Radiation Survival Curve for Pediatrics Rhabdomyosarcoma Cells

Alexander F. Ibrahim[(1)*], Siddig T. Kafi[(1)] and Omer F. Idris[(2)].

1)Dept. of Medical Physics, School of Physics, Faculty of Science, Al-Neelain University, P. O. Box: (12702), Code: (11121), Fax: + 249-183-789055, Khartoum. Sudan.

2) Bio Chemistry Department, Faculty of Science, Al-Neelain University, P. O. Box: (12702), Code: (11121), Fax: + 249-183-789055, Khartoum. Sudan.

Author e-mail: AlexanderFadul@yahoo.com

ABSTRACT

We report on the survival curve of cultured pediatric rhabdomyosarcoma (RD) cells derived from children. X-ray radiation of variable doses (i.e. 4 Gy up to 20 Gy) was used for this purpose. Cell death resulted from chemical alterations in the genome. Both single-hit multi-target model and linear-quadratic model which base on the target theory allowed for the description of the survival curve, and for the calculations of characteristic parameters such as mean lethal dose (D_0), quasi-threshold (D_q), extrapolation number (n), dose at which the contribution of linear and quadratic components of cell killing are equal (α/β) and the radiosensitivity of the cells (SF_4 value). From the results obtained the survival curve was constructed, and it was found that D_0 = 3.2 Gy, D_q= 9.2 Gy, n = 18, α/β = 9 Gy and SF_4 = 0.79. Low radiosensitivity of cells under study was concluded.

Key Words: Cell Survival Curve/ Pediatric Rhabdomyosarcoma/ In Vitro Cell Culture/ Target Theory.

INTRODUCTION

Rhabdomyosarcoma (RD) is the most common soft tissue sarcoma of childhood. It exhibits the skeletal muscle differentiation [(1-4)]. The embryonal RD has spindle cells and tends to occur in head and neck area, bladder, vagina, and in or around the prostate and testes [(4-6)]. To choose adequate criteria of cell radiosensitivity and to calculate the characteristic parameters for comparative radiation studies, a survival curve is needed [(7,8)]. A cell survival curve describes the relationship between the radiation absorbed dose and the proportion of cells that survive (surviving fraction) [(7-10)]. Cell death or the lethal effect of irradiation means that the cell loses the ability to proliferate (reproductive death) [(8)]. A survivor cell that has retained its reproductive integrity and is able to proliferate indefinitely to produce a large colony is said to be *clonogenic* [(7)]. For a tumor to be eradicated in single fraction in radiotherapy, it is only necessary that cells be killed in the sense that they are rendered unable to divide and cause further growth and spread of the malignancy [(7)].

* Corresponding author e-mail: *AlexanderFadul@yahoo.com*.

In 1956 the first radiation survival curve for cultured *HeLa* cells exposed to X-ray was demonstrated by Puck and Marcus [11]. Since then it has remained the basic method used in quantitative radiobiology. A lot of research work on the survival curve parameters [12-20] was done for different normal and malignant cell organisms including bone marrow stem cells, cartilage, epithelial cells, intestinal mucosa, parenchymal hepatocytes, hyperthermia, lung, skin colonies, skin fibroblast, mammary cells, etc… both *in vitro* and *in vivo*. Knowledge of the radiosensitivity and the mechanisms of regulation promise most important practical results. It aims at the possibility of using special agents to control the dose response of tissues, i.e. weaken them when dealing with the protection of an organism or increase them when irradiating malignancy tumors, inactivation bacteria, etc… [8]. The objective of the present study was to determine the intrinsic radiosensitivity of the RD cell type and determine the parameters related to survival curve used for the calculations of tumor cell kill in radiotherapy of pediatric tumors, of the type RD sarcoma. This is necessary when treating patients with radiotherapiutic techniques.

MATERIALS AND METHODS

Materials

The reagents and equipment used for cell culture and irradiation were as follows: RD cells, trypsin (or trypsin/versene), phosphate buffered saline (PBS), cell culture tubes and flasks, bio safety cabinet (BSC), inverted microscope, incubatator (36^{o}C), mixer (Denley Spiramix), linear accelerator (ELEKTA 75/15, 6 MV photon, output:1.00 cGy/MU), haemocytometer or cell counting chamber based on freshney (improved Neubauer type, 0.1 mm depth, 25 squares per cm^2, 0.2 mm long and 0.2 mm wide), pipettes, fine Pasteur pipettes and slides [21-23].

Sample Preparation

Culture flasks with confluent monolayers of RD cells derived from human RD (American Type Culture Collection, Manassas) provided by *National Health Laboratory – Sudan* were grown as monolayer cultures in growth medium (GM) supplemented with 10% serum (Fetal calf serum is the serum of choice). Growth medium from the cell culture flask was decanted and the twice confluent cell layers were washed with Ca and Mg free PBS. 0.25% trypsin solution (or equal parts of 0.25% trypsin and 1:5000 Versene solution) in PBS was added to the monolayer. The flask was then placed in a 36°C incubator until the cells were detached from the surface. Complete detachment of cells was checked by an inverted microscope. The cells were re-suspended in the growth medium, and the desired concentration was adjusted to (1600 cell/ml) for each cell culture tube of RD cell lines following WHO procedures [24] .

Method

After one hour of incubation the cell culture tubes of RD cell lines were irradiated to single dose X-rays 4 Gy, 8 Gy, 12 Gy, 16 Gy and 20 Gy in *Radiation and Isotopes Centre – Khartoum - Sudan (RICK)* by linear accelerator machine (ELEKTA).One tube was kept free of irradiation as a control. Cell irradiation was done at room temperature and the calculations were done by Radiation Oncology Computer System (ROCS) [1, 23].

The irradiated fresh culture tubes of RD cell line were sub-cultured for a period of 5–7 days and placed in a 36°C incubator supplied with CO_2 by cell metabolism and 7.2 pH usually included in basal growth media from proprietary sources to divide and grow until the surviving cells produces macroscopic colonies.

Cells were calculated following chamber counting method [23]. Accurate numbers of cells in suspension were calculated by counting the cells in a haemocytometer based on Freshney. The cell culture tubes of RD cells were mixed well with a fine Pasteur pipette, and a sufficient volume was aspirated to fill both sides of the haemocytometer chamber. The viable cell concentration was calculated. The plating efficiency of the RD cells culture (PE) is the ratio of the number of colonies observed to the number of cells plated, and the surviving fraction (SF) of the treated cells is the percentage of the number of colonies counted to the number of cells seeded, normalized to the plating efficiency of the control cells [21-24].

RESULTS AND ANALYSIS

A radiation survival curve was generated using an *in vitro* cell culture technique, and the surviving fraction SF was taken on logarithmic scale versus the absorbed dose on linear scale. Two descriptions of the shape of survival curves were discussed.

Single-hit Multi-target Model

In this model the cells were supposed to contain **n** distinct and identical targets which can be individually inactivated by the passage of a charged particle. Inactivation of a target represents a sub-lethal event and the cell is killed when all **n** targets have been inactivated. The surviving fraction of cells corresponding to this type of lethality is given by the relation [25]:

$$S = 1 - \left(1 - e^{-D/D_0}\right)^n \qquad (1)$$

Where:

S: is the fraction of cells surviving a dose D.

D_0: is the mean lethal dose for which the mean number of lethal events per cell is equal to one.

n: is the extrapolation number and it corresponds to the number of sub-lethal targets.

The three parameters **n**, D_0, and D_q are related by the expression:

$$\log_e n = D_q / D_0 \qquad (2)$$

D_q: is the *quasi-threshold* dose or the dose below which there is no effect.

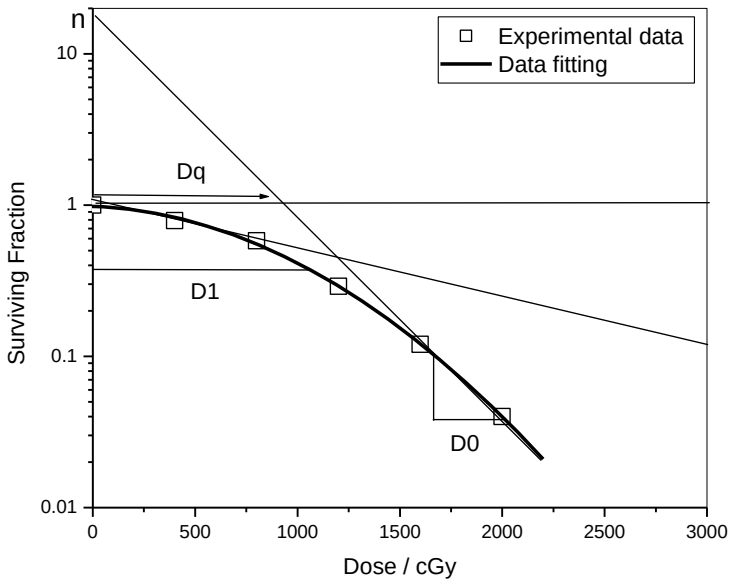

Fig. (1): Shape of survival curve for RD cells exposed to X-ray. The curve is described by the one lethal target and **n** sub-lethal targets model. (D_0=3.2 Gy, D_q=9.2 Gy, and **n**=18).

D_0 the **final slope** (mean lethal dose) interpreted as multiple event killing is the dose required to reduce the fraction of survivals from 0.1 to 0.037 or from 0.01 to 0.0037 and on so. Some quantity to describe the width of the shoulder, the extrapolation of the final slope D_0, back to the y axis yields **n** the *extrapolation number*. The *quasi-threshold dose* is almost a threshold dose D_q and it is defined as the dose at which the straight portion of the survival curve extrapolated backward intercepts the dose axis drawn through a surviving fraction of unity.

Linear-quadratic Model

The linear quadratic model assumes a cell can be killed in two ways, either by a single lethal event or by the accumulation sub-lethal events. If these modes of cell death are taken to be independent, the probability of survival (S) is the product of the probabilities of each type of lethal event [25]:

$$S = e^{-\alpha D - \beta D^2} \qquad (3)$$

Where:

S: is the fraction of cells surviving a dose D.

α: is a constant representing the linear component of cell killing.

β: is a constant representing the quadratic component of cell killing.

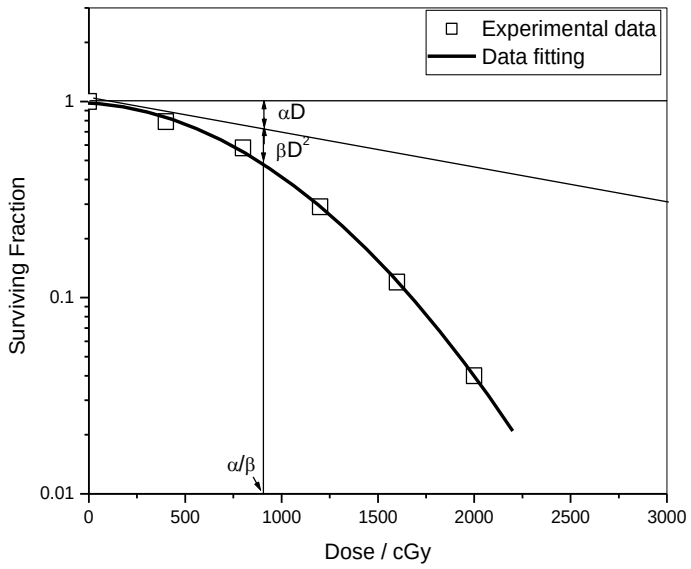

Fig. (2): Shape of survival curve for RD cells exposed to X-ray. The experimental data were fitted to a linear quadratic function. (α/β = 9 Gy).

The ratio α/β is the dose at which the contribution of the linear and the quadratic component are equal. One can then write; $\alpha D = \beta D^2$ and $D = \alpha / \beta$.

DISCUSSION

When the RD cell lines were acutely exposed to X-rays, the ability to divide was indefinitely declined as a function of radiation dose. The shape of the survival curve depends on the density of ionizations, for sparsely ionizing irradiation (X- and γ-rays) the logarithmic response is linear quadratic. The radiosensitivity was evaluated by a clonogenic test. A **final slope** or mean lethal dose (a dose that given an average of one hit per cell) that is (D_0=3.2 Gy), the *quasi-threshold* dose (D_q=9.2 Gy), and the *extrapolation number* **n** is a measure of the width of the shoulder (**n**=18) indicates that the size of the shoulder of the survival curve for RD cell type has a broad shoulder and reflects the ability of the cells to repair sub-lethal radiation damage [24]. The α/β ratio (α/β=9 Gy), and the survival fraction after 2 or 4 Gy (SF_4) were determined for RD cell lines (SF_4= 0.79%). The high SF_2 value for RD cell lines correlates with the low clinical radiosensitivity of RD [21]. From results α-component increased for RD sarcoma cells type. This resulted in high α/ß ratio of approximately 9 Gy [26]. Cells displaying a high α/ß ratios more than 6 Gy for radioresistant cells, conventional doses per fraction of 2 Gy should provide therapeutic gain with an increased probability of tumor cure and sparing of late-responding normal tissues [24,27]. Following exposure to radiation RD cells may die attempting the next or a subsequent mitosis (mitotic death), or there may be programmed cell deaths (apoptosis death). The RD cells have a large dose-rate effect and the mitotic death is dominant (mechanism of death). In cells that die a mitotic death, there is a one-to-one correlation between cell survival and the average number of putative (lethal) chromosomal aberrations per cell. That is asymmetric exchange type aberrations such as dicentrics and rings [7].

CONCLUSION

Radiation survival curve for RD cell lines was constructed using an *in vitro* clonogenity test. The plating efficiency PE of the RD cell lines culture was 75%. The mean lethal dose D_0 of RD cells was found to be 3.2 Gy, and the quasi-threshold dose D_q was found to be 9.2 Gy. The *extrapolation number* **n** measure the width of shoulder was found to be 18 indicates that the survival curve for RD cells has a broad shoulder. RD could be classified as low radiosensitive or radioresistant cells [(22)]. Conventional doses per fraction of 2 Gy should provide therapeutic gain for RD cells with an increased probability of tumor cure and sparing of late-responding normal tissues. The mechanism of death of the RD cells is the mitotic death (cells die attempting the next or a subsequent mitosis).

ACKNOWLEDGMENTS

We thank Mr. Ali Sid Ahmed Mohammed – Al Neelain University for helpful comments and discussions and Mr. M. A. Halato. And we are grateful to Mr. Hatim B. Osman - *National Health Laboratory* for providing the RD cell lines and reagents, the staff of *Radiation and Isotopes Center - Khartoum (RICK)*, and Mr. Mamdouh Yassin – *Sudan Atomic Energy Commission (SAEC)* for facilities and suggestions.

REFERENCES

1) Jane Dobbs, Ann barrett and Dan Ash; "Practical Radiotherapy Planning", Arnold, London, 3rd Ed., (1999).
2) Pappo AS, Shapiro DN, Crist WM, et al; "Biology and therapy of pediatric rhabdomyosarcoma", J Clin Oncol 13: 2123-2139, (1995).
3) Crist W, Kun L; "Common solid tumors of childhood", N Engl J Med 324: 461-471, (1991)
4) http⁄⁄www.cancer.org. (2008).
5) Porth, Carol; "Pathophysiology of altered health state", Carol Mattson Porth; with 28 contributors, Lippincott, 5th Ed., (1998).
6) Vinay Kumar, Ramzi S. Cotran, Stanley L. Robbins; "Basic Pathology", parkins, 6th Ed., (1997).
7) HALL, E. J.; "Radiobiology for the Radiologist", Lippincott Williams and Wilkins, Philadelphia, 5th Ed., (2000).
8) Yarmonenko S. P., "Radiobiology of Humans and Animals", Translated from the Russian by G. Leib, Mir Publishers, Russia, 1st Ed., (1988).
9) IAEA; "Review of Radiation Oncology Physics", A Handbook for Teachers and Students, (2004).
10) Tubiana, Maurice; "Introduction to Radiobiology", Maurice, Jean Dutreix, Andre Wambersie; translated by David Bewley, Burgess, France, (1990).
11) Puck TT, Markus PI; "Action of X-ray on mammalian cells", J Exp Med 103:653-666, (1956).

12) J. E. Till, E. A. McCulloch, “A direct measurement of the radiation sensitivity of normal mouse bone marrow cells", Radiat. Res., (1961), 14:213-222.
13) N. F. Kember, “An in vivo cell survival system based on the recovery of rat growth cartilage from radiation injury", Nature, (1965), 207:501-507.
14) H. R. Withers, “The dose survival relationship for irradiation of epithelial cells ofd mouse skin", Br. J. Radiol., (1967), 40:187-194.
15) H. R. Withers, M. M. Elkind, “Microcolony survival assay forr cells of intestinal mucosa exposed to radiation", Int. J. Radiat. Biol., (1970), 17:261-267.
16) R. L. Jirtle, G. Michalopoulos, J. R. Mclain, J. Crowley, “Transplantation system for determing the clonongenic survival of parenchymal hepatocytes exposed to ionizing radiation", Cancer Res. (1981), 14:3512-3518.
17) Rofstad, E. K, and Brustand T.; “Hyperthermic radiosensitization of cells from a human melanoma Xenograft”, Radiat, Res. 99:59-72, (1984).
18) G. B. Gerber and J. Maes; “The *in vitro* radiosensitivity of hemopoietic stem cells from control and preirradiated infant mice”, Springer Berlin, German, (1981), vol. 19, n°3, pp. 173-179.
19) J.E.Coggl; “Biological Effict of Radiation”, 2nd Ed., Tylor and Froncis, London, (1983).
20) C. S. Parkins, J. F. Fowier, “The linear quadratic fit to lung function after irradiation with X-rays at smaller doses per fraction than 2 Gy" Br. J. Cancer, (1986), 53: VI, 320-323.
21) WHO, “POLIO LAB NETWORK”, Quarterly Update, Volume VIII, Issue 4, Dec. (2002).
22) KHAN, FAIZ M.; “The Physics of Radiation Therapy”, Lippincott Williams and Wilkins, USA, 2nd. Ed,(1994).
23) WHO; “Polio laboratory manual”, Department of Vaccines and Biologicals. Geneva: World Health Organization, (2001).
24) Van den Aardweg, GJMJ, Naus, NC, Verhoeven, AAC, de Klein, A. Luyten, GPM. (2002); “Cellular radiosensitivity of primary and metastatic human uveal melanoma cell lines Invest”, Ophthalmol Vis Sci 43,2561-2565
25) Karin H. Yohem, Donald J. Slymen, Marvin D. Bregman, Frank L. Meyskens, JR.,“ Radiosensitivity of Murine and Human Melanoma Cells: A Comparative Study With Different Models", (DIO), California, (1990).
26) HONG A., LEIGH B; “Induction of interleukin-6 and oncostatin M by radiation in Kaposi's sarcoma cell lines”, International journal of radiation oncology, New York, (2001), vol. 50, n°2, pp. 533-540.
27) Malaise, EP, Fertil, B, Chavaudra, N, Guichard, M.; “Distribution of radiation sensitivities for human tumor cells of specific histological types: comparison of *in vitro* to *in vivo* data”, Int J Radiat Oncol Biol Phys, (1986), pp. 12,617-624.