Radiobiological characterization of different energy-photon beams used in radiotherapy from linear accelerator

A. Elata[1]
P.CALZOLARI[2]
A. M. E. Hassan[1]
D. BETTEGA[2]
E. Alli[1]
1- Sudan Atomic Energy Commission
2-Departmento di Fisica, Universita degli Studi di Milano and INFN, Via Celoria 16, 20133 Milano,Italy.

تعيين الخصائص البيولوجية والإشعاعية لحزمتين من الفوتونات ذات طاقة مختلفة تنتج من المسرع الخطى وتستخدم في العلاج الاشعاعى للأورام

عباس العطا حسين الطيب[1]
باولا كالزولارى[2]
عمار محمد الأمين حسن[1]
دانيلا بيتقا[2]
الطيب احمد الطيب على[1]
1- هيئة الطاقة الذرية السودانية
2- جامعة ميلان للدراسات، شعبة الفيزياء، ايطاليا، ميلان

الملخص

أجريت هذه الدراسة بغرض تعيين الخصائص البيولوجية والإشعاعية لحزمتين من الفوتونات ذات طاقة مختلفة (6 و15 ميغافولت) تنتج من المسرع الخطى وتستخدم في العلاج الاشعاعى للأورام لمعرفة مدى التاثيرالمتاخرللاشعاع وذلك من خلال دراسة ناتجين نهائيين لمنحنى البقاء والنويات الصغيرة (Survival Curve and Micronuclei) على نوع معين من خلايا القوارض (V79Rodent cell line).تم تحديد عدد الصبغيات(Karyotype) في الخلايا المستخدمة ووجد أن العدد المثالي 22 كروموسوم لكل خلية بعد حساب 60 خلية لحساب منحنى البقاء والنويات الصغيرة تم تزريع الخلايا في وسط غذائي مناسب حضانتها لفترة يومين ومن ثم تعريضها للحزم الفوتونية الإشعاعية وبعد ذلك تم إزالة الوسط الغذائي وعد الخلايا بواسطة

عداد خلايا (cell coulter counter) واعادة تزريعها باعداد معينة لكل جرعة إشعاعية.تم حضانة الخلايا في درجة حرارة 37°C لمدة 6ايام لتحديد منحنى البقاء كما تم تزريع جزء من الخلايا لكل جرعة لتحديد النويات الصغيرة وتم حضانتها لمدة 24 ساعة ثم اضافة مادة (Cytochalasin-B) لإيقاف الانقسام الخلوي في مرحلة الانقسام النووي (Cytokinesis). تم عد النويات الصغيرة بمعدل 3000 خلية لكل جرعة واخذ الوسط الحسابى لها وبمقارنتها بعينات التحكم وجد أنها تزداد بزيادة الجرعة وبمقارنة 6 ميغا الكترون فولت مع 15 ميغا الكترون فولت وجد ان عدد النويات الصغيرة يزداد بزيادة طاقة الفوتون المستخدم مما يدل على وجود فوتونيوترون (Photoneutrons) تنتج من استخدام فوتونات ذات طاقة عالية.

Abstract:

The main objective of this study was to perform a radiobiological characterization of different energy photon beams (6 MV and 15MV) from linear accelerator used in radiotherapy, and comparison of different treatment modalities, with special regard to late effects of radiation. Using two end-points, cell survival and micronucleus induction, in the biological system (Chinese hamster V79 cell line). Chromosomes number was counted and found to be 22 chromosomes per cell. Cells were kept in confluent growth for two days and then exposed to two photon beams and immediately after irradiation were counted and reseeded in different numbers for each dose. For evaluation of surviving fraction samples were incubated at 37°C for 6 days, five samples were counted for each dose. At the same time three samples were seeded for the micronuclei frequency and incubated at 37°C after 24 hours cytochalasin-B was added to block cells in cytokinesis. The survival curve showed similar curves for the two beams and decreased with dose. The micronuclei frequency was positively correlated with dose and the energy of the photon. This indicates the presence of low dose of photoneutrons produced by using high energy photon beams.

Introduction:

Induction of cancers in human by radiation was first documented by the appearance of cancers among scientists and radiologists exposed to radium salts and workers in X-ray generators in the first few years after discovery of X-rays and radioactivity. During the period 1900-1950, radiation-induced cancers were observed in persons exposed accidentally or inadvertently, patients treated by radiation or after

عداد خلايا (cell coulter counter) واعادة تزريعها باعداد معينة لكل جرعة إشعاعية.تم حضانة الخلايا في درجة حرارة 37°C لمدة 6ايام لتحديد منحنى البقاء كما تم تزريع جزء من الخلايا لكل جرعة لتحديد النويات الصغيرة وتم حضانتها لمدة 24 ساعة ثم اضافة مادة (Cytochalasin-B) لإيقاف الانقسام الخلوي في مرحلة الانقسام النووي (Cytokinesis). تم عد النويات الصغيرة بمعدل 3000 خلية لكل جرعة واخذ الوسط الحسابى لها وبمقارنتها بعينات التحكم وجد أنها تزداد بزيادة الجرعة وبمقارنة 6 ميغا الكترون فولت مع 15 ميغا الكترون فولت وجد ان عدد النويات الصغيرة يزداد بزيادة طاقة الفوتون المستخدم مما يدل على وجود فوتونيوترون (Photoneutrons) تنتج من استخدام فوتونات ذات طاقة عالية.

Abstract:

The main objective of this study was to perform a radiobiological characterization of different energy photon beams (6 MV and 15MV) from linear accelerator used in radiotherapy, and comparison of different treatment modalities, with special regard to late effects of radiation. Using two end-points, cell survival and micronucleus induction, in the biological system (Chinese hamster V79 cell line). Chromosomes number was counted and found to be 22 chromosomes per cell. Cells were kept in confluent growth for two days and then exposed to two photon beams and immediately after irradiation were counted and reseeded in different numbers for each dose. For evaluation of surviving fraction samples were incubated at 37°C for 6 days, five samples were counted for each dose. At the same time three samples were seeded for the micronuclei frequency and incubated at 37°C after 24 hours cytochalasin-B was added to block cells in cytokinesis. The survival curve showed similar curves for the two beams and decreased with dose. The micronuclei frequency was positively correlated with dose and the energy of the photon. This indicates the presence of low dose of photoneutrons produced by using high energy photon beams.

Introduction:

Induction of cancers in human by radiation was first documented by the appearance of cancers among scientists and radiologists exposed to radium salts and workers in X-ray generators in the first few years after discovery of X-rays and radioactivity. During the period 1900-1950, radiation-induced cancers were observed in persons exposed accidentally or inadvertently, patients treated by radiation or after

diagnostic radiological procedures (including those receiving Thorotrast), radium dial painters, miners exposed to pitchblende and uranium and others. Increased incidences of cancer after low radiation dose has been extensively documented by the analysis of mortality due to solid cancers and leukemia among survivors of the atomic bomb explosion in Japan in1945(1).

A linear relation exists from about 0.1Gy up to about 2.5Gy these data represent the gold standard for our knowledge concerning radiation induced cancer. Many studies on second malignancy after radiotherapy reported that approximately 10% of patients who present at major cancer centers have a second malignancy. Causes may relate to treatment of previous malignancy. In radiotherapy patients the induced tumor include carcinomas, that may appear in sites adjacent to or remote from the treated area, the number of carcinomas is relatively large but the relative risk is small. In addition sarcomas may appear in heavily irradiated tissues, either within or close to the treatment field. In radiotherapy patients sarcomas are small in numbers but are characterized by a high relative risk. The prime sites for developing a second malignancy are colon, lung and stomach, which are characterized by high probabilities. Radiation induced tumor in radiotherapy patients will gain increasing importance as younger patients are treated and improved cure rates obtained (2).

The incidence of second malignancy by conventional radiotherapy include incidence of carcinomas in tissues such as the gastrointestinal tract, breast, thyroid and bladder, which in linear relation with dose up to about 2.5 Gy. There is great uncertainty concerning the higher dose response relationship, some data suggest a decrease at higher dose, usually attributed to cell killing. Radiotherapy patients also show an incidence of carcinomas, often in sites remote from the treatment field and sarcomas in heavily irradiated in field tissues. Radiotherapy has been shown to be associated with a statistically significant, though very small enhancement in the risk of second malignancies, particularly in long term survivors. Childhood cancer survivors are at >19-fold increased risk for developing another malignancy. The increased incidence of breast cancer in women after previous radiotherapy for Hodgkin's disease is well described and is attributed to the incidental inclusion of portions of the breast in the portals used to irradiate the mediastinum with or without infraclavicular/axillary

regions. Radiation induced fibrosarcoma and osteosarcoma as a side effect of therapeutic irradiation for breast carcinoma were reported (3).

The study of DNA damage at the chromosome level is an essential part of genetic toxicology because chromosomal mutation is an important event in carcinogenesis. The observation that chromosome damage can be caused by exposure to ionizing radiation or carcinogenic chemicals was among the first reliable evidence that physical and chemical agents can cause major alterations to the genetic material of eukaryotic cells. Micronuclei (MN) are small, extranuclear bodies that arise in dividing cells from acentric chromosome/chromatid fragments or whole chromosomes/chromatids that lag behind in anaphase and are not included in the daughter nuclei in telophase. The cytokinesis-block micronucleus (CBMN) assay is the most extensively used method for measuring MN in human lymphocytes, and can be considered as a cytome assay covering cell proliferation, cell death and chromosomal changes. The key advantages of the (CBMN) assay lie in its ability to detect both clastogenic and aneugenic events and to identify cells which divided once in culture (4).

Materials and methods:

The biological system used was the Chinese Hamster V79 cell line (passage 31). Cells were routinely cultured in Eagle's Minimal Essential Medium (MEM), supplemented with 10% Foetal calf serum, 2mM glutamine, 50ug/L gentamycine and 10mM HEPES Buffer. V79 cells were maintained in confluent growth at 37°C in humidified 2%CO_2 and 95% air. About 24hours before irradiation, V79cells were seeded into T-25 flasks with about 15,000cells/flask

The cells were irradiated with full medium in a water phantom at room temperature. The doses were between 0.0, and 7.0 Grays for both 6MeV and 15MeV photon beams. Samples were irradiated at ISTITUTO NAZIONALE DEI TUMORI in Milano using the linear accelerator LINAC-VARIAN DHX MLC. Samples were irradiated in a water phantom in a field size 20X20cm^2 and at a depth of 5cm in the isocenter position and SSD 100cm and the suitable monitor units were calculated for each dose.

Dosimetry was performed using ionizing chamber IONEX DOSIMETER 2590 and FARMER NE 2581 according to the routinely used procedure for patients' treatment.

For Micronuclei assay, immediately after irradiation cells were harvested by trypsinization, diluted and counted (by coulter counter), and plated into T 25- flasks at about 30,000 cells/flask, containing completed medium with cytochalasin-B (final concentration of 3μg/ml) and incubated at 37°C for 24 hours. After this period the medium was removed and cells were rinsed with PBS, fixed with 70% ethanol for 7minutes, dried overnight and stained with 10% GIEMSA solution for about 15minutes, washed by tap water three times and dried. A minimum of 10^3 binucleated cells were scored using a ZEISS light microscope. Following the scoring criteria previously described (5).

For chromosomes scoring assay, V79cells were seeded into two T-25 flasks with ($2x10^5$) cells/flask and complete Medium, maintained in exponential growth at 37°C with 2% CO_2 and 95% air. After 24hours Colcemid was added at final concentration of (0.1μg/ml) and samples were incubated at37°C. After 3 hours medium was removed and cells were harvested by trypsinization and resuspended in new medium, centrifuged for 10mins at 2000r/min then medium was removed and cells were detached by shaking, rinsed in PBS solution and centrifuged at 2000r/min for 10mins PBS was removed. Hypotonic solution 0.075m KCl was added and samples were incubated at 37°C. After 20 minutes samples were centrifuged for 5 minutes at 800r/min, fixed using (acetic acid: methanol-1:3) and allowed to stand for 20minutes at room temperature again centrifuged at 1000r/mn for 5min. Fixation step was repeated then fixative was removed and 2 drops of fresh fixative was added. Samples were resuspended by gentle shaking and spreaded on slide and dried at room temperature for 1 day. Samples were stained with10%GIEMSA solution. Kept at room temperature for 15-20 minutes. Slides were covered with covering glass and stay for 1day at room temperature. Samples were scored under light microscope using magnification 100 and SIGMA immersion oil.

The Cell Survival was carried out in parallel with the micronuclei assay. Aliquots of the suspensions were also plated into T 25-flasks (five flasks for each dose) at suitable numbers for survival assay and

incubated at 37°C. After 6 days, the samples were fixed with 70% ethanol (for 10 minutes). Dried and stained with 10% GIEMSA solution for 20 minutes and dried overnight.

Colonies with more than 50 cells were scored as survivors. Plating efficiency (PE) was calculated by dividing counted colonies by cells seeded. Survival fractions were evaluated by the following formula:

$$(SF) = \frac{\text{colonies counted}}{\text{Cells seeded X PE/100}}$$

Results

Karyotyping of Chinese hamster cell line (V79):

Twenty two chromosomes were found to be most abundant karyotype for V79 strain with frequency more than 80% compared to the other types; 21 and 20 as shown in figure 1, which is similar to previously reported data.(5)

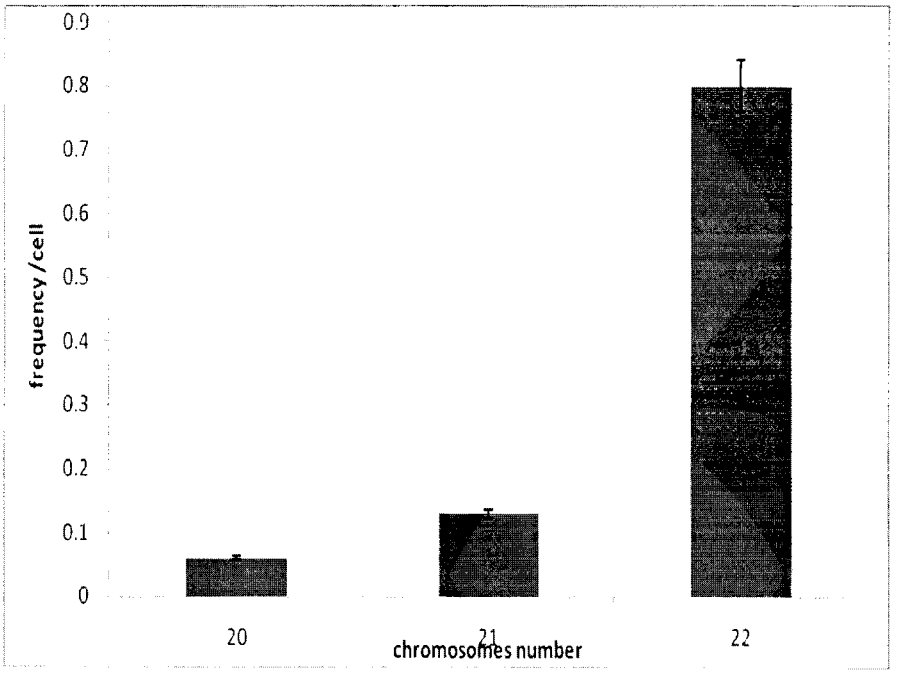

Figure 1: Distribution of chromosomes number for Chinese hamster V79 strain.

Survival curve:
The survival fraction was observed to decrease with dose. No difference in the survival fraction against dose was observed for both 6MV and 15MV.

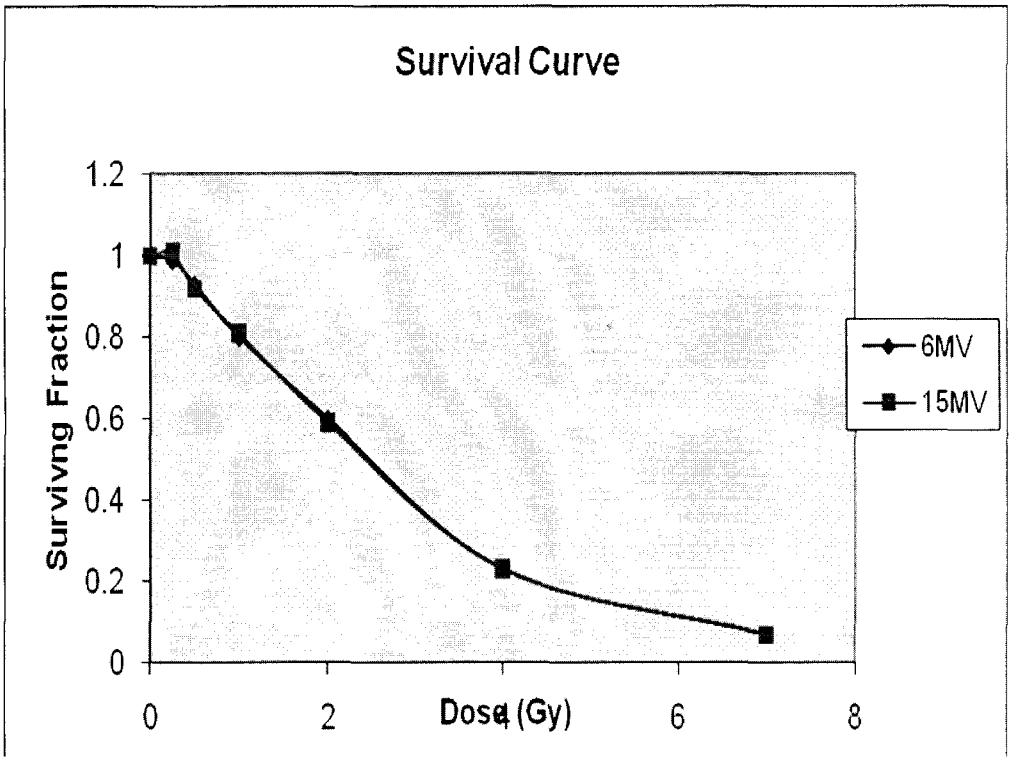

Figure 2: Survival fraction versus Dose in Gy.

Micronuclei frequency:
The frequency of micronuclei was observed to be positively correlate with dose (r Sq = 0.994) accompanied with high micronuclei frequency in high energy photon 15 MeV as shown in figure 3.

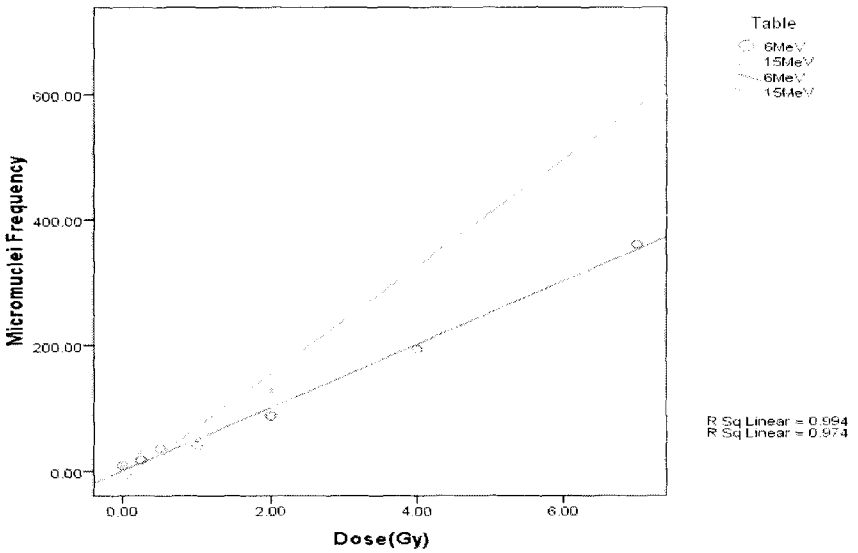

Figure 3: Frequency of micronuclei for two different Photon energies at different doses.

Discussion:

Karyotyping:
Chromosome frequency of Chinese hamster strain V79 cell line was determined by counting of 60 cells and the model number was found to be 22 chromosomes with the highest frequency about 85% more than 21 and 20 chromosomes, which was similar to the data reported by (5).
Survival Curve:
Plotting of survival curve was done by using Excel software; from figure 1 it was clear that survival fraction is decreasing by increasing the dose increased i.e. inverse relation, this is most probably due to cell killing by ionizing radiation as similar to the previously reported data (6).

Micronuclei:
Pearson correlation test which show positive correlation of Micronuclei frequency with dose with correlation coefficient (r) value 0.997 and the ionizing radiation was the leading cause of micronuclei

frequency in V79 cells, with significant P value 0.00, and a determining factor r^2 value is 0.994. Higher frequency of micronuclei was found in cells exposed to 15MV than that exposed to 6MV which indicated the presence of low dose of photoneutrons, and this may give a clue for late effect and the possibility of secondary cancer in long term survivors having radiotherapy.

Conclusion:

From this study we conclude that ionizing radiation is the leading cause of mammalian cell killing, decreasing cell survival curve, and the main cause that increased micronuclei frequency in V79cells. Comparing 6MV and 15MV photon beams we found that micronuclei frequency was higher in cells exposed to 15MV beam compared to cells exposed to 6MV which indicated the presence of low dose of photoneutrons.

References

1- **Suit, H.,** Goldberg, S., Niemierko, A., Ancukiewicz, M., Hall, E., Goitein, M., Wong, W., Paganetti, H., (2007). Secondary carcinogenesis in patients treated with radiation A review of data on radiation induced cancer in human, non human, primate Canine and rodents. Radiation Research. 167; 12-42.
2- **Hall, E. J.,** Phil D., (2006). Intensity-modulated radiation therapy, protons and the risk of second cancers. Int. J. Radiation Oncology Biol. Phys. 65; 1-7.
3- **Deutsch M.,** Gerszten, K., Bloomer, W., Avisar, E., (2001). Lumpectomy and Breast Irradiation for Breast Cancer Arising After Previous Radiotherapy for Hodgkin's disease or Lymphoma. 24; 33–34.
4- **Mateuca, R.,** Lombaert, N., Aka, V. P., Decordier, I., Volders, M. K., (2006). Chromosomal changes: induction, detection methods and applicability in human biomonitoring.
5- **Trott,** K. R., Teibe, A., Lack of specificity of chromosomes breaks resulting from radiation –induced genomic instability in Chinese hamster cells.

6- **Fenech, M.,** Chang, W. P. M., Volders, N. K., Zeiger, B. E., (2003) HUMN project detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. Mutation Research, 534; 65-75.
7- **Dickerman, J. D.,** (2007). The Late Effects of Childhood Cancer Therapy. 119; 554-568.
8- **Fenech M.,** (2000).The in vitro micronucleus technique Mutation Research. 455; 81–95.
9- **Bonassi, S.,** Znaor, A., Ceppi, M., Lando, C., Chang, W. P., Holand, N., Volders, M. K., Zeiger, E., Ban, S., Barale, R., Bigatti, M. P., Bolognesi, C., Wasilewska, A. C., Fabianova, E., Fucic, A., Hagmar, L., Joksic, G., Martelli, A., Migliore, L., Mirkova, E., Scarfi, M. R., ZijnoA., Norppa, H., Fenech, M., (2007). An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. 28; 625-631.
10- **Ulrich, W. M.**, Rode, A., (2006). The micronucleus assay in human lymphocytes after high radiation doses (5–15 Gy). Mutation Research, 502; 47–51.