photon and neutron irradiation. This study is the only reported experiment to describe the clonogenic survival curves of human IGNHL cell lines following neutron

Table 1. The radiobiological characteristics of the examined cell lines

	WSU-DLCL2		SK-DHL2B	
Parameter	Neutrons	Photons	Neutrons	Photons
$\alpha(Gy^{-1})$	1.20 ± 0.012	0.18 ± 0.044	2.43 ± 0.55	0.43 ± 0.066
$\beta(Gy^{-2})$	0.22 ± 0.013	0.11 ± 0.019	0	0.05 ± 0.028
α/β(Gy)	5.45 ± 0.018	1.64 ± 0.05	—	8.6 ± 0.072

and photon irradiation. There is no agreement as to what is the most appropriate endpoint to measure the RBE of these two types of ionizing radiation. We have calculated the RBE for neutron irradiation using four different methods. The α_n/α_p ratio expresses the potential benefit of neutron therapy as suggested by Britten *et al.* (18). The α_n/α_p values for the examined IGNHL cell lines were 5.65 and 6.67 for SK-DHL2B and WSU-DLCL2 cells, respectively. These values are higher than the average RBE reported by Courdi *et al.* (2.94) after studying 41 tumor cell lines of different histological types (19). In a similar study, Warenius *et al.* examined 26 human tumor cell lines (these cell lines were also included in the study by Courdi *et al.* cited above) and found that only four cell lines had $\alpha_n/\alpha_p \geq 5$ (15). As demonstrated in Table 2, the highest RBE for both cell lines was noticed when the ratio α_n/α_p was considered the relevant biological endpoints. As expected, the RBE for both cell lines was smaller when the 10% survival was chosen as the biological point of interest than when survival after 2 G_{yp} was considered. The RBE_{mid} values were between those of RBE 2 G_{yp} and $RBE_{10\%}$. We noticed, however, that all values of RBE were considerably > 1. MID is a value that describes the radiosensitivity of the cells at multiple dose levels and its use is recommended by the International Commission on Radiation Units and Measurements (ICRU) (20). The RBE_{mid} for both cell lines (2.97 Gy and 2.81 Gy), are also well above the mean RBE reported by Courdi *et al.* (2.15 Gy). When 10% survival was chosen as the endpoint used to calculate the RBE, a similar finding was obtained. These results challenge the belief that the RBE for all lymphoma cells should be low, and indicate that WSU-DLCL2 and SK-DHL2B would be among the most likely cell lines to be treated more efficiently by neutron irradiation. It should be noticed that both cell lines exhibit *in vitro* chemoresistance to doxorubicin and L-Pam, and are able to repair sublethal radiation damage (11). The relationship between cellular radiosensitivity and chemosensitivity is not clearly defined, as there are several experimental studies that report a positive correlation, while many others demonstrate the absence of such a correlation (21–26). This conflicting result can be explained by realizing that although the mechanisms of radioresistance appear to be distinct from those of chemoresistance, a considerable overlap between these two resistance mechanisms exist (27). To the best of our knowledge, our previously reported study (11) is the only one that examined the correlation of radiosensitivity and chemosensitivity in human lymphoma cell lines. We are currently examining this question in many more lymphoma cell lines.

Warenius *et al.* noticed that there is a wide range of intrinsic cellular radiosensitivity within any histological group, and they emphasized the need for developing a predictive assay for individual tumors (15). Similarly, Steel and Wheldon demonstrated that even leukemia cells show a broad spread of radiosensitivity, with most leukemic cell lines being more sensitive than the average of a series of carcinoma cell lines, but a significant minority of leukemic cell lines being more resistant than a typical carcinoma (28). Drewinko *et al.* investigated a human cell line established from a patient with lymphocytic lymphoma and they observed a distinct shoulder and ability to recover from sublethal damage (29). Also, Song *et al.* have reviewed the literature and concluded that the repair capacity of some hemopoietic cell lines may be greater than what had previously been thought (30). We suggest the use of the lack of clinical response to chemotherapy in selecting patients with IGNHL who may benefit more from neutron irradiation than photon irradiation. We base this concept on both clinical and laboratory observations. We have previously demonstrated, in a retrospective clinical study, a very high "in-field" failure rate in patients who did not respond or progressed on systemic chemotherapy (31). Also, a report on a recent Eastern Cooperative Oncology Group (ECOG) randomized trial indicated that only 28% of patients who achieved partial remission following eight cycles of CHOP chemotherapy were converted to complete responders after receiving 4,000 cGy to sites of residual disease (32). IGNHL that responds well to chemotherapy is typically radi-

Table 2. RBE values for cell lines

Cell line	α-Neutron/ α-Photon	RBE (2 G_{yp})	RBE (10%)	RBE (MID)
WSU-DLCL2	6.67 ± 0.045	3.34	2.54	2.97
SK-DHL2B	5.65 ± 0.55	3.05	2.59	2.81

osensitive, with high control rate within the radiation field; therefore, there is no need to investigate different methods of treating them by radiotherapy.

Courdi *et al.* has described a trend for RBE increase for cell lines characterized by relative photon radioresistance (18) and our findings are consistent with this observation, as the RBE for both lines were above the average RBE reported in the literature as discussed above.

Our hypothesis is also supported by the observation of Britten *et al.* who described melphalan-resistant human ovarian tumor cells that were cross resistant to photons but not to high LET (linear energy transfer) neutrons (33). A possible potential benefit of neutron irradiation is also the inherent acceleration in the neutron fractionation regimen, where the overall treatment time is usually shorter than that of photon irradiation, as increased tumor proliferation kinetics has been described in a mouse lymphoma model to cause radiation treatment failure (34).

We believe that the result of this study indicates that further investigations of the potential role of neutron irradiation in the management of chemotherapy-resistant IG-NHL is warranted.

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