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Cell Transformation and Radiation-induced Cancer

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Contents

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Chapter 9

Comparison of transformation efficiencies of gamma-rays, soft x-rays and alpha particles

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ABSTRACT: Soft and ultrasoft x-rays have been shown to be more effective than conventional x-rays or γ-rays in the inactivation of mammalian cells, in the production of chromosome aberrations, and in the induction of mutations, but there are, up to now, no data on their transformation efficiency. We have therefore in a comparative investigation determined, transformation rates of C3H 10T1/2 mouse embryo-fibroblasts after exposure to characteristic Cr-K_α x-rays, Co-γ-rays, and Am-α-particles. We have confirmed the enhanced effectivity of the soft x-rays with regard to cell inactivation, and we have found that approximately the same enhancement factor applies to the transformation efficiency. The relative biological effectiveness for soft x-rays versus γ-rays was approximately 1.3 in the range of soft x-ray doses from about 2 Gy to 5 Gy. There was no recognizable dependence of the RBE on dose, which contrasts with the finding for α-particles where we find RBE values of about 16 at an α-ray dose of 0.25 Gy and a substantially smaller value of about 6 at 1 Gy.

1. INTRODUCTION

Transformation studies have been performed with a variety of ionizing radiations of different ionization density. There are studies with sparsely ionizing γ-rays and conventional x-rays by Borek and Hall (1973), Terzaghi and Little (1976), Miller at al (1979), Han et al (1980), Miller and Hall (1978), and by other authors. The information about the transformation effectiveness of densely ionizing radiations has been derived from studies with α-particles (Lloyd et al 1979, Robertson et al 1983, Hall and Hei 1985, Hieber et al 1987), with neutrons (Borek et al 1978, Barendsen and Gaiser 1985, Hill et al 1979, 1984, 1985, Miller et al 1988, Balcer-Kubiczek et al 1988), and with heavy ions by Yang et al (1985) and Hieber et al (1989). But, up to now, there have been no data on the transformation effectiveness of soft x-rays, which are intermediate in ionization density and are of particular interest because of the highly localized energy deposition they produce.

The short ranged, monoenergetic electrons produced by soft x-rays have been found to be more effective than conventional x-rays in a variety of radiobiological studies. First results on the action of soft x-rays came from experiments with Drosophila (Timofeeff-Ressovsky 1934, Wilhelmy et al 1936, and Timofeeff-Ressovsky and Zimmer 1938, 1939), with procaryotic systems (Lea et al 1941, Lea and Smith 1942), and with plant cells (Neary et al 1964). Data on the effectiveness of various soft and ultrasoft x-rays for the inactivation of mammalian cells and for the induction of mutations and chromosomal aberrations were given by Goodhead and Thacker (1977), Goodhead et al (1979, 1981), Cox et al (1977), and Virsik et al (1977, 1980). As a general rule, the relative biological effectiveness increased with decreasing energy, i.e. with increasing ionization density of the x-rays.

The aim of the present study was to compare the effectiveness of chromium K_α x-rays of 5.4 keV with that of cobalt-γ-rays for the induction of neoplastic transformations in C3H 10T1/2 cells. A further comparison was made with americium α-particles.

2. MATERIAL AND METHODS

The transformation studies were performed with C3H 10T1/2 mouse-embryo fibroblasts, established by Reznikoff et al (1973). The cells were from a stock of Hall and Miller and were used in passages 12 to 14. The cells were maintained in Eagle's basal medium (BME, BRL Karlsruhe) supplemented with 10% foetal bovine serum (Boehringer Mannheim), 50 units/ml penicillin, and 50μg/ml streptomycin (BRL Karlsruhe). The cells were irradiated during exponential growth either in 25cm^2 flasks (Greiner Nürtingen) with γ-rays or in special dishes with a foil bottom (Hostaphan, Kalle Wiesbaden) of 2μm thickness with Cr-K_α x-rays or with α-particles.

γ-ray exposures were performed with a cobalt-60 unit at a dose rate of 0.5 Gy/min. The doses were determined with a therapy dosimeter unit (PTW, Freiburg).

The 5.4 keV chromium K_α characteristic x-rays were produced by a tube with chromium anode and berylium window (type AGCR61, Siemens). The tube was operated at 10kV and the radiation was filtered by a 20μm Cr foil to reduce the bremsstrahlung (Modler et al 1984). The dosimetry was performed with an ionization chamber (type M 23342) and the same dosimeter unit that was employed for the γ-rays. The dose rate was 0.43 Gy/min, the half value layer was 0.23 mm in water. The cells were exposed to soft x-rays through the bottom foil of the dishes. The dose distribution inside the cell and the cell nucleus is substantially constant; therefore, there was no need to apply corrections for changes of absorbed dose with depth, as they are essential in studies with ultrasoft x-rays, for example of the Al-K_α and the C-K_α line.

For the α-particles studies the cells were irradiated from an americium-241 source through the bottom foil of the dishes. The α-irradiator has been described in detail, elsewhere

(Hieber et al 1987, Roos and Kellerer 1986, 1989). Exposures were performed at a dose rate of 0.2 Gy/min. The most frequent energy of α-particles emerging from the bottom foil was 2.7 MeV, their dose mean unrestricted LET was 147 keV/μm.

After exposure the cells were trypsinized, counted, and plated for the survival and the transformation assays. Survival was determined by the ability of single cells to form colonies. The cell numbers for plating were chosen to attain about 80 colonies per 25 cm^2 culture flask after 12 days of incubation. The cells were fixed with methanol and stained with 10% Giemsa. Colonies with more then 50 cells were counted as survivors. In the transformation studies about 300 viable cells were plated in 25 cm^2 flasks, and the flasks were incubated for 6 to 7 weeks. After about 2 weeks of incubation with no medium change the cells reached confluency, subsequently the cells were re-fed once a week. Foci of type 2 and 3 were scored as transformants in the fixed and Giemsa-stained samples. Further technical details have been reported earlier (Hieber et al 1987).

3. RESULTS AND DISCUSSION

3.1 Survival Studies

In the studies with γ-rays and Cr-K_α characteristic x-rays survival curves for C3H 10T1/2 cells with pronounced shoulders were obtained, whereas the experiments with α-particles lead to purely exponential relations $-\ln S(D) = \alpha D$, with $\alpha = 1.65/Gy$ (see Figure 1). The survival curves for the γ-rays and soft x-rays were consistent with the linear-quadratic equation $-\ln S(D) = \alpha D + \beta D^2$ with the values $\alpha = (0.158 \pm 0.027)/Gy$ and $\beta = (0.040 \pm 0.005)/Gy^2$ for Co-γ-rays and $\alpha = (0.235 \pm 0.019)/Gy$ and $\beta = (0.060 \pm 0.004)/Gy^2$ for the soft x-rays. The mean inactivation doses, $\bar{D}$, were 2.99 Gy and 2.27 Gy for the γ-rays and the soft x-rays; the D_{37} (= $\bar{D}$) for α-particles was 0.606 Gy.

It is difficult to recognize from the data for soft x-rays and γ-rays a possible dose dependence of the relative biological effectiveness. Estimating an overall value from the ratio of the mean inactivation doses one obtains the relative biological effectiveness 1.3 for the soft x-rays versus γ-rays, and the data are not inconsistent with a constant RBE of this magnitude. On the other hand, one obtains from the linear-quadratic fit the slightly larger RBE of 1.5 and a value of 1.28 at a γ-ray dose of 7 Gy. For the α-particles one estimates an RBE versus γ-rays of 10.4 at low doses and a value of 3.7 at an α-ray dose of 2 Gy. In survival studies with Cr-K_α x-rays and with other mammalian cells of rodent and human origin we have found RBE values versus γ-rays that appear to be slightly larger than the value 1.3 for 10T1/2 cells (unpublished results). A quantitative comparison to the variety of data obtained by different authors with soft x-rays between 0.3 keV and 18.5 keV for the inactivation of human and Chinese hamster cells (Raju et al 1987, Goodhead and Thacker 1977, Cox et al 1977, and Hoshi et al 1988) shows that our RBE values fit the general trend of the dependence on x-ray energy.

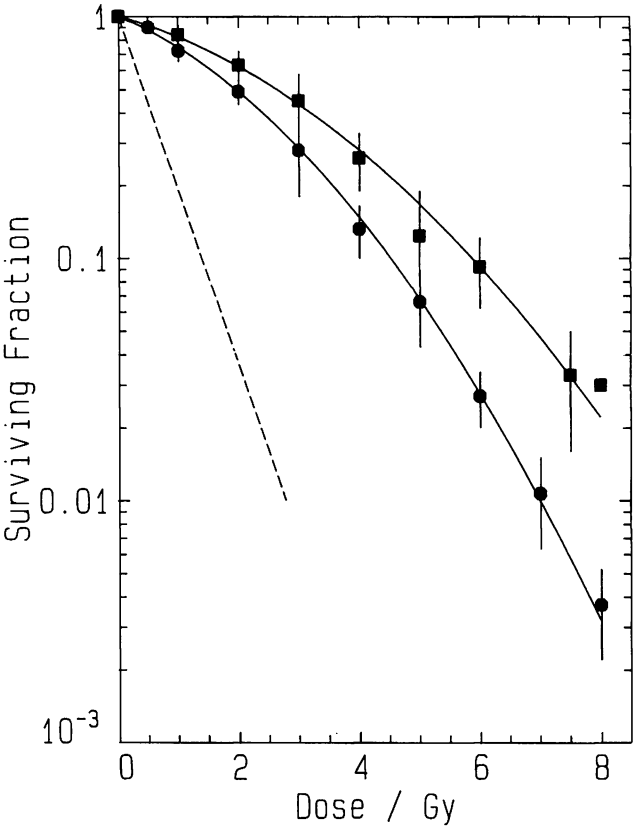

Fig.1. Inactivation of 10T1/2 cells by cobalt-γ-rays (squares), Cr-K_α x-rays (circles), and α-particles (broken line).

3.2 Transformation Studies

The induction of cell transformations by Cr-K_α x-rays and Co-γ-rays was studied in the dose range from 1 to 7 Gy and 1 to 8 Gy, respectively, i.e in a dose range where the surviving fraction of C3H 10T1/2 cells decreased to about 1 and 3 per cent. Transformation experiments with α-particles were performed over the dose range from 0.125 to 3 Gy, i.e at surviving fractions down to about 1 per cent.

The results in Figure 2 show that Cr-K_α x-rays induce transformations more effectively than γ-rays, and they suggest further that the relative biological effectiveness for transformations is nearly the same as that obtained in the inactivation studies. A constant value of 1.3 is consistent with the data over the entire dose range that has been utilized. There is no indication of a dose dependence of the relative biological effectiveness; the statistical uncertainties at low doses do not permit to exclude a certain change of the RBE at low

doses. The finding of nearly the same RBE values for cell inactivation and transformation applies essentially also to the α-particle studies. At an α-particle dose of 1 Gy one obtains an RBE of about 6 versus γ-rays that is similar to the value inferred in the inactivation studies (RBE≈5). There is, on the other hand, an indication that the RBE varies somewhat more strongly with dose in the transformation studies than in the survival studies. This corresponds to the consistent observation in our transformation studies that the transformation yield for α-particles is nearly proportional to the square of the dose, while the frequency of transformations after exposure to soft x-rays or γ-rays increases with a substantially higher power of dose in excess of 4.

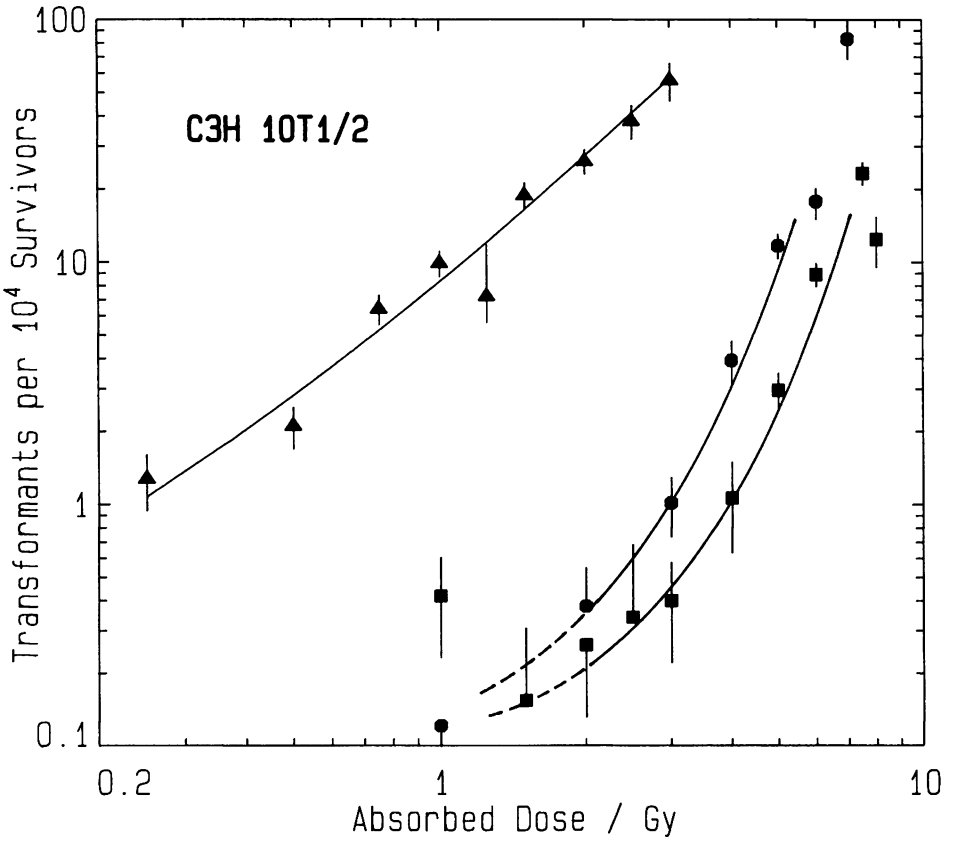

Fig.2. Transformation frequencies per surviving cell after exposure to Co-γ-rays (squares), Cr-K_α x-rays (circles), and α-particles (triangles). (Note the scale difference by a factor of two between abscissa and ordinate).

The essential result of our soft x-ray studies is that electrons of low energy and of ranges less than 1 μm are more effective than fast electrons, not only for cell inactivation but also for cell transformation. This finding is in agreement

with earlier data on the enhanced effectiveness of soft and ultrasoft x-rays for cell inactivation (Goodhead et al 1977, 1981, Cox et al 1977, Raju et al 1987, Hoshi et al 1988), micronucleus induction (unpublished data from our laboratory), the induction of DNA double strand breaks (Frankenberg and Binder 1985), and the production of chromosome aberrations (Virsik at al 1977, 1980). An extension of these studies would be required to establish the trend with x-ray energy and to determine whether it parallels the one obtained for other types of cellular damage.

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REFERENCES

Balcer-Kubiczek E K, Harrison G H, Zeman G H, Mattson P J and Kunska A 1988 *Int. J. Radiat. Biol.* 54 531
Barendsen G W and Gaiser J F 1985 *Radiat. Protest. Dosim.* 13 145
Borek C and Hall E J 1973 *Nature* 243 450
Borek C, Hall E J and Rossi H H 1978 *Cancer Res.* 38 2997
Cox R, Thacker J and Goodhead D T 1977 *Int J. Radiat. Biol.* 31 561
Frankenberg D and Binder A 1985 *Radiat. Protect. Dosim.* 13 157
Goodhead D T and Thacker J 1977 *Int. J. Radiat. Biol.* 31 541
Goodhead D T, Thacker J and Cox R 1979 *Int. J. Radiat. Biol.* 36 101
Goodhead D T, Thacker J and Cox R 1981 *Phys. Med. Biol.* 26 1115
Hall E J and Hei T K 1985 *Radiat. Protect. Dosim.* 13 149
Han A and Elkind M M 1979 *Cancer Res.* 39 123
Han A, Hill C K and Elkind M M 1980 *Int. J. Radiat. Biol.* 37 585
Hieber L, Ponsel G, Roos H, Fenn S, Fromke E and Kellerer A M 1987 *Int. J. Radiat. Biol.* 52 859
Hieber L, Ponsel G, Fenn S and Kellerer A M 1989 *Adv. Space Res.* in press
Hill C K, Buonoguro F M, Myers C P, Han A and Elkind M M 1982 *Nature* 298 67
Hill C K, Han A and Elkind M M 1984 *Int. J. Radiat. Biol.* 46 11
Hill C K, Carnes B A, Han A and Elkind M M 1985 *Radiat. Res.* 102 404
Hoshi M, Antoku S, Nakamura N, Russell W J, Miller R C, Sawada S, Mizuno M and Nishio S 1988 *Int. J. Radiat. Biol.* 54 577
Lea D E and Smith K M 1942 *Parasitology* 34 227
Lea D E, Haines R B and Bretscher E 1941 *J. Hyg.* 41 1
Lloyd E L, Gemmell M A, Henning C B, Gemmell D S and Zabransky B J 1979 *Int. J. Radiat. Biol.* 36 467
Miller R C and Hall E J 1978 *Nature* 272 58

Miller R C, Hall E J and Rossi H H 1979 *Proc. Nat. Acad. Sci. USA* **76** 5755
Miller R C, Brenner D J, Geard C R, Komatsu K, Marino S A and Hall E J 1988 *Radiat. Res.* **114** 589
Modler H, Blohm R, Hermann K-P and Harder D 1984 *Med. Physik* ed T Schmidt pp 375-8
Neary G J, Savage Y R K and Evans H J 1964 *Int. J. Radiat. Biol.* **8** 1
Raju M R, Carpenter S G, Chmielewski J J, Schillaci M E, Wilder M E, Freyer J P, Johnson N F, Schor P L, Sebring R J and Goodhead D T 1987 *Radiat. Res.* **110** 396
Reznikoff C, Bertram J, Brankow D W and Heidelberger C 1973 *Cancer Res.* **33** 3239
Robertson J B, Koehler A, George J and Little J B 1983 *Radiat. Res.* **96** 261
Roos H and Kellerer A M 1986 *IMSK* **108**
Roos H and Kellerer A M 1989 *Phys. Med. Biol.* submitted
Roos H, Thomas W-H, Fitzek M and Kellerer A M 1988 *Radiat. Res.* **116** 292
Terzaghi M and Little J B 1976 *Cancer Research* **36** 1367
Timofeeff-Ressovsky N W 1934 *Biol. Rev. Cambridge* **9** 411
Timofeeff-Ressovsky N W and Zimmer K G 1938 *Naturwissenschaften* **26** 108
Timofeeff-Ressovsky N W and Zimmer K G 1939 *Strahlentherapie* **66** 684
Virsik R P, Harder D and Hansmann I 1977 *Radiat. Environ. Biophys.* **14** 109
Virsik R P, Schäfer C and Harder D 1980 *Int. J. Radiat. Biol.* **38** 545
Wilhelmy E, Timofeeff-Ressovsky N W and Zimmer K G 1936 *Strahlentherapie* **57** 521
Yang T, Craise L, Mei M and Tobias C 1985 *Radiat. Res.* **104S** 177