AD_____

Award Number: DAMD17-00-1-0052

TITLE: Permanent Implantation Brachytherapy for Prostate Cancer Using A Mixture of Radionuclides with Different Half Lives

PRINCIPAL INVESTIGATOR: Ravinder Nath, Ph.D.

CONTRACTING ORGANIZATION: Yale University School of Medicine
New Haven, Connecticut 06520-8047

REPORT DATE: March 2001

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	March 2001	Annual (1 Mar 00 - 28 Feb 01)

4. TITLE AND SUBTITLE
Permanent Implantation Brachytherapy for Prostate Cancer Using A Mixture of Radionuclides with Different Half Lives

5. FUNDING NUMBERS
DAMD17-00-1-0052

6. AUTHOR(S)
Ravinder Nath, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Yale University School of Medicine
New Haven, Connecticut 06520-8047

E-Mail: ravinder.nath@yale.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
This Report Contains Colored Photos

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

The project objective is to test whether the effectiveness of brachytherapy for prostate cancer can be improved by using a combination of short and long half life radionuclides simultaneously. Key research accomplishments in the past year are: (i) a theoretical model based on incomplete repair of radiation damage at low dose rates was developed for addressing the questions raised in the project; (ii) cell survival curves for both ^{125}I and ^{103}Pd were measured using monolayers of cells in a petri dish irradiated at low dose rates using ^{125}I and ^{103}Pd sources; (iii) an afterloading seed applicator was designed and fabricated in order to produce a consistent dose distribution to irradiate tumors transplanted to different animals and to minimize the radiation exposure to personnel handling radioactive seeds. In summary, we have made considerable progress towards the development of a theoretical model for continuous low dose rate irradiation using a mixture of radionuclides. Relative biological effectiveness of ^{125}I and ^{103}Pd at low dose rates in vitro was determined. An applicator for in vivo irradiations was designed and fabricated. Animal care procedures during the long low dose rate irradiations were developed. We are now ready to launch in vivo experiments with radioactive sources.

14. SUBJECT TERMS
cancer therapy, radiation therapy, radiobiolgy, radiation physics

15. NUMBER OF PAGES
15

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

INTRODUCTION

The objective of the project is to test whether the therapeutic effectiveness of permanent implant brachytherapy for prostate cancer can be improved by using a combination of short and long half life radionuclides simultaneously. Specific aims of the proposed project are:

1. To test theoretically the potential of a mixture of radionuclides in permanent implants, using the linear quadratic model, as a function of T_{pot}, potential tumor doubling time.

2. To test experimentally the validity of this concept by in vitro irradiation of BA1112 sarcoma cells at a continuous low dose rate (CLDR) with ^{125}I (60 d half life), ^{103}Pd (17 d half life) and a 50:50 mixture of ^{125}I and ^{103}Pd under aerobic conditions leading to exponential growth at different rates (from near quiescence to full exponential growth at a maximal rate, with a doubling time of approximately 14 hours).

3. To measure the radiobiology parameters such as alpha, beta, half life of repair for the BA1112 sarcoma cells under different growth conditions and develop a theoretical model to predict expected levels of cell killing using ^{125}I, ^{103}Pd or a mixture of these isotopes.

4. To use immunohistochemical techniques to measure, in solid BA1112 tumors in vivo, the proportion of cells in S phase, the proportion proliferating and non-proliferating cells and the tumor doubling time.

5. To test the therapeutic effectiveness of ^{103}Pd, ^{125}I and a Pd/I mixture in the BA1112 in vivo tumor system;

6. To test the therapeutic effectiveness of ^{103}Pd, ^{125}I and a Pd/I mixture in human prostate carcinoma xenografts in nude mice, using a slow growing and a fast growing carcinoma.

7. To evaluate the clinical potential and feasibility of this approach in the treatment of human prostate cancer.

BODY OF THE REPORT

The overall strategy of our project has been to focus our efforts on determining the therapeutic effectiveness of ^{125}I and ^{103}Pd implants by performing tumor cure experiments in a rat solid tumor model. Short of human clinical studies, the best model to investigate our hypothesis of using a mixture of short and long half life radionuclides simultaneously is to use an in vivo animal model. In order to plan animal experiments and to enhance our understanding of the radiobiology underlying the effects observed, we have made an intensive effort in developing a theoretical radiobiology model and in vitro experiments using continuous low dose rate irradiation (CLDRI). Theoretical studies were performed to investigate the hypothesis and to plan animals' studies. Some

experiments have been performed using BA1112 tumor cells and Chinese Hamster cells growing in vitro and BA1112 cells growing in vivo as solid tumors in WAG/rij rats.

The linear-quadratic model of cell-killing by radiation

One of the models that have gained popularity for the understanding of time-dose fractionation and dose rate effects is the linear quadratic model (the alpha-beta model). Ionizing radiation destroys cells by depositing energy directly in a critical target within the cell or indirectly by producing highly reactive radicals in the aqueous medium around the cells. Substantial evidence exists to show that the critical target or targets are within the cellular nucleus and that ionizing radiation can cause single strand breaks (SSBs) and double strand breaks (DSBs) in the DNA. Much of the radiation-induced damage is sublethal and is repaired with a time constant of a few hours. The alpha-beta model of radiation action includes two types of cell damage: (i) the alpha damage is represented by a linear coefficient per unit dose, that may reflect death due to single lethal events such as DSBs in the cell, (ii) the beta damage is represented by a quadratic term, that reflect death due to the summation of individually sublethal events that occurred within a critical time and volume, such as two SSBs separated by a few bases and occurring within a short time. In this model the surviving fraction of cells, S, irradiated with a dose D is given by Poisson distribution as

$$S = e^{-(\alpha D + \beta D^2)} \text{ or } -\ln S = \alpha D + \beta D^2 .$$

The above equation holds when radiation is delivered at a high dose rate in a short time period, but does take into account the repair of sublethal damage through the quadratic term, but does not take into account the repair of beta-type damage over long irradiations. The alpha/beta ratio for tumors and early responding normal tissues is about 10 Gy; the ratio for the late responding tissues is about 2.5 Gy.

If irradiation is delivered continuously at a low dose rate over a time period of hours or days, as is typical in brachytherapy, repopulation of tumors and early responding normal tissues can have significant effects on the cell population (Dale 1985, 1989). This is taken into account by adding an exponential term, which depends on the irradiation time, T, as $-\ln S = \alpha D + \beta D^2 - (0.693T / T_{pot})$, where T_{pot} is the potential tumor doubling time of the cell population.

It is also possible to include the repair of beta-type damage during a long irradiation (in low dose rate brachytherapy) by introducing a term, G,

$$-\ln S = \alpha D + G\beta D^2 - (0.693T / T_{pot}),$$

where $G = (2/\mu T)\left(1 - \left[1 - e^{-\mu T}\right]/\mu T\right)$ and μ is the time constant for sublethal repair. Typically, half time for repair ($= 0.693/\mu$) is about 0.5 hr for tumors and 1.5 hr for late responding normal tissues (Dale 1989).

For a course of fractionated radiotherapy or brachytherapy, the concept of

biologically effective dose (BED) has been defined as the dose that results in cell survival given by the equation $-\ln S = \alpha \cdot \mathrm{BED}$.

For CLDRI, the BED for a constant dose rate of R is

$$BED = D\left\{1+2(\beta/\alpha)\frac{R}{\mu}\left[1-\frac{1}{\mu T}(1-e^{-\mu T})\right]\right\}-0.693T/(\alpha T_{pot})$$

This has been further extended to consider the change in dose rate occurring with irradiation from a decaying radionuclide, as for prostate seed implantation

$$BED = D(t)\left[1+2(\beta/\alpha)\frac{R_0\gamma\cdot}{(\mu-\lambda)}\right]-0.693t/(\alpha T_{pot})$$

and $\gamma = \dfrac{1}{1-e^{-\lambda t}}\left\{\dfrac{1}{2}(1-e^{-2\lambda t})-\dfrac{\lambda}{\mu+\lambda}(1-e^{-(\mu+\lambda)t})\right\}$

where D(t) the total dose delivered in the time period of t, R_0 the initial dose rate, λ the decay constant of the radionuclide. Using this model, Ling et al. (1995) reported BEDs for ^{198}Au (2.7 day half-life), ^{103}Pd (17 day half-life) and ^{125}I (60 day half-life) seed implants. For low dose rate permanent implants, beyond a certain effective time (t_{eff}) there is no further increase in BED and no further decrease in the surviving fraction, because repopulation overcomes cell killing. Surviving fractions as a function of time up to (t_{eff}) have been calculated, and show different levels of cell survival for different implants.

Ling et al (1995) conclude that the most important parameter determining the ultimate low level of cell survival is T_{pot}, and conclude that there is an advantage for ^{103}Pd over ^{125}I for T_{pot} greater than 10 d and an advantage for ^{125}I over ^{103}Pd for T_{pot} of less than 10 days.

The current alpha-beta model has been modified for addressing the questions raised in the project. The BED for implants with a mixture of two radionuclides is derived as

$$BED = D(t)\left[1+2(\beta/\alpha)\frac{\gamma}{D(t)}\right]-0.693t/(\alpha T_{pot})$$

where

$$\gamma = \frac{R_1^2}{\mu-\lambda_1}\left\{\frac{1}{2\lambda_1}(1-e^{-2\lambda_1 t})-\frac{1}{\lambda_1+\mu}(1-e^{-(\mu+\lambda_1)t})\right\}$$
$$+\frac{R_2^2}{\mu-\lambda_2}\left\{\frac{1}{2\lambda_2}(1-e^{-2\lambda_2 t})-\frac{1}{\lambda_2+\mu}(1-e^{-(\mu+\lambda_2)t})\right\}$$
$$+\frac{R_1 R_2}{\mu-\lambda_1}\left\{\frac{1}{\lambda_1+\lambda_2}(1-e^{-(\lambda_1+\lambda_2)t})-\frac{1}{\lambda_2+\mu}(1-e^{-(\mu+\lambda_2)t})\right\}$$
$$+\frac{R_1 R_2}{\mu-\lambda_2}\left\{\frac{1}{\lambda_1+\lambda_2}(1-e^{-(\lambda_1+\lambda_2)t})-\frac{1}{\lambda_1+\mu}(1-e^{-(\mu+\lambda_1)t})\right\}$$

and $D(t)=\frac{R_1}{\lambda_1}(1-e^{-\lambda_1 t})+\frac{R_2}{\lambda_2}(1-e^{-\lambda_2 t})$. This equation for BED states that the BED for implants with a mixture of radionuclides does *not* equal to the simple addition of the BEDs from each radionuclide type alone.

BA1112 in vitro studies

A BA1112 tumor was grown between the ears of the a 14 week old male WAG/Rij Y rat by interdermal inoculation from a single cell suspension of BA1112 cells obtained from a 21 day BA1112 tumor growing on the head of a previously inoculated rat. A tumor cell suspension was made from the BA 1112 tumor and between $1.5x10^5$ and $5.0x10^5$ cells were plated into petri dishes. These cells were allowed to settle and reach logarithmic growth, (48-72 hrs), before they were used in a continuous low dose rate experiment.

Monolayers of Chinese hamster lung cells (CCL-16) and rat rhabdomyosarcoma cells (BA1112) were irradiated in vitro by ^{103}Pd and ^{125}I sources in a polystyrene phantom. Colony formation ability of irradiated cells under aerobic conditions was measured for graded doses, at dose rates of 6 to 20 cGy/hr. Dose to the cell monolayers was determined using $FeSO_4$ Fricke dosimetry, with a calculated correction for interface effects due to photoelectric effect in the tissue culture dishes. The sources (up to 60 in one experiment) were arranged in concentric circles in such a way as to provide a dose uniformity of better than ± 5% across the dishes.

After continuous low-dose-rate irradiations *in vitro*, cell survival curves were determined using our *in vitro* colony formation assay. Immediately following irradiation treatment, each petri dish was washed with 5 ml of Hanks basal salt solution (w/o calcium and magnesium) to remove any sera that may be attached to the cells. The Hanks solution was removed and fresh trypsin is added and the dishes put back into a 37° C incubator until the cells gently detach from the petri dish to yield a single-cell suspension. The trypan-blue excluded cells were counted using a phase-contrast microscope and diluted to produce approximately 80 colonies per dish. The cells were plated into a dish of preincubated nutrient medium (Dulbecco's modified Eagle's medium with 1000 mg/liter of glucose, supplemented with16% fetal bovine serum, 0.1 ml nonessential amino acids, 1.1 mg/liter sodium pyruvate, and 1000 U/liter each of penicillin and

streptomycin. The cultures were incubated for 2 weeks in a humidified 37° C atmosphere composed of 95% air and 5% CO_2. The resultant colonies were then fixed, stained and counted. Surviving fractions were calculated based upon the ratio of plating efficiency of irradiated tumor cells to the plating efficiency of untreated tumor cells.

Cells were in exponential growth during the irradiation and a correction for cell loss during the irradiation period was applied to the cell survival data. Cell survival curves for both ^{125}I and ^{103}Pd were observed to be linear at all dose rates studied (Figure 1). The slopes of the survival curves for ^{125}I increased as the dose rate increased from 6.89 to 19.1 cGy/hr, indicating a large dose rate effect over this range (Figure 2). Similarly, the ^{103}Pd survival curve for 12.6 cGy/hr was considerably steeper than that for 6.86 cGy/hr. The dose sparing effect of 7 cGy/hr relative to 12 cGy/hr can be expressed by dose modifying factors of 2.4 ±0.6 and 1.5 ±0.5 for ^{103}Pd and ^{125}I, respectively. The RBEs of ^{103}Pd relative to ^{125}I were 1.2 ± 0.4 and 2.0 ± 0.5 for 7 and 12 cGy/hr, respectively (Table 1). For rapidly growing Chinese hamster cells under aerobic conditions, a profound dose rate effect is observed over the dose rate range of 6 to 19 cGy/hr. Because ^{103}Pd implants are generally prescribed at a higher initial dose rate (19.7 cGy/hr) than the corresponding ^{125}I implants (7.72 cGy/hr), effects of both dose rate and photon energy on biological response should be considered together. In our system, the RBE of ^{103}Pd at 19.7 cGy/hr relative to ^{125}I at 7.72 cGy/hr is estimated to be 3±1.

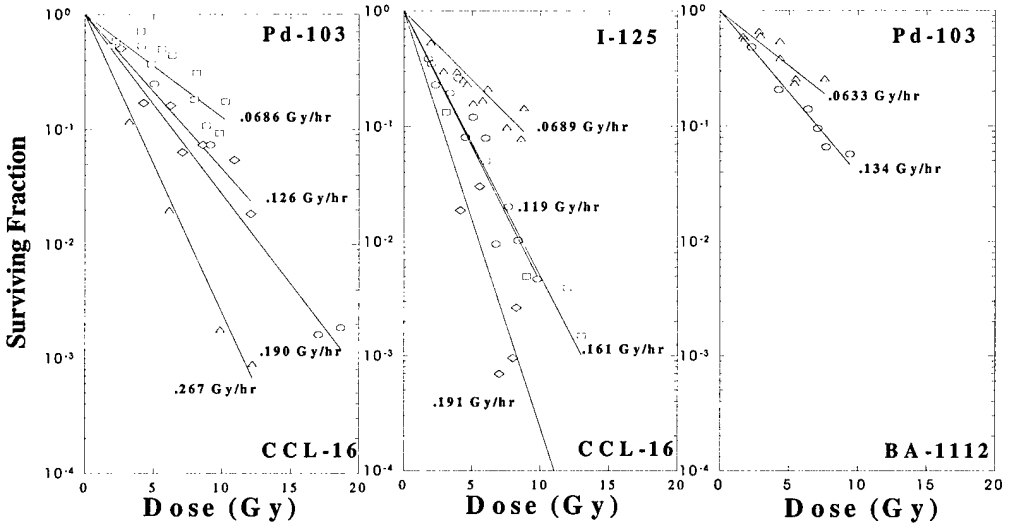


Figure 1. The survival curves for the cells from Chinese hamster cells and from BA 1112 tumors treated *in vitro* with continuous low-dose-rate irradiation at dose rates of 0.06 and 0.134 Gy/hr respectively using ^{103}Pd and ^{125}I are shown in the graphs above. Points are surviving fractions determined for individual tumors; lines were fitted by regression analysis.

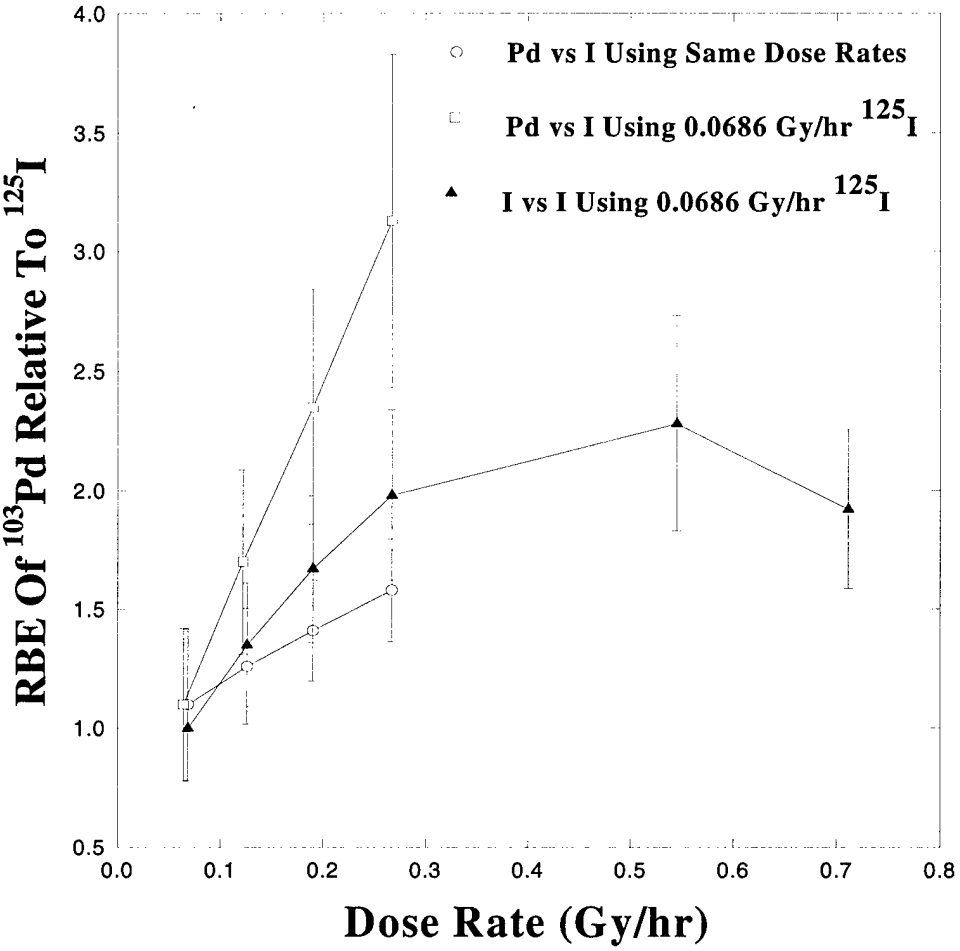


Figure 2. RBE effect of ^{103}Pd vs ^{125}I using the same dose rates (open circles), using 0.06Gy/hr ^{125}I as reference (open squares), and the RBE effect of ^{125}I at one dose rate vs ^{125}I at another dose rate using 0.06 Gy/hr ^{125}I as reference (filled triangles).

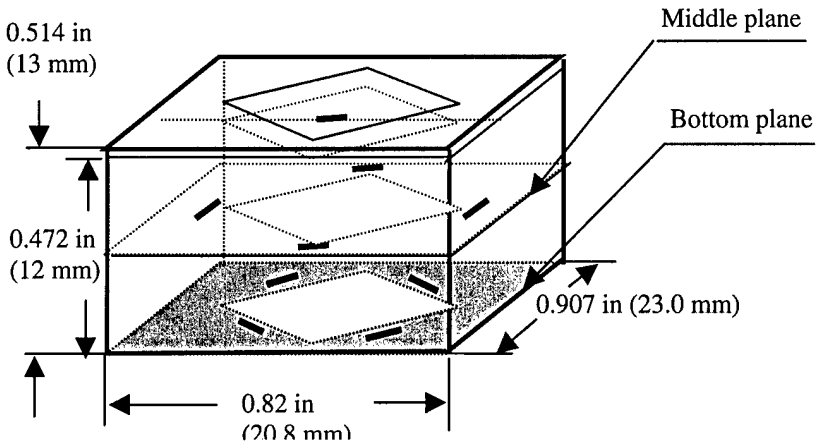
Dose rate (Gy/hr)	RBE of ^{103}Pd relative to ^{125}I at same dose rate	RBE of ^{103}Pd relative to ^{125}I at 0.0686 cGy/hr	RBE of ^{125}I relative to ^{125}I at 0.0686 cGy/hr
0.0683	1.1 ± 0.3	1.1 ± 0.3	1.0 ± 0.3
0.126	1.3 ± 0.2	1.7 ± 0.3	1.4 ± 0.3
0.190	1.4 ± 0.2	2.3 ± 0.5	1.7 ± 0.4
0.267	1.6 ± 0.2	3.1 ± 0.7	2.0 ± 0.4
0.545			2.3 ± 0.5
0.711			1.9 ± 0.4

Table 1: Relative Biological Effectiveness (RBE) of ^{103}Pd photons relative to ^{125}I.

Afterloading applicator for in vivo studies

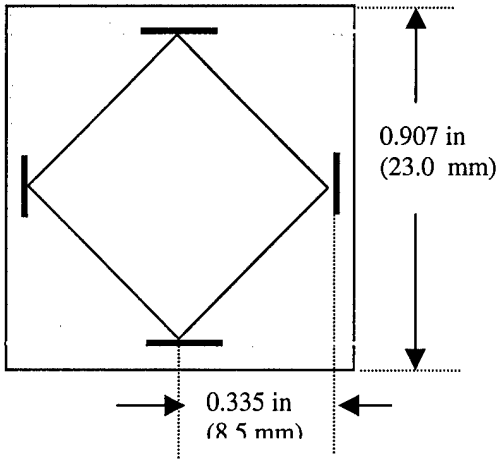
In order to produce a consistent dose distribution to irradiate the tumors transplanted to different animals and to minimize radiation exposure to personnel handling the radioactive seeds, an afterloading seed applicator was designed. Figure 3 depicts the applicator and the designed seed locations. The applicator was made of polystyrene with loading ports for nine seeds. The central portion of the applicator was open and has a dimension large enough for tumor to grow. Equal source strength was assigned to all nine seeds in order to minimize the possible confusion of handling variable source strengths. The seeding configuration was optimized to produce an, as uniform as possible, dose distribution to the central portion of the applicator and to be usable for both ^{125}Iand ^{103}Pd seeds. Sample isodose distributions in the planes parallel to the base of the applicator are shown in Figure 4 for ^{125}I seeds for a planned total dose of 145 Gy.

Figure 3. ^{125}I and ^{103}Pd afterloading applicators



Middle Plane:

(0.236 in (6mm) from bottom)



Bottom plane:

(0.039 in (1 mm) from bottom)

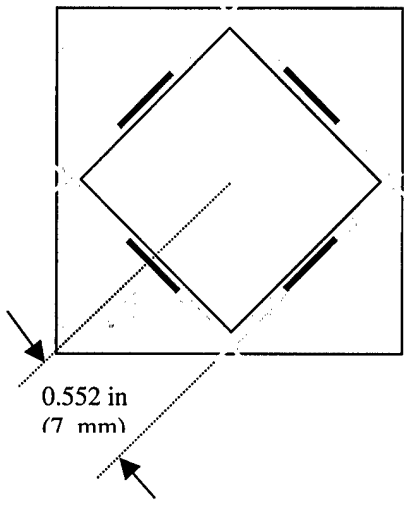
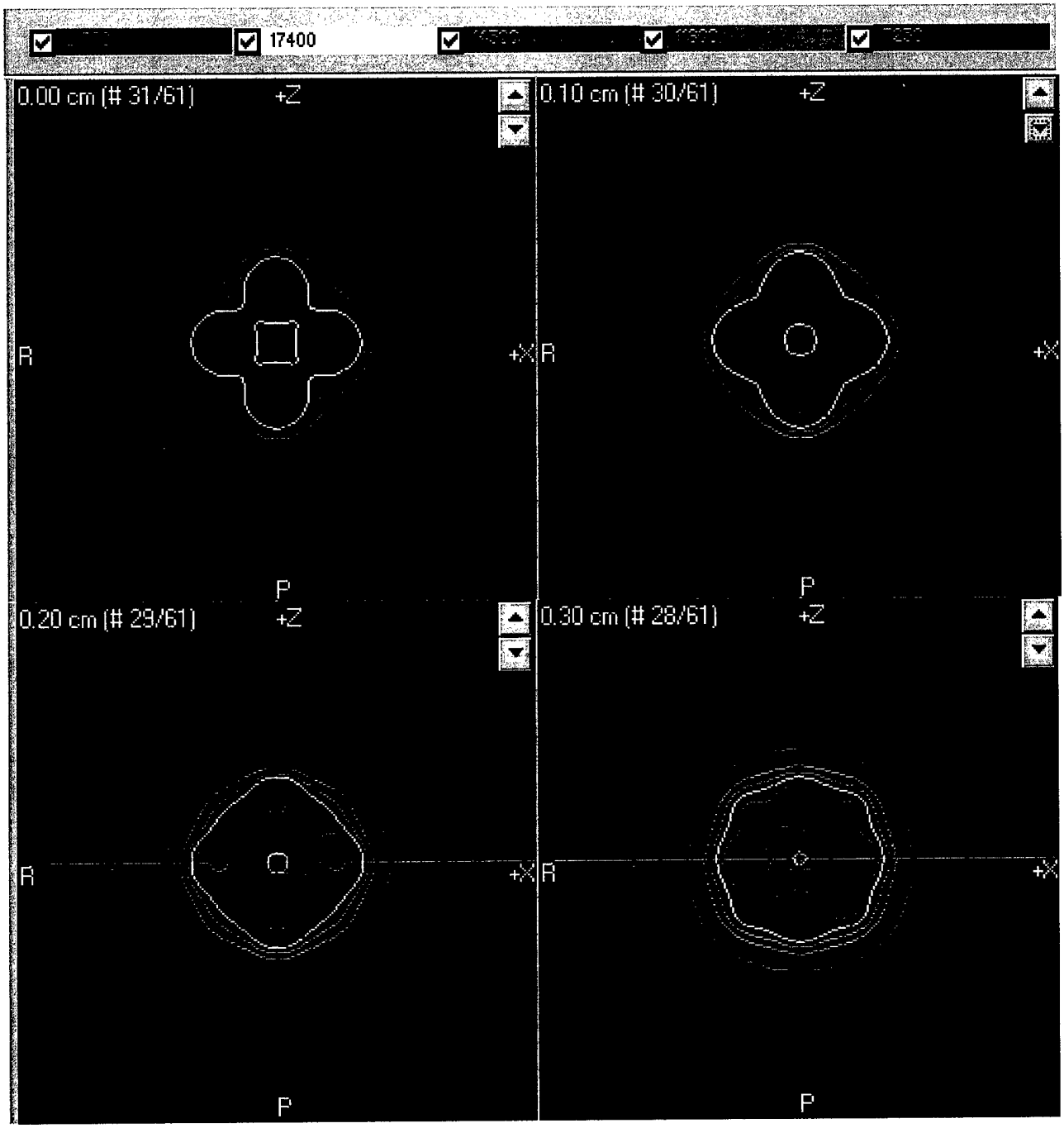


Figure 4: I-125 Isodose Distribution for a Prescription of 145 Gy



BA1112 tumor in vivo studies

Prototype applicators were built to conduct a sham tumor cure experiment. This was necessary to see if the rats could tolerate the long irradiation times that were necessary to reproduce the permanent interstitial brachytherapy dose rates used in the clinical setting. The prototype applicators were loaded with dummy $^{125}I/^{103}Pd$ seeds to simulate the experimental conditions of the experimental applicators.

In the live-source experiments, all applicators will hold the maximum 9 seeds as shown the Figure I and will be loaded initially in the Hot lab, then transported to the animal facility to be afterloaded into the helmet. Initial dosimetry measurements with the $^{103}Pd/^{125}I$ seeds will be performed for each applicator. This will ensure accurate dose rates and total doses delivered to the tumors.

A light-weight metallic helmet measuring 2.2 cm (cranial-caudal length) x 2.15 cm (side-side width) x 2.2 cm (height) was sutured to the rat's head by two stitches through the cartilage of the ears and two more stitches just behind the head at the neck. A fifth suture placed under the tumor will be tied to the central bar that across the top of the helmet, thus ensuring the tumor is pulled up into the center of the treatment volume. Treatment volumes will be 2.3 cm^3 for ^{125}I or ^{103}Pd. The lightweight metallic helmet was afterloaded with the prototype seed applicator.

The clinical doses that will be delivered in the experimental applicators are 145 Gy for the ^{125}I sources and 115 Gy for the ^{103}Pd sources.

For these experimental rat applicators, the initial doses and source strengths that will be used are the following:

Dose Rate (cGy/hr)	**Total Activity ^{125}I (mCi)**	**Total Activity ^{103}Pd (mCi)**
32.0	26.7	18.7
16.0	13.4	9.4
8.0	6.7	4.7

In order to obtain the prescribed dose of 145 Gy at the three dose rates of interest, 32, 16 and 8 cGy/hr, the helmets must remain on the animal for approximately 22, 40, and 80 days, respectively. The initial results on long-term in-vivo irradiations show that the long irradiation times up to 44 days can be obtained for these experiments.

Prior to the initiation of these experiments, the animal research reported here was reviewed and approved by the Yale University Animal Care and Use Committee. After an inspection by the Institutional Animal Care & Use Committee (IACUC) and new

Federal Regulations being implemented, concerns were raised about the housing of the animals in the existing shielded cages. These concerns related to the light, airflow and length of time in the boxes. The shielded boxes were redesigned and changes made to allow more light and air into the box for the rat's comfort during the long irradiations. New technology, Clear-Pb Plastic, was used to make the sliding outer door. The 46 mm thickness, 2.0mm lead equivalence, doors allows the animals to be viewed during the irradiation procedure while giving maximum radiation safety. The Yale Animal Care and Use Committee has approved all the modifications in the boxes.

Tumors for in vivo studies have been implanted by the subcutaneous inoculation of 5000 tumor cells into the subcutaneous tissues on the heads of WAG/rij rats. Rats are bred and maintained in our breeding colony under SPF conditions. Tumors used for experiments have a volume of 100-200 mm^3, approximately 3 weeks after inoculation. Two techniques have been developed to measure the response of tumors to treatment: a tumor cell survival assay and a tumor growth/cure assay. In the first assay, rats are killed at the end of the irradiation and the tumor cells are suspended, counted, and assayed for viability using the same colony formation assay used for cells from cultures. Analyses of cell yield are performed to allow for the loss of cells during the protracted irradiations. This endpoint will be used to measure cell survival curves after relatively short, graded treatment times (hours to days). To measure the effects of more intensive treatments, tumors will be measured 3 times per week until each tumor reaches a volume of 1000 mm^3 or until the animal has been free of tumor for 100 days. The pattern of tumor growth will be analyzed and TCD_{50} will be calculated to compare the effects of different treatments.

The response of the tumors to treatment will be analyzed by implanting the tumors with ^{103}Pd, ^{125}I or mixed implants, as described above, and measuring the tumors twice weekly until each tumor has reached a maximum volume of 1 cm^3 or until the tumor has regressed and the animal has been free of tumor for 100 days.

KEY RESEARCH ACCOMPLISHMENTS

- A theoretical model based on incomplete repair during CLDRI has been developed for addressing the questions raised in the project. The BED for implants with a mixture of two radionuclides has been derived as an analytical expression (see Appendix for details).

- Cell survival curves for both ^{125}I and ^{103}Pd were measured using monolayers of cells in a petri dish irradiated at low dose rates using ^{125}I and ^{103}Pd sources. The dose sparing effect of 7 cGy/hr relative to 12 cGy/hr can be expressed by dose modifying factors of 2 ±0.6 and 1.5 ±0.5 for ^{103}Pd and ^{125}I, respectively. The RBEs of ^{103}Pd relative to ^{125}I were 1.2 ± 0.4 and 2.0 ± 0.5 for 7 and 12 cGy/hr, respectively. In our system, the RBE of ^{103}Pd at 19.7 cGy/hr relative to ^{125}I at 7.72 cGy/hr is estimated to be 3±1.

- In order to produce a consistent dose distribution to irradiate the tumors transplanted to different animals and to minimize the radiation exposure to personnel handling the

radioactive seeds, an afterloading seed applicator has been designed and fabricated.

- Animal care procedures during long low dose rate irradiations have been developed.

REPORTABLE OUTCOMES

None

CONCLUSIONS

We have made considerable progress towards the theoretical model for continuous low dose rate irradiation using a mixture of radionuclides and in vitro experiments. We have designed and fabricated an applicator for in vivo irradiations as well as developed the animal care procedures. We are now ready to launch in vivo experiments with radioactive sources.

REFERENCES

1. R.G. Dale, Br. J. Radiol. 62, 241-244 (1989).

2. R.G. Dale, Br. J. Radiol. 58, 515-528 (1985).

3. C.C. Ling, Int. J. Radiat. Oncol. Biol. Phys. 23, 81-87 (1992).

4. C.C. Ling, W.X. Li, L.L. Anderson, Int. J. Radiat. Oncol. Biol. Phys. 32, 373-378 (1995).