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CRADA FINAL REPORT

ORNL98-0502

"Development and Evaluation of Rhenium-188-labeled Radioactive Stents for Restenosis Therapy and Development of Strategies for Radiolabeling Brachytherapy Sources with Palladium-103"

F. F. (Russ) Knapp, Jr., Ph.D.
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ORNL-27 (4-00)

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ABSTRACT -

This project involved collaboration between InnerDyne, Inc., and radiopharmaceutical research programs at the Oak Ridge National Laboratory (ORNL) and Brookhaven National Laboratory (BNL) which explored new strategies for the development and animal testing of radioactive rhenium-188-labeled implantable stent sources for the treatment of coronary restenosis after angioplasty and the development of chemical species radiolabeled with the palladium-103 radioisotope for the treatment of cancer. Rhenium-188 was made available for these studies from radioactive decay of tungsten-188 produced in the ORNL High Flux Isotope Reactor (HFIR). Stent activation and coating technology was developed and provided by InnerDyne, Inc., and stent radiolabeling technology and animal studies were conducted by InnerDyne staff in conjunction with investigators at BNL. Collaborative studies in animals were supported at sites by InnerDyne, Inc. New chemical methods for attaching the palladium-103 radioisotope to bifunctional chelate technologies were developed by investigators at ORNL.

OBJECTIVES -

Specific Project Objectives -

Development of the stent radiolabeling technology, coating technology and initial animal studies were conducted in conjunction with ORNL through a "*Memorandum of Purchase*" at the Medical Department of Brookhaven National Laboratory (BNL), where InnerDyne, Inc. staff worked closely with BNL staff on these aspects of the project. Other animal studies were supported by InnerDyne, Inc., at collaborating institutions. Studies at ORNL focused on providing the tungsten-188/rhenium-88 generators and concentration technology to BNL and in developing and characterizing new palladium-103 bifunctional chelates.

The Specific Project Objectives were as follows:

a. Development and evaluation of methods for preparation of rhenium-188-labeled stents for inhibition of coronary restenosis following balloon angioplasty (InnerDyne, Inc./BNL). Tungsten-188/rhenium-188 generators are being provided for those aspects of the project which require rhenium-188 (ORNL).

b. Development and optimization of the *in vivo* compatible post-radiolabeling stent coating materials (InnerDyne, Inc./BNL).

c. Evaluation of the effectiveness of the rhenium-188-labeled stents in animal models (InnerDyne, Inc./BNL).

D. Development and synthesis of new bifunctional chelates for attachment of the palladium-103 radioisotope to biodegradable implants and targeting agents for potential applications for restenosis and cancer therapy (ORNL).

BENEFITS TO THE FUNDING OF DOE OFFICE OF SCIENCE MISSION -

The primary benefit of this CRADA research was the opportunity for DOE laboratory programs to use their technology, expertise and resources for interaction with an industrial partner working a project of expected importance to the U.S. health care community. InnerDyne, Inc., the industry partner, had been able to access laboratories at BNL suitable for the handling and use of rhenium-188, the required animal laboratories and other support facilities (e.g. health physics, etc.), without having to undergo the high expense of setting up such laboratories. The High Flux Isotope Reactor (HFIR) and associated hot cell processing and radiochemical facilities at ORNL were important resources that had provided the opportunity to provide the tungsten-188/rhenium-188 generators to InnerDyne, Inc. for use at BNL and other collaborating institutions for this project.

A major interest of the DOE since formation of the*Atomic Energy Commission (AEC)* continues to be the peaceful applications of nuclear technology for medical applications. The clinical specialty of nuclear medicine was developed and grew from AEC support of such programs at the national laboratories. The DOE continues to support major program elements in nuclear medicine and radiopharmaceutical research at the national laboratories and the research supported by this CRADA complements and further expands the capabilities of these important programs. In addition, the ORNL HFIR represents a unique DOE resource required for production of the tungsten-188 and palladium-103 that were required for this project. The availability of the extensive facilities and resources at the BNL were important for the animal studies associated with this project.

The initial development of this project grew out of the availability of the tungsten-188/rhenium-188 generator system and radiopharmaceutical research capabilities at ORNL and the extensive animal testing capabilities at BNL, which were both supported by the DOE *Office of Biomedical and Environmental Research*. The radiopharmaceutical research programs at ORNL and BNL have international recognition for the development and testing of a large variety of tissue-specific diagnostic and therapeutic radiopharmaceuticals and have had an effective collaboration for over a decade.

OVERVIEW OF SCIENTIFIC WORK - TECHNICAL DISCUSSION -

Summary of Work Performed at ORNL -

Synthetic and radiochemical development studies were performed at ORNL by Dr. D. W. McPherson, Dr. H. Luo and Mr. A. L. Beets and included the following:

a. Production and processing tungsten-188 and fabrication of tungsten-188/rhenium-188 generators
b. Synthesis and characterization of a variety target bifunctional chelates for palladium(II)
c. Binding and stability studies with Pd(II) by NMR and chromatography
d. Optimization of palladium-103 radiolabeling of best candidate ligand

Summary of Work Performed at Brookhaven National Laboratory (BNL) -

Dr. P. Som (BNL) and Dr. P. O. Zamora (InnerDyne, Inc.) participated in work done at BNL. Over the course of the CRADA, InnerDyne, Inc. provided salary and travel and housing funds for Dr. Zamora to work at BNL as a guest scientist. Work at BNL facilities involved:

e. Development of methodology to attach chelating agents to stent material suitable for use with rhenium-188
f. Development and optimization of rhenium-188 radiolabeling
g. Development of sealants to be used for rhenium-188 stents
h. Preparation and supply of rhenium-188 radiolabeled stents at institutions collaborating with InnerDyne, Inc.

Summary of Work Performed by InnerDyne, Inc. (Salt Lake City, Utah) -

Drs. G. Osaki and M. Chen participated in work performed at InnerDyne's Salt Lake City facility. Over the course of the CRADA and in collaboration with Dr. Zamora (InnerDyne, Inc.) at BNL, this group performed the following:

a. Preparation and physicochemical characterization of stents and stent surrogate surfaces,
b. Coating of stents for use in animal studies
c. Oversight of contracted animal studies involving implant of both radioactive and non-radioactive stents
d. Oversight of contract histomorphometry studies involving implant of both radioactive and non-radioactive stents.

Rhenium-188-Labeled Stents for Restenosis Therapy -

Rhenium-188 (radioactive half-life = 16.9 hours) emits beta particles with a maximal energy of 2.12 MeV which has an ideal soft tissue penetrating range for use in intravascular brachytherapy for the inhibition of restenosis after coronary angioplasty and for the treatment of and certain cancer with rhenium-188-labeled devices. The focus of this part of the project was the development of stent coating technologies, rhenium-188-labeling methods and testing of the rhenium-188-labeled stents in animal models. Rhenium-188 was available from the ORNL alumina-based tungsten-188/rhenium-188 generators. Tungsten-188 was produced in the ORNL High Flux Isotope Reactor (HFIR), processed and the tungsten-188/rhenium-188 generators fabricated and shipped to BNL for these studies. Methods were developed and provided to BNL and InnerDyne investigators for concentration of the rhenium-188 solution obtained from the tungsten-188/rhenium-188 generator.

Rhenium-188 was attached to metal wafers and stents *via* a chelating microfilm, and these brachytherapy sources were characterized *in vitro* and *in vivo*. To prepare the sources, a siloxane film containing reactive amines was plasma deposited on the metal, a chelating microfilm conjugated to the amines, and the chelating microfilm used to attach rhenium-188, which was selectively bound to materials coated with the chelating microfilm. Binding correlated with the amount of radioactivity used. Wafers (1 cm^2) bound up to 62.9 MBq (1.7 mCi) of rhenium-188 with

yields generally near 30%. Stents bound up to 26.6 MBq (720 mCi). Typically, stents were labeled to bind 4-12 MBq to deposit 10-30 Gy at 2 mm in the arterial wall. In phantom studies, the longer nitinol stents deposited doses of 2.3 Gy/MBq (0.085 Gy/mCi) while shorter stainless steel stents deposited 4.62 Gy/MBq (0.171 Gy/mCi). After placement in arteries of pigs, radioactivity from rhenium-188 was only detected by scintigraphy associated with the stents at times up to 24 hrs. Scintigraphy did not detect activity in other organs. Blood sampling (0.1-24 hrs) detected maximum radioactivity (up to 388 cpm/ml/100mCi) at 6 hours, which demonstrated that on-demand radiolabeling of stents and other brachytherapy sources with rhenium-188 could be performed routinely. A pilot dose study was conducted using two candidate sealants for rhenium-188 stents. Implantation of the rhenium-188 stents decreased the percentage of arterial occlusion compared to historical controls when above a 200 mC/stent threshold. Inflammation was slightly higher than historical controls even at high doses.

An expanded series of animal studies were conducted to evaluate the efficacy of using rhenium-188 stents using the sealant with the best results from the pilot study. Stents were labeled at BNL "on-demand" in a format suitable for assembly into a radiolabeling kit. The rhenium-188 stents were sealed with a biocompatible polymer and delivered by courier to InnerDyne, Inc. collaborators for implantation. The stents were implanted in porcine coronary arteries and after one month the stented arteries evaluated by histomorphometry.

As with the initial studies, the stents were easily, reproducibly radiolabeled with rhenium-188 using methods developed under the CRADA. The rhenium-188 stents were routinely implanted and no significant radiation safety concerns were encountered. *In vivo* the rhenium-188 remained associated with the stent, although a small amount entered the blood pool. At one month post implantation, there was an increased arterial patency in arteries implanted with rhenium-188 compared to non-radioactive stents as determined by histomorphometric analysis. This increased patency was found in studies with three different stent types. There was a statistically significant increase in intimal fibrin in the arteries receiving radioactive stents.

As many of the effects from radioactive treatments are chronic, another animal study was conducted wherein the animals were survived for 2 months post-implantation. In this study two different stents from two different manufacturers were used. Histomorphometric analysis indicted no significant difference in the rhenium-188 stented animals and those receiving non-radioactive stents. Many of the rhenium-188 stents had inflammatory reactions around what appeared to be coating material that was sloughed and encapsulated. This sloughing had not been observed in one month studies and was considered to be the most likely cause for intimal thickening in the experimental group. Animal studies with rhenium-188 were placed on hold pending solution of the sloughing problem. As the coating on the rhenium-188-stents was found to inadequate, a series of studies were implemented to develop a coating that would be compatible with the radiolabeling process.

Since the rhenium-188 stent labeling technology had progressed rapidly and detailed stability and animal studies were in progress at BNL and in conjunction with InnerDyne collaborators earlier than expected in the project period, a joint decision was made in October 1998 between the ORNL and InnerDyne, Inc. partners to pursue the development of bifunctional chelate technology at ORNL focused on methods which could be used to attach the palladium-103 radioisotope to therapeutic agents and devices for the treatment of various types of cancer. Palladium-103 encapsulated metal implants had already been developed, commercialized and widely used as implantable seeds for

the treatment of inoperable/refractory prostatic cancer. The development of versatile chemistry which would allow the introduction of palladium-103 into a wide variety of therapeutic agents and devices was the goal of this research.

Development of Bifunctional Chelates Labeled with Palladium-103 for Cancer Therapy -

Palladium-103 is an attractive reactor-produced radioisotope for a variety of therapeutic applications and has been shown to be one of the most effective methods for treatment of refractory prostatic cancer by the use of permanent palladium-103 implants. The low energy emission results in localized radiation damage to the tumor tissue minimizing exposure to the surrounding healthy tissue, and thus simplifies radiation protection issues. Modification of CRADA # ORNL98-31 in October 1998 refocused the research efforts at the Oak Ridge National Laboratory to explore the synthesis of palladium-103(II) bifunctional chelates, that would effectively and strongly complex the Pd(II) ion and also afford a second functional group that could then be attached to a therapeutic implant material.

Because of its attractive therapeutic properties and availability*via* reactor-production, palladium-103 is thus of broad interest for a variety of therapeutic applications in nuclear medicine, oncology and interventional cardiology. For the research discussed in this report for oncology applications, studies focused on the development of organic synthetic strategies for the preparation of bifunctional chelates that strongly bind palladium-103 and can be attached to tissue targeted agents and radioactive sources that can be implanted for tumor therapy. The initial stages of this project involved the evaluation of several divergent approaches for the synthesis of model N_4 and N_2S_2 ligands [i.e. either four nitrogen, or two nitrogen and two sulfur atoms in the acyclic ring] for palladium(II) binding. Based on our understanding of the chelate binding properties for Pd(II) in the literature, a variety of both N_4 and N_2S_2 model ligands were synthesized and several convergent synthetic approaches were evaluated. These synthetic approaches were based on ligands into which flexible heteroatom-containing arms were introduced which allow a stable and strong binding of Pd(II). Functionalities were also introduced which provided anchors for attachment of the ligand to the biodegradable implant materials. These ligands and intermediates were fully characterized by NMR, mass spectroscopy and chromatographic methods.

The successful approach for the model acyclic bifunctional molecule that contain four nitrogen atoms for palladium chelation (N_4 ligand) involved alkylation of diethylmalonate with 6-bromohexanol, oxidation to the desired carboxylate and condensation with ethylendiamine. The Pd(II) chelate was also prepared and characterized by TLC and NMR. Palladium-103 chelation and stability studies were performed with a total of eight ligands (i.e. six acyclic and two cyclic). As expected, the model acyclic ligands examined (including the model N_2S_2) formed chelates having varying levels of stability under a variety of conditions for up to five hours post labeling, while two cyclic N_2S_2 and N_2S_4 ligands examined were unstable under the chelation conditions. The N_4 ligand was shown to be have strong Pd(II) binding and contained the requisite functional group for attachment to targeting species and conditions for synthesis were optimized.

The availability of the various acyclic ligands in comparison with similar "irrelevant" cyclic N_4 and N_2S_2 ligands which do not have the geometrical alignments required for Pd(II) binding provided an

opportunity to evaluate the formation and stability of the palladium-103-labeled chelates. The ligands were reacted with palladium-103 chloride standard solution, with a ligand/Pd(II) stochiometry was about 5:0.15 in these initial studies. The solutions were analyzed by ITLC SG and activity in the chelate peaks estimated using a radioTLC scanning device. Because of the very polar nature of the ligands, an ethanol-ammonium hydroxide solvent system was required. The stability of the radiolabeling mixture was evaluated over time. As expected, the acyclic ligands which were expected to strongly bind Pd(II)-103, showed well-defined complexes which were stable over the 24 hour period, compared with the instability of cyclic ligands which cannot specifically bind Pd(II). In some cases the chelate represented a higher level after 30-60 minutes, demonstrating the slow kinetics of chelate formation under these conditions.

The bifunctional target N_4 ligand that we synthesized by the new route also showed good binding of Pd(II)-103. The details of these chemical synthesis and characterization of these chelates are described in the ***Interim Report, March 2000***, entitled, "Development of palladium-103-Labeled Bifunctional Chelates for Therapeutic Applications." Final stages of these studies involved the large scale preparative synthesis and purification of the model acyclic N_4-functionalized ligand [6-(5-carboxypentyl)-5-7-dioxo-1,4,8,11-tetraazaundecane], which would be available to InnerDyne for radiolabeling/device binding studies and will also be available for ORNL DOE-supported future studies for attachment to tumor cell-specific peptides for tumor therapy studies. In addition, the improved chemistry developed for the synthesis of this ligand is now available and represents a simple general method which can be used for the synthesis of a series of ligands with altered carboxy-alkyl linker chains which will allow for the first time a structure-activity evaluation of this chelator with the goal to optimize the binding of this type of palladium-103-labeled bifunctional chelate moiety to tumor targeting agents.

Technical Milestones Achieved -

Rhenium-188 radiolabeling of stents and stent surrogates was reduced to practice.
The method for radiolabeling stents with rhenium-188 was optimized.
The radiation dose delivered from rhenium-188 stents was established.
Rhenium-188 stents were implanted in ileac and coronary arteries of pigs.
The sealant used on the rhenium-188 stents was optimized.
Additional chelates were attached to stent surrogates.
Proof of principle for therapeutic efficacy has been established.
Improved methods were developed for synthesis of bifunctional chelates for palladium-103
A series of new chelates were synthesized and characterized and labeled with palladium-103
The most promising bifunctional chelate [6-(5-carboxypentyl)-5-7-dioxo-1,4,8,11-tetraaza undecane] was prepared in preparative quantities

Publications/Presentations Connected with this Project -

Kuan, H. M., Zamora, P. O., Ferretti, J. A., Choi, J., Singletary, Pollack, W. M., Osaki, S., Stern, R. and Oster, Z. H. "Radiation Dosimetry and Safety of Rhenium-188-Labeled Metal Stents in treating Restenosis," *Med. Phys.*, 26, 1748 (1999).

Luo, H., McPherson, D. W., and Knapp, F. F., Jr. "Improved Synthesis of Carboxy-Alkyl-Substituted N_4 Bifunctional ligands for Palladium-103 labeling of Therapeutic Agents,"*for, Applied Radiation and Isotopes, in preparation.*

P. O. Zamora, S. Osaki, P. Som, J. A. Ferretti, J. Choi, C.-z Hu, R. Tsang, H. M. Kuan, S. Singletary, R. Stern, and Z. H. Oster, "Radiolabeling brachytherapy sources with Rhenium-188*via* Chelating Microfilms: Application to Stents," *submitted for publication.*

P.O. Zamora, J. A. Ferretti, P. Som, J. Choi, S. Osaki, P. Kuan, S. Singletary, R. Stern, and Z. H. Oster, "On-demand Labeling of Metal Stents with Re-188 for Use in Treating Restenosis,". Accepted for presentation, 46th Annual Society of Nuclear Medicine Meeting, Los Angeles, CA, June 6-10, 1999; *J. Nucl. Med.*, 40, 185P (1999).

INVENTIONS -

None reported

COMMERCIALIZATION POSSIBILITIES -

At the termination of the efficacy studies for the rhenium-188 stents an InnerDyne corporate project review was held and a strategic decision was made not to pursue the rhenium-188 stent labeling technology. This decision was based on the poor biological performance of the rhenium-188-labeled stents in two month animal studies and problems reported in the literature by other investigators with radioactive stents. This decision was compounded by a number of non-scientific issues including the acquisition of InnerDyne, Inc. by Tyco International, and patent positions on radioactive stent technology. There are thus no corporate plans by InnerDyne for further development of the rhenium-188 stents.

PLANS FOR FUTURE COLLABORATION -

Some of the technology developed in pursuit of the rhenium-188-stent project was found to have potentially useful applications and these applications, all of which are non-radioactive technologies, are being developed for potential commercialization. It is anticipated that some of this work will continue to be performed at BNL in collaboration with Dr. Som and other researchers.

CONCLUSIONS -

The interaction between ORNL and InnerDyne in conjunction with collaborators at BNL provided an important opportunity to explore an important applications of reactor-produced radioisotopes for new therapeutic applications. Progress in development of the rhenium-188-stent radiolabeling technology and the associated animal studies had shown proof of principal for the first time that stents can be successfully radiolabeled with rhenium-188, implanted in arteries of experimental animals and could inhibit the restenosis which normally would occur after such vessel injury. The support provided by InnerDyne, Inc. for the rhenium-188 stent radiolabeling studies and collaborative animal studies had made possible the rapid progress of these aspects of the project. Another aspect of the CRADA allowed for the evaluation, optimization and demonstration of the

sefulness of a new improved approach for the preparation of bifunctional chelate molecules for tumor targeting of palladum-103 labeled therapeutic agents. The interest of the industrial partner and the opportunity to also explore the development of implantable sources radiolabeled with other therapeutic radioisotopes provided an opportunity to explore and develop other useful aspects of this important technology.

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