III. 10  Studies on Myocardial Fatty Acid Metabolism of Normal and Adriamycin-Treated Rats

Department of Clinical Cancer Chemotherapy, Research Institute for Tuberculosis and Cancer, Tohoku University
Cyclotron and Radioisotope Center, Tohoku University*

Introduction

Adriamycin(ADR) is an anthracycline antibiotics which is used for a variety of malignancies. The lethal cardiotoxicity caused by ADR is one of the clinical problems, however there is no certain predictions of the causative factor in cardiotoxicity of this drug. The only way for the protection is restriction of total dosage. We reported that the decrease in 2-deoxy-2-fluoro[18F]-D-glucose(18FDG) uptake was observed in the heart of the rats both in acute and chronic toxicities caused by ADR1). By utilizing 18FDG, myocardial dysfunction brought about by ADR may be predicted in terms of cardiotoxicity. Free fatty acids such as glucose are a major energy source for the myocardium. Fatty acids have been estimated to provide 65% of the total energy requirement for heart muscle2). This study deals with fatty acids metabolic dysfunction of the myocardium induced by ADR, since the administration of ADR induces dysfunction of myocardial glucose metabolism. Our trial to detect ADR-induced cardiomyopathy used fatty acids.

We investigated fatty acids myocardial metabolism by normal and ADR-treated rats with 18F-palmitic acid and β-methyl[1-11C]heptadecanoic acid (11C-BMHDA), a labeled fatty acid analog that is partially metabolized and trapped in the myocardium.

Materials and Methods

Male Donryu rats weighing 140-160 g were used. ADR was administered through tail vein at a dose of 4.25mg/kg. The rats were fed ad libitum. 18F-palmitic acid and 11C-BMHDA were synthesized by the method developed by Livni E. et al.2) and Takahashi T. et al.3).

Experiment 1: 18F-palmitic acid were injected intravenously through a lateral tail vein of the normal rat at a dose of 20 μCi/0.3ml/rat.
The rats were killed by cervical dislocation at 1, 5, 10, 30, 60, 90 or 120 min after 18F-palmitic acid injection. The blood, heart, lung, liver, kidney, thigh muscle and brain were removed, bottled, weighed and counted by auto-gamma counter. The radioactivity of 18F-palmitic acid in tissues was expressed as % injected dose/g tissue.
Experiment 2: On days 1, 3, 5 & 7 after the injection of 4.25mg/kg of ADR, the rats were killed 60 min after the injection of $^{18}$F-palmitic acid. On the 3rd day after the injection of ADR, similar studies were made 1, 5 or 10 min after the injection of $^{18}$F-palmitic acid. The distribution of $^{18}$F-palmitic acid in heart was counted and expressed like experiment 1. The uptake of $^{18}$F-palmitic acid in the heart of the ADR-treated rats was compared to that of the heart of untreated rats and the uptake ratio was defined as follows: Uptake ratio = [\% injected dose/g tissue of the heart (ADR-treated)] / [mean of \% injected dose/g tissue of the heart (normal)].

Experiment 3: $^{11}$C-BMHD A was injected through a lateral tail vein of normal rats at a dose of 100μCi/0.3ml/rat. The rats were killed by cervical dislocation at 5, 10 or 20 min after $^{11}$C-BMHD A injection. Similar study was made as described in experiment 1.

Experiment 4: On days 1, 3 & 5 after the injection of 4.25 mg/kg of ADR, the rats were killed 20 min after the injection of $^{11}$C-BMHD A. This experiment was similar to experiment 2.

Experiment 5: 4.25 mg/kg ADR were injected through a lateral tail vein of the normal rat.
On days 1, 3, 5 & 7 after the injection of ADR, serous triglyceride, β-lipoprotein and free fatty acid of arterial blood of the rats were measured. Triglyceride and β-lipoprotein were measured by autoanalyzer (OLYMPUS AU500). Free fatty acid was measured by ACS-ACO enzymatic method (NEFA kit U, Nihon Syoji).

Results

Experiment 1: The tissue distribution of $^{18}$F-palmitic acid in normal rat is shown in Fig. 1. There observed rapid washout of activity from the liver, kidney, heart and other tissues due to the beta-oxidation process. The accumulation in the heart was not observed.

Experiment 2: On days 1, 3, 5 & 7 after the injection of ADR, the uptake ratio of $^{18}$F-palmitic acid in the heart was not changed. On the 3rd day, the uptake ratio of $^{18}$F-palmitic acid is shown in Table 1. This study showed no change in uptake ratio of the heart.

Experiment 3: The tissue distribution of $^{11}$C-BMHD A in normal rat is shown in Fig. 2. There is a fast washout of the activity from the liver and kidney, but the heart activity is in a state of equilibrium at 10 min to 20 min.

Experiment 4: The uptake ratio of $^{11}$C-BMHD A in the heart is shown in Fig. 3. The uptake ratio reached the nadir 5 days after the injection of ADR.

Experiment 5: Serous triglyceride, β-lipoprotein and free fatty acid of arterial blood from ADR-treated rats is shown in Fig. 4. Triglyceride and β-lipoprotein are above the average at 5 days after the injection of ADR compared with that of the normal rat. On the other hand, free fatty acid is below the average at 5 days.
Discussion

The principle of metabolic trapping has been used to assess the energy requirements of the heart with $^{18}$FDG which is metabolic trapping agent$^{1}$,$^{4}$. $^{11}$C-BMHDHA is designed to inhibit the beta-oxidation process by preventing the formation of the corresponding beta-ketoacyl SCoA. Beta-ketoacyl SCoA could not be produced because the presence of the methyl group results in an inhibition of the metabolic process.

In this study with $^{18}$F-palmitic acid, there is a fast washout of activity from the heart due to the beta-oxidation process. $^{18}$F-palmitic acid exhibits the normal pattern of metabolism with a fast washout, whereas $^{11}$C-BMHDHA is trapped in hydroxyacyl-CoA.

In the previous study we have demonstrated that the decrease in $^{18}$FDG uptake was observed in the heart of the rats in acute toxicity caused by ADR$^{1}$. The uptake ratio after a single administration of ADR was below in the groups given 3mg/kg and 4.25mg/kg of ADR. The $^{18}$FDG uptake ratio reached the nadir 3 days after the injection of 4.25mg/kg of ADR. But $^{11}$C-BMHDHA reached the nadir 5 days after the injection of ADR.

These results suggest that there is a lag between glucose ($^{18}$FDG) and fatty acid ($^{11}$C-BMHDHA) metabolism in the heart of the ADR-treated rats. That is to say, at first glucose metabolism is suppressed and then fatty acid metabolism is suppressed after the injection of ADR. A causative factor, mechanism and progress of cardiotoxicity of this drug may be indicated. Further studies are necessary to elucidate the effect of ADR on myocardial metabolism.

Acknowledgements

This work was supported in part by the Grant-in-Aid for Cancer Research (S61-21) from Ministry of Health and Welfare, and for Scientific Research (61570299) from the Ministry of Education, Science and Culture.

References

Table 1. On the 3rd day after the injection of ADR(4.25mg/Kg), the uptake ratio of $^{18}$F-palmitic acid is shown.

<table>
<thead>
<tr>
<th>min</th>
<th>Uptake, %injected dose/g tissue</th>
<th>ratio=a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a:ADR(4.25mg/kg)</td>
<td>b:Normal</td>
</tr>
<tr>
<td>1</td>
<td>3.03 ± 0.67</td>
<td>3.04 ± 0.22</td>
</tr>
<tr>
<td>5</td>
<td>1.95 ± 0.21</td>
<td>1.71 ± 0.29</td>
</tr>
<tr>
<td>10</td>
<td>1.38 ± 0.17</td>
<td>1.36 ± 0.18</td>
</tr>
</tbody>
</table>

Fig. 1. Tissue distribution of $^{18}$F-palmitic acid in rats. Each point represents the mean of 6 rats.
Fig. 2. Tissue distribution of $^{11}$C-BMHDA in rats. Each point represents the mean of 6 rats.

Fig. 3. Uptake ratio of $^{11}$C-BMHDA into hearts of rats injected ADR(4.25mg/kg). Each point represents the mean of 6 rats.
Fig. 4. Changes in fatty acids of arterial blood from rats injected ADR(4.25mg/kg). Each point represents the mean of 6 rats.