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9. Effects of Auger-electron-emitting Radionuclides on Human Cells

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Introduction

Auger-electron-(AE)-emitting radionuclides have the potential to be used in adjuvant therapy to augment the clinical efficacy of the conventional radiotherapy of tumors [4]. The main reasons for this are the emission of the large number (average 21 per decay in the condensed phase) of low-energy electrons [1] (from a few eV up to some tens of keV) and their extremely short range (from a few nm up to 10–20 μm). Therefore, when AE-emitting radionuclides such as ^{125}I are present in cells of neoplastic tissue, they would significantly damage these cells and spare the cells of the surrounding normal tissue. However, the clinical superiority of ^{125}I-based radiotherapy would depend on how efficiently the radionuclide is transported to the critical targets of the cells, for example, DNA [6]. Several ^{125}I-bound carriers are currently under examination to evaluate their biological efficiency.

The transport of AE-emitting radionuclides is also critical for proper dosimetry. For dosimetric calculation, ^{125}I nuclides would create problems if they were exclusively incorporated into DNA and the doses should be applicable for the cellular nucleus or the entire cell. The definition of radiation dose (energy deposition per mass of target) necessitates the energy being homogeneously distributed in the target. If ^{125}I is incorporated into DNA one can only determine the number of decays per target valid for the nuclide during a certain time range. Therefore, a suitable carrier molecule must be used which uniformly distributes ^{125}I inside the nucleus or the whole cell.

This investigation was designed to evaluate three different carriers for ^{125}I-induced cell damaging efficiency and dosimetric calculations. The experiments were carried out with asynchronous cultures of human kidney T1 cells at 37°C (physiological conditions). The following ^{125}I-labelled carrier molecules were used:

- ^{125}Iododeoxyuridine (^{125}IUdR), which would be incorporated into the DNA of S-phase cells,
- ^{125}Iodoantipyrine (^{125}I-AP) which would be distributed uniformly inside the cell, and
- Na ^{125}I, which would remain extracellularly located.

The damage induced by ^{125}I was measured by two biological end points: survival assay (clonogenic survival) and Comet assay (single cell electrophoresis) [2]. These ^{125}I-induced effects were compared with that found after ^{137}Cs-γ-irradiation.

Materials and Methods

Incorporation of ^{125} IUdR

Exponentially growing monolayer cultures of kidney T1 cells were incubated at 37°C and 5 percent CO_2 for 30 hours (approx. one population doubling time) with various ^{125}I concentrations of $^{125}IUdR$ (0, 74–15 kBq/ml medium). During this period of time the medium was renewed twice to prevent saturation of $^{125}IUdR$ uptake which was found to start between 8 and 10 hours. Fluorodeoxyuridine (FUdR) was added (10^{-8} M) to the medium to promote the incorporation of $^{125}IUdR$ into DNA by inhibition of the cellular thymidilate synthetase. FUdR increased $^{125}IUdR$ incorporation by a factor of ten. After the incubation period a single cell suspension was prepared and aliquots were measured in a γ-counter to calculate the ^{125}I decays per cell.

Exposure to ^{125}I-AP

Cells (5×10^6) were suspended in 2 ml medium of various ^{125}I concentrations of ^{125}I-AP (1.8-9.3 MBq), seeded into Leighton tubes and incubated for 28 hours at 37°C. It was assumed that ^{125}I-AP had uniformly distributed immediately after addition [8]. Calculations of decays per cell were based on the volume of T1 cells ($V = 3051\ \mu m^3$, $\varnothing = 18\ \mu m$).

Exposure to Na^{125} I and^{137}Cs-γ-rays

Corresponding to the $^{125}IUdR$ experiments T1 monolayers were exposed to various ^{125}I concentrations of $Na^{125}I$ (1.9–15 kBq/ml medium) without any further additives. The growth medium was also renewed twice during the period of ^{125}I exposure. At the end of this time no radioactivity was measured in the cells. For reference, 1×10^6 cells in suspension were irradiated on ice with ^{137}Cs-γ-rays (0.88 Gy/min) accumulating doses of up to 10 Gy.

Survival and Comet Assays

The cellular damage of the entire cell population was measured by survival assay reflecting the clonogenic ability of the cells. Eleven days after seeding, the colonies were stained with haematoxilin and counted (minimum of 50 cells). The alkaline Comet assay detected molecular damage mainly as DNA single-strand breaks. The assay was performed by a modified method of Singh [7]. Briefly, suspended cells in low-melting-point agarose were plated on agarose-precovered slides and lysed for 70 minutes at 4°C (lysing solution: 2.5 M NaCl, 100 mM Na_2-EDTA, 10 mM tris, one percent Na-sarcosinate, one percent Triton-X, pH 10). Subsequently DNA was allowed to unwind for 25 minutes in electrophoresis buffer (0.3 M NaOH, 1 mM Na_2-EDTA, pH >12) followed by a horizontal electrophoresis (0.8 V/cm) for 25 minutes. The negatively charged DNA fragments moved towards the anode and generated the so-called Comet

tail. After neutralization with 0.4 M tris-HCl (pH 7.5) the fragments were stained with propidium iodide (20 μg/ml) and analyzed by a fluorescence microscope connected to a PC hosting software (Komet 3.1, OPTILAS). The Olive Tail Moment (OTM) was chosen as the parameter to quantify the damage. It represents the product of percentage of DNA in the tail and the distance between the mean of the head and the mean of the tail [5]. The higher the DNA damage the more DNA fragments leave the head and migrate into the tail. This enhances the OTM value.

Results and Discussion

Effects of ^{125} IUdR and^{125}I-AP

The survival curves (Fig. 1a) showed a steep decrease of the surviving fraction after ^{125}IUdR incorporation and a shoulder after ^{125}I-AP exposure. The D_{37} values calculated from the graphs were 90 d/c for ^{125}IUdR and 1160 d/c for ^{125}I-AP. The survival results after ^{125}IUdR incorporation were comparable with those found by Sedelnikova *et al.* [6]. A characteristic feature of the ^{125}IUdR survival curve was the 'tailing' in the second decade of the logarithmic scale starting from approx. 1000 d/c [3]. The reason for this was a certain fraction of the asynchronous cell culture which did not pass the S-phase during incubation and was, therefore, responsible for preventing a further decline of surviving fractions. The consequence of this was an inhomogeneous distribution of ^{125}IUdR among the cells of the entire population. Theoretically, this resistant fraction could possibly be eliminated by an additional application of ^{125}I-AP which is independent of cell cycle specific problems of ^{125}IUdR incorporation. The steep linearly increasing functions of the Comet assay depicted stronger damage to DNA after ^{125}IUdR incorporation than after ^{125}I-AP exposure (Fig. 1b). Unlike the results obtained with the survival assay no 'tailing' was recognised by using the Comet assay after ^{125}IUdR incorporation (Figs. 1a & 1b). The non-labelled cell fraction

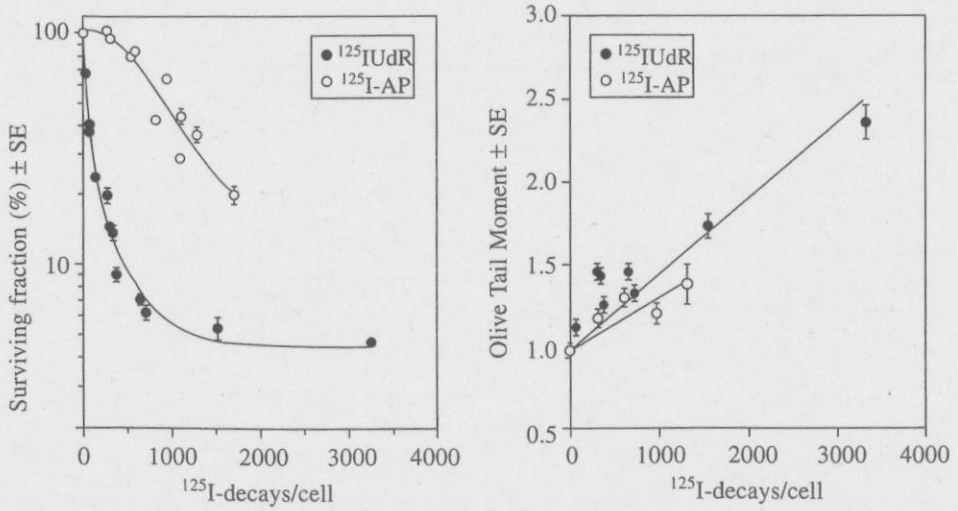

Figure 1. (a) Survival after ^{125}IUdR and ^{125}I-AP exposure (n = 3); (b) Comet assay after ^{125}IUdR-and ^{125}I-AP exposure (n = 200)

did not influence the Comet results. Whereas the survival assay determined the changed reproducibility of the entire cell population relative to the unaffected population, the Comet assay quantified the DNA damage of each individual cell as an absolute OTM value. This distinction is important After exposure of cells to ^{125}I-AP an acceptable correlation ($r = 0.76$) was found between the decreasing survival data and those of increasing DNA damage with the Comet assays (Figs. 1a & b).

Effect of Na^{125}I

After exposure to the highest radioactive concentration of Na^{125} I the surviving fraction only decreased to 90 percent of that of the controls (Fig. 2a). Also no significant differences of the OTM values compared with those of the controls could be analyzed with increasing Na ^{125}I concentrations (Fig. 2b). The slight decrease of the survival curve is probably caused by the 35.4 keV gamma rays which are emitted at a rate of 7 percent per ^{125}I decay of the extracellulary deposited Na ^{125}I. These photons and some released conversion electrons of higher energy can affect the intracellularly located DNA. This clearly demonstrates that the short-distant low energy AEs (93 percent up to 3.5 keV ≙ 400 nm) will reach the DNA if the AE-emitting nuclide is positioned close enough to the DNA depending on the carrier molecule. In the case of Na^{125}I the DNA target was hardly reached.

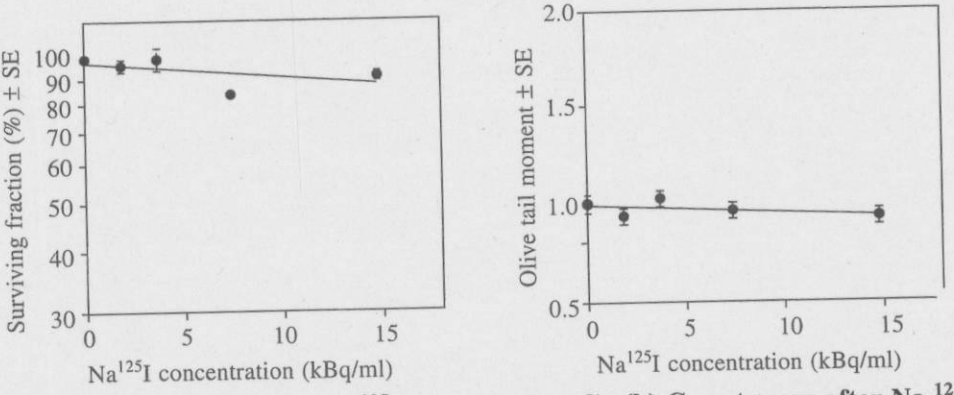

Figure 2. (a) Survival after Na ^{125}I exposure (n = 3); (b) Comet assay after Na ^{125}I exposure (n = 200)

Effect of^{137}Cs-γ-irradation

Parallel experiments were carried out with ^{137}Cs-γ-irradiation in order to compare the ^{125}I induced results with those after exposure to γ-rays. The data obtained with the survival assay showed a decreasing curve down to the fourth decade of the logarithmic scale with a small shoulder and a D_0 of 0.9 Gy (Fig. 3a). The Comet assay results revealed an OTM-function increasing strictly linearly with the dose (Fig. 3b). Moreover, a correlation between the two assays (Fig. 3a and 3b) resulted in a high correlation coefficient of $r = 0.99$. Therefore it is postulated that after γ-irradiation the clonogenic survival of cells depends strongly on DNA/chromatin damage, particularly DNA strand breaks.

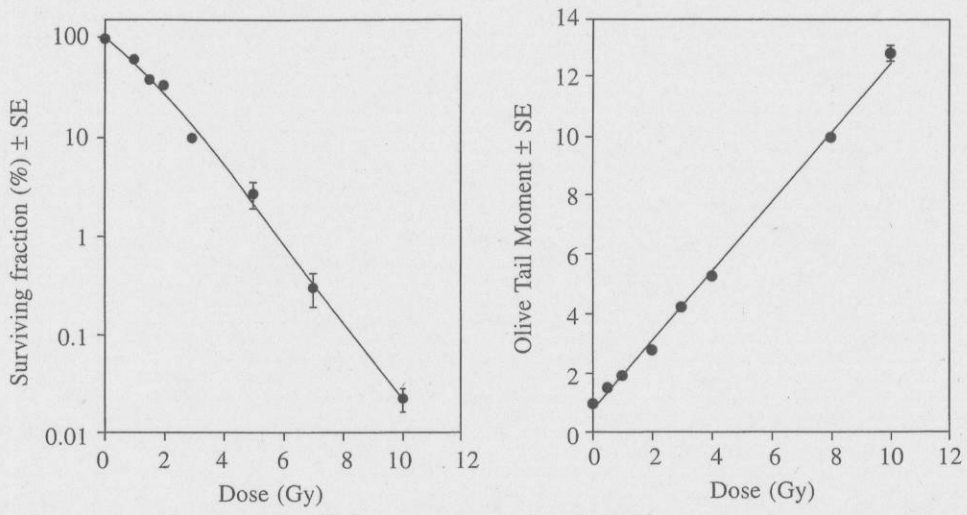

Figure 3. (a) Survival after ^{137}Cs-γ-irradiation (n = 3); (b) Comet assay after ^{137}Cs-γ-irradiation (n = 200)

Conclusion

Among the three different carrier molecules, IUdR, I-AP and NaI, each labeled with ^{125}I, ^{125}IUdR was found to be the most efficient in relation to DNA/chromatin damage and to the clonogenic survival of T1 and 86HG39 cells. The reason was the direct incorporation of ^{125}IUdR into the DNA whereas ^{125}I-AP distributed in the whole cell and Na^{125}I located only extracellularly were not bound to the target and were, therefore, less efficient in inducing destructive effects in the DNA and the cell. These results indicate that the selection of carrier is extremely important, especially for medical purposes. In the case of tumour therapy, ^{125}IUdR seems to be suitable as an adjuvant radiotherapeutic agent because the relative amount of S-phase cells of the fast proliferating tumour tissue is higher than in normal tissue and the strong cytotoxic effect of ^{125}IUdR can be generated even with small amounts of radioactivity. However, clinical use requires exact knowledge of the injected ^{125}I dose. An essential prerequisite for dosimetric calculation is the homogeneous energy deposition by uniform distribution of the radiopharmaceutical in the target. This condition is not fulfilled by ^{125}IUdR, but ^{125}I-AP is uniformly distributed over the entire cell including its nucleus. If the same number of decays per nucleus/cell is determined for both ^{125}I-AP and ^{125}IUdR and the correct dose (Gy) is calculated for the homogeneously distributed ^{125}I-AP then a comparable dose of ^{125} IUdR is obtained.

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