Radiosensitivity of Prostate Cancer Cell Lines for Irradiation from Beta Particle-emitting Radionuclide ^{177}Lu Compared to Alpha Particles and Gamma Rays

JÖRGEN ELGQVIST[1], OSKAR VILHELMSSON TIMMERMAND[1], ERIK LARSSON[2] and SVEN-ERIK STRAND[1]

[1]*Department of Medical Radiation Physics, Institute of Clinical Sciences, Lund University, Lund, Sweden;*
[2]*Department of Radiation Physics, Skåne University Hospital, Lund, Sweden*

Abstract. *Aim: The purpose of the present study was to investigate the radiosensitivity of the prostate cancer cell lines LNCaP, DU145, and PC3 when irradiated with beta particles emitted from ^{177}Lu, and to compare the effect with irradiation using alpha particles or gamma rays. Materials and Methods: Cells were irradiated with beta particles emitted from ^{177}Lu, alpha particles from ^{241}Am, or gamma rays from ^{137}Cs. A non-specific polyclonal antibody was labeled with ^{177}Lu and used to irradiate cells in suspension with beta particles. A previously described in-house developed alpha-particle irradiator based on a ^{241}Am source was used to irradiate cells with alpha particles. External gamma-ray irradiation was achieved using a standard ^{137}Cs irradiator. Cells were irradiated to absorbed doses equal to 0, 0.5, 1, 2, 4, 6, 8, or 10 Gy. The absorbed doses were calculated as mean absorbed doses. For evaluation of cell survival, the tetrazolium-based WST-1 assay was used. After irradiation, WST-1 was added to the cell solutions, incubated, and then measured for level of absorbance at 450 nm, indicating the live and viable cells. Results: LNCaP, DU145, and PC3 cell lines all had similar patterns of survival for the different radiation types. No significant difference in surviving fractions were observed between cells treated with beta-particle and gamma-ray irradiation, represented for example by the surviving fraction values (mean±SD) at 2, 6, and 10 Gy (SF2, SF6, and SF10) for DU145 after beta-particle irradiation: 0.700±0.090, 0.186±0.050 and 0.056±0.010, respectively. A strong radiosensitivity to alpha particles was observed, with SF2 values of 0.048±0.008, 0.018±0.006 and 0.015±0.005 for LNCaP, DU145, and PC3, respectively. Conclusion: The surviving fractions after irradiation using beta particles or gamma rays did not differ significantly at the absorbed dose levels and dose rates used. Irradiation using alpha particles led to a high level of cell killing. The results show that the beta-particle emitter ^{177}Lu as well as alpha-particles are both good candidates for radionuclide-therapy applications in the treatment of prostate cancer.*

Correspondence to: Jörgen Elgqvist, Ph.D., Associate Professor, Department of Medical Radiation Physics, Institute of Clinical Sciences, LundUniversity, Barngatan 2B, SE-221 85 Lund, Sweden. E-mail: jorgen.elgqvist@gmail.com

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Prostate cancer (PCa) is one of the most frequently diagnosed type of cancer in men, at the same time being the second leading cause of cancer-related mortality in the USA. According to the International Agency for Research on Cancer approximately 345,000 men were diagnosed with PCa in Europe during 2012, and 72,000 men died of the disease. The corresponding numbers for the USA for 2012 were 233,000 and 30,000, respectively. The American Cancer Society estimates that 221,000 men will be diagnosed with PCa during 2015 in the USA, and approximately 28,000 of them will die from the disease (1). In Europe, PCa is the most commonly diagnosed cancer type.

Therefore, improvement of already established therapies and the development of new ones is of utmost importance. Prostate cancer is most often treated using radiotherapy (external irradiation or brachytherapy), chemotherapy or radical prostectomy, with or without androgen-deprivation therapy (2). Regarding radiotherapy, almost 30% treated by using potentially curative absorbed dose levels relapse at the irradiation site (3, 4), and very little is known on how certain prostate tumor cells seem to be more radioresistant than others. As death from PCa is most often the consequence of a metastatic spread of the disease, new targeted treatment strategies, such as radionuclide therapy (RNT) based on radiolabeled specific antibodies (radioimmunotherapy) or peptides (peptide radionuclidetherapy) are under development and could be a way forward to cure or prolong survival. In the development of such new treatments, knowledge on radiosensitivity, and the causes of radioresistance, is important. The area of radioimmunotherapy is progressing and has so far

resulted in two Food and Drug Administration (FDA)-approved drugs using beta-particle emitters: Zevalin® and Bexxar® (^{90}Y Ibritumomab and ^{131}I Tositumomab, respectively), both used to treat refractory low-grade B-cell non-Hodgkin lymphomas (5-8). Another newly FDA-approved drug is Xofigo®, based on the alpha-particle emitter ^{223}Ra, is aimed for RNT of metastatic PCa (9). Another very intriguing future therapy approach for avoiding normal organ toxicity and increasing the absorbed dose to the tumor is to combine RNT with external beam therapy (10). Herein, knowledge of the tumor radiosensitivity will be of utmost importance in calculating the prescribed absorbed doses (11).

Generally, radiosensitivity is related to the rate of cell division and the level of cell differentiation. Cells that are most radiosensitive in general terms are those having a high metabolic rate, good access to nutrients, and a relatively high division rate, *i.e.* highly proliferative, actively dividing cells (12). But also cells not fully mature or less differentiated (*e.g.* stem cells or stem cell-like cells) have increased radiosensitivity (12). The part of the cell cycle that is least sensitive to radiation is the S-phase (especially the latter part of the S-phase), at which the synthesis and replication of DNA occurs. The most radiosensitive parts of the cell cycle are the G_2- and M-phase, at which the chromatin is condensed and the cell starts to divide, respectively. However, these general statements on radiosensitivity cannot be made specific for certain cell types. Therefore, investigations, such as the present study, need to be performed in order to determine the radiosensitivity of specific cells. Moreover, as radiosensitivity is dependent not only on the cells, but also on which type of radiation is being used, it is important to investigate the radiosensitivity to different types of radiation, *e.g.* beta particles, alpha-particles, or gamma rays, all used in this study.

Alpha particles differ, for example, from beta particles and gamma rays in their ability to create densely ionizing tracks when passing through tissue (13). This characteristic is expressed as the linear energy transfer (LET), which indicates the ability of radiation to transfer energy to the surrounding media per unit travelled length (14). Typically, alpha particles have an average LET of ~100 keV/μm, depending both on the initial alpha-particle energy as well on where on the Bragg curve the individual alpha-particle is along its ionization track. Beta particles in comparison, emitted for example from radionuclides such as ^{177}Lu, ^{131}I, or ^{90}Y, have an LET in range of 0.2-0.3 keV/μm. The same LET range is also applicable when considering gamma rays, *e.g.* emitted from ^{137}Cs. Different LET values have different impacts on the irradiated cells. Irradiation with high LET causes more double-strand breaks and more severe damage to DNA compared to that with low LET (12). The biological effect from radiation with high LET is also less dependent on the cell cycle, oxygenation, and absorbed dose rate compared to that with low LET. Hence, inclusion of different kinds of radiation, *e.g.* with different LET and different dose rates, is important during investigations of the radiosensitivity of cells in order to obtain as broad a picture as possible as to how specific cell types behave when irradiated.

In the context of radiosensitivity, a comment on radiosensitizers and the determinants of the radiosensitivity of PCa is important. Examples of radiosensitizers of PCa are baicalein and BMD122, both inhibitors of platelet-type 12-lipoxygenases (12-LOX), which play an important role in the progression of PCa (15). It was recently shown that both these sensitizers increase the radiosensitivity of PCa cells, without resulting in the same effect on normal cells (16). For the androgen-dependent LNCaP and androgen-independent PC3 cell lines, it has been shown that these 12-LOX inhibitors, when combined with radiation, have a synergistic inhibitory effect on survival *in vitro*, and also resulted in a significantly reduced tumor growth *in vivo* (16). Another interesting approach regarding radiosensitizing relates to growth factors, which are the basic regulators of differentiation, cellular proliferation, and neoplastic transformation (17). Poor prognosis and advanced PCa are often associated with expression of the epidermal growth factor receptor (EGFR) (18, 19). It has been shown that EGFR expression is high in many PCa cells, and that EGFR activation reduces the radiosensitivity of tumor cells in general (20-23). Besides being associated with resistance to radiation therapy, EGFR expression is also important for processes such as angiogenesis, inhibition of apoptosis, and metastasis (17, 24, 25). Therefore, EGFR is a relevant candidate for targeted therapies. A number of EGFR-blocking antibodies have been developed, *e.g.* cetuximab (C225), which significantly enhances radiosensitivity and synergistically inhibits cell growth in combination with radiation (17, 26-28). Regarding specific determinants of the radiosensitivity of PCa, the tumor-suppressor protein retinoblastoma (RB) should be mentioned. This protein regulates cell proliferation and has proven to be inactivated in 25-30% of all PCa, leading to a castration-resistant phenotype of PCa (29). Loss of RB also reduces the ability of the cell to repair DNA damage induced by radiation, by down-regulating G_1-S cell-cycle arrest, and might therefore be used as a biomarker for prediction regarding response to radiation (29). Another determinant for the radiosensitivity of tumors in general, but also for PCa, is the *TP53* gene or the p53 protein level, for which a study has shown that the wild-type TP53 is a stronger determinant for radiosensitivity than mutant TP53 (30).

In the present study, we investigated the radiosensitivity of the PCa cell lines LNCaP, DU145, and PC3 when irradiated with beta particles emitted from ^{177}Lu, and compared the effect using alpha particles or gamma rays. Cells were irradiated to mean absorbed doses equal to 0, 0.5, 1, 2, 4, 6, 8, or 10 Gy. For evaluation of cell survival, the tetrazolium-based WST-1 assay was used. In short conclusion, irradiation using alpha particles showed a high level of cell killing and the surviving fractions for irradiation using beta particles or gamma rays showed no significant difference for the absorbed dose levels and dose rates used.

Materials and Methods

Cancer cells. The prostate cancer cell lines LNCaP, DU145, and PC3 were purchased from the American Type Culture Collection (Manassas, VA, USA) and grown in Roswell Park Memorial Institute-1640 medium (Sigma-Aldrich, St. Louis, MO, USA), Eagle's minimum essential medium (LGC Standards, Wesel, Germany), and Kaighn's modification of Ham's F-12 medium (Thermo Fisher Scientific, Waltham, MA, USA), respectively, supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (100 U/ml) (Thermo Fisher Scientific). The cells were grown in T-75 or T-150 flasks as a monolayer and kept at 37˚C in a humidified atmosphere in an incubator with 5% CO_2. Culture media were changed at least twice per week and the cells passaged before reaching confluence using trypsin-EDTA. All experiments were carried-out with cells in passage numbers 12-28, 12-25, or 12-24 and the cell-doubling times were determined to be 50, 35, and 30 h for LNCaP, DU145, and PC3 cells, respectively. All cell counting was performed using The Countess™ automated cell counter (Life Technologies, Paisley, UK) that utilizes trypan-blue staining and image-analysis algorithms to identify the total number of cells and the fraction of viable cells.

Labeling of non-specific antibody with ^{177}Lu. The isothiocyanate functional group of the chelator p-SCN-Bn-CHX -A"-DTPA (B-355; Macrocyclics, Dallas, TX, USA) was conjugated to a non-specific polyclonal IgG antibody derived from mouse serum (I876510.76, mg/ml; Sigma-Aldrich, St. Louis, MO, USA) in 0.07 M sodium borate buffer (Sigma-Aldrich) adjusted to pH 9.2 containing 5.38 mg of IgG with a chelator to antibody molar ratio of 3:1. The solution was incubated at room temperature overnight and conjugated antibody was separated from free chelate, and eluted with 1 ml ammonium acetate buffer (0.2 M, pH 5.5; Sigma Aldrich), on an ammonium acetate buffer equilibrated NAP-5 column (GE Healthcare Life Sciences, Little Chalfont, UK). Aliquoted samples of conjugated antibody were stored at 4˚C.

For radiolabeling with ^{177}Lu, about 3 µl $^{177}LuCl_3$ (312 MBq; IDB Holland, Baarle-Nassau, the Netherlands) was added to 400 µl of ammonium acetate buffer. The pH was tested with pH strip (Sigma-Aldrich) and a pH of 5.0-5.5 was confirmed. Subsequently, 100 µl of conjugated non-specific antibody in ammonium acetate buffer (5.83 mg/ml) was added, giving a total volume of approximately 500 µl. The sample was incubated for 2 h, after which the labeling was terminated and the sample purified, eluated with 1 ml PBS, on a PBS-equilibrated NAP-5 column. The stability in PBS of the labeled immunoconjugate was monitored up to 5 days after labeling using instant thin layer chromatography strips (Biodex, Shirley, NY, USA), eluted with 0.2 M citric acid (Sigma-Aldrich). In this system, the radiolabeled conjugate remains at the origin, while free radionuclide or radionuclide-chelate complexes migrate with the solvent front. The strips were analyzed with a Cyclone Storage Phosphor System using the Optiquant as quantification software (both from Perkin Elmer, Waltham, MA, USA).

Irradiation and dosimetry. Alpha particles: The alpha-particle irradiations were performed using a previously described in-house developed alpha-particle irradiator based on a ^{241}Am source on which cells growing in monolayers in well inserts were placed (31). The dosimetry was performed using Monte Carlo (MC) simulations of the source and irradiation geometry. All details for the dosimetry calculations and description of the alpha-particle irradiator can be found in the methodological work by Nilsson *et al*. (31). The cells were seeded into two removable well inserts (24-well plate; Thermo Fisher Scientific, Waltham, MA, USA) for each absorbed dose level at an approximate cell density of 20,000 cells/well in 100 µl culture medium. Two additional wells were filled with the same amount of medium without any cells present and used as reference. After 2-3 days of recovery, the medium was changed and the cells were irradiated to the desired absorbed-dose level. The absorbed-dose rate was 1.05 Gy/min. Controls were treated in exact same manner, without being irradiated. Before each irradiation, it was confirmed that the cells grew in a monolayer by inspection using light microscopy. The above procedure was carried out three times for each cell line and absorbed dose level.

Beta particles: The beta-particle irradiations were performed using the non-specific polyclonal antibody I8765 labeled with ^{117}Lu, which was added to single-cell suspensions in Eppendorf vials containing cell medium. Added total activity, volume, and irradiation time determined the final absorbed dose to the tumor cells, which was calculated as the mean absorbed dose. Mean absorbed dose (D) calculations were performed using the MIRD formula (32):

$$D = \tilde{A}S = \int_0^T A_0 e^{-\lambda t} dt * S = \frac{A_0}{\lambda}\left(1 - e^{-\lambda T}\right)S$$

where $\tilde{A}$ is the cumulative activity, S is the mean absorbed dose per cumulative activity, T the irradiation time, A_0 the added activity at t=0, and λ the decay constant. The S factor was derived from the Olinda spheres (33), which were recalculated to the absorbed energy and then divided by the actual weight of the total cell solution to obtain the S factor for this volume. Additionally, control S values were calculated by the MCNP5 MC-code package (Los Alamos National Laboratory, Los Alamos, NM, USA). The MC simulations showed a good agreement with the Olinda spheres for a 0.5 ml cell suspension volume. The absorbed-dose contribution from photons was 0.43% compared to that from beta particles.

The added activity, volume, and irradiation time were in the range of 5-10 MBq, 0.3-0.5 ml, and 4-18 h, respectively. The mean±SD absorbed-dose rate during the irradiations was 1.3±0.4 Gy/h. To end irradiation, the vials were placed in a centrifuge (Eppendorf 5417C; Eppendorf GmbH, Hamburg, Germany) at 468 × g for 4 min, after which the supernatant was removed and replaced by fresh cell medium. The cells were then resuspended in this medium. This centrifugation and resuspension process was repeated three times in total, after which the cells were seeded onto 96-well plates and incubated for a time period equal to the specific cell-doubling time, plus one day. All irradiations were performed in triplicates for each cell line and absorbed dose level.

Gamma rays: For the gamma-ray irradiations, 96-well plates were placed in a ^{137}Cs irradiator (Gammacell 40 Exactor; Best Theratronics, Ashford, Kent, UK) and irradiated. The cells were seeded onto 96-well plates (Thermo Fisher Scientific) at an approximate cell density of 10,000 cells/well in 100 µl culture medium. Two additional wells were filled with the same amount of medium without any cells present and used as reference. After 2-3 days of recovery, the medium was changed and the cells were irradiated to the desired absorbed-dose level. The absorbed-dose rate during the ^{137}Cs irradiations was 0.86 Gy/min, calibrated against a national reference source. Controls were treated in the exact same manner, but without being irradiated. All irradiations were performed in triplicates for each cell line and absorbed dose level.

Radiosensitivity measurements. After irradiation, the cells were left in an incubator for a time period equal to the specific cell-doubling time, plus at least one day. After the alpha-particle irradiations, the cells in each well insert were gently washed with cell media twice and then very carefully trypsinized and transferred to three individual wells on a 96-well plate in 100 ml of fresh medium, after which a protocol for cell-viability measurements was carried out (see below). The cells in the 96-well plates used for the beta-particle and gamma-ray irradiation were subjected to the same protocol.

Cell viability was determined by a tetrazolium salt-based calorimetric assay using the Cell Proliferation Reagent WST (water soluble tetrazolium)-1 kit (Roche Applied Science GmbH, Pensberg, Germany). The WST-1 kit is especially manufactured for non-radioactive spectrophotometric quantification of, for example, cell viability. Reduction of tetrazolium salt to formazan by mitochondrial dehydrogenase gives a direct correlation to the number of metabolically active or viable cells in the solution.

To each well on the 96-well plates, 10% of WST-1 solution was added and incubated for up to 24 h at 37˚C in 5% CO_2 in a humidified atmosphere. Repeated measurements were performed during that time interval, and at least three of these measurements were used. The absorbance of the dissolved formazan product was measured at 450 nm on a Labsystems Multiscan Plus plate reader using the DeltaSoft JV software (BioMetallics, Princeton, NJ, USA). The mean value from measurements of fresh medium containing 10% WST-1 was used for correction of background absorbance, and untreated cells were used as reference value for zero absorbed dose.

Results

Labeling the polyclonal non-specific antibody with ^{177}Lu gave a radiochemical purity of ~99% and a radiochemical yield of about 55%. The stability of the radioimmunocomplex after 5 days was high, indicated by a ~99% radiochemical purity after NAP-5 size-exclusion chromatography.

The surviving fraction of LNCaP cells as a function of the mean absorbed dose for the three different types of radiation is shown in Figure 1A. There was a strong cell response when cells were irradiated with alpha particles, exemplified by a surviving fraction at 2 Gy (SF2) of 0.048±0.008 (mean±SD). The response of the LNCaP cells when irradiated with beta particles emitted from ^{177}Lu or gamma rays emitted from ^{137}Cs were almost identical, exemplified by SF10 values of 0.100±0.045 and 0.116±0.041, respectively.

The results for DU145 cells are shown in Figure 1B. As for LNCaP cells, DU145 responded strongly when irradiated with alpha particles, exemplified by an SF2 value of 0.018±0.006. The response of DU145 cells when irradiated with the beta particles or gamma rays are almost identical, as for LNCaP cells, exemplified by SF10 values of 0.056±0.001 and 0.036±0.013, respectively.

The surviving fraction as a function of the mean absorbed dose for PC3 cells is shown in Figure 1C. Again, there was a strong response of cells when irradiated with alpha particles, exemplified by an SF2 value of 0.015±0.005. The response of PC3 cells when irradiated with beta particles or gamma rays were similar, exemplified by SF6 values of 0.158±0.022 and 0.220±0.034, and SF10 values of 0.019±0.008 and 0.029±0.006, respectively.

SF2, SF6, and SF10 values for all three cell lines and the three types of radiation are presented in Table I.

Discussion

Irradiation set-up and dosimetry. During irradiation, different geometries and set-ups were used: monolayers of cells for alpha-particle irradiation, single-cell suspensions for the beta-particle irradiation, and cells grown on 96-well plates for the gamma-ray irradiation. These set-ups were used in order to achieve as homogenous an absorbed dose distribution as possible. Regarding the gamma irradiation, they also could have been performed using single-cell suspensions. However, since the measurements of survival using the calorimetric assay were performed on 96-well plates, and previous experiments (not shown) have not revealed any difference in survival data using singe-cell suspensions in Eppendorf vials or wells on a 96-well plate regarding gamma-ray irradiation, the fastest method was chosen, *i.e.* 96-well plates.

Regarding the absorbed-dose calculations, they also differ somewhat between the different types of irradiation. For the alpha particles, the dosimetry was performed using MC simulations of the whole radiation set-up (31). For the beta particles, the absorbed dose was calculated as the mean absorbed dose in the 1.5-ml Eppendorf vials. For the gamma-ray irradiation, an established calibration curve for the ^{137}Cs Gammacell-40-Exactor machine was used to determine the irradiation time needed for each absorbed-dose level. Regarding alpha particles, statistical uncertainties can be created when considering low absorbed doses. For example, depending on the initial energy of the alpha particle, where on the Bragg curve the alpha particle is when it enters the cell, and how large the cell nucleus is, the energy imparted to a cell nucleus by one alpha particle is often of the order of 0.2 Gy. And if then, as in this study, absorbed doses of 0.5, 1, and 2 Gy are used, the average number of hits per cell nucleus is about 2, 5, and 10, respectively, *i.e.* very small numbers. The true number of hits for each individual cell is not known, and will vary. Some nuclei will receive zero hits and some will receive a number of hits exceeding the average value. In order to be able to estimate the fraction of cells in this set-up receiving, for example 0, 1, 2, and 3 hits, a microdosimetric approach should be used (34). An upcoming study will therefore investigate the statistical variation in the number of hits for the absorbed-dose levels used in present study.

The surviving fractions are presented as a function of the mean alpha-particle absorbed dose. The contribution to the absorbed dose from photons emitted from ^{241}Am during the alpha-particle irradiation was of the order of 10^{-5} to that by the alpha particles and could therefore be ignored. The estimated error in the absorbed-dose calculations for the

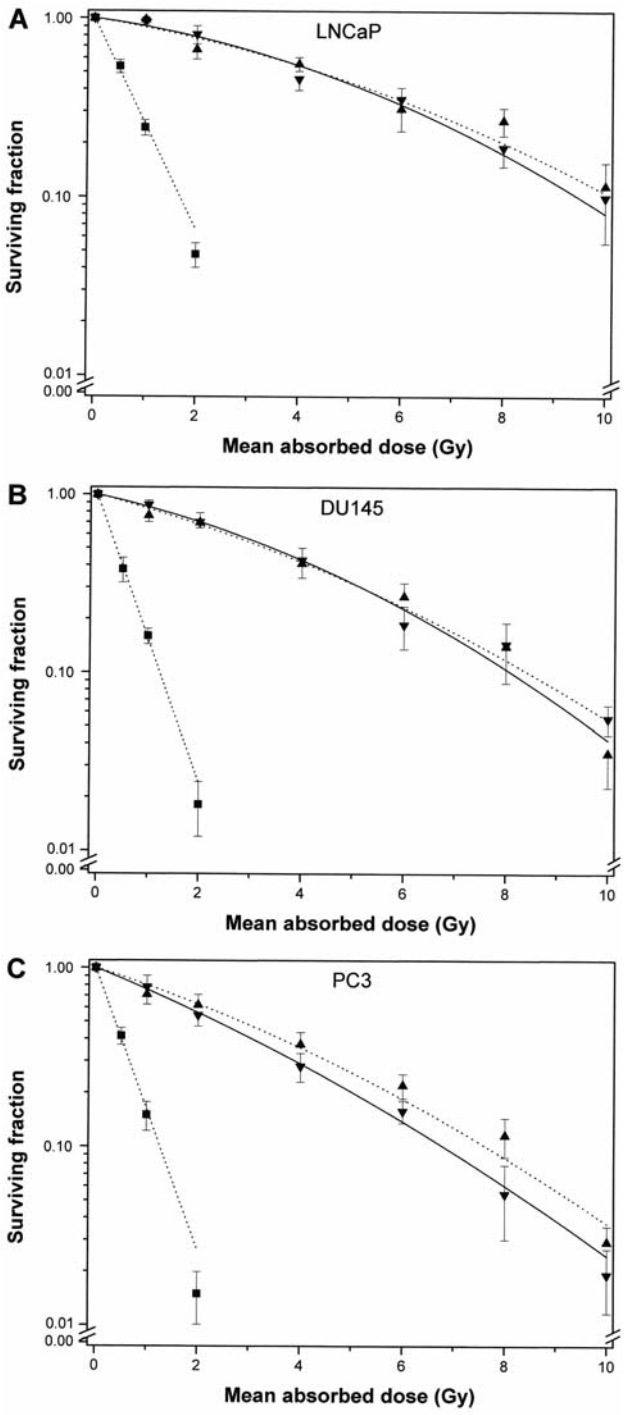

Figure 1. *Surviving fraction (SF) of LNCaP (A), DU145 (B) and PC3 (C) cells as a function of the absorbed dose after irradiation with ^{241}Am (alpha particles) (■), ^{177}Lu (beta particles) (▼), and ^{137}Cs (gamma rays) (▲). The mono-exponential curve fitted to the alpha-particle irradiation is of the form $SF=exp(-k_1D)$, where k_1 is a constant and D the absorbed dose. The bi-exponential curves fitted to the beta-particle and gamma-ray irradiations are of the form $SF=exp(-k_2D-k_3D^2)$, where k_2 and k_3 are constants. The curve fitted to the ^{177}Lu data points is solid, whereas the other two curves are dotted. Values are presented as mean±SD.*

Table I. *Surviving fraction after 2, 6, and 10 Gy (SF2, SF6, SF10) for alpha-particle, beta-particle, and gamma-ray irradiation of prostate cancer cells LNCaP, DU145, and PC3. Values are presented as mean±SD.*

	Irradiation type		
SF2	Alpha	Beta	Gamma
LNCaP	0.048±0.008	0.813±0.094	0.670±0.079
DU145	0.018±0.006	0.700±0.090	0.696±0.047
PC3	0.015±0.005	0.543±0.072	0.620±0.089
SF6			
LNCaP	–	0.354±0.057	0.312±0.077
DU145	–	0.186±0.050	0.268±0.051
PC3	–	0.158±0.022	0.220±0.034
SF10			
LNCaP	–	0.100±0.045	0.116±0.041
DU145	–	0.056±0.010	0.036±0.013
PC3	–	0.019±0.008	0.029±0.006

alpha-particle irradiation is less than 10% (31). Regarding the irradiation using beta particles emitted from ^{177}Lu, we consider the uncertainty in the mean absorbed-dose calculations to be less than 5%, only due to uncertainty in the total amount of activity added to the cell suspension. The continuous slowing-down approximation range in water of the most abundant beta particles is 1.8 mm, and the radioactivity will, therefore, create a highly uniform absorbed-dose distribution in the 0.3-0.5 ml solutions used.

The absorbed-dose rates differ between the irradiation modalities in this study; 1.05 and 0.86 Gy/min for the alpha-particle and gamma-ray irradiation, respectively; and 1.3±0.4 Gy/h (mean±SD) for the beta-particle irradiation. The dose rates of the alpha-particle (high-LET) and gamma-ray (low-LET) irradiation were fixed and could not be changed/decreased, and the dose rates for beta-particle (low-LET) irradiation were maximized, taking the amount of available radioactivity into account. High-LET radiation is known to induce the same amount of mutagenic effect and effect on survival, disregard to at which dose rate the absorbed dose is delivered. Low-LET radiation, on the other hand, often induces an increasingly amount of damage to the cell when increasing the dose rate, for a certain absorbed dose level. For the alpha-particle irradiation, even if we had been able to alter the dose, it would have had no or very little effect on the survival data. But for the low-LET irradiation, the survival data might be altered using other dose rates. In our case, if we had reduced the dose rate during the gamma irradiation by using ^{137}Cs, the SF values in Figure 1 would probably have increased to some degree, *i.e.* indicating an increase in survival when reducing the dose rate. If we had

increased the dose rate for the beta-particle irradiation by reducing the volume of the single-cell suspension, the SF values in Figure 1A would probably have decreased to some degree, *i.e.* indicating a decrease in survival when increasing the dose rate. A study of dose-rate sensitivity of the LNCaP, DU145, and PC3 cell lines is planned. It should also be noted that during the decay of ^{177}Lu, during which beta particles are predominantly emitted, a small fraction of Auger electrons are also emitted. However, the contribution from the emitted energy per decay from Auger electrons, compared to that of the beta particles, is less than 1‰, and can therefore be neglected, even though the LET-value for beta particles and Auger electrons average ~0.3 and ~16 keV/μm, respectively. The irradiation using ^{177}Lu in the present study was performed using a polyclonal antibody, not directed towards any specific antigen on the cells. The radio-immunocomplex did not indicate any significant binding to, or internalization into, the cells. If, however, internalizing antibodies had been used, even a fraction as small as 1‰ of emitted energy from Auger electrons might have influenced the results, due to the approximately 50-times higher average LET value for the Auger electrons compared to the beta particles, which could have affected DNA, for example causing double strand breaks.

Radiosensitivity measurements. Using the tetrazolium-based WST-1 assay during the radiation-sensitivity measurements has several advantages, but also some drawbacks. Advantages are that it is easy to use and possible to repeat measurements for the same well without killing cells, as opposed to for example the tetrazolium-based MTT assay. This is explained by the fact that the tetrazolium salt used in the WST-1 is not internalized into the cell, as opposed to the tetrazolium salt used in the MTT assay. The WST-1 assay also produces a result relatively fast with a limited amount of work, and is operator-independent, in contrast to the colony-forming assay. The drawbacks are that the WST-1 assay is not as sensitive as the colony-forming assay for higher absorbed-doses values, and can only be used for surviving fractions down to approximately two orders of magnitude; this is true for all tetrazolium-based assays. It has been shown that tetrazolium-based assays overestimate the surviving fraction for absorbed doses exceeding approximately 8-10 Gy (35). In the present study, we limited the absorbed doses to a maximum of 10 Gy, and used the WST-1 assay for all measurements, so we believe that the comparisons of SF values between the different irradiation types are valid. By using the WST-1 assay, it was possible to obtain SF data for a large number of measurements in less time, with less effort, and operator independence, compared to the colony-forming assay. Something that should be further investigated is the effect of senescent cells on data acquired with this method senescent cells remain metabolically active and this is believed to be a major path for the regression of prostate cancer tumors (36). In this case, the result could potentially differ between the WST-1 assay and clonogenic assays, with the WST-1 assay underestimating clonogenic death due to remaining metabolically active senescent cells. This could be especially true for the relatively lower LET of gamma-ray irradiation, and additionally the lower absorbed dose-rate of β-particle irradiation.

Conclusion

The surviving fractions for cells after irradiation using beta particles or gamma rays did not significantly differ by absorbed-dose level and dose rate used. Irradiation using alpha-particles lead to a high level of cell killing. The results show that the beta-particle emitter ^{177}Lu, as well as alpha-particles, are both good candidates for radionuclide-therapy applications in the treatment of prostate cancer.

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