Evaluation of Fatty Acid and Glucose Metabolism in Myocardium of Heart Using Dual Radiopharmaceutical Approach

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***Abstract*—** *Understanding the metabolic consequences of Heart Failure (HF) is important in evaluating potential mechanism for disease progression in the heart, based on that we investigated whether changes of myocardial uptake of fatty acid and glucose tracer after induction of heart failure in various conditions in rats' heart were closely related to alterations in myocardial fatty acid and glucose metabolism. The dual radiopharmaceuticals, ^{31}I-15-(p-iodophenyl)-9-methylpentadecanoic acid (9MPA) and ^{14}C- 2-deoxy-D-[1-^{14}C] glucose (2DG) used to evaluate the changes of fatty acid and glucose metabolism of myocardium respectivelyin normal and HF stages with time.The myocardial uptake of whole heart and left ventricular were measured by Differential Absorption Ratio (DAR) and dual-tracer autoradiography in normal, acute and Chronic Heart Failure (CHF) at 5, 10, 30 and 60 minutes. Accumulation properties were compared with normal, HF stages and among HF stages using independent two sample t- test. Accumulation of ^{132} I 9MPA in acute and CHF stages at 5, 30 minutes, normal and CHF stages at 30 minutes did not show any significant difference (p>0.05). in normal stage accumulation of 14 C-2DG with CHF and acute stages at 30 and 60 minutes respectively did not show any significant different (p>0.05). In acute condition left ventricular myocardial accumulation of ^{131}I-9MPA was high and it deviated significantly from other part of myocardium in specific acute stage of heart. Metabolic functions of the heart vary with time in normal and HF stages. Mainly acute stage shows significant changes of metabolic function in whole and left ventricular myocardium. When the metabolic function of heart in normal and HF stages are compared, they do not differ significantly with time.*

***Keywords*—** *Heart failure, Radiopharmaceutical, Autoradiography.*

I. INTRODUCTION

Primarily two types of carbon substrates are used for myocardial ATP synthesis: Fatty acids and Glucose (sugar) in the heart. Long chain fatty acids are the predominant substrate used in the heart and generate the most ATP through beta (β) oxidation (Alam*et al*., 1998). Glycolysis is the metabolic pathway that oxidizes Glucose into pyruvate (Allard et al., 1994). ATP is released in both β oxidation of fatty acid and glycolysis of glucose. In disease conditions primary energy sources and metabolic process are changed significantly in myocardium to produce energy to maintain continuous contractile function and viability (Robert *et al*., 2004). There are two main types of heart failure, acute (myocarditis) and chronic (myocardial fibrosis). Radionuclide imaging is suggested as a good way to find out deviation of functional assessment of energy sources in normal and HF conditions (Takahiro H*et al., 2013*). In nuclear imaging field, to evaluate the status of human heart, Single Photon Emission Computed Tomography (SPECT) and Positron Emission Computed Tomography (PET) are the most suitable methods. In animal experiment, Autoradiography is good way to assess heart in vitro (Nieminen *et al*., 2005).

Using human model to evaluate fatty acid and glucose metabolism in various conditions of the human heart is an intricate process: due to time consumption and inability to find exact stage of disease condition. Further, ethical issues restrict the evaluation of human heart even in vitro conditions. On the other hand animal models are widely used to evaluate heart metabolic activity of heart in various conditions. With these facts in mind, we have evaluated how primary energy sources change with time according to various conditions of rat hearts in normal and HF.

II. METHODOLOGY AND EXPERIMENTAL DESIGN

A. Experimental design

In this study, 9 weeks old rats of Lewisstrazwere used (n=25). Their mean weight was 300±60 grams. Heart failure was induced by intravenous administration of 0.2-1ml myosin through tail vein. The rats were grouped as normal (n=5), acute (n=5) and chronic (n=5) according to the degree of HF. (0.9-1.5)MBq^{131}I- MPA and (4-185)MBq^{14}C-2DG was injected to all rats at the same time

after their weight was measured. The total amount of injected activity was 1083.9 MBq. After the injection of dual tracer 0.2-0.3 ml, pentobarbital sodium was injected intraperitoneally (lower left quadrant) to anesthetize those rats at 5, 10, 30 and 60 minutes. The hearts were excised immediately at specific times, weighted and specific activity was measured to calculate differential absorption ratio. The excised hearts were frozen and cut into 20 µm slices using a cryostat and these were kept with imaging plate (IP) immediately after the procedure. After 8 days and 6 months particular slices were imaged by Fujifilm BAS 5000 Image Analyser to evaluate ^{131}I-MPAdistribution and ^{14}C-2DG distribution respectively to generate dual tracer autoradiograms. Cross-talk between the two tracers was less than 3% so cross-talk between ^{131}I and ^{14}C could be considered as negligible.

B. Differential absorption ratio (DAR)

Differential absorption ratio was calculated for each group of rats by measuring total heart activity, specific slice activity, total weight of rat and heart weight. DAR is depicted by the following formula.

$$\text{DAR} = \left(\frac{\text{Observed tissue activity}}{\text{Injected activity}} \times \frac{\text{body weight}}{\text{Tissue Weight}} \right)$$

C. Autoradiography method

Autoradiography images (1.5MB) were analysed by *imageJ*image processing software to evaluate the intensities of the dual radiopharmaceutical distribution in left ventricular myocardium because red colour represent highest accumulation. Region of interest (ROI) was drawn in the centre of left ventricular myocardium with value of 50×50 pixels. ROI was analysed by measuring intensity changes of red colour of images of heart slices.

D. Statistical analysis

The results were expressed as mean ± SD. Data were analysed by using Minitab 16 Version and expressed as mean±SD. Groups comparison were made with independent two sample t-test with 95% confident interval. *P*<0.05 was considered as statistically significant.
CHF Acute

III. RESULTS

A. Cardiac uptake of ^{131}I- MPA and ^{14}C-2DG autoradiograms

As shown in figures 1 and 2: ^{131}I- MPA accumulation and ^{14}C-2DG changed with theCHF and acute stages of heart. It was clearly depicted by changes of colour intensities in particular images.

B. Measurement and comparisons of DAR of ^{132}I- 9MPA

As shown in Table1, whole myocardial uptake measured by DAR of ^{131}I-9MPA changed according to the condition of heart.

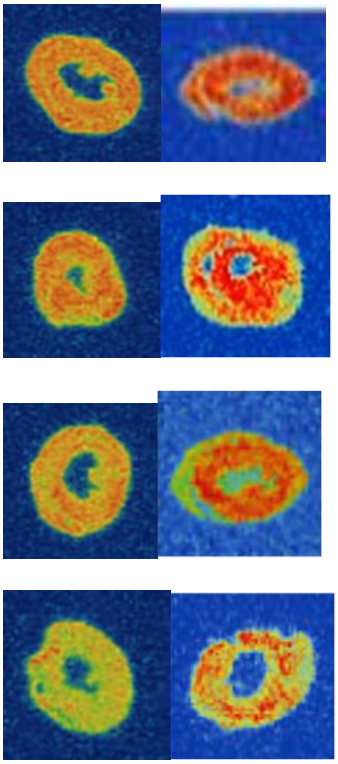


Figure1. Representative examples of heart slices, myocardial ^{131}I-MPA uptakeshown by dual tracer autoradiography images in CHF and acute stages. CHF Acute

Comparisons of DAR of ^{132}I- 9MPAwere made between the normal and HF stages and among the HF stages. There was a significant difference (*P*<0.05) between the absorption and accumulation of ^{131}I-9MPA between normal rats with acute heart failure ones at different times.

There was no significant difference (*P*>0.05) between the accumulation of ^{131}I 9MPA between normal and CHF at 30 minutes. Except these, the other groups showed a different of accumulation of ^{131}I-9MPA in whole heart between normal and CHF conditions with time. Among the HF stages, at 5 and 30 minutes the DAR values were not significantly different, but, at 10 and 60 minutes significant changes in absorption and accumulation of ^{131}I -9MPA (*p*<0.05) were evident.

According to the Graph 1, Normal stage of heart always had higher DAR of ^{131}I-9MPA than CHF and acute stages, with time. According to the graph after 30 minutes patterns of changes were same in all heart conditions and did not show any significant fluctuations with time.

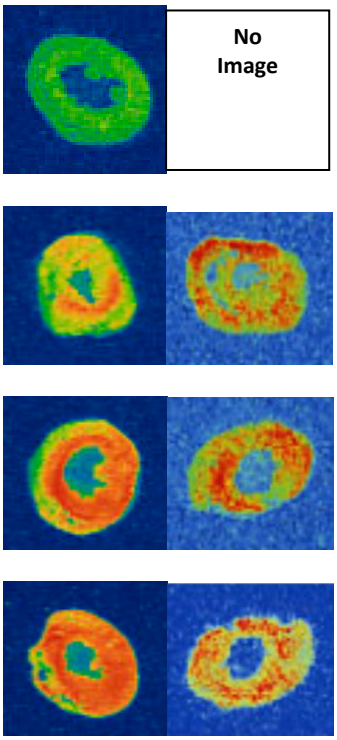


Figure2. Representative examples of heart slices, myocardial ^{14}C-2DG uptake shown by dual tracer autoradiography images in CHF and acute stages.

C. Measurement and comparisons of DAR with ^{14}C-2DG

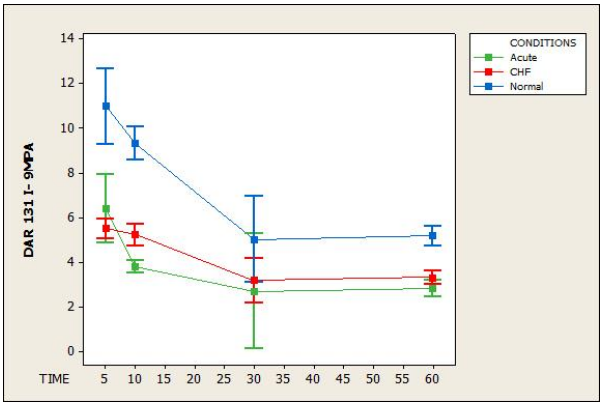
As shown in Table 2 and Graph 2, whole myocardial uptake was measured by DAR (mean) ± SD of ^{14}C-2DG; it was changed according with the heart. Comparisons were made between normal and HF stages and among HF stages.

DAR changes of ^{14}C-2DG between normal and acute group at 60 minutes and comparison between normal and CHF conditions at 30 minutes were not shown any significant different (P>0.05). At all specific times among the HF stages, normal and acute at 10, 30 minutes , normal and CHF at 5, 60 minutes were shown significant different of accumulation of ^{14}C-2DG (P<0.05). Especially at 5 minutes were unable to measure the heart activity because those acute rats were failure to survive during the procedure.

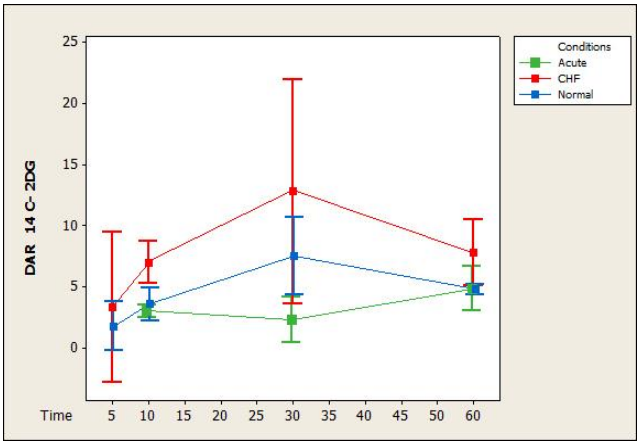
According to graph 2, CHF stage was shown higher DAR changes than normal and acute stages with time. After 30 minutes pattern of DAR changes in CHF and normal hearts were similar with time but acute stage was differ significantly.

Table 1. Comparison of ^{131}I-9MPA DAR changes between stages of heart with time.

Time (minutes)	DAR (mean) ± SD	
	Normal	**Acute**
5	10.99 ± 1.60	6.418 ± 0.96
10	9.34 ± 1.03	3.820 ± 0.37
30	5.048 ± 0.78	2.729 ± 0.28
60	5.190 ± 0.54	2.838 ± 0.30
	Normal	**CHF**
5	10.99 ± 1.60	5.523 ± 0.27
10	9.34 ± 1.03	5.247 ± 0.64
30	**5.048 ± 0.78**	**3.204 ± 0.11®**
60	5.190 ± 0.54	3.328 ± 0.25
	Acute	**CHF**
5	**6.418 ± 0.96**	**5.523 ±0.27®**
10	3.820 ± 0.37	5.247 ±0.64
30	**2.729 ± 0.28**	**3.204 ± 0.11®**
60	2.838 ± 0.30	3.328 ± 0.25
	®P>0.05	



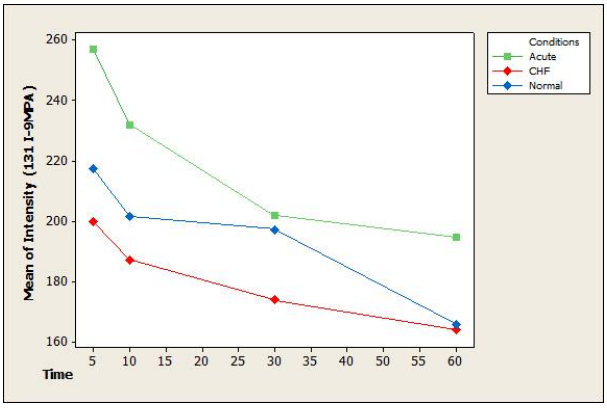
Graph 1. Pattern of DAR changes of ^{131}I-9MPA with time.



Graph 2. Pattern of DAR changes of ^{14}C-2DG with time.

Table 2. Comparison of ^{14}C-2DG DAR changes bet Ween stages of heart with time.

Time (minutes)	DAR (mean) ± SD	
	Normal	**Acute**
5		
10	3.62 ± 1.09	3.046 ± 0.43
30	7.537 ± 0.35	2.320 ± 0.21
60	**4.803 ± 0.18**	**4.880 ± 0.72®**
	Normal	**CHF**
	3.62 ± 1.1 0	5 7.00 ± 1.38
5	**7.537 ± 0.35**	**12.80 ± 1.02®**
10	4.803 ± 0.18	7.79 ± 1.08
30		
60	**Acute**	**CHF**
	3.046 ± 0.43	7.00 ± 1.38
5	2.320 ± 0.21	12.80 ± 1.02
10	4.880 ± 0.72	7.79 ± 1.08
30		
60	®*P*>0.05	



Graph 3. ^{131}I -9MPAChanges of RGB colour intensity of heart stages with time.

E. RGB colour intensity changes of ^{14}C-2DG autoradiographs with time

As shown in Graph 4, red colour intensity was increased with time in all stages of the heart. Specially, CHF was showed a significant increment of intensity changes within first 10 minutes. Pattern of changes were almost similar in other two stages of heart.

IV. DISCUSSION

Major finding of the present study were as follows. First, generally the accumulation and the absorption of fatty acid were higher than glucose in normal hearts. Autoradiographs clearly depicted those processes visually; and according to the colour intensity images. ^{131}I -9MPAand^{14}C-2DG distribution represent fatty acid and glucose distribution respectively in autoradiographs.

Second, accumulation and uptake of primary energy sources changed significantly with time in HF to compensate the energy production in heart. Graphs 1 and 2 clearly showed that DAR changes with time. In HF fatty acid uptake was reduced compared to the normal. Further, acute stage showed a significant reduction of fatty acid accumulation than CHF among HF stages. But, glucose uptake increased with time in HF stages. Moreover, in CHF a higher accumulation of glucose was evident than other two stages of heart with time.

Third, when we compared accumulation of fatty acid with each type of heart at 5 minutes not shown any significant different (p>0.05). Comparison made between acute HF and CHF stages at 30 minutes and the normal and acute HF stages with CHF; was not shown any significant different (*p>0.05*). CHF at 10 minute and acute HFat 60 minute with normal heart; were not

D.RGB colourintensity changesof^{131}I -9MPA autoradiographs with time

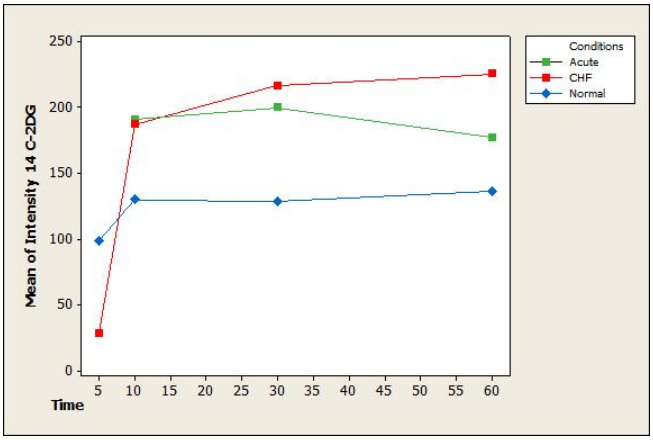
As shown in Graph 3, red colour intensity was decreased almost in all stages with time. Pattern of changes was depending on types of heart. However acute stage was shown higher colour intensity than other two stages with time. End of the 60 minutes normal and CHF were shown same intensity of colour.

shown any significant different (*p>0.05*) of accumulation of glucose.

Fourth, as shown graphs 3 and 4 represent fatty acid and glucose uptake within the left ventricular myocardium of heart; Fatty acid metabolism was reduced and glucose metabolism was increased in HF with time. Glucose metabolism was increased rapidly in CHF stage of heart within first 5 minutes.

Similar finding were reported , normal tracer uptake early after reperfusion following transient myocardial ischemia may not always imply unimpaired intracellular fatty acid oxidation, especially of 9MPA (Norio Igarashi *et al*.,2005) Both sub maximal and maximal exercise were unaffected by either myocardial infarction or training while no differences was observed between sedentary and trained rats with infarction. These results demonstrated that an exercise training program of moderate intensity produces beneficial hemodynamic and metabolic effects in rats with moderate compensated heart failure and energy sources (Timothy., 1986).

In an another study conducted through PET imaging, it has been revealed that myocardial fatty acid uptake rates in heart failure are higher than expected for the normal heart, whereas myocardial glucose uptake rates are lower. This shift in myocardial substrate use may be an indication of impaired energy efficiency in the failing heart (Michael Taylor *et al*., 2001).



Graph 4. ^{14}C-2DG Changes of RGB colour intensity of heart stages with time.

Some methodological limitations deserve comments. First, heart pressure and regional myocardial flow was not measured in this experiment. Those factors primarily determine the amount of primary energy sources in blood.

Second, the sample size was low in this study. If we increased the sample size we can produce more reliable results.Third, we used *imagej* image processing software in our study. There are other specialised softwares available in animal experiment to analyse the autoradiographs. Those are very expensive and we restricted to use them.

CONCLUSION

Heart diseases conditions significantly alter the uptake and metabolism of fatty acid and glucose in heart.Changes of primary energy sources are potential bio markers for disease conditions of heart. Mainly acute stage show significant changes of metabolic function in whole and left ventricular myocardium. In addition, metabolic functions of the heart in normal and HF stages did not change significantly with time.

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