

Targeting Apoptosis in Various Cancer Cells Using ^{90}Y

Penelope Felipe, Sun-Ha Yoon, So-Young Lee, Young Dong Hong, and Sunju Choi
Radioisotope Research and Development Division, Korea Atomic Energy Research Institute
Daedok-daero 1045, Yuseong-gu, Daejeon 305-353, Korea
*Corresponding author: enel@kaeri.re.kr

1. Introduction

Despite several interests in cancer therapy with radioisotopes, the choice of the optimal radionuclide energy remains uncertain and probably depends on the nature of the tumor. Certain physical properties have been proposed for an ideal radionuclide to be used in cancer therapy. These include a physical half-life ($t_{1/2}$) of 1–3 days, major energy deposition between 0.5 and 30cm cell diameters, and single decay-to-ground state. ^{90}Y is a radiometal that fits these criteria and may, thus, be a better choice than other radionuclide such as ^{131}I . ^{90}Y has a greater energy emission than ^{131}I (β , $E_{\text{max}} = 2.27$ MeV *versus* β , $E_{\text{max}} = 0.81$ MeV, respectively) and a shorter half-life ($t_{1/2} = 2.67$ days *versus* $t_{1/2} = 8.06$ days, respectively). In addition, ^{90}Y deposit its energy out to 1.81 cm *versus* only 0.55 cm for ^{131}I . Of particular note, when conjugated to an internalizing antibody, ^{90}Y will be retained within the tumor cell for longer periods of time than will ^{131}I . The residualizing property of radiometals such as ^{90}Y may allow for a higher radiation dose to the tumor than could be achieved by ^{131}I . Considering the potential advantages of using a residualizing radionuclide, we examined the antitumor efficacy of ^{90}Y for the treatment of various cancer cells.

2. Methods and Results

2.1 Cell lines and culture condition

The non-small cell lung carcinoma Calu6, hepatocellular carcinoma cell line Hep3B and, the glioblastoma cell line T98G were grown as monolayers and maintained at 37°C in a humidified incubator with 5% CO_2 in Modified Eagle's Medium supplemented with 10% FBS and 1% antibiotics (GIBCO BRL, Paisley, UK).

2.2 Cytotoxicity Assay

Approximately 5×10^4 cells were seeded in each well of 96-well tissue culture plates and fresh stock solutions of ^{90}Y (NRG, Netherlands) were prepared in media and aliquots of the stock solutions were added to wells of the plates at desired activity. After 48-h incubation with ^{90}Y , surviving cells in wells were determined by the MTT assay. Twenty microliters of a 5mg/ml stock solution of MTT was added to each well, and after 2h incubation at 37°C,

100 μl of the solubilization buffer was added. After 30mins of incubation, absorbance was measured at 570 (ref. 690) nm using a microplate reader. The cell viability was calculated as the ratio of absorbance in wells containing ^{90}Y compared with control (con) cells. IC_{50} values were calculated from a linear regression from dose-dependent curves.

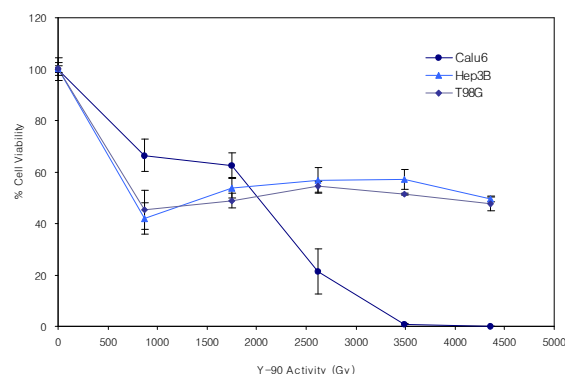


Figure 1. The effect of ^{90}Y activity on different cancer cells. Cells were incubated with various activities of ^{90}Y (0 to 4 KGy) for 48 h. Cell viability was determined by the MTT assay. Values are means (\pm S.D.) of 3 experiments.

2.3 Evaluation of DNA synthesis (BrdU incorporation assay)

This was measured by BrdU Assay kit (Chemicon, USA) according to the manufacturer's instructions. Briefly, 5×10^4 cells/well were seeded into a 96-well microtiter plate. ^{90}Y was prepared in media and aliquots of the stock solution were added to wells of the plates at desired activity. After 44-h incubation, cells were labeled with BrdU labeling reagent and reincubated further for 4 h. During this period, the pyrimidine analogue BrdU is incorporated instead of thymidine into the DNA of the proliferating cells. The cells were fixed and the DNA was denatured by adding FixDenat for 1 h. Monoclonal anti-BrdU peroxidase-conjugated antibody was added for 90 min. Cells were washed three times with wash buffer and the quantity of BrdU incorporation was detected by the monoclonal antibody from mouse-mouse hybrid cells conjugated with peroxidase (anti-BrdU-POD) which binds to the BrdU in newly synthesized cellular DNA. These immune complexes were detected by the subsequent substrate reaction and after addition of 1M H_2SO_4 , the colorimetric measurement of BrdU was read in an ELISA reader 450 nm (ref. 690 nm).

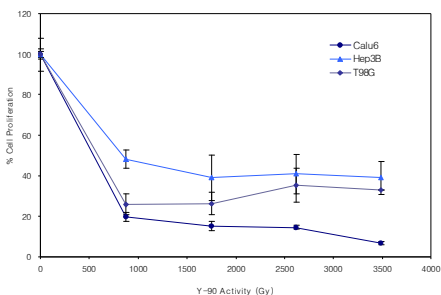


Figure 2. The effect of ^{90}Y activity on the DNA synthesis of lung cancer cells. Cells were incubated with various activities of ^{90}Y (0 to 3500 Gy) for 48h. DNA synthesis was determined by the BrdU assay. Values are means (\pm S.D.) of 3 experiments.

Table 1. The calculated IC_{50} values of ^{90}Y based on MTT and BrdU assay.

Cancer cells	^{90}Y (IC_{50})			
	MTT (uCi)	MTT (Gy)	BrdU(uCi)	BrdU (Gy)
Calu6	401	1748	179	780
Hep3B	855	3727	102	445
T98G	778	3392	204	889

The calculated IC_{50} for the MTT for lung, liver and brain cancer cells are 1750, 3730 and 3392 Gy, respectively. While for BrdU assay showed lower calculated IC_{50} 780, 444 and 890 Gy, respectively, which suggests that the cellular metabolism is not completely inhibited at concentrations where almost no cell division occurred, indicating likewise a higher sensitivity of the DNA synthesis measurement.

2.4 Western blot analysis

Following 48-hour exposure to treatment with 1750Gy of ^{90}Y , cells were harvested. Equal amounts of protein (30 μg) were resolved on 10% polyacrylamide gels. Gels were transferred onto nitrocellulose membranes that were incubated overnight at 4°C with antibodies against p53, p21 (Santa Cruz Biotechnology, Santa Cruz, CA) and cyp32 (BD laboratories). The immunoreactive proteins were detected using the enhanced chemiluminescence method (Amersham, Piscataway, NJ).

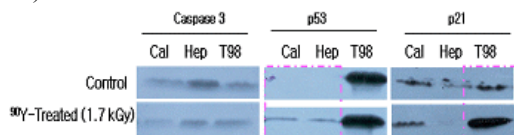


Figure 3. The apoptotic effect of ^{90}Y activity on lung, liver and brain cancer cells. Cells were incubated with 1750 Gy of ^{90}Y for 48h. Apoptotic signals were increased as shown by western blot assay. Where: Cal: Calu6; Hep: Hep3B; and T98: T98G cancer cell lines.

2.5 Terminal Transferase dUTP Nick End Labeling (TUNEL)

We used the In Situ Cell Death Detection Kit, with fluorescein, according to manufacturer's (Roche

Diagnostics, Mannheim, Germany) protocol. Briefly, controls and ^{90}Y treated cells were fixed and permeabilized. Cells were added with TUNEL reaction mixture and incubated in the dark for 1 hour. Apoptotic cells were analyzed using fluorescent microscope (40x mag).

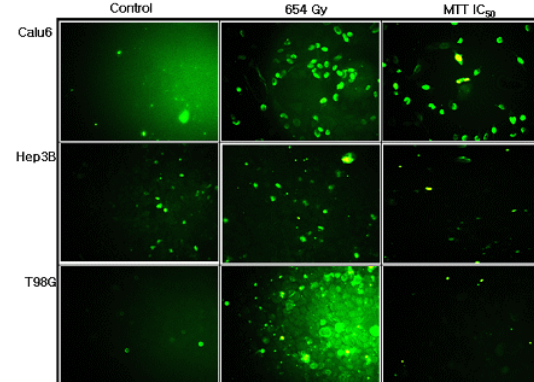


Figure 4. The apoptotic effect of ^{90}Y activity on lung, liver and brain cancer cells. Cells were incubated with 654 Gy and calculated IC_{50} (based on MTT assay for respective cells) of ^{90}Y for 48h. Apoptotic signals increased after treatment with low activity of ^{90}Y as shown by TUNEL assay.

3. Conclusion

Our study shows that ^{90}Y can inhibit proliferation of various cancer cell lines *in vitro* in an activity-dependent manner by inducing apoptosis. Our observations also suggest that the responsiveness of various cancers to ^{90}Y was due to the activation of apoptosis cascade proven by the increase in apoptotic signals such as p53, caspase3, and p21. The DNA was proven to be damaged by its laddering and positive apoptosis signal assessed by TUNEL assay. This isotope proves to be a good choice for future therapeutic studies.

REFERENCES

- [1] F.H. Miller; A.L. Keppke; D. Reddy; J. Huang; J. Jin; M.F. Mulcahy and R.Salem, Response of Liver Metastases After Treatment with Yttrium-90 Microspheres: Role of Size, Necrosis, and PET, *Am J Roentgenol.* 188 (3) p.776-783, 2007.
- [2] JYC Wong, DZ Chu, DM Yamauchi, et al. A phase I radioimmunotherapy trial evaluating 90yttrium-labeled anti-carcinoembryonic antigen (CEA) chimeric T84.66 in patients with metastatic CEA-producing malignancies. *Clin Cancer Res.* 6 p. 3855–3863, 2000.
- [3] DJ Macey, EJ Grant, L Kasi, et al. Effect of recombinant alpha-interferon on pharmacokinetics, biodistribution, toxicity, and efficacy of ^{131}I -labeled monoclonal antibody CC49 in breast cancer: a phase II trial. *Clin Cancer Res.* 3 p. 1547–1555, 1997.
- [4] RM Sharkey, WJ McBride, H Karacay, et al. A universal pretargeting system for cancer detection and therapy using bispecific antibody. *Cancer Res.* P 63:354–363, 2003.