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DOE/ER/60460--TH

Harvard-MIT Research Program in Short-lived Radiopharmaceuticals
Final Report
(1977-1994)

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The Harvard-MIT Research Program in Short-lived Radiopharmaceuticals was established in 1977 to foster interaction among groups working in radiopharmaceutical chemistry at Harvard Medical School, the Massachusetts Institute of Technology, and the Massachusetts General Hospital. To this was added a group at The Children's Hospital. From these collaborations and building upon the special strengths of the participating individuals, laboratories and institutions, it was hoped that original approaches would be found for **the design of new, clinically useful, radiolabeled compounds.**

The original thrust of this proposal included: (a) examination of the coordination chemistry of technetium as a basis for rational radiopharmaceutical design, (b) development of an ultrashort-lived radionuclide generator for the diagnosis of congenital heart disease in newborns, (c) synthesis of receptor-site-directed halopharmaceuticals, (d) improved facile labeling of complex molecules with positron-emitting radionuclides. Our 1986 proposal was oriented toward organs and disease, emphasizing radiolabeled agents that delineate specific functions and the distribution of receptors in brain, heart, and tumors. In 1989, we further refined our purposes and focussed on two major aims: (a) synthesis and utilization of neutral technetium and rhenium complexes of high specific activity, and (b) development of new approaches to the radiolabeling of proteins, peptides, immunoglobulins, and their fragments. In 1992, we amended this proposal to concentrate our efforts on biologically active peptides and proteins for targeted radiodiagnosis and therapy.

A. Specific Project Objectives

The Harvard-MIT Research Program in Short-lived Radiopharmaceuticals was established in 1977 to foster interaction among groups working in fields related to radiopharmaceutical chemistry at Harvard Medical School, the Massachusetts Institute of Technology, and the Massachusetts General Hospital. To this was added a group working at The Children's Hospital. From these collaborations and building upon the special, but different, strengths of the participating individuals, laboratories, and institutions, it was hoped that original approaches would be found for **the design of new, clinically useful, radiolabeled compounds.**

1. 1977-1989

The original thrust of this proposal was eclectic. Projects included: (a) an examination of the coordination chemistry of technetium as a basis for rational radiopharmaceutical design, (b) the development of an ultrashort-lived radionuclide generator for the diagnosis of congenital heart disease in newborns, (c) the synthesis of receptor-site-directed halopharmaceuticals, (d) an improved facile synthesis of complex molecules labeled with positron-emitting radionuclides. Our 1986 proposal was oriented toward organs and disease as well as the science and technology required to produce more effective radiopharmaceuticals. It emphasized agents that delineate specific functions (blood flow, metabolism) and the distribution of receptors (transmitter, hormone, immune) in brain, heart and tumors.

2. 1989-1992

In 1989, we further refined our purposes and organized our proposal around two major aims, both subsumed as the chemical basis of radiopharmaceutical action: (a) studies in technetium chemistry to develop synthesis and utilization of neutral technetium and rhenium complexes of high specific activity, and (b) development of binding-site-directed radiopharmaceuticals to radiolabel proteins, peptides, immunoglobulins and their fragments and assessment of the biologic consequences of these techniques.

3. 1992-1993 (extension to 1994)

In our 1992-1993 proposal, still retaining the chemical thrust of the research, we proposed to concentrate our efforts on the radiolabeling of biologically active peptides and proteins for targeted radiodiagnosis and therapy. The importance of biologically active peptides for a host of functions has now become clear. These molecules, of varying size and composition, play essential and specific roles as hormones, growth factors, and neurotransmitters. Their specificity derives, in part, from particular receptors found on the surface of cells that are their targets, e.g. islet cells of the pancreas, fibroblasts of wounded tissue, fields of neurons in the central nervous system. Aside from the immunoglobulin receptors, little effort has been made to explore the possibility of utilizing these specific sites for targeted radionuclide diagnosis and therapy.

We focussed attention on methods for labeling peptides with single-photon- and annihilation-photon-emitters, testing their effectiveness in appropriate biologic models. The labeling of peptides with trace levels of technetium for diagnosis or rhenium for therapy is a formidable process. First, stable complexes of the transition elements with the peptide must be formed. Second, this must not

interfere with the distribution and binding of the peptide or, alternatively, of its labeled antagonist. Davison and Jones proposed to accomplish this by labeling the N-terminal end of peptides which is relatively inert in biologic functioning. Initially they planned to explore the use of N_2S_2 complexes with which they already had considerable experience. Fischman and Strauss proposed to continue experiments with chemotactic peptides conjugated at the C-terminal end to hydrazinonicotinamide. These conjugates, which bind specifically to chemotactic leukocytes, were to be labeled with ^{99m}Tc and used to localize sites of inflammation and infection. Elmaleh proposed to label the chemotactic peptide with the positron emitters ^{18}F and ^{124}I. The fluorine label was to be attached by way of an activated aromatic tetraalkylammonium ester or through the synthesis of a fluorocyclohexyl derivative. Kassis proposed to continue work with immunoglobulins and immunoglobulin fragments, developing indirect methods for radiolabeling antibodies and in-vitro and in-vivo techniques for assessing the immunocompetence of these radiopharmaceuticals.

B. Relationship to DOE Mission

This project was supported by the Department of Energy, Office of Energy Research, under the Office of Health and Environmental Research, Division of Medical Applications and Biophysical Research. The proposal addresses the second area of the program objectives: "the nuclear medicine component that is aimed at enhancing the beneficial applications of radiation, radionuclides, and stable isotopes in the diagnosis, study, and treatment of human diseases." It has fostered basic and applied studies for the development of radioactive agents for use in both the diagnosis and the treatment of disease and the understanding of human disorders. The research has been motivated by the recognition that diagnostic imaging is a sensitive and powerful technique for examining physiologic and pathologic processes in man. Moreover, increased knowledge in the design of binding-site-directed radiopharmaceuticals has suggested their potential application in cancer therapy. This proposal has been designed to combine the fruits of modern biologic research with nuclear technology for these purposes.

C. Relationship to Other DOE-Funded Projects

Alun G. Jones and Alan Davison, two of the investigators on Project 1, were partially supported from 01/87 to 01/90 (extended to 06/91 without funding) by DOE, DE-FG02-87ER60526, A New Approach to the Analysis of Radiopharmaceuticals. The grant was designed to investigate the application of specific techniques to radiopharmaceutical chemistry, including various forms of mass spectrometry, ^{99}Tc NMR and resonance Raman spectroscopy. The research funded represents the development of an analytical base for this (DE-FGO2-86ER60460) and other grants.

D. Results

1. 1977-1989

Studies in Technetium Chemistry - to facilitate the design of radiopharmaceuticals incorporating technetium-99m

a. first identification and characterization of oxotechnetium(V) complexes isolated from aqueous solution
b. elucidation of the systematics in technetium complexation
c. first application of high resolution field desorption mass spectroscopy to the structure of these complexes
d. design and synthesis of new agents for heart, brain, and kidney imaging based on the chemistry of the element; n.b. isonitriles for cardiac imaging with SPECT
e. development of new classes of cationic and neutral complexes
f. determination that the uptake of $Tc(AcAc)_3$ complexes by the brain has a maximum that depends on lipophilicity

Binding-Site-Directed Radiopharmaceuticals - to improve the specificity or image quality and to decrease the radiation dose through the use of short-lived and disease-specific radiopharmaceuticals

a. preparation of radioiodinated vinylestradiol derivatives with high affinity for estrogen sites, having clinical application for detection of mammary tumors
b. synthesis of adrenergic neuron indicators of the phenylpiperazinium type that can be labeled with either ^{123}I or ^{11}C
c. development of novel cerebral perfusion imaging agents based on phenylpiperazines and phentermines labeled with either ^{123}I or ^{11}C
d. development of radiohalodestannylation as a method for rapid and efficient site-specific incorporation of radioiodine and radiobromine
e. radiolabeling of insulin with ^{123}I for biokinetic studies and preparation of high-specific-activity ^{123}I[Tyr-A14] for evaluation in human studies
f. determination that in-vitro radioimmunoassay does not necessarily predict in-vivo behavior of a radiolabeled antibody

Osmium-191--Iridium-191m Radionuclide Generator - to decrease the radiation dose through the use of short-lived radiopharmaceuticals

development to the level of clinical utility of the ^{191}Os--^{191m}Ir generator for use in first pass and continuous infusion radionuclide angiography

Labeling of Complex Molecules with Fluorine-18, Nitrogen-13 and Carbon-13 - to provide short-lived, positron-emitting radiopharmaceuticals with advantages in transverse-section imaging of regional physiologic processes

a. construction of a semiautomated modular system for preparation of reactive intermediates such as $^{11}CO_2$, $^{11}CH_2O$ and $^{11}CH_3I$
b. development and construction of a target box for production of $^{18}F^-$ and $^{18}F_2$
c. synthesis of a new series of ^{11}C and radioiodinated branched-chain fatty acids

for myocardial and metabolic imaging

d. improvement of $^{13}NH_3$ production and its use as an intermediate for preparation of ^{13}N-labeled amino acids such as ^{13}N-L-glutamate (enzymatic synthesis) and ^{13}N-L-asparagine (chemical synthesis) for heart and tumor imaging

e. development of a chemical synthesis of ^{11}C-L-methionine for studying tumor growth and response to radiation therapy

f. synthesis of ^{18}F-labeled sugar analogs, 2-[^{18}F]fluoro-2-deoxy-D-glucose and 3-[^{18}F]fluoro-2-deoxy-D-glucose, for studying brain and heart energy metabolism

2. 1989-1992

References in parentheses refer to papers not yet submitted to the Department of Energy and, therefore, included in alphabetical order in the appendix.

Studies in Technetium Chemistry - to develop synthesis and utilization of neutral technetium and rhenium complexes of high specific activity

a. characterization of N_2S_2 chelates; attachment of metal chelates to a progestin skeleton and conversion to oxo-rhenium complexes; assessment of diastereoisomers indicating two anti diastereomers and the syn mixture of the 11β-N_2S_2 system have high affinity for the progesterone receptor suggesting potential as imaging agents for steroid-positive tumors (DiZio, Fiaschi, Davison, Jones, Katzenellenbogen, 1991)

b. studies with human tumor cell lines and the cation ^{99m}Tc-hexakis(2-methoxyisobutylisonitrile) (MIBI) demonstrating that uptake is a temperature- dependent process restricted to living cells and is sensitive to changes in cytosolic and mitochondrial membrane potentials (Delmon-Moingeon, Piwnica-Worms, Van den Abbeele et al, 1990)

c. characterization in cultured chick heart cells of the accumulation of various cationic and neutral technetium complexes indicating that cationic Tc complexes (e.g. MIBI) are taken up by mitochondria with modest washout rates and uptake is sensitive to changes in membrane potential, while neutral complexes (e.g. boronic acid adducts of technetium tris(dioxime)) are taken up by nonspecific hydrophobic partitioning with rapid biexponential washout and uptake is only slightly influenced by metabolic or cationic membrane transport inhibitors (Kronauge, Chiu, Cone et al, 1992)

d. synthesis and identification of technetium(I) hexakis(isonitrile) complex Tc(CPI) containing functionalized alkyl isocyanide ligands with a terminal methyl ester group and characterization of its hydrolysis products (Kronauge, Davison, Roseberry et al, 1991); pharmacokinetic properties of this compound including rapid clearance from the lung and liver and moderate washout from heart muscle suggest potential as a myocardial imaging agent

with use in same-day stress/rest imaging protocols; examination in human volunteers and guinea pigs of its pharmacokinetics by dynamic SPECT imaging and HPLC analysis of blood samples indicate that enzymatic hydrolysis of the terminal ester group occurs in blood at a moderate rate producing new species not accumulated in heart tissue and that after extraction by the heart, liver and kidneys the Tc(CPI) complex undergoes metabolism at a slower rate giving different products suggesting a different mechanism of hydrolysis (Kronauge, Leon, Silvia Verdera et al, 1992)

e. synthesis and characterization of new Tc(V) phenylimido complexes $[TcCl_3(NPh)(PPh_3)_2]$ and $[TcCl_3(NPh)(Ph_2PCH_2CH_2PPh_2)]$ as part of an ongoing project to examine organohydrazines as ligands for Tc and Re (Nicholson, Davison, Jones, 1991; Nicholson, Storm, Davis, Davison, Jones, 1992)

Binding-Site-Directed Radiopharmaceuticals - to elaborate new approaches to the radiolabeling of proteins, peptides, immunoglobulins and their fragments and to assess the biologic consequences of these techniques

a. demonstration that preadsorption of radiolabeled antibodies to liver and spleen tissues results in significantly decreased uptake in certain normal tissues and higher tumor-to-normal-tissue ratios and that protection of the antigen-binding site during radioiodination leads to improved immunoreactivity

b. development of indirect radiolabeling methods for monoclonal antibodies including biotinyl-m-[^{125}I]iodoaniline (Khawli, Kassis, 1992) and N-(m-[^{125}I]iodophenyl)maleimide (Khawli, Van den Abbeele, Kassis, 1992)

c. development of a simple remote system for high-level radioiodination of monoclonal antibodies (Weadock, Anderson, Kassis, 1989) and an immunoradiometric assay for antibodies recognizing antigenic gangliosides (Baranowska-Kortylewicz, Berman, Kaldany, Eisenbarth, Kassis, 1990)

d. demonstration that incubation of ^{125}I-3G5 (an IgM monoclonal antibody against a membrane ganglioside on RINm5F cells) with rat insulinoma RINm5F cell monolayers leads to cell-bound radioactivity detectable within 10 min, with electron microscopy over time confirming the presence of radiolabeled antibody on the plasma membrane followed by distinct capping and diffuse radioactive deposits within the cells (Mariani, Ito, Nayak et al, 1991)

e. involvement in two on-going clinical studies: phase I therapeutic trial examining the feasibility of intraperitoneal radioimmunotherapy in refractory ovarian carcinoma utilizing escalating doses of ^{131}I labeled OC125 $F(ab')_2$ (Muto, Finkler, Kassis, Lepisto, Knapp, 1990; Muto, Finkler, Kassis et al, 1992), and detection of focal infection with ^{111}In-human polyclonal IgG

f. improvement of methods for labeling peptides and proteins with ^{18}F, including reductive alkylation with the use of tetrafluorophenyl and pentafluorophenyl esters (Herman, Elmaleh, Fischman, Hanson, Strauss

1991; Shen, Hanson, Elmaleh, 1991)

g. demonstration that multiple PET measurements can be used to assess pharmacokinetics of fluconazole labeled with ^{18}F in rabbits; direct measurement of partition coefficient in muscle agrees closely with predictions of kinetic model hypothesizing that compound is compartmentalized in blood and tissue with rate constants describing transition between compartments (Fischman, Alpert, Livni et al, 1991)

h. characterization of uptake and retention of tritiated tetraphenylphosphonium in certain human and animal nervous-system-tumor cell lines demonstrating that certain of these tumors retain lipophilic compounds with a decentralized positive charge which may be useful in the intraoperative delineation of tumor margins and in photodynamic therapy (Steichen, Weiss, Elmaleh, Martuza, 1991)

i. characterization and comparison of various radiolabeled proteins for detection of focal sites of infection using rat and rabbit E. coli, soft-tissue infection models: demonstration that ^{99m}Tc- labeled monoclonal antigranulocyte antibody and ^{111}In-labeled IgG are equivalent, nonimmunogenic agents (Juweid, Fischman, Rubin, Baum, Strauss, 1991), and ^{111}In-labeled IgG and ^{125} I-labeled albumin accumulation are not significantly different indicating binding of IgG to Fc receptors on inflammatory cells is not its mechanism of localization in infection (Tompkins, Fischman, Yarmush, 1991)

j. conformational studies of the endogenous brain peptide N-Tyr-MIF-1 in aqueous solution by ^1H nuclear magnetic resonance spectroscopy as a basis for designing new analogs (Petersheim, Moldow, Halladay, Kastin, Fischman, 1992)

Completion of Work Begun During 1977-1989 Funding Period

a. assessment of potential clinical utility of fatty acid imaging and comparison of ^{11}C and ^{123} I labeled straight-chain and branched-chain fatty acids using PET and single photon imaging, respectively (Fischman, Saito, Dilsizian et al, 1989)

b. demonstration in mouse myocardium by microautoradiography and electron microscopy that ^3H-beta-methylheptadecanoic acid is found in mitochondria and lipid droplets and by thin-layer chromatography that ^{14}C-beta-methylheptadecanoic acid is found in various lipid pools, indicating distribution similar to physiologic fatty acids (Livni, Ito, Kassis, Elmaleh, 1990)

3. 1992-1993 (extension to 1994)

Studies in Technetium Chemistry - to develop synthesis and utilization of neutral technetium and rhenium complexes of high specific activity

a. evaluation of pharmacokinetics of ^{99m}Tc-CPI in isolated contractile rat atrial

tissue demonstrating temperature-sensitive and concentration-dependent linear accumulation over time and slow washout with 75% of the retained activity the original Tc-CPI complex (Vedera, Leon, Kronauge et al, 1993)

b. development of a method for labeling peptides/ proteins by introducing N_2S_2 chelates that possess amine amide dithiols as the coordinating atoms; use of this method with a final N-alkylation at the amino nitrogen for conjugation to other molecules of interest; complexation with Tc or Re by using pertechnetate or perrhenate with trityl-protected ligands and $SnCl_2$-HCl as the reducing agent or alternatively by deprotection of the thiols followed by complexation with prereduced metal oxo precursors (Mahmood, Wolff, Davison, Jones, in press)

c. development of a method using hydrophilic, polycationic poly-D-lysine modified by N-acetyl homocysteine thiolactone with high affinity for reduced Tc to label white blood cells (Mahmood, Delmon-Moingeon, Limpa-Amara, Davison, Jones, 1993; Mahmood, Delmon-Moingeon, Limpa-Amara, Davison, Jones, 1994; Mahmood, Delmon-Moingeon, Limpa-Amara, Davison, Jones, submitted); evaluation of integrity and immune functions of lymphocytes labeled with this carrier indicating radiolabeling procedure does not alter these characteristics (Delmon-Moingeon, Mahmood, Davison, Jones, submitted)

Binding-Site-Directed Radiopharmaceuticals - to elaborate new approaches to the radiolabeling of proteins, peptides, immunoglobulins and their fragments and to assess the biologic consequences of these techniques

a. synthesis and evaluation of hydrazinonicotinamide-derivatized chemotactic peptide analogs labeled by ^{99m}Tc-glucoheptonate (^{99m}Tc-HP): demonstration that ^{99m}Tc-HP maintain biologic activity and receptor binding when assayed in human leukocytes; assessment in E. coli, soft-tissue models of infection indicating ^{99m}Tc-HP are cleared rapidly from blood in rats with low levels of accumulation in normal tissues and produce good images of infection sites in rabbits at 15 hours (Babich, Solomon, Pike et al, 1993); assessment in rhesus monkeys indicating that ^{99m}Tc-HP induce significant transient reductions in peripheral leukocyte levels only at high peptide doses, and that in rhesus monkeys with focal sites of mild sterile inflammation good target/nontarget ratios are achieved at 3 hours (Fischman, Rauh, Solomon et al, 1993); comparison of one ^{99m}Tc-HP with ^{111} In-leukocytes (WBC) in rabbits demonstrating that the ^{99m}Tc-labeled compound achieves better absolute levels of accumulation and target/nontarget ratios at all imaging times (Babich, Graham, Barrow et al, 1993)

b. development of a method for labeling immunoglobulins with bifunctional, sulfhydryl-selective, and photoreactive coumarins (Baranowska-Kortylewicz, Kassis, 1993)

c. development of a semipreparative isoelectrophoresis method for separating antibody isoforms and DTPA-antibody conjugates on the basis of pI,

permitting isolation of antibody isoform fractions from immunologically irrelevant and low-affinity antibodies (Gangopadhyay, Saravis, Van den Abbeele, Kassis, in press) and DTPA-antibody isoform fractions with desired numbers of DTPA and specific negative charges without a measurable diminution in immunoreactivity (Gangopadhyay, Saravis, Kassis, 1994)

d. determination that radioiodination changes the pI of proteins differentially depending on the oxidant used and its concentration, and that changes in electrophoretic mobility are a function of the iodide/protein ratio (Gangopadhyay, Van den Abbeele, Kassis, 1994)

e. rapid synthesis of N-(4-[$^{127/125/123}$I]iodobenzyl) biotin amides via the trazine of N-(4-aminobenzyl)biotin amide to produce a high specific activity, biochemically intact biotin molecule (Kortylewicz, Baranowska-Kortylewicz, Adelstein, Carmel, Kassis, 1994)

f. development of a one-step method for radiolabeling proteins using pentafluorophenyl derivatives and tetrabutylammonium [^{18}F]fluoride exchange (Herman, Fischman, Tompkins et al, 1994)

g. preparation of ^{18}F-labeled biotin derivatives in high specific activity for PET applications: achievement of infection localization in E. coli-infected rats less than 1 hour after injection (Shoup, Fischman, Jaywook et al, 1994)

Completion of Work Begun During 1977-1989 Funding Period

a. synthesis and evaluation of 1-[^{11}C]methyl-4-aryl-piperazinium salts as myocardial imaging agents; biodistribution studies in rats and imaging in dogs indicating high concentrations in heart obtained at 5 min and level of activity at 20 min maintained over 1-hour period (Elmaleh, Padmanabhan, Hassan et al, 1993)

b. preparation of a series of phentermine analogs including the unsubstituted, para-F, -Cl, -Br and -I, and the meta-CF_3, their [^{11}C]methylation, and evaluation in rats to determine structure-localization relationships for cerebral blood-flow imaging; N-[^{11}C]methyl-p-iodophentermine with best biodistribution (Elmaleh, Kizuka, Hanson et al, 1993); method for rapid preparation of N-[^{11}C-methyl]-chlorphentermine (Kizuka, Elmaleh, 1993)

c. determination of rates of fatty acid metabolism in a dog myocardium model from steady-state concentration of 1-[^{11}C]betamethylheptadecanoic acid (Elmaleh, Livni, Alpert et al, 1994)

E. Project Output: Complete List of Publications Supported Completely or Partially by DE-FG02-86ER60460 (Formerly DE-AC02-76EV04115)

1977

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Appendix

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