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ENHANCEMENT OF RADIATION SENSITIVITY IN IODODEOXYURIDINE LABELLED CELLS EXPOSED TO LOW ENERGY X-RAYS

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Abstract

In order to investigate if low-energy x-rays induce Auger cascades by photoelectric absorption in iodine present in DNA, CHO cells were labelled with iododeoxyuridine (IUdR) for 72 hours. Following labelling, the cells were either irradiated with low-energy x-rays (75 kV, 4 mm Al) or ^{137}Cs-gamma-rays. The radiation response was measured using clonogenic survival, and the survival parameters were analyzed according to the linear quadratic model. The dose modifying factors were determined as the ratios of the α-coefficients. The IUdR labelled cells were found to be about 3.2 times as sensitive as the control cells when irradiated with low-energy x-rays. For ^{137}Cs-gamma the ratio was about 1.5. The standard deviations were estimated by Gauss' approximation to be about 0.5 for both irradiation conditions.

Key words: Radiobiology, iododeoxyuridine, clonogenic survival, x-rays.

Halogenated thymidine analogues such as 5-bromodeoxyuridin (BrUdR) and 5-iododeoxyuridine (IUdR) incorporated into DNA of mammalian cells is known to sensitize the cells to sparsely ionizing radiation (1–7). The potential of using these compounds as clinical radiosensitizers has been proposed and some clinical trials have been performed (8–10). However, the results obtained have been partly discouraging which may depend on poor drug delivery techniques and difficulties in selecting suitable types of tumors (11, 12). Additionally, the fundamental mechanism of radiosensitization is unknown, which may impair the possibilities to design better optimal clinical studies.

A new approach in using IUdR in radiation therapy has been suggested by Fairchild et al. (13, 14). The technique, called photon activation therapy, PAT, involves irradiation of cells, prelabelled with IUdR, with photons of energies slightly above the K-shell electron binding energy of iodine. These photon-energies will cause a higher probability for the photoelectric effect in the K-shell of iodine especially, yielding higher energy absorption in the iodine atom compared to the surrounding atoms (13). In addition, and probably more important, the absorption event may create a K-shell vacancy, thus stimulating emission of cascades of Auger electrons similar to the decay of ^{125}I.

Disintegration of ^{125}I by electron capture leads, on the average, to the emission of about 21 low-energy electrons, most of them with very short ranges in tissues (15, 16). In fact, the mean energy deposition from an ^{125}I decay has been calculated to be 335 eV within a sphere of 1 nm radius from the site of the decay (15). This highly localized energy absorption is potentially very effective in causing subcellular damage in a critical cell structure. The radiotoxicity of ^{125}I incorporated into DNA as ^{125}IUdR in mammalian cells has been manifested by measurements of DNA strand breaks (17, 18), chromosomal aberrations (19), cell survival (20, 21) and induction of mutations (22).

The number of electrons emitted when filling the K-shell vacancies in iodine has been suggested to be about 10–12, i.e. half of an ^{125}I-decay (14). This high number of electrons emitted in the proximity of DNA would give rise to severe biological effects. Survival studies have shown IUdR-labelled cells to be more sensitive to x-irradiation than normal cells, but most data are obtained with x-rays produced at high tube voltages (e.g. 23, 24). The probability of producing K-shell vacancies will increase using low energy x-ray photons, as suggested by Fairchild et al. (13, 14).

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In order to evaluate the potential of PAT we have investigated the survival of CHO cells labelled with IUdR and irradiated with low-energy x-rays or high energy gamma radiation (^{137}Cs).

Material and Methods

Chinese hamster ovary cells, CHO, were maintained in Ham's F10 medium supplemented with 10% newborn calf serum and antibiotics. The cells were grown as monolayers at 37°C in 5% CO_2:95%. The experimental cultures were grown in thymidine-depleted F-10 medium containing 10^{-6} mol/l IUdR for three days in darkness. The IUdR solution was stored frozen in a weak alkaline stock solution of 1 mmol/l.

In order to estimate the amount of IUdR uptake, cells were incubated with ^{125}IUdR of known specific activity for 72 h. Following incubation, the DNA content and the incorporation of ^{125}IUdR per cell were determined. From the DNA content data the number of thymidine moieties was calculated. The replacement of thymidine with IUdR under these circumstances was theoretically estimated to be approximately 10%.

X-irradiation was performed with a Siemens Stabilipan therapy x-ray unit, 75 kV, 20 mA with a total filtration of 4 mm Al. The absorbed dose and radiation quality were measured using an ionization chamber. The absorbed dose was also measured with thermoluminescence dosimeters (TLD)(Harshaw Extruded LiF) which were placed in the same geometry as the cells.

Cells were trypsinized for 5 min in 0.25% trypsin, suspended in medium in small test tubes, irradiated on ice and then immediately plated into 50 mm plastic dishes.

Cells were gamma-irradiated at room temperature with a ^{137}Cs source (Scanditronix, Uppsala, Sweden) at a dose rate of 0.8 Gy per min. In these experiments the cells were trypsinized, diluted and plated in 50 mm plastic dishes, and then incubated at 37°C for at least 2 h prior to irradiation.

The number of cells plated was chosen so that 100–200 colonies would appear, taking into account loss of survival due to the radiation dose. After 7–8 days of incubation, cells were fixed and stained with methylene blue and colonies were counted.

Results

The half-value layer (HVL) for the 75 kV x-rays with a total filtration of 4 mm Al was measured to be 4.3 mm Al. This corresponds to a mean photon energy of 38 kV. The average dose rate in the experiments was measured to be 0.16 Gy per min.

The dose-survival data were analyzed using the linear quadratic model

$$Sf = e^{-(\alpha D + \beta D^2)}$$

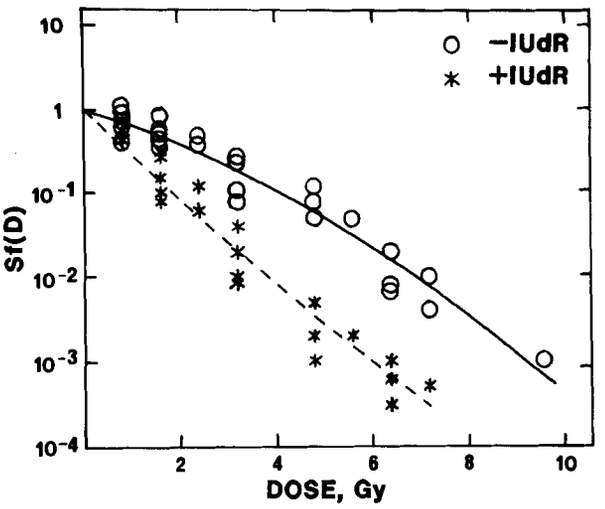

Fig. 1. Survival curves of CHO cells after exposure to various doses of 75 kV x-rays. Cells had been labelled with 10^{-6} mol/l IUdR for 72 h before irradiation. The different curves represent control cells (○) and IUdR labelled cells (✳). The survival data were analyzed according to the linear-quadratic model.

where Sf is the surviving fraction at absorbed dose D, α is the linear, and β is the quadratic inactivation coefficient (25). Fig. 1 shows the estimated survival curves of IUdR labelled and control cells irradiated with low-energy x-rays at 0°C. The major difference between the two curves was found in the shoulder region, i.e. between the α-coefficients. Compared to control cells, IUdR-labelled cells showed an almost linear decrease in the survival over the entire dose range. In the Table the coefficients, α and β, estimated by OLS (Ordinary Least Squares) and their standard deviations are presented. The difference between the IUdR labelled and the control cells was significant at 5% level.

The estimated value of the β-coefficient (Table) is negative for x-irradiated IUdR labelled cells although not significantly different from zero. This change in sign may be caused by fraction of cells in the original cell population that had incorporated less IUdR, resulting in a slightly positive slope of the curve at higher doses (23).

Table

The constants α and β of the equation, $Sf = e^{-(\alpha D + \beta D^2)}$ with standard deviation, for +/− IUdR-labelled cells following x- or γ-irradiation

Treatment	Irradiation	α	SD	β	SD
−IUdR	x-ray	0.40	0.062	0.038	0.0092
+IUdR	x-ray	1.27	0.11	−0.022*	0.018
−IUdR	^{137}Cs-gamma	0.18	0.057	0.013	0.006
+IUdR	^{137}Cs-gamma	0.27	0.071	0.018	0.008

All estimates except (*) were considered significantly different from zero at 5% level.

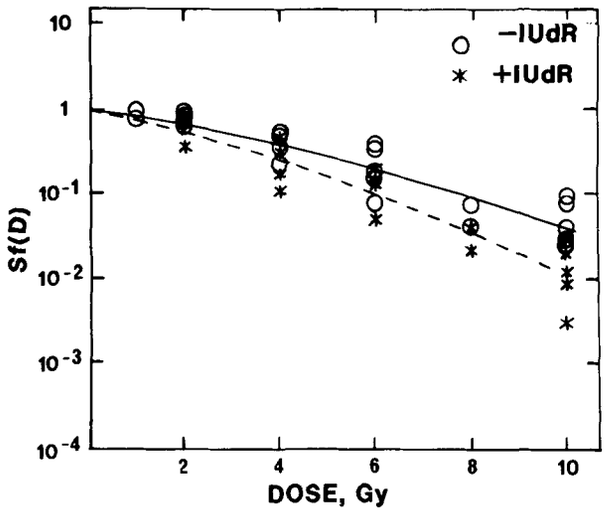

Fig. 2. Survival curves of ^{137}Cs-gamma irradiated CHO cells: control cells (○); cells labelled with 10^{-6} mol/l IUdR for 72 h (*) before irradiation. The linear quadratic model were used to analyze data.

The effect of IUdR on survival following gamma-irradiation was less than after x-irradiation as depicted from Fig. 2. In this case there are no significant difference in the radiation response of the two groups.

The dose modifying factor (DMF) defined as the ratio

$$\alpha_{\text{IUdR}}/\alpha_{\text{control}}$$

was calculated to be 3.2 for x-irradiated cells, with a standard deviation of 0.5 as estimated by Gauss' approximation. For gamma irradiation the DMF estimated in the same way was found to be about 1.5 and the standard deviation 0.5.

Discussion

The incorporation of IUdR into DNA of CHO cells was found to sensitize the cells to low energy x-radiation. The degree of radiosensitization was much less for gamma irradiated IUdR-labelled cells. The incorporation of IUdR was assumed to be analogous with both types of radiation since the IUdR concentrations and the duration of the labelling period were the same for the two irradiation procedures. In contrast cells were treated differently during the irradiations but this could scarcely have any significant effect on the obtained α-quotients. The survival of IUdR labelled cells was in all experiments slightly affected, resulting in a 10–20% reduction of plating efficiency.

The level of the DMF includes both the effects of chemical sensitization by IUdR alone and the photon activation process yielding the Auger effect. It is assumed that the major part of the damage by gamma irradiation of the cells may be due to the chemical sensitization since most photons have energies that are too high to stimulate release of Auger electrons. Others have reported a DMF of about 1.5 for chemical sensitization with IUdR which is in accordance with our observations (4, 13, 26). In principle, neglecting a possible contribution from low energy photons present in the field from high energy radiation sources might give rise to an overestimation of the chemical sensitization.

For x-irradiation of the cells at low average energies the DMF increased to 3.2. This would indicate that the radiation damage is accomplished by some other mechanisms than those occurring as a result of ^{137}Cs-gamma irradiation. The α-coefficient in the linear-quadratic model is supposed to account for the formation of double-strand breaks in one single radiation event, implicating higher values of the α-coefficient for high LET radiations, i.e. in case of high local energy transfer (25). It is thus conceivable to believe that the radiation response of the x-irradiated IUdR labelled cells involves a similar component due to the high energy transfer. Other possible interpretations of the results can be offered, such as impairment to the repair of DNA damages representative of low LET radiation. However, the major difference between the curves was the loss of the shoulder for IUdR labelled cells which points to the occurrence of severe DNA damage. The possibility that different photon energies would interfere differently with the DNA repair mechanism seems unlikely.

The assumption that x-irradiation of IUdR labelled cells induces critical DNA lesions for cell lethality has also been suggested by Shinohara et al. (27). They have demonstrated that the rate of radioprotection by cysteamine of IUdR labelled cells, changed from 82% for x-rays (average energy 40 keV) to 89% for ^{60}Co-gamma-rays which is indicative of different damage following x-irradiation.

The radiosensitization of IUdR is expected to increase almost linearly with the degree of thymidine replacement (4). However, variations in the incorporation and replacement of thymidine by IUdR may occur during the experiments. It has been shown that IUdR are excised from DNA of mouse tongue keratinocytes with a turnover time of 3.8 days (28). High concentrations of sera seem to accelerate the degradation of IUdR (29). We have some indications that this process also occurs in CHO-cells, but at a much lower degree (K. J. Johanson, unpublished results).

We conclude that IUdR labelling sensitizes the cell to radiation. The effect seems to be more pronounced for soft x-rays than for hard gamma-rays as determined by the ratio of the α-factors. The probable reason for this higher sensitivity might be a high local energy transfer when IUdR-labelled cells are irradiated with low-energy x-ray photons.

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