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**Microimaging Studies of Myocardial Substrate Utilization and
Perfusion in Two Models of Non-Coronary Heart Disease**

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Introduction

The study of coronary heart disease (CHD) using radiolabeled compounds, including fatty acids, has received the greatest attention because of the high incidence of this condition. However, non-coronary artery heart diseases such as congestive and hypertensive cardiomyopathies are also of clinical importance and the diagnosis is difficult and often very invasive.

We have studied two animal models of non-coronary heart disease. The salt-sensitive Dahl strain hypertensive rats and their genetically matched normotensive controls and the cardiomyopathic BIO 53.58 (CM) strain Syrian hamsters with age and sex-matched RB strain controls. The CM strain hamster seems to be a very good model of human congestive cardiomyopathy (1) and the Dahl strain hypertensive rats have also been found to be good models for studying the effects of hypertension on the myocardium (2). In our studies we compared the utilization of various metabolic substrates, viz., fatty acids, glucose analogs, and the early distribution of ^{201}Tl , as an indicator of myocardial flow.

The routine studies involving dissection of animals for assaying the radioactivity following the injection of radiopharmaceuticals is not suitable for assessing regional changes in metabolism and flow. The use of quantitative autoradiographic microimaging (ARG) enables the visualization of discrete regional as well as global changes from normal and to quantitate them.

This paper describes the methodology and results of these investigations.

Materials and Methods

Radiopharmaceuticals

The following compounds were used in various experiments: Branched chain fatty acids ^{14}C -Beta-methyl heptadecanoic acid ($[^{14}\text{C}]\text{BMHDA}$) obtained from

commercial sources, (15-p-iodophenyl)-3-R,S-methyl pentadecanoic acid ($[^{131}\text{I}]$ BMIPP) was prepared by decomposition of piperidyltriazene derivative of p-amino analog or by thallation of 15-phenyl-3(R,S) methylpentadecanoic acid followed by treatment with potassium iodide yielding specific activities of 8-10 Ci/mmol (3), (15-p-iodophenyl)-3,3-dimethylpentadecanoic acid ($[^{131}\text{I}]$ -DMIPP) was prepared as described earlier (4), 19-Iodo-3,3-Dimethyl-18-nonadecanoic acid ($[^{131}\text{I}]$ DMIVN) was prepared by the method described (5,6) elsewhere.

^{14}C -2-fluoro-2-deoxy-D-glucose ($[^{14}\text{C}]$ DG) and ^{201}Tl chloride (Tl) were obtained from commercial sources (NEN., North Billerica, MA) and ^{18}F -2-fluoro-2-deoxy-D-glucose ($[^{18}\text{F}]$ FDG) was prepared according to the method described before (7).

Animal Models

The Dahl strain salt-sensitive (S) rats developed from Sprague-Dawley ancestors have been studied extensively (8). After weaning at two weeks of age the rats are given 8% NaCl in the food and by 5 weeks they develop hypertension at levels of 214 ± 18 mm Hg. Rats fed 0.4% NaCl in food did not develop hypertension (BP 138 ± 6 mm Hg) and served as controls. The rats were given food and water ad libitum until 2 hrs before the experiment. No significant difference in the body weights of the hypertensive (203 ± 18 g) and normotensive (201 ± 12 g) rats was observed.

The CM BIO 53.58 golden Syrian hamster was used as a model of congestive cardiomyopathy. This strain of hamsters develop congestive cardiomyopathy and muscular dystrophy. The genetic predisposition is transmitted as an autosomal recessive trait with 100% phenotypical expression in homozygots. Age and sex-matched RB strain hamsters served as controls (1). Food and water

was given ad libitum and no fasting protocols were used in order to avoid the fluctuations in glucose levels known to occur when fasted. Equal numbers of male and female hamsters were used in CM and RB groups, although no sex-related differences could be observed. Studies were performed at the age of 40-48 days, early phase and at 120 days, mid-stage of CM. The early phase is characterized by minimal changes visible only on electron microscopic sections. In the mid-stage, there is moderate cardiac hypertrophy with focal myocytolysis, fibrosis, and calcium deposition. Prior to sacrificing, blood tests for glucose, free fatty acids and cholesterol were obtained.

Experimental Protocols

Comparison of [^{14}C]BMHDA, [^{18}F]FDG, ^{201}Tl , [^{14}C] DG and ^{201}Tl in hypertensive and normotensive rats was carried out as follows. An i.v. dose of [^{14}C]BMHDA, 12.5 μCi was given followed 10 min later by 500 μCi of ^{201}Tl and 5 min later the animals were sacrificed. A group of hypertensive rats and their age matched, normotensive controls received 12.5 μCi [^{14}C]DG followed 40 min later by 500 μCi ^{201}Tl and 5 min later were sacrificed. A third group of hypertensive rats and controls was given 3 mCi [^{18}F]FDG; 30 min later 12.5 μCi [^{14}C]BMHDA was given and the animals were sacrificed 15 min later.

Comparison of [^{14}C]BMHDA with [^{131}I]BMIPP was performed on groups of hypertensive rats and age matched normotensive controls. All rats were given an i.v. injection of 10 μCi [^{14}C]BMHDA and 170 μCi [^{131}I]BMIPP simultaneously. BMIPP reaches equilibrium in the myocardium 30 min p.i. (9) and therefore the animals were sacrificed at this time interval. Six hypertensive rats and six controls were studied by tissue distribution after the heart and lungs were removed for microimaging (ARG). Small pieces of heart and lung were taken for counting. Results were expressed as percent injected activity per gr of

tissue. Two hypertensive and two normotensive rats were sacrificed and processed for whole body microimaging (ARG).

Evaluation of [^{131}I]DMIVN for detecting changes in myocardium due to hypertension and the effect of verapamil was studied in weaned (2-week-old) salt-sensitive rats. One group was given food containing 8% NaCl and developed hypertension. The control group, normotensives, was given food containing 0.4% NaCl and remained normotensive. A third group was given food with 8% NaCl and started on 0.5 mg of verapamil S.C. twice daily for two weeks. At the end of two weeks, group (1) was hypertensive and groups (2) and (3) had normal blood pressure values. IV injections of [^{131}I]DMIVN were given and the animals were sacrificed after 15, 30, 60, 120, 240 min and 24 hrs later. Microimaging (ARG) and tissue distribution was performed.

Comparative studies of substrate utilization, [^{131}I] DMIPP, [^{14}C]DG and blood flow, using ^{201}Tl were carried out. One group of CM hamsters was treated with verapamil 0.5 mg/0.1 ml S.C. twice daily for 41 days; a second group was given 0.1 ml saline twice daily for 41 days. RB strain hamsters matched for age and sex served as normal controls. The animals received 150 $\mu\text{Ci}/0.25$ ml DMIPP and 4 $\mu\text{Ci}/0.1$ ml [^{14}C]FDG and were sacrificed 1 hr later. A similar number of CM animals treated with verapamil or saline and RB hamsters were injected with [^{14}C]FDG 4 $\mu\text{Ci}/0.1$ ml and ^{201}Tl 180 $\mu\text{Ci}/0.2$ ml. This group was killed 60 min after the FDG injection and 5 min after ^{201}Tl injection.

Microimaging (ARG)

The heart and lungs were frozen in isopentane cooled in dry ice followed by embedding in methyl cellulose. For whole body microimaging, animals were frozen in liquid nitrogen before embedding. Sections of 20-30 μm were cut and exposed on Lo-dose mammography (DuPont) film along with graded step-wedge

standards. The first exposure (^{201}Tl 6 hrs, [^{18}F]FDG 3 hrs) for short-lived isotopes was immediately after sections were cut and dried. A second exposure of the tissue or whole body sections was started following the elapse of 10 half-lives of the shorter-lived isotopes. The exposure time for ^{14}C -labeled BMHDA or FDG was 2 weeks whereas the exposure time for ^{131}I -labeled compounds was shorter because larger doses were injected. When dual tracer studies with ^{131}I and ^{14}C were performed, higher doses of ^{131}I were given in order to maintain short exposures so as to minimize the contribution of ^{14}C . Eighty days later, the sections having undergone decay for 10 half lives for ^{131}I , were exposed for ^{14}C . The exposed films were processed and the images were digitized along with serial step-wedge standards. Digitization was performed using a videodensitometer so that a 2 cm^2 of the image was digitized to a 128×128 pixel matrix. The resolution limited by the pixel size was therefore $0.15 \times 0.15\text{ mm}$. It was possible to quantitate nCi/g for ^{14}C and cts/pixel for ^{131}I and ^{201}Tl , by creating response curves of radioactivity/film density using the graded standards exposed and digitized together with the tissue or whole body sections (10,11).

Results

Our earlier experiments (2) clearly showed that the utilization of the ^{14}C -labeled monomethyl-branched-chain fatty acid-BMHDA and the glucose analogs [^{18}F]FDG and [^{14}C]FDG was homogeneous in normotensive rats. The uptake of the fatty acid in the myocardium was higher than glucose (Table 1).

Table 1: BMHDA to FDG Ratios

	<u>RV</u>	<u>SEPTUM</u>	<u>LV Endocard</u>	<u>LV Epicard</u>
Normotensives	9.4	5.0	5.5	10.0
Hypertensives	1.2	0.5	0.4	0.78

In the hypertensive rats both glucose and fatty acid utilization becomes non-homogeneous. Glucose utilization increases in the endocardium and free wall of the left ventricle and fatty utilization decreases in the same regions. Because of these changes the ratio of BMHDA/FDG decreases by a factor of 10 (Table 1). At the same time no marked changes occurred in Tl distribution.

The next step was to compare a ¹⁴C-labeled methyl-branched-chain fatty acid [¹⁴C]BMHDA, to a ¹³¹I-labeled methyl-branched chain analog [¹³¹I]BMIPP (9). This comparative study was carried out in normal and hypertensive rats, using similar microimaging (ARG) and tissue distribution techniques. A major difference between the ¹⁴C-labeled fatty acid-BMHDA and the ¹³¹I-labeled BMIPP was evident: the liver uptake of the radioiodinated fatty acid was much higher 2.72-2.87 (normotensives, hypertensives) as compared to the ¹⁴C-labeled compound, 0.7-0.8 (normotensive, hypertensive). The blood levels of BMHDA in normotensive rats was slightly lower (0.25% d/g) as compared to BMIPP (0.7% d/g) but the difference becomes much smaller in the hypertensive rats. The global heart uptake of BMHDA (5.1 vs 2.4) was higher in the normotensive rats than in the hypertensive rats. Although BMIPP myocardial concentration appears to be lower than BMHDA, the spread of latter values makes this difference not statistically significant.

Table 2: Heart-to-Blood-Ratios of [^{14}C]BMHDA and [^{131}I]BMIPP

	[^{14}C]BMHDA	[^{131}I]BMIPP
Normotensive Rats	20.0	5.4
Hypertensive Rats	3.8	3.1

As a result of the changes described above, the heart/blood ratios for [^{14}C]BMHDA in the normal rats are significantly higher (Table 2). In the normotensive rats the utilization of [^{14}C]BMHDA and [^{131}I]BMIPP is homogeneous (Fig. 1). In hypertensive rats both radiopharmaceuticals show heterogeneous distribution in the LV myocardium (Fig. 2).

A newer fatty acid analog, DMIVN, labeled with ^{131}I was shown to have advantages over the betamethyl-iodovinyl-nonadecanoic acid (5,6). This compound showed homogeneous utilization in normotensive rats as opposed to the nonhomogeneous utilization in the hypertensive animals. The concentration in the heart and clearance from the blood were rapid. Weaning salt-sensitive rats given a high salt diet and treated with verapamil (0.5 mg s.c. for 2 weeks) did not develop hypertension (Fig. 3). The DMIVN utilization was homogeneous, and similar to the controls.

Microimaging (ARG) and tissue distribution studies carried out in the BIO 53.38 (CM) and RB Syrian hamsters showed that before the onset of severe significant histological changes in the myocardium (early phase), glucose utilization and myocardial flow were homogeneous (Fig.4) whereas the DMIPP utilization was moderately non-homogeneous.

Table 3: Regional FFA and FDG Utilization and Myocardial Flow

(Homog - Homogeneous, NH - Nonhomogeneous)

	<u>DMIPP</u>	<u>FDG</u>	<u>Tl</u>
Early Cardiomyopathy	NH+	Homog	Homog
Mid Stage Cardiomyopathy	NH+++	NH+	NH+

The whole body distribution patterns of DMIPP, FDG and Tl were similar in CM and RB control hamsters. The highest DMIPP concentration was in the liver (11-12% μ d/g) followed by heart (6-7% μ d/g) and adrenals (4-5% μ d/g). The DMIPP concentration in all other organs was less than 2% μ d/g. At 60 min after injection blood DMIPP activity was low, 0.5% μ d/g and the heart/blood ratio was 12:1. The microimaging data were in agreement with the direct counting of tissue samples.

Animals treated with verapamil showed homogeneous DMIPP and FDG utilization and flow, similar to the non CM prone, RB control hamsters. (Fig. 5).

Discussion

Fatty acids constitute the major energy source in the normal myocardium. Decreased oxygenation favors increased glucose utilization which is less efficient for energy production. It is also known that metabolic changes precede changes in blood flow (2).

The newer fatty acid analogs were synthesized in order to improve their suitability for metabolic imaging by promoting rapid myocardial extraction, longer intracellular persistence and faster blood clearance without release of radiolabeled metabolites and/or free iodine. A radiolabel suitable for SPECT imaging should also be considered as a desirable characteristic.

These goals were partly achieved by using long chain fatty acids and by the addition of branched methyl or dimethyl groups in the beta position in order to slow beta-oxidation and the subsequent release into circulation of radiolabeled metabolites (12). Deiodination was prevented by stabilizing the radioiodine label on a terminal phenyl or vinyl group (5,6,13,14). The animal models (1,8) and autoradiographic microimaging techniques have been described earlier (10,11).

Our studies of the changes in myocardial substrate utilization and blood flow induced by hypertension were conducted in order to answer the following questions. First, whether a ^{14}C -labeled, monomethyl fatty acid (BMHDA) will be suitable for studying the myocardial changes due to hypertension as compared to glucose analogs and a blood flow marker (2). By using the microimaging technique it was clearly shown that the distribution, i.e., utilization and flow, of the tracers was homogeneous in the myocardium of normotensive rats. In the hypertensive myocardium the most pronounced changes from normal were seen in the decrease in regional utilization of BMHDA and increase in FDG uptake, as seen in the BMHDA/FDG ratios. These changes occurred mostly in the free wall of the LV. The possible causes for these changes in metabolism in the hypertension-induced hypertrophy of the LV are a decrease in capillaries-to-sarcomere ratio, a defect in membrane transport favoring glucose incorporation, or defects in energy production or utilization.

The second step in our investigations was to compare ^{14}C -BMHDA with an iodinated fatty acid analog ^{131}I -BMIPP (9). The results showed that the single photon ^{131}I -BMIPP had similar biological behaviour to ^{14}C -BMHDA and that it was therefore suitable for SPECT imaging. The latest fatty acid

analog studied by us was ^{131}I -DMIVN. The results demonstrate very clearly that this compound shows a similar pattern to the ^{14}C -BMHDA studied earlier. It was also possible to show that the prevention of salt-induced hypertension by the administration of verapamil is paralleled by a normal metabolic pattern: utilization of ^{131}I -DMIVN in the myocardium of these animals is homogeneous as compared to the non-treated animals.

The studies of cardiomyopathic hamsters indicate that the ^{131}I -DMIPP utilization became abnormal in the early phase of the disease. At that point there are no changes visible with the light microscope and only minimal changes can be detected with the electron microscope. Glucose utilization and blood flow remain homogeneous. Later in the course of the disease foci of myocytolysis, fibrosis, and calcification are seen. At this stage, prior to the development of terminal congestive cardiomyopathy, lesions in the myocardium could be detected by microimaging. Some of these lesions showed matched decreases in DMIPP, FDG and Tl concentration. Other lesions, probably containing viable cells, still showed normal Tl and FDG and decreased DMIPP concentration indicating progressive disease.

By treating the animals with the calcium-channel blocker verapamil, the metabolic and morphological expression of this hereditary trait could be prevented.

Our data thus indicate that the newer iodine-stabilized, beta-methyl-branched, long-chain fatty acids are suitable for studying early metabolic changes in two non-coronary artery heart disease models. Microimaging autoradiography provides unique information for studying regional blood flow and substrate utilization and these findings are probably good predictors of

the potential of these compounds for diagnostic purposes in humans using SPECT imaging.

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Legends to Figures

- Fig. 1 Dual tracer ARG after [^{14}C]BMHDA (left) and [^{131}I]BMIPP (right) of same heart section from normotensive rat.
- Fig. 2 Dual tracer ARG after [^{14}C]BMDHA (left) and [^{131}I]BMIPP (right) from heart section of hypertensive rat.
- Fig. 3 ARG of heart section of hypertensive saline-treated rat (left) and verapamil-treated rat (right) following administration of [^{125}I]DMIVN.
- Fig. 4 Dual tracer ARG of hearts of early phase and mid-stage cardiomyopathy in the CM Syrian hamster. The most prominent abnormalities are noted in the DMIPP images although less prominent non-homogeneity is noted in mid-stage sections of FDG and ^{201}Tl .
- Fig. 5 ARG of myocardial uptake of DMIPP in CM-prone verapamil-treated and CM-prone saline-treated controls. The marked non-homogeneity and decrease in DMIPP concentration in saline-treated CM hamsters is evident.

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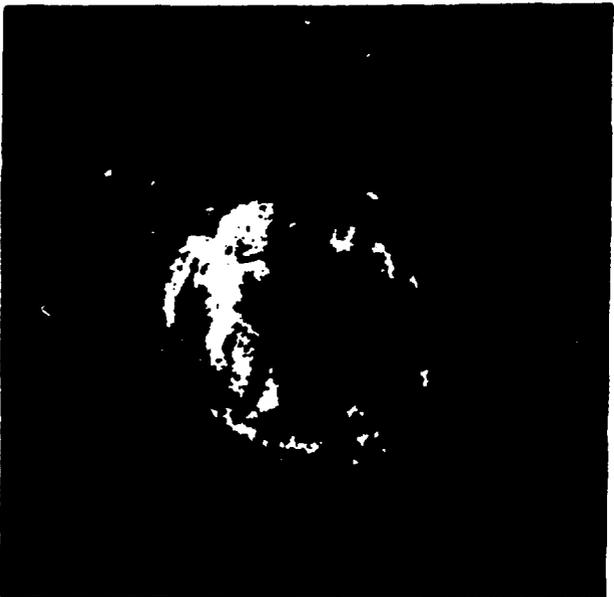
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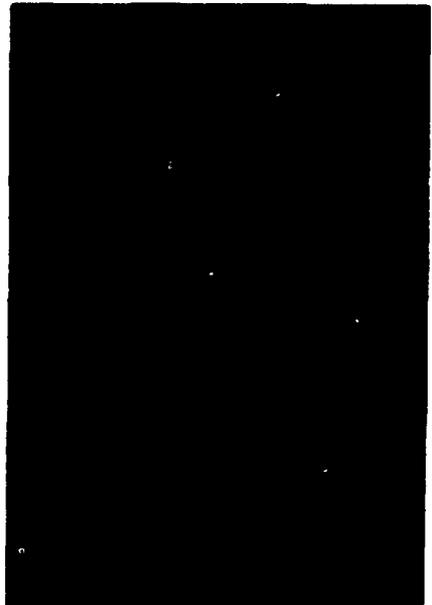




C-14 BPHDA



I-131 BHPDA



DMIPP

FDG

201-Tl



Normal Hamsters

DMIPP

FDG

201-Tl



Cardiomyopathy: Early Stage

DMIPP

FDG

201-Tl



Cardiomyopathy: Advanced Stage

DMIPP

Verapamil



Normal hamsters

Saline



Verapamil



Cardiomyopathy

Saline

