8/10-22-75
LA-6049-MS

Informal Report

UC-48

Reporting Date: August 1975

Issued: September 1975

**Survival of Cultured Mammalian Cells
Irradiated at Various Depths in the
LAMPF Negative Pion Therapy Beam**

by

P. W. Todd
J. Dicello
G. West
C. Shonk
M. M. Kligerman
M. R. Raju

MASTER

los alamos
scientific laboratory

of the University of California

LOS ALAMOS, NEW MEXICO 87545

An Affirmative Action/Equal Opportunity Employer

UNITED STATES
ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
CONTRACT W-7405-ENG. 36

UNCLASSIFIED

In the interest of prompt distribution, this report was not edited by the Technical Information staff.

Work partially supported by U.S. Public Health Service grant No. CA-14052 from the Division of Research Resources and Centers of the National Cancer Institute to the University of New Mexico Cancer Research and Treatment Center.

Printed in the United States of America. Available from
National Technical Information Service
U S Department of Commerce
5285 Port Royal Road
Springfield, VA 22151
Price: Printed Copy \$4.00 Microfiche \$2.25

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Energy Research and Development Administration, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights.

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Energy Research and Development Administration, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

SURVIVAL OF CULTURED MAMMALIAN CELLS IRRADIATED AT VARIOUS DEPTHS IN THE LAMPF NEGATIVE PION THERAPY BEAM

by

P. W. Todd, J. Dicello, G. West, C. Shonk,

M. M. Kligerman, and M. R. Raju

ABSTRACT

Cultured human kidney T-1 cells were irradiated in flasks or on coverslips at different depths in a water phantom using the Clinton P. Anderson Meson Physics Facility (LAMPF) negative pion beam. Studies of cell survival and division delay as a function of depth and recovery between doses indicate RBEs of 1.8 ± 0.3 and an ability of cells to recover between two doses of stopping pions.

I. MATERIALS AND METHODS

Human kidney T-1 cells were cultured in Eagle's BME containing 10% fetal bovine serum and were irradiated while attached to T-25 culture flasks (Falcon Plastics) or coverslips (LUX Plastics). Colony development was allowed to take place without further post-irradiation disturbance of the cells. Survival was determined as previously described.¹ The configurations of irradiation are shown in Fig. 1. For brevity and consistency, the results reported here

were obtained from experiments in which the cells were irradiated in flasks.

The depth-dose and transverse beam profiles for which biological data are currently available are shown in Fig. 2. The double-beam profile shown in Fig. 3 was also used in the irradiation of coverslip cultures, and the colony-count data are reported elsewhere.²

Fig. 1. Irradiation configuration for human T-1 cells in flasks and on coverslips.

Fig. 2. Depth-dose profile (left) and transverse beam profile (right). The horizontal bars represent dimensions of the irradiated pairs of flasks in the x and y beam planes.

Fig. 3. Depth-dose profile calculated for double irradiation of cultures by adding 50% more dose at a 2.5-cm shallower position than the first exposure (used in irradiation of the coverslip holders to evaluate change in RBE through the modulated peak).

II. RESULTS

Figure 4 shows the results of experiment 800 in which cells in flasks were irradiated with 100 rads (bottom panel) and 200 rads (top panel) of negative pions at the position of maximum dose (in the profile of Fig. 2). In this experiment, an aluminum oxide target was employed for pion production, and the peak dose rate occasionally reached 6 rads/min.

In experiment 801, cells of another cell line were used (Chinese hamster M3-1), as this cell line is more resistant than T-1 cells to both high- and low-LET radiations.³ Figure 5, which shows the

Fig. 4. Depth-survival profile for cultured human T-1 cells irradiated in flasks with 200 peak rads pions (top) and 100 rads (bottom).

Fig. 5. Depth-survival profile for cultured Chinese hamster M3-1 cells irradiated in flasks with 200 peak rads pions.

depth-survival profile for these cells, indicates that they are also resistant to stopping pions. Although the dose peak occurred at a depth of 18.4 to 18.7 cm, this figure also indicates a rather flat survival profile all the way back to 19.6 cm. The lethality peak is expected to be broader than the dose peak because the dose-average LET (and hence the biological effect) is greater beyond the depth at which the maximum dose occurs.

This biological broadening of the dose peak is also suggested in Fig. 6 which presents a portion of the results of experiment 820. This experiment was a repeat of the above experiments with T-1 cells, but a carbon target was used for pion production, and the average dose rate tended to be around 3 rads/min at the dose peak (located 18.4 to 18.7 cm into the water phantom). Survival was very nearly constant over a breadth of ~ 1 cm at the doses used (100, 200, 300, and 400 rads). In addition, a fractionated exposure was made in which a set of cultures received 400 rads in four 100-rad fractions delivered exactly every 3 h.

Dose-response curves were generated from the data of experiments 800 and 820 using doses and survivals at 18.75 cm depth, and these are plotted in Fig. 7 along with an estimate of the x-ray survival

Fig. 6. Depth-survival profiles for human T-1 cells irradiated with 100 (-O-), 200 (-X-), 300 (-O-), and 400 (-Δ-) peak rads pions.

Fig. 7. Dose-survival curves derived from Figs. 3 and 5 compared with x-ray survival (-X-) and with survival of cells receiving 400 rads as four 100-rad treatments every 3 h (-X-X-).

curve under the same conditions at low doses and at acute dose rates. At the higher pion doses, the survival curve of experiment 800 (Fig. 4) lies below that of the later experiment (Fig. 6). It is possible that this difference is due to a dose-rate effect. In the region of 100 peak pion rads, the two curves do not differ significantly. Comparing the combined curves to cell survival following a 200-rad dose of x rays yields an average relative biological effectiveness (RBE) of 1.8 ± 0.2 . The error limits on this figure are still too broad for serious clinical application. The cells that received 400 rads in 100-rad increments survived as if they had received 340 rads in a single dose. Therefore, the existence of a small amount of sublethal damage repair following pion irradiation in this dose range is apparent.

Additional end points studied included post-irradiation growth. The scoring of cells per colony

Fig. 8. Post-irradiation growth curves for human T-1 cells exposed to pions in the peak and plateau regions.

by phase-contrast microscopy of the irradiated cultures yielded the growth curves of Fig. 8. The division delay per rad appeared to be very nearly the same at the two positions studied.

III. DISCUSSION

A single irradiation with 100 rads of peak pions does not appear to kill any cells in the plateau portion of the depth-dose profile. This may not be true in the case of daily 100-rad fractions, and it would need to be tested in an *in vivo* system. A 10 to 15% reduction in cell survival in the plateau region occurred after four 100-rad fractions were delivered at the peak; however, in this case, only 3 h intervened between exposures.

An unmodulated beam should be adequate for irradiating a 1-cm treatment volume, and two overlapping peaks may be adequate for treating a 4-cm treatment volume to a uniform dose. Biological results to date are consistent with this conclusion.

The downward curvature of the survival curves implies that some pion damage is sublethal and must be accumulated. The fractionation experiment suggests that this sublethal damage can be repaired. When these preliminary data are used to determine the exponent of N (the number of daily fractions) in the "NSD" (Nominal Standard Dose) formula, the value of the exponent becomes 0.11, similar to that determined for neutrons used for radiotherapy at the Texas A and M University cyclotron (TAMVEC) but greater than ~ 0.04 estimated for the Hammersmith Hospital⁴ and the Naval Research Laboratory⁵ neutrons. In neutron experiments, no evidence has indicated that the exponent of T (the time over which treatment is administered) should change,^{4,5} and pion experiments with synchronous cells to investigate division delay and other nonlethal effects are needed. $D = NSD \times N^{0.11} \times T^{0.11}$ can be used as an initial estimate of the time-dose relationship for stopping pions in the configuration used in these experiments. In other words, the Kligerman formula, $D = SD \times T^{0.33}$, for daily irradiations, would become $D = SD \times T^{0.22}$.

An analysis of the data presented here is still incomplete. For example, a thorough consideration of the higher RBE on the "back" side of the dose peak has yet to be made. An RBE in the vicinity of 2.0 can perhaps be anticipated.

The biological action of pions in the peak of this beam configuration may or may not resemble that of TAMVEC neutrons. It may be worthwhile to explore other factors than RBE and fractionation to determine to what extent such a resemblance exists. Experiments determining the fractionation response of mouse jejunal crypts and the OER for *Vicia faba* root growth inhibition, both of which have been done very well at TAMVEC, may make an entire set of parameters from the TAMVEC experience available to the LAMPF pion program.

An initial estimate of the equivalent of 200 x-ray rads daily for 7 wk was made from the results of this study. It appears that approximately 120 rads of peak pions per day on the same schedule would lead to the same level of cell lethality.

REFERENCES

1. P. Todd, "Heavy Ion Irradiation of Cultured Human Cells," *Radiation Res. Suppl.* 7, 196-207 (1967).
2. P. Todd, G. West, C. Shonk, J. Dicello, and M. M. Kligerman, "Spatial Distribution of Cultured Human Cell Survival in a Negative Pi Meson Beam," *Radiology* 116, 179-182 (1975).
3. P. Todd, "Heavy-Ion Irradiation of Human and Chinese Hamster Cells *in Vitro*," *Radiation Res.* 61, 288-297 (1975).
4. S. B. Field, "The Ellis Formulae for X-rays and Fast Neutrons," *Brit. J. Radiol.* 45, 315 (1972).
5. P. Todd, C. B. Schroy, F. H. Attix, and R. B. Theus, "Fractionated Irradiation of Cultured Human Cells with Therapeutic Doses of Fast Neutrons," *Radiation Res.* 59, 123-124 (1974).