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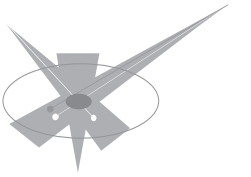

Original Paper

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Effect of depth on radiation-induced cell damage in a water phantom

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Summary

Aim The paper describes the effect of medium depth on radiation-induced genetic damage to SK-mel cultured cells placed inside a water phantom as measured by the number of cells with micronuclei formed and apoptoses seen in these cells.

Materials/Methods The energy of photon radiation used was 6 MV; cells placed at various depths were irradiated with the same dose (5Gy).

Results/Conclusions Depending on medium depth, differences were noted in the number of damaged cells: ca.30% more affected cells were seen at a depth of 25 cm as compared to a depth of 5 cm. With increasing depth also the radiation energy spectrum changes. This is probably responsible for the observed effect. It should be taken into consideration in both clinical practice and in treatment planning.

Key words **scattered radiation • energy spectrum • cytogenetic damage**

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Background

Linear electron accelerators generate photon and electron radiation with a nonmonoenergetic spectrum. In a medium such as water (assumed equivalent to human soft tissues) both photon and electron ionizing radiation is scattered. The scattering increases with penetration depth [1]. The energy of scattered radiation is lower than that of the incident beam. It is known that the biological effectiveness of ionizing radiation depends on its energy; the lower the energy the greater its biological effectiveness [2]. An identical dose can bring about different biological effects depending on the energy delivered. In other words, the same biological effect can be seen for varying doses, depending on delivery energy. This relationship has been expressed in terms of RBE (Relative Biological Effectiveness) [3]. Ionizing radiation causes damage to cellular DNA that leads to structural changes such as the appearance of micronuclei. Micronuclei originate from acentric chromosome fragments or from whole chromosomes that were not incorporated, during cell division, into the nuclei of daughter cells. It also induces cell death *via* apoptosis [4,5]. Both appearance of micronuclei and apoptosis are genetic events that lead to cell death [4,6,7].

Available literature data [5,8–11] point to a relationship of radiation-induced damage with both the energy and type of ionizing radiation.

Radiation generated by linear electron accelerator is nonmonoenergetic [12,13]. In addition, energy broadening increases with penetration depth in the irradiated medium, for example water (Figure 1). With increasing penetration depth the number of interactions with the surroundings also increases and so does the fraction of radiation that becomes scattered. A larger portion of scattered radiation with energy lower than that of the incident beam, should increase the fraction of damaged cells. For example, following administration of a 5 Gy dose at depths of 5 cm and 25 cm, the respective fractions should differ.

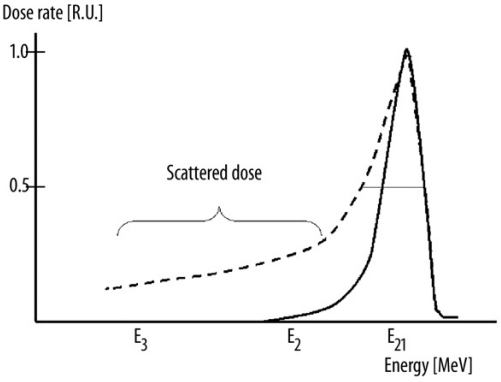

Figure 1. The distribution of radiation dose generated by a linear electron accelerator, at various depths (in water). The solid line shows energy distribution at smaller depth compared to that depicted by the dotted line. At a greater depth the fraction of radiation with lower energy (E_3) is increased. Energy (E_1)>(E_3) [1].

Aim

The purpose of this study assess the damaging effect of scattered radiation upon cancer cells that had been placed in a medium assumed to be equivalent to human soft tissues.

Materials

An SK-mel cell line derived from human colon carcinoma was used throughout this study. The melanotic human melanoma cell line (SK-MEL) was donated by Dr. K. Urbanska from Jagiellonian University in Cracow. The water phantom was equipped with suitable cell culture flask holder. A Clinac 600 CD linear electron accelerator, with 6 MeV maximum photon energy served as a radiation source.

Methods

SK-mel cells were grown routinely in monolayer cultures using Ham's F-10 medium (Sigma) supplemented with 10% fetal bovine serum (FBS) (GIBCO), 1% penicillin (10000 UI/ml) and streptomycin (10 mg/ml). The cultures were incubated at 37°C in humid atmosphere containing 5% CO_2. Exponentially growing cultures were trypsinized. Trypsin was

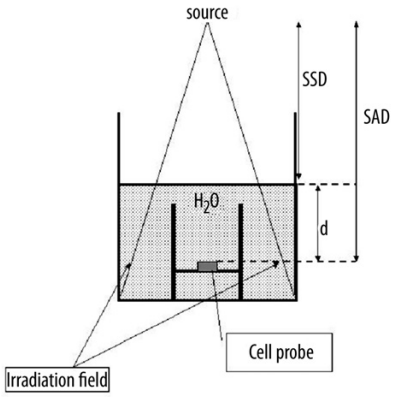

Figure 2. The experimental system. A culture flask containing growing cells is placed in a water phantom at varying depths but always at the same distance from the radiation source. Cells are always irradiated using an identical dose (5 Gy) irrespective of penetration depth. Irradiation area is held constant. SAD – Source Axis Distance, SSD – Source Skin Distance, d – depth.

inactivated by addition of an equal volume of medium. Cells were centrifuged and resuspended in fresh medium at the concentration of 2×10^5/ ml. 24 hours following the last passage cell cultures were exposed to radiation. Subsequently, the cultures were incubated for 48 hours at 37°C in 5% CO_2. The choice of this time point was based on the results of preliminary experiments. The induction of micronuclei and apoptosis was optimal at 48 h of incubation; longer induction caused cell detachment. Cells were then collected, washed with PBS, and slides for microscopy studies were prepared. Air-dried preparations were fixed for 20 min. in a cold solution of 1% glutaraldehyde in 1/15 M phosphate buffer (pH=7.5). Slides were rinsed in distilled water and were stained according to the Feulgen method.

Genetic alterations in cells exposed to radiation as well as in controls (unexposed cells) were expressed as the fre-

Table 1. Cellular damages produced by 100 MU/min. and 600 MU/min. dose rate; mean of three measurements.

Dose rate: 100 MU/min.		Cells with micronuclei [per thousand] mean range		Cells undergoing apoptosis [per thousand] mean range		Total cell damage [per thousand]
	Control	**6**	**(5–7)**	**0.7**	**(0–1)**	**6.7**
Depth (cm)	5	61.3	(58–66)	8.3	(8–9)	69.6
	10	80.7	(79–83)	14	(11–18)	94.7
	15	133.7	(126–141)	28	(26–29)	161.7
	20	165	(158–174)	40	(36–40)	205.0
	25	195.7	(183–210)	42.7	(40–45)	238.4
Dose rate: 600 MU/min.	**Control**	**6**	**(5–7)**	**0.7**	**(0–1)**	**6.7**
Depth (cm)	5	50	(46–54)	6.7	(6–8)	56.7
	10	72	(69–77)	11.6	(9–15)	83.6
	15	106	(96–113)	23	(8–27)	129.0
	20	137	(130–147)	31	(29–31)	168.0
	25	152	(142–168)	36	(32–38)	188.0

Table 2. Micronuclei formation and apoptosis for medium depths of 5 and 25 cm. varying dose rates [Gy/MU] and irradiation times [min].

Depth [cm]	Dose rate [MU/min]	Irradiation time [min]	Dose rate [Gy/min]	Cells with micronuclei [per thousand] mean range		Cells undergoing apoptosis [per thousand] mean range		Total cell damage [per thousand]
5	100	4.92	1.02	68	(66–71)	16.5	(15–18)	84.5
5	200	2.46	2.03	72.5	(68–77)	15.5	(14–17)	88.0
25	300	3.05	1.64	92.5	(90–95)	29.5	(29–30)	122.0
25	600	1.53	3.28	93.5	(89–98)	26.5	(25–28)	120.0

quency of cells forming micronuclei and the frequency of cells showing signs of chromatin condensation, characteristic for apoptotic processes. Microscopic preparations were analyzed using criteria described by Abend et al., (1995) [6] and Guo et al., (1998) [14]. Each experiment was performed three times; to obtain each datapoint 1000 cells were scored for the presence of micronuclei and apoptotic bodies. Total cell damage was also calculated and it represents the sum of cells exhibiting micronucleation and apoptotic events (Abend et al., 1995). The control values were not subtracted.

Flasks with growing cells were initially placed in a water phantom at the following depths: 5, 10, 15, 20 and 25 cm, while keeping the 100-cm distance from the radiation source constant (i.e. using the SAD irradiation technique), irrespective of penetration depth. The area irradiated was 35×35 cm in isocentre position. The radiation dose was 5 Gy, the same for each penetration depth (Figure 1). Measurements were made for two dose rates: 100 MU/min. and 600 MU/min. In the remaining experiments cells were irradiated using only two depths (5 and 25 cm) and varying dose rates in order to obtain comparable irradiation times (in min.). Under such conditions the dose rates (doses delivered within a given time period) at the 5- and 25-cm depths were comparable (Figure 2).

Results

The results are presented in the Tables. Table 1 shows data concerning number of cells with formed micronuclei and cells showing signs of apoptosis, both in relation to medium depth and for 100 MU/min dose rate (MU – Monitor Units) (Table 1) and for 600 MU/min dose rate.

Table 2 contains data concerning micronuclei formation and apoptosis, obtained for equal dose rates at depths of 5 and 25 cm so that irradiation times become comparable.

Figure 3 shows average numbers of cells with micronuclei and apoptosis as a function of the medium depth. The performed measurements indicate that the number of cells with formed micronuclei depends not only on the initial dose rate but that it also increases with medium depth.

With increasing depth in water the radiation dose rate decreases. Therefore, in order to apply identical doses, the irradiation period must be extended. For example, if a dose

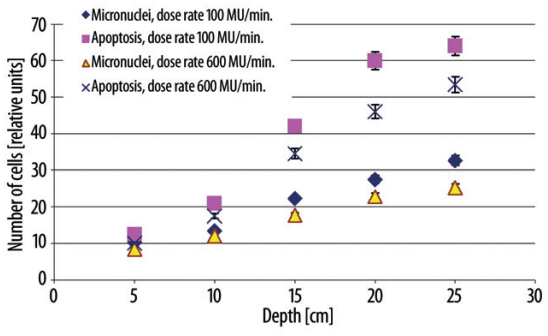

Figure 3. Number of cells with micronuclei and apoptosis as a function of medium depth for 100 MU/min and 600 MU/min dose rates. $p<0.05$ according to Friedman's Repeated Measures ANOVA; Kendall's tau >0.9. Each point represents the mean value of three experiments.

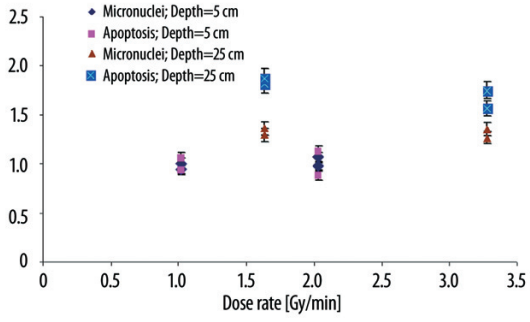

Figure 5. Number of cells forming micronuclei and apoptosis as a function of dose rate at the indicated depth in the water phantom.

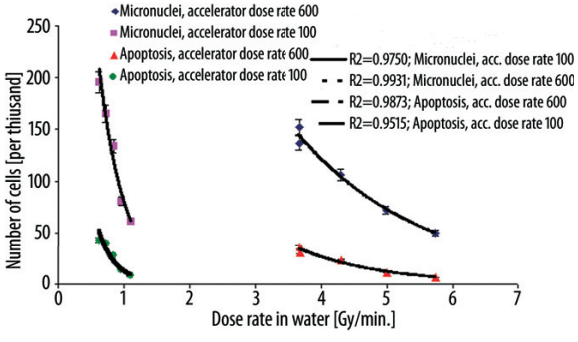

Figure 4. The number of cells with micronuclei and apoptosis as function of "real" dose rates at various water depths for two different (100 and 600 MU/min.) accelerator dose rates of generated radiation. Pearson's correlation (R2) is >0.95 for all four schemes analyzed.

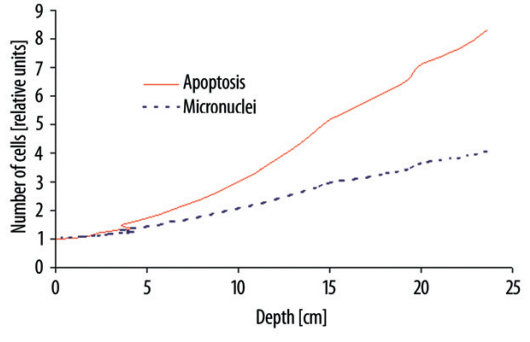

Figure 6. The number of cells with signs of irradiation-induced micronuclei and apoptosis as a function of medium depth. Statistical analysis by nonparametric ANOVA (Friedman's test) and Kendall's tau shows a correlation between the number of cells with micronuclei formed and apoptoses ($p<0.00005$).

of 5 Gy is applied at a depth of 5 cm (for 100 MU/min dose rate), then the irradiation time required is 4.57 min. In order to reach the equivalent dose at a depth of 25 cm, irradiation requires 8.16 min. From the value of the absolute dose rate (2.7 Gy/min) for SAD=100 cm, a depth of 5 cm and a 10x10 cm surface area, one can calculate dose rates at a given depth for both 100 or 600 MU/min dose rates of incident radiation. This relationship is illustrated in Figure 4 (formation of micronuclei and apoptosis).

Figure 5 shows numbers of examined cells exhibiting signs of micronuclei or with apoptosis present following irradiation at medium depths of 5 and 25 cm. Varying dose rates and irradiation times were chosen in order to assure identical doses at these depths but different dose rates [Gy/min]. Statistical analysis using the non-parametric Kendall's tau test revealed no correlation between dose rate and the number of micronuclei and apoptoses (correlation coefficent = 0.27; $p<0.13$).

If the number of cells showing micronuclei or the number of cells showing apoptosis found for the 100 MU/min. dose rate are extrapolated to a dose rate of 600 MU/min. one obtains a difference reflecting the energy spectrum change as a function of medium depth (Figure 6).

Measurements made at depths of 5 and 25 cm indicate that, although a comparable dose rate (5 Gy in 3 minutes) was used, the resulting damage to cells located at a 25-cm depth is 30% greater than at a 5-cm depth. The above conditions were assumed to have assured that differences in cell survival (based on formation of micronuclei or apoptosis) were associated only with medium depth or, more precisely, with respective differences in the radiation energy spectrum. At a depth of 5 cm the portion of scattered radiation is substantially smaller than at a depth of 25 cm.

Conclusions

The results of measurements make it clear that increased depth of placement in the phantom medium is related to increased numbers of damaged cells. Also, with increasing depth the radiation energy spectrum is changed and less energy is delivered, which is probably responsible for the observed effect. This should be taken into consideration in both clinical practice and in treatment planning. Further measurements are required to characterize various conditions of both electron and photon irradiation. One might expect the observed effect to depend on the magnitude as well as the type of energy initially delivered.

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