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Eds. RW Howell, VR Narra, KSR Sastry, DV Rao

THE BIOLOGICAL EFFICACY OF INDUCED AUGER CASCADES: COMPARISON OF IODINE AND BROMINE AS TARGET ATOMS

BRENDA H. LASTER[1,5], WILLIAM C. THOMLINSON[1,2], JOHN KALEF-EZRA[1,3], VILI BENARY[1,4], EDWIN A. POPENOE[1], VICTOR P. BOND[1], CHRIS GORDON[1], LYNNE WARKENTIEN[1], NICHOLAS GMÜR[2], NANCY LAZARZ[2], and RALPH G. FAIRCHILD[1]

[1]Medical Department, [2]National Synchrotron Light Source
Brookhaven National Laboratory, Upton, NY 11973

[3]University of Ioannina, Ioannina, 451.10 Greece

[4]Tel Aviv University, Tel Aviv, Israel

[5]Department of Radiation Oncology
University Hospital at Stony Brook, Stony Brook, NY 11794

ABSTRACT

Photon Activation Therapy (PAT) has been postulated as a binary system for the treatment of malignant brain tumors. PAT proposes the clinical administration of iododeoxyuridine (IUdR) followed by irradiation with radioactive sources (^{145}Sm, 40 keV γ rays) interstitially implanted via brachytherapy techniques. The radiosensitizing properties of halogenated pyrimidines are well known (1-3). This study was undertaken to compare the biological efficacy of the Auger cascades following an induced photoelectric effect in iodine or bromine target atoms using synchrotron radiation.

To document if Auger electron emission would increase the effectiveness of a dose (in addition to the enhancement from sensitization) delivered to mammalian cells, a series of experiments were conducted at the Brookhaven National Laboratory (BNL). Either iodine (K absorption edge = 33.17 keV) or bromine (K edge = 13.47 keV) was introduced into the DNA of exponentially growing V79 Chinese hamster cells during cell replication through the analog nucleosides, iododeoxyuridine (IUdR) or bromodeoxyuridine (BrUdR), replacing the DNA precursor thymidine. The percent replacement of thymidine by the substituted halogenated pyrimidines was determined by neutron activation analysis of digested cell samples. Control cells, without incorporated halogen, and halogenated cells irradiated below the K absorption edge of the halogen, were used to separate the radiosensitization enhancement component as distinct and apart from any observable enhancement attributable to the induced Auger cascades. An Auger effectiveness factor (AEF) resulting from the combined effect of the X rays and the Auger electron emission, was obtained by comparing the ratios of the D_{10} and D_0 values for the halogenated cells irradiated above and below their respective absorption edges. Monochromatic photons above and below the K absorption edge of each halogen were obtained at the National Synchrotron Light Source (NSLS) at the Brookhaven National Laboratory. Cells were irradiated at a dose rate of 0.65 Gy/min. Data analyses were based on curve fits using both the linear-quadratic and single-hit multi-target models.

An Auger Effectiveness Factor of 1.4 was observed for iodinated cells when comparing irradiations with 33.4 and 32.9 keV photons. However, brominated cells, irradiated at 13.5 and 13.4 keV failed to show a similar response. The AEF observed for bromine was 1.0. Sensitization enhancement due to the incorporated halogen was 2.2 for both iodine and bromine. The total therapeutic gain factor for iodinated cells was, therefore, 1.4 X 2.2 = 3.1, and for brominated cells, 2.2. These results indicate that IUdR is the better of these two halogens for both demonstrating the Auger effectiveness in biological experiments, and for providing a clinical therapeutic advantage for PAT.

INTRODUCTION

The halogenated pyrimidines, 5-bromo-2'-deoxyuridine (BrUdR) and 5-iodo-2'-deoxyuridine (IUdR), are incorporated in cellular DNA during cell

replication as analog nucleosides replacing the natural DNA precursor, thymidine (Thd). Having demonstrated radiosensitizing properties (1,2), it was anticipated that ^{125}IUdR would serve as an endoradiotherapeutic agent, seeking and being taken up by malignant tumor cells during cell replication, thus, providing radiation directly to the DNA, the presumed critical target of cells. However, uptake of the analogs was not restricted to tumor cells alone, since all rapidly proliferating tissue competed for IUdR uptake as avidly as did the tumor. Normal proliferative tissue accrued severe biological damage due to the incorporation of the ^{125}I label. This was attributed to the emission of Auger electrons as an inherent part of the photoelectric process initiated by the electron capture and internal conversion events during the radioactive decay of the iodine-125 (3). While the use of the radiolabeled analog nucleoside was precluded due to its severe biological toxicity, it was postulated that irradiation with photons above the K absorption edge of stable halogens could induce a photoelectric effect and concommitant Auger cascades (4). To propose a feasible radiotherapeutic modality, it was necessary to consider a means of delivering the radiation locally to avoid the problem of poor penetration associated with the low energy of the photons required for inducing a photoelectric effect.

Photon Activation Therapy was proposed by Fairchild *et al.* (5) as a radiotherapeutic technique for the treatment of malignant brain tumors. Stable IUdR, in addition to radiosensitizing tumor cells, would be activated by photons above the K absorption edge of iodine (33.2 keV); samarium-145, emitting 40 keV photons, would be interstitially implanted into the tumor via brachytherapy techniques.

The present work was undertaken to document the therapeutic gain which might be achieved from the high-LET type damage in DNA resulting from the induction of a photoelectric effect and the release of Auger electrons. It was designed to separate the contribution to cellular damage from radiosensitization alone and from any increased effectiveness resulting from the combined effect of the X rays and the Auger electrons. V79 Chinese hamster cells, with either BrUdR or IUdR incorporated in DNA, were irradiated with monochromatic photons obtained from the National Synchrotron Light Source at the BNL. Irradiations were carried out at energies above and below the respective edges of the halogens, 33.4 and 32.9 keV for iodine, and 13.5 and 13.4 keV for bromine. The cell preparation, irradiation procedure and geometry was identical in both cases. The plating efficiency was similar. The data were analyzed and fit using the same software (6).

MATERIALS AND METHODS

Incorporation of Halogenated Pyrimidines in Cellular DNA

V79 Chinese hamster cells in exponential growth were incubated with Dulbecco's Modified Eagle's growth medium[1], consisting of 10% dialyzed fetal bovine serum[1], 0.1 mM hypoxanthine[2], 1.0 μM aminopterin[2], 2.0 μM L-glutamine[1], 6.0 μM IUdR or BrUdR[2], and 4.0 μM thymidine[2], for one cell cycle (~14 h) at 37°C in a humidified environment, 95% air, 5% CO_2. After this "pulsing" period, cell monolayers were washed 3X with 1% PBS[1], trypsinized[1], harvested and counted. Control cells were treated similarly, receiving 10 μM thymidine, and no halogen. Aliquots corresponding to a cell density of 3.0×10^5 cells/ml were prepared and 120 μl of this cell suspension were placed in 250 μl polypropylene tubes for irradiation. Cells from the same iodinated or brominated dish were taken for the above and below K edge irradiations to assure identical uptake of the halogen. All cell processing, including the irradiation, was carried out under amber light to minimize any effects of photosensitization. Replicate dishes were harvested similarly and processed for neutron activation analysis to determine the amount of halogen present. This procedure is described below.

Cell Digestion Procedure for Neutron Activation Analysis

After counting the cells in suspension, cells were centrifuged at 1500 RPM and pelletized 3X, following their resuspension in TCA (5% w/v) and EtOH, then finally digested in 2.5 ml formic acid (88%) for 10 min in a 60°C hot water bath. A 1.2 ml aliquot of this TCA-insoluble fraction was placed in a 1.8 ml cryogenic vial for activation. Following activation, 1 ml of the sample was transferred to a new, non-activated 1.5 ml Eppendorf tube to increase the signal-to-noise ratio.

Neutron Activation Analysis (NAA) for Quantifying Halogen Uptake

The 1.8 ml cryogenic vial was placed in a transporting sleeve or "rabbit" for pneumatic passage through a tangential tube to a position adjacent to the core of the reactor. A 2 μg/ml iodine or bromine standard was treated

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[1]Gibco
[2]Sigma

similarly and activated simultaneously. Subjected to a thermal neutron fluence of 3.0×10^{12} thermal neutrons cm^{-2} at a reactor power level of 1 Megawatt for 10 minutes (iodine) and 1.25 Megawatt for 30 minutes (bromine), ^{127}I atoms were converted to ^{128}I ($\tau_{1/2}$ = 25 min). The 443 keV γ ray from ^{128}I was counted with a sodium iodide detector. The stable isotopes of bromine (^{79}Br = 50.6% abundance and ^{81}Br = 49.4% abundance) produced ^{80}Br and ^{82m}Br upon activation. The two peaks (554.3 and 776.5 keV) were counted 18.5 h post-activation to allow for the decay of radionuclides having interfering energies.

Calculation of Percent Uptake of Halogen

Assuming that an asynchronously growing population of cells has 8×10^{-12} g of DNA/cell (personal communication, S.L. Commerford), the average molecular weight of all DNA bases is 309, the molar amount of all DNA bases is 2.8×10^{-14} moles/cell and that Thd constitutes 29.4 mole percent of all DNA, the thymidine content is 7.51×10^{-15} moles/cell. The molar concentration of iodine in the cell sample was established by taking the ratio of counts in the sample and in the standard, and converting the mass of halogen in grams to a molar concentration. The ratio between the molar concentration of halogen and the molar concentration of Thd calculated to be present in the sample determined the percent incorporation in DNA.

Irradiation Procedure

The 250 µl tapered tube containing the cell suspension was positioned horizontally in a vertically oscillating scanner, designed for these experiments, with a stroke of 0.96 cm at 4.73 sec intervals. This stroke assured that the cells oscillated completely through the collimated beam, thus providing a uniform dose to all cells. For the bromine experiments, the cells were rotated about the horizontal axis at 330 rpm to assure uniform penetration of the photons. The photon source for the iodine experiments was the X17B1 wiggler magnet operating at a magnetic field of 4.9 Tesla; the X18B bending magnet (1.2 T) beam line was used for the bromine experiments. A bent Laue geometry Si (111) monochromator selected the above and below K edge X rays for the iodine experiments. A Si (220) double crystal monochromator was used for the bromine. The beam dimensions at the position of the cells were 0.8 mm X 27 mm, and 2 mm X 25 mm for iodine and bromine, respectively. The cell scanner was positioned downstream of a fast computer-activated shutter, slits which collimated the monochromatic

beam, and a 10 cm long ion chamber. The ion chamber was filled with 1 atmosphere of argon and an accelerating voltage of 300 V was used. The samples and the cell scanner were inside a Pb shielding enclosure in the beamline hutch. The energy of the monochromator was calibrated by scanning the monochromator through the absorption edge of an iodine or bromine sample. The inflection points of the absorption curves were defined to be exactly 33169 and 13474 eV for iodine and bromine, respectively.

All the experiments were performed in the mode in which dose was delivered for a predetermined number of ion chamber scaler counts. The ion chamber signal was amplified by a Keithley 427 Current Amplifier at a gain of 10^7, and converted to scalar counts by a Voltage/Frequency at 1 volt/103 Hz. This mode of operation automatically compensated for the effect of the decreasing storage ring current.

The number of scalar counts at each energy necessary for delivery of a predetermined dose to the cells was calculated from measured coefficients. With the monochromator set at an energy, LiF thermoluminescent dosimeters (TLDs) were put inside a sample tube and oscillated through the beam for a predetermined number of scalar counts. The TLDs were then read and the dose/count coefficient was thereby determined. The accuracy of the coefficient was improved by repeating this procedure many times at both energies. The TLDs were distributed inside the tubes to measure any variation in dose with position. No such variations were found. Dosimetry calibration runs were conducted before and after each experiment to verify consistency of experimental parameters. Dosimetry runs in which the monochromatic beam was prevented from impinging on the TLDs showed no dose delivered from scattered radiation in the hutch. The maximum error in the dose delivered due to the indeterminate starting point of oscillation was less than 2% for the lowest doses and less than 0.25% for the highest. Closure time for the fast shutter was less than 0.1 s, so the maximum additional dose delivered after the closure command was less than 0.8%.

Irradiation Dosimetry

Dose measurements were performed with thermoluminescent dosimeters (TLD-700, 1 mm X 1 mm X 6 mm) inserted in the same irradiation tubes and irradiated under the same conditions as the cell suspensions.

The average absorbed dose to water was calculated using the following relation:

$$D_{H_2O} = \left(\frac{\mu_{en}}{\rho}\right)_{H_2O/TLD} \bullet \frac{S(H_2O)}{S(TLD)} \bullet \frac{f^{\gamma}(D)}{f^{x}(D)} \bullet D_{TLD}$$

where $(\mu_{en}/\rho)_{H_2O/TLD}$, or the ratio of the mass energy absorption coefficient of the photons in water and in the TLD,

$S(H_2O)$, the self-shielding factor $1\text{-}e^{-\mu d}/\mu d$ for the water in the tube

S(TLD), the self-shielding factor $1\text{-}e^{-\mu d'}/\mu d'$ for the TLD
$f^{x}(D)$ and $f^{\gamma}(D)$ the supralinearity factor for the X and γ field, respectively, and

D(TLD), the absorbed dose for the calibration ^{60}Co field that is required to produce the same TL-signal as the X ray field, corrected for energy dependence.

Since the photon beam is plane parallel, the average path length d' of the photon in the dosimeter was assumed to be 82% of the thickness of the dosimeter (*i.e.*, 0.82 mm), and d is $2^{-1/2}$ times the diameter of the water in the tube. The dosimeters were calibrated individually by irradiation in a known field (^{60}Co source) both before and after the measurements. Pre-irradiation annealing was carried out at 400°C for one hour, post-irradiation annealing at 100°C for 15 min. A one-hour cooling time was used in both procedures. Twenty eight TLD dosimeters were irradiated before and in between cell irradiations.

Colony Assay for Cell Survival

After irradiation, cells were plated in 60 mm petri dishes with DME complete growth medium (FBS[3] , pen-strep-fungizone[1]), and permitted undisturbed colony growth for 5 days. After washing with PBS, colonies were fixed with EtOH, and stained with Giemsa. Colonies were counted opto-electronically on an Artec counter, adjusted to exclude colonies consisting of

[3] Hyclone

fewer than fifty cells. Survival curves were fit using an iteratively-weighted least squares analysis program as described in Ref. 6. Both the linear quadratic (LQ) and single-hit-multi-target (SHMT) models were used.

RESULTS

Cell survival curves obtained after irradiation with monochromatic photons (33.4 and 32.9 keV for above and below the K absorption edge of iodine, respectively) with a ~16% IUdR replacement in DNA are shown in Fig. 1 (LQ) and Fig. 2 (SHMT). Table I summarizes the data obtained from three experiments in composite form. The AEF is 1.4 when comparing iodinated cells irradiated above and below the K edge using the ratio of doses

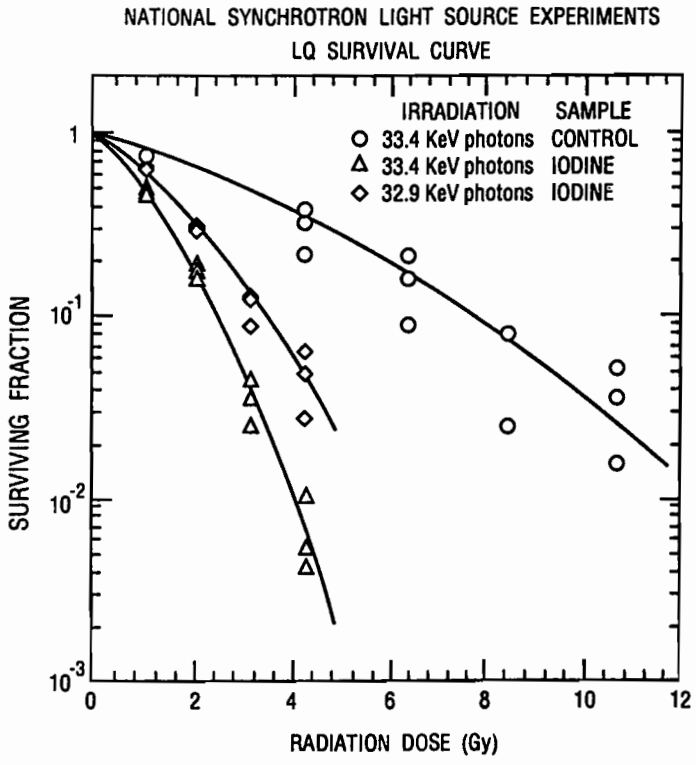

FIG. 1. V79 Chinese hamster cell survival assay post irradiation with monochromatic photons above and below the K absorption edge of iodine. Curves were least squares fit with the linear-quadratic model as in Ref. 6. IUdR incorporation ~16%.

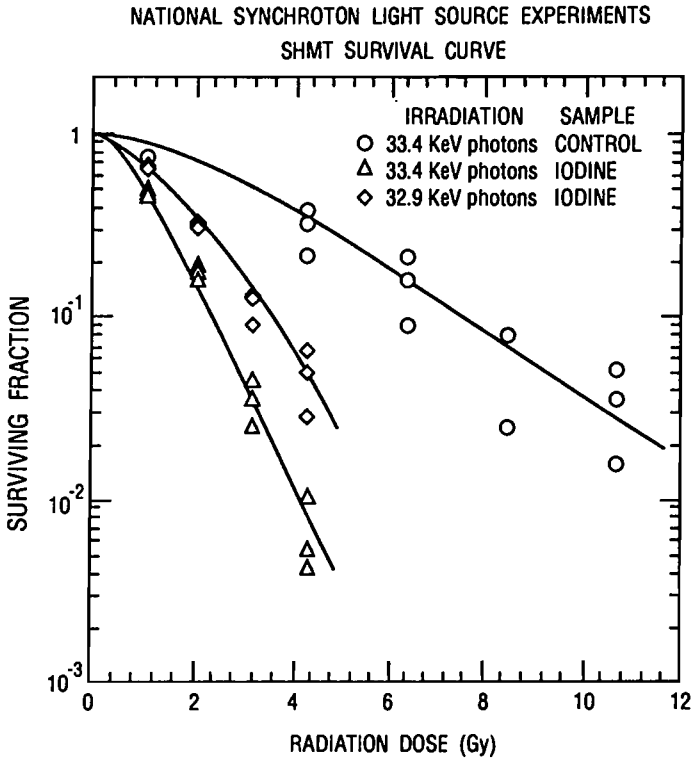

FIG. 2. V79 Chinese hamster cell survival following irradiation with monochromatic photons above and below the K absorption edge of iodine. Curves were fit with single-hit multi-target model as in Ref. 6. IUdR incorporation ~16%.

at a 10% level of survival (LQ). Using the ratio of doses at the D_0 values the factor is ~1.5. The sensitization enhancement ratio (SER) compares the ratio of doses between non-iodinated control cells and iodinated cells irradiated below the K absorption edge. At 10% survival, the SER is 2.2. Thus, the total therapeutic gain factor is the product of the SER and the AEF, 1.4 X 2.2 = 3.1.

Survival curves from cells with a ~60% BrUdR replacement in DNA are shown in Figs. 3 (LQ) and Fig. 4 (SHMT) after irradiation with monochromatic photons. Table II is a summary of these experiments. Photon energies of 13.5 and 13.4 keV were used for the above and below K edge

TABLE I
Monochromatic X ray Irradiation of IUdR Labeled V79 Cells

Sample	Dose (Gy) @ 10% Survival		D_o[a]	Therapeutic Enhancement @ 10% Survival		Gain Enhancement from Ratio of D_o Values	Auger Effectiveness Factor[b]	
	LQ	SHMT		LQ	SHMT		@ D_{10}	D_o[a]
Control	7.63	7.49	2.39					
Iodinated, above K[c]	2.53	2.46	0.74	3.02 ± 0.23	3.05 ± 0.49	3.20 ± 0.51	1.38	1.51
Iodinated, below K[d]	3.49	3.46	1.1	2.19 ± 0.16	2.17 ± 0.33	2.13 ± 0.32		

[a]SHMT only
[b]Ratio of doses or slopes (iodine below: iodine above)
[c]at energy above K edge
[d]at energy below K edge

irradiations, respectively. The SER determined at 10% survival is 2.2, or 1.94 if based on the ratio of the D_0 values. The total therapeutic gain factor is 2.2 (SER) X 1.0 (AEF) = 2.2.

DISCUSSION

While the IUdR showed a significant and demonstrable enhancement of the dose effectiveness resulting from the release of the Auger electrons, the results with the BrUdR were rather tenuous. The bromine data fell within the statistical error which interferes with the ability to distinguish an effect. However, the observed sensitization enhancement comparing unhalogenated control cells with their iodinated or brominated counterparts at 10% survival, when irradiated below their respective K edges, is 2.2 for both. This value was obtained despite a difference in halogen uptake, the bromine uptake being a factor of 4 greater than the iodine. Sensitization has

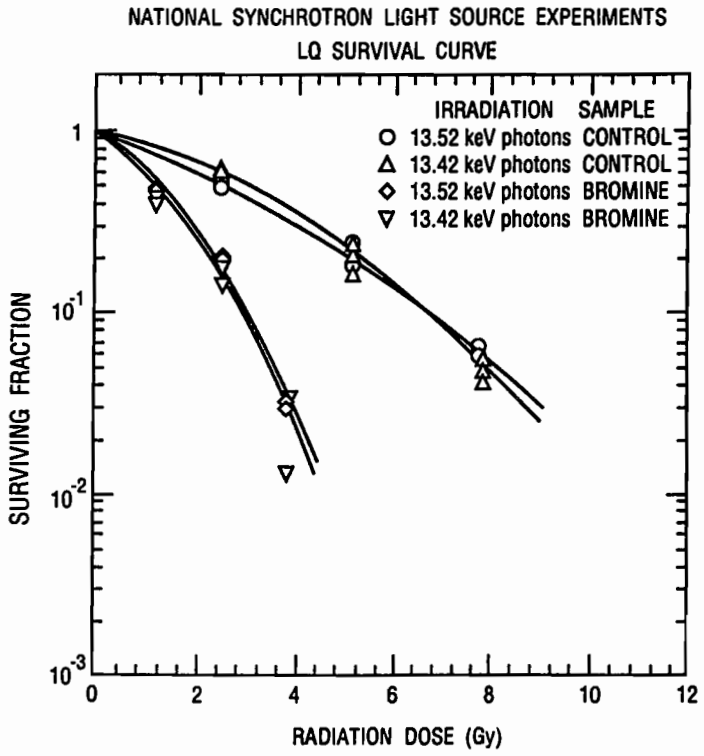

FIG. 3. V79 Chinese hamster cell survival assay post irradiation with monochromatic photons above and below the K absorption edge of bromine. Curves were fit with the linear-quadratic model as in Ref. 6. BrUdR incorporation ~60%.

been shown to increase with increased incorporation of the halogen (1). Our results show neither increased sensitization with bromine, nor any increased effectiveness attributable to Auger electron emission. This result will have to be investigated further.

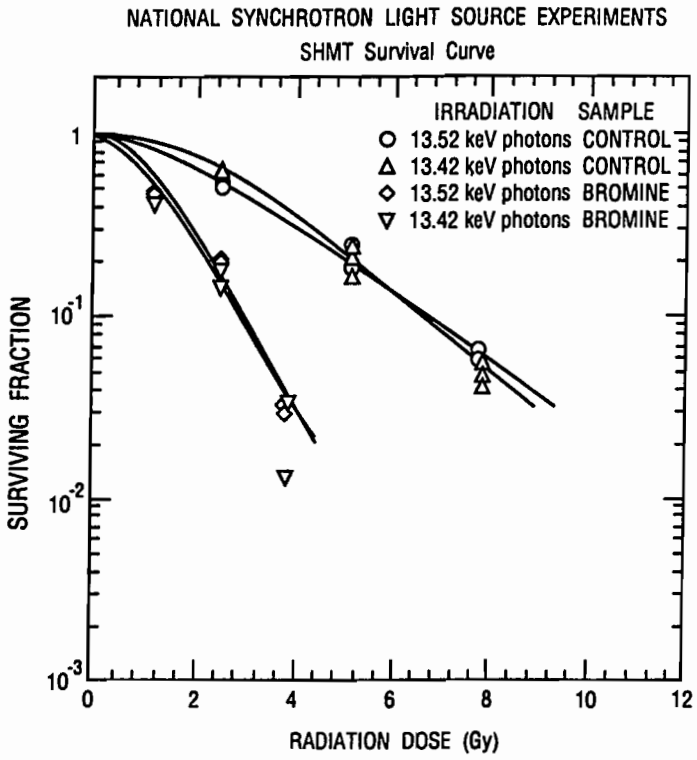

FIG. 4. V79 Chinese hamster cell survival after irradiation with monochromatic photons above and below the K absorption edge of bromine. Curves were fit with the single-hit multi-target model as in Ref. 6. BrUdR incorporation ~60%.

TABLE II
Monochromatic X ray Irradiation of BrUdR Labeled V79 Cells

Sample	Dose (Gy) @ 10% Survival		D_o[a]	Therapeutic Enhancement @ 10% Survival		Gain Enhancement from Ratio of D_o Values	Auger Effectiveness Factor[b]	
	LQ	SHMT		LQ	SHMT		@ D_{10}	D_o[a]
Control	6.83	6.74	2.23					
Iodinated above K[c]	3.13	3.08	0.89	2.18 ± 0.09	2.19 ± 0.38	2.51 ± 0.44	0.99	1.1
Iodinated below K[d]	3.09	3.04	0.98	2.21 ± 0.11	2.21 ± 0.41	1.94 ± 0.36		

[a]SHMT only
[b]Ratio of doses or slopes (bromine below: bromine above)
[c]at energy above K edge
[d]at energy below K edge

CONCLUSION

IUdR, demonstrating a 40-50% increased effectiveness of the dose from the combined effect of X rays above the K absorption edge of the iodine and the concommitant Auger electron emission, appears to be superior to BrUdR for use in PAT.

ACKNOWLEDGMENTS

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DISCUSSION

Goodhead, D. T. Have you calculated the number of photons absorbed in incorporated iodine per cell (above the K shell edge) and compared this with the amount of extra killing that you see? I am surprised that there are sufficient absorptions to cause such an effect.

Laster, B. H. No, we have not. However, I would welcome someone more experienced to assist in this approach.

Halpern, A. What is the dose you are talking about? Exposure or absorbed dose? How do you determine it?

Laster, B. H. TLD's were inserted in to lucite holders and placed in the irradiation vessel (250 µl polypropylene tube). Calculating the average absorbed dose to H_2O:

$$[(\mu_{en}/\rho)_{H_2O/TLD}]\,[S_{H_2O}/S_{TLD}]\,[f^{\gamma}(D)/F^{x}(D)]\,[D_{TLD}]\,,$$

where, $(\mu_{en}/\rho)_{H_2O/TLD}$ = ratio of mass energy absorption coefficient of the photons in water and in the TLD, S_{H_2O} = self-shielding factor $(1-e^{-\mu d})/(\mu d)$ for the water in the tube, S_{TLD} = self-shielding factor $(1-e^{-\mu d'})/(\mu d')$ for the TLD, $f^{x}(D)$ and $f^{\gamma}(D)$ = supralinearity factor for x and γ field, D_{TLD} = the absorbed

dose for the calibration in the ^{60}Co that is required to produce the same TL signal as the X ray field. The average path length d' of photons in the dosimeter was assumed to be 0.82 mm, and d is 2.5 times the diameter of the tube in H_2O. The X ray energy is 13.424 keV. D_{H_2O} = 208.6 ± 6% for 1.324353×10^6 counts from ionization chamber = 157.5 rad/10^6 counts at 13.54 keV. D_{H_2O} = 213.9 ± 6% = 161.5 rad/10^6 counts.