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Nuclear Medicine Progress Report for Quarter Ending June 30, 1986

F. F. Knapp, Jr.
K. R. Ambrose
M. M. Goodman
P. C. Srivastava

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Health and Safety Research Division

NUCLEAR MEDICINE PROGRESS REPORT FOR QUARTER ENDING JUNE 30, 1986

F. F. Knapp, Jr.

K. R. Ambrose
M. M. Goodman
P. C. Srivastava

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SUMMARY

In this report the development of a new method of protein radiolabeling involving the attachment of radioiodinated N-substituted maleimides to the sulfhydryl groups of proteins is described. The radioiodine is stabilized by attachment on a phenyl ring. Maleimides are known to form strong, sulfide bonds by reaction with organic thiols, and this method thus represents a new alternative to minimize the deiodination of radiolabeled proteins. As a model radioiodinating agent, N-(p-[^{125}I]-iodophenyl)maleimide (IPM), was prepared by coupling p-iodoaniline with maleic anhydride, followed by subsequent ring closure. For this synthesis, the p-iodoaniline was prepared by iodination of a p-aminophenylmercuric acetate substrate. When administred to rats, the [I-125] IPM resulted in the retention of high levels of radioactivity in the blood pool. Further analysis demonstrated about 90% binding to red blood cells. It was also demonstrated that this agent effectively radiolabels model proteins such as BSA and IgG.

An evaluation of the relative lipid pool distribution in rat heart extracts after intravenous administration of the straight-chain 15-(p-iodophenyl)pentadecanoic acid (IPP) and 3,3-dimethyl-branched terminal (DMIPP) iodophenyl-substituted fatty acid analogues to normotensive and hypertensive Dahl rats has been evaluated. Although earlier autoradiographic studies have demonstrated differences in the regional uptake patterns of DMIPP, the global lipid distribution patterns are similar in the two groups of rats.

During this period a variety of research products were evaluated in conjunction with Medical Cooperative investigators. Six shipments of osmium-191 were made to the University of Liege, Belgium, for preparation of radionuclide generators for clinical use and two generators were supplied to the Massachusetts General Hospital for continuous infusion studies in animals. In addition, seven samples of radioiodinated fatty acids were supplied to collaborators in Amsterdam, Boston and Brookhaven National Laboratory, and copper-64 was supplied to the Oak Ridge Associated Universities.

RADIOIODINATED N-SUBSTITUTED MALEIMIDES — NEW SULFHYDRYL BINDING PROTEIN-LABELING AGENTS

Radiolabeled proteins, red blood cells, and other blood compounds (leukocytes, platelets, etc.) are useful for the study of hematological disorders and other diseases. Radiolabeled erythrocytes are useful agents for the determination of total red blood cell volume, red blood cell survival time, red blood cell destruction sites and visualization of the intravascular blood pool by external imaging technqiues. Radiolabeling of leukocytes, which are important for defense against infection, provides useful agents for the localization of infection sites. Platelets play an important role in hemostasis and thrombosis mediated by ADP (adenosine 5'-diphosphate) induced platelet aggregation following vascular injury. Platelet aggregation thus leads to thromboembolism and plaque formation that can result in coronary heart disease and stroke. Thus, the radiolabeling of platelets could potentially be useful to identify early stages of plaque formation by imaging and to study the platelet phenomenon in general. In addition, these protein labeling techniques may have potential applications for the radiolabeling of antibodies used in tumor diagnosis and therapy.

For radiolabeling of such blood components, the physical properties (e.g., half-life, mode of decay and radiation energy) and availability of the radionuclide, stability of the substrate under radiolabeling conditions, ease of radiolabeling techniques and in vivo stability of the radiolabel are important factors. A number of radionuclides including tritium, carbon-14, phosphorus-32, sulfur-35, and chromium-51 have been used for the radiolabeling of proteins, antibodies and blood components. These radioisotopes do not have suitable half-lives or radiation energies for in vivo or external imaging applications. Agents labeled with such radioisotopes are limited only to in vitro determinations using scintillation counting techniques, etc. Other radionuclides such as gallium-67, gallium-68, selenium-75, technetium-99m, indium-111, and rubidium-82, have also been used for radiolabeling. Carbon-11 (half-life 20 min) is useful for PET (positron emission computerized tomography) imaging, but the necessity of an onsite cyclotron for the production of this short half-life positron emitting radionuclide may limit its usefulness.

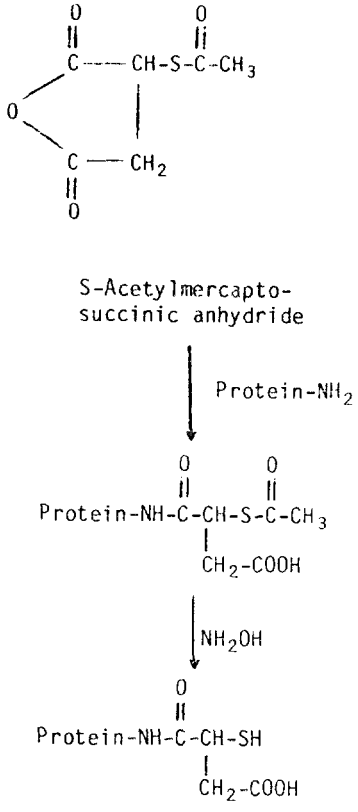
The radionuclides of gallium, indium, rubidium and technetium, etc., are useful for imaging, but often form less stable bonds with organic molecules. Thus, radiopharmaceuticals radiolabeled with these radionuclides are usually complexes or chelates. The stability of these complexes is often pH dependent making the radiopharmaceutical prone to decomposition on mild chemical interactions. This results in the in vivo release and diffusion of the radiolabel from the radiopharmaceutical into the extravascular fluid compartment, accumulation in non-target organs and association of metallic radioisotopes with endogenous proteins.

As an alternative, the radioisotopes of iodine are useful for radiolabeling such agents. Iodine can be attached covalently to organic molecules in a variety of ways. A number of procedures are available for the radiolabeling of antibodies and blood proteins. The use of chloramine-T. which oxidizes I^- into I^+, is a widely used procedure and presumably labels the phenyl ring of the tyrosine residues of proteins. The phenyl ring as the only site of radioiodination, however, has not been established unequivocally. Non-specific labeling at other sites could result in in vivo deiodination and accumulation of radioactivity in non-target organs and the exposure of the substrate to the radioiodination conditions is undesirable.

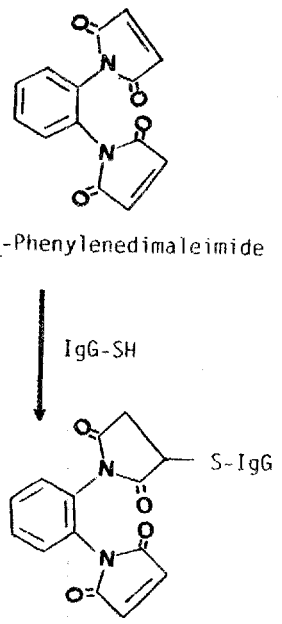
Another strategy for radiolabeling proteins involves attachment of the radiolabeled group to the free sulfhydryl group of proteins. Some bismaleimides have been radiolabeled with tritium or sulfur-35, and used to identify thiol-bearing proteins on the external and internal surfaces of the red cell membrane.[1] The bismaleimide agents are not commercially available and have not been applied to platelets. The synthesis of more useful, radioiodinated maleimides for labeling of biological substrates for diagnostic, therapeutic and imaging applications, however, has remained unexplored. The object of the current study was to provide a process for the radiolabeling of thiol-containing biological substrates including proteins and blood constituents as new agents for a variety of diagnostic, therapeutic and biochemical research applications.

The sulfhydryl (thiol-seeking) property of maleimides has been used for glutathione receptor site labeling in aqueous buffer. The thiol seeking rate depends upon the type of N-substituent on the maleimide analogue. Sulfhydryl-malemide coupling is a technique for the preparation

of protein to protein conjugates which occurs through very stable amide and thioether bonds with the necessary modification reactions usually causing very little disruption to the function of proteins.[2] The proteins to be radiolabeled must have at least one sulfhydryl group for conjugation, or it is necessary to incorporate such a group for binding with maleimides. The methods for incorporating a sulfhydryl group in the protein are reported in the literature[2,3] (Scheme I). Maleimides have also been used for enzyme-labeling of insulin and rabbit antibodies.[4-6] A disulfide bond is present in rabbit IgG and was converted to thiol groups by mild reduction with 2-mercaptoethylamine without affecting other disulfide bonds in IgG molecule. IgG with thiol groups thus generated was treated with a dimaleimide and conjugated with β-D-galactosidase[5,6] Scheme II).

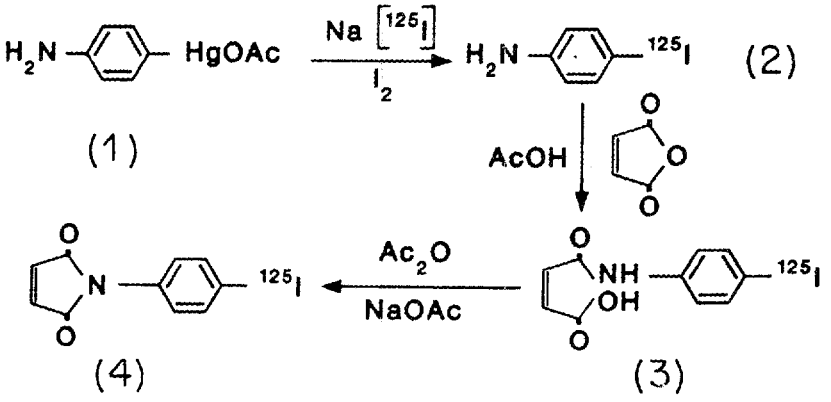


Scheme I[2]



Scheme II[2]

The synthesis of the new maleimide agent, N-(p-[^{125}I]iodophenyl)-maleimide (IPM) (4), and its evaluation in various biological systems as a potential thiol binding radiolabeling agent has now been explored. Iodine-125-labeled N-(p-iodophenyl)maleimide has been synthesized using p-[^{125}I]iodoaniline (2) substrate prepared from p-aminophenylmercuric acetate (1). Condensation of p-[^{125}I]iodoaniline with maleic anhydride in glacial acetic acid gave the n-(p-[^{125}I]iodophenyl)-acid-amide (3) intermediate which after ring annulation in the presence of acetic anhydride and anhydrous sodium acetate gave the desired [^{125}I]-IPM (4) (Scheme III). The radiolabeled IPM was synthesized in 50-70% yields with specific activities ranging from 700 mCi/mmole to 2500 mCi/mmole.



Scheme III

Rats were injected with [^{125}I]4 and the uptake of radioactivity in different tissues is given in Table 1 and 2. The data demonstrated that high levels of radioactivity were associated with the blood, and fractionation studies indicated 88.9% of the radioactivity to be associated with red blood cells. These studies demonstrate the potential utility of [^{125}I]4 for radiolabeling of red blood cells.

For protein labeling studies, samples of bovine albumin and human γ-globulin were prepared in phosphate buffer and 0.15 M NaCl solution (PBS). [^{125}I]-IPM (10 μCi) in 100 λ DMSO was added to each sample and incubated for 30 min at 37°C. The samples were repeatedly extracted with ethyl ether to remove the unbound activity (as [^{125}I]-IPM). The activity remaining in the aqueous portion was considered to be bound with the proteins. In these preliminary studies, the majority of the radioactivity was recovered in ether from the control sample, while the λ-globulin and albumin samples showed ~ 40% and ~ 60% activity, respectively, remaining in the aqueous portions. Future studies with this new protein labeling agent will include the evaluation of a "kit" procedure which will allow a one-step labeling of proteins and antibodies, and as the evaluation of interaction with a variety of model proteins such as the ADP-dependent platlet aggregating factor.

Table 1. The distribution of radioactivity in tissues of female Fischer 344 rats after intravenous administration of [^{125}I]4.[a]

Time after Injection	Mean percent injected dose/gm (range)					
	Tissue					
	Blood	Liver	Kidneys	Heart	Lungs	Thyroid
5 min	8.84 (7.98-9.51)	1.90 (1.60-2.59)	2.15 (1.67-2.52)	1.45 (1.16-1.83)	2.04 (0.90-2.77)	13.66 (8.93-17.64)
30 min	8.44 (7.87-9.13)	1.73 (1.47-2.11)	2.12 (1.62-2.49)	1.81 (1.43-2.42)	2.52 (1.90-3.00)	21.73 (15.5-25.64)
60 min	7.56 (7.22-7.75)	1.50 (1.42-1.65)	2.07 (1.75-2.40)	1.74 (1.41-1.70)	2.31 (2.19-2.47)	20.49 (14.69-28.51)

[a]Animals had body weight range of 121-139 gm. Each animal received 3.7 μCi of [^{125}I]4 by tail vein injection.

Table 2. The distribution of radioactivity in tissues of female Fischer 344 rats after intravenous administration of [^{125}I]4.[a]

Time after Injection	Mean percent injected dose (range)					
	Tissue					
	Blood	Liver	Kidneys	Heart	Lungs	Thyroid
5 min	57.58 (55.49-59.64)	10.16 (7.79-15.82)	2.17 (1.84-2.67)	0.61 (0.44-0.86)	1.76 (1.46-2.28)	0.13 (0.09-0.17)
30 min	54.07 (51.53-57.21)	7.85 (7.06-9.27)	2.18 (1.70-2.74)	0.79 (0.64-1.02)	1.89 (1.32-2.36)	0.20 (0.15-0.25)
60 min	48.56 (46.22-50.76)	7.05 (6.04-7.93)	2.21 (1.91-2.61)	0.74 (0.61-0.85)	1.74 (1.62-1.85)	0.19 (0.15-0.28)

[a]Animals had body weight range of 121-139 gm. Each animal received 3.7 μCi of [^{125}I]4 by tail vein injection.

DISTRIBUTION OF RADIOIODINATED FATTY ACIDS IN MYOCARDIAL LIPIDS FROM NORMOTENSIVE AND HYPERTENSIVE DAHL RATS

As a continuation of collaboration with investigators at the Brookhaven National Laboratory (Drs. P. Som and A. B. Brill), salt-sensitive hypertensive Dahl strain rats were obtained in order to determine the effects of hypertension on myocardial uptake and lipid distribution of iodophenyl radioiodinated-substituted fatty acids analogues. The Dahl rats are derived from the Sprague-Dawley rat strain and have a genetic predisposition to develop fatal hypertension when extra salt (i.e. 8% NaCl) is included in their diet. Autoradiographic studies using the hypertensive Dahl rats[7] have shown differences in the regional distribution of methyl-branched iodophenyl fatty acid uptake, especially in the endocardium, when compared to the uptake in normotensive rat hearts.

The radioiodinated iodophenyl-substituted fatty acids, 15-(p-iodophenyl)-3,3-dimethylpentadecanoic acid ([^{125}I]-DMIPP) and 15-(p-iodophenyl)pentadecanoic acid (^{131}I]IPP), were injected intravenously into hypertensive rats maintained on the high salt diet and normotensive Dahl rats maintained on the low salt diet. Both groups of animals were fasted 24 h prior to injection. Hearts were removed at various periods between 1-60 min after injection and assayed for total heart uptake of the

radiolabeled fatty acids. The hearts were then homogenized and lipids extracted for determination of the lipid pool distribution of the radioactivity. Both the hypertensive and the normotensive Dahl rats showed similar kinetic patterns of myocardial uptake and retention of the straight chain IPP and dimethyl-branched DMIPP analogues. As expected, the IPP showed rapid washout and DMIPP showed prolonged heart retention. These heart uptake and retention patterns were similar to those observed earlier in normotensive Fischer strain rats (ORNL/TM-9609).

In addition to the lack of any observed difference in global uptake in the hearts of hypertensive rats, the distribution of the IPP and DMIPP analogues in the triglyceride and the free fatty acid pools of the hypertensive rat hearts was similar to that observed in normotensive hearts (Table 3). It thus appears that although hypertensive rat hearts

Table 3. Distribution of radioactivity into the triglyceride (TG) and free fatty acid (FFA) lipid pools of the hearts of normotensive and hypertensive Dahl rats following injection with 15-(p-[^{131}I]iodophenyl)-pentadecanoic acid (IPP) and 15-(p-[^{125}I]iodophenyl)-3,3-dimethyl-pentadecanoic acid (DMIPP).[a]

	Time after injection (min)	IPP		DMIPP	
		TG	FFA	TG	FFA
Normotensive rats	1	25	6	22	45
	3	38	7	31	44
	5	47	6	45	30
	10	49	5	57	16
	30	67	4	63	10
	60	59	4	63	8
Hypertensive rats	1	32	2	22	68
	3	39	3	28	61
	5	54	2	47	41
	10	54	2	61	21
	30	49	3	63	9
	60	47	3	62	8

[a]TG = triglycerides; FFA = free fatty acids

may show differences in the regional uptake of methyl-branched fatty acids when compared with normotensive hearts, these differences cannot be detected in the global heart uptake and lipid distribution patterns. Potential differences in the regional lipid pool distribution of methyl-branched fatty acids in hypertensive rat hearts should also be explored.

The normotensive Dahl strain rats were also used to investigate the effect of dietary status (i.e. fasting vs feeding) on the uptake and lipid distribution of the ^{125}I-labeled dimethyl-branched fatty acid, DMIPP. Hearts and blood samples were removed at 5 and 30 min after injection of the animals, and lipid distribution studies performed on the hearts removed at 5 min post injection. Comparison of the heart uptake and blood level values (% dose/gm) in fasted and fed rats showed no significant difference in blood levels at either time point, but a significantly increased heart uptake of DMIPP was observed in the fasted rats at 5 min post-injection (Table 4). At 30 min post injection the hearts of the fasted and the fed rats had similar levels of ^{125}I-DMIPP. Lipid analysis of the hearts removed at 5 min showed that the majority of the ^{125}I label from the hearts of fasted rats was in the triglyceride pool (58%) versus free fatty acid pool (31%). In nonfasted rats, the free fatty acid pool contained more of the label (63%) than the triglyceride pool (29%). The difference in the ratios of triglyceride:free fatty acid in hearts of fasted versus fed rats at 5 min post-injection suggests a faster incorporation of DMIPP into the hearts of fasted rats versus fed rats, since there is a shift with time to a predominance of the radiolabel in the triglyceride fraction. Whether this is the result of substrate competition in the fed rats is not known at present.

Table 4. Effect of dietary status on the uptake and retention of radioactivity in the heart and blood of normotensive Dahl rats injected intravenously with 15-(p-[^{125}I]iodophenyl)-3,3-dimethyl-pentadecanoic acid (DMIPP).

	Heart		Blood	
Dietary status	5 min	30 min	5 min	30 min
Fasted	2.83	2.78	0.99	0.28
Fed	1.73	2.67	0.90	0.24

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AGENTS FOR MEDICAL COOPERATIVE PROGRAMS

Osmium-191

Six shipments of osmium-191 were made during this reporting period. Three shipments went to the Cyclotron Center, Liege, Belgium (Drs. M. Guillaume and C. Brihaye); two shipments to Massachusetts General Hospital, Boston, MA (Dr. H. W. Strauss); and one shipment to Children's Hospital, Boston, MA (Dr. S. Treves).

Iodine-125-Labeled Agents

A total of seven shipments of iodine-125-labeled agents as research products were made to collaborators. Three shipments of radioiodinated fatty acids were made to the Department of Cardiology, Free University Hospital, Amsterdam, The Netherlands (Dr. F. C. Visser); two shipments to Massachusetts General Hospital, Boston, MA (Dr. H. W. Strauss), and one each to Brookhaven National Laboratory, Upton, NY (Drs. A. B. Brill and P. Som), and Temple University, Philadelphia, PA (Dr. D. C. B. Mills).

Copper-64

One shipment of copper-64 was made to the Oak Ridge Associated Universities (Dr. J. Crook) during this reporting period.

AGENTS DISTRIBUTED ON COST-RECOVERY BASIS

Platinum-195m

Eight shipments of ^{195m}Pt-labeled *cis*-dichlorodiammineplatinum(II) (*cis*-DDP) were made on a cost recovery basis. One shipment each was made to the University of Iowa, Iowa City, Iowa (Dr. C. P. Burns); University of California, San Diego, CA (Dr. P. Andrews); City of Hope National Medical Center, Duarte, CA (Dr. K. J. Scanlon); National Institutes of Health, Bethsda, MD (Dr. C. Litterst), University of Massachusetts, Amherst, MA (Dr. K. S. R. Sastry), University of Medicine and Denistry of New Jersey, Neward, NJ (Dr. V. V. Rao), University of Colorado Health Science Center, Denver, CO; and Harvard Medical School, Boston, MA (Dr. A. G. Jones).

OTHER NUCLEAR MEDICINE GROUP ACTIVITIES

Publications

Brechner, R., Wolf, W., Dahalan, R., D'Argenio, Butler, T. A. and Knapp, Jr., F. F. "Non-Invasive Estimation of Bound and Mobile Platinum Compounds in the Kidneys." Radiopharmaceuticals and Labelled Compounds 1984. Conference Proceedings, Tokyo, October 22-26, 1984, P. 451, International Atomic Energy Agency, Vienna, 1985.

Knapp, Jr., F. F., Goodman, M. M., Callahan, A. P. and Kirsch, G. "Radioiodinated 15-(p-Iodophenyl)-3,3-Dimethylpentadecanoic Acid: A Useful New Agent to Evaluate Myocardial Fatty Acid Uptake," J. Nucl. Med., 27, 521-531 (1986).

Visitors

A group of nuclear medicine technology students from Vanderbilt University visited the Nuclear Medicine Group and toured its facilities on April 10.

Mr. John Kuperas, director of the radiopharmacy at UCLA Medical Center, visited the Nuclear Medicine Group on April 29-30, to learn the operation of ORNL's new carbon-based prototype osmium-191/iridium-191m generator. This new system will be evaluated by Dr. Ismael Mena, M.D., at UCLA, for pediatric nuclear cardiology applications.

Staff members, Gann Ting, Ph.D., and Haw-Jan Chen, Ph.D., of the Institute of Nuclear Energy Research Atomic Energy Council, Lung-Tan, Taiwan, visited the Nuclear Medicine Group and toured its facilities on April 28.

Miscellaneous

F. F. Knapp, Jr. was a guest lecturer at the Institute for Reactor Research in Wurenlingen, Switzerland, on April 24 and the Cardiology Department, University of Geneva, on April 25, and presented lectures describing the development and applications of new iodine-123-labeled fatty acids developed at ORNL.

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