III. 14. Experimental Studies on Comparison among Changes of 18FDG Uptake of the Myocardium, ECG and Histological Findings in Doxorubicin-Administered Rats


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Introduction

2-deoxy-2-[18F]fluoro-D-glucose (18FDG) has been used as a tracer for detecting cancer or analysing the brain and myocardium function. This report deals with possibility to detect dysfunction of the myocardium under chemotherapy with an anti-cancer agent, Doxorubicin (DXR), which often induces cardiotoxicity clinically, by comparing to electrocardiographic (ECG) findings and histological ones especially by electron microscopy in DXR-administered rats.

Materials and Methods

1. Animals

Male Donryu rats, 5 weeks old, weighing 120-150g were used in the following experiments.

2. Induction of cardiac toxicity with DXR

DXR was dissolved in physical saline and intravenously injected into the tail vein at the dose of 0.2ml/100g of animal weight at a time.

Since it was known in our experiment that a single optimal dose inducing 18FDG uptake decrease in the heart was 4.25mg/kg of DXR 1), this dose of the agent was administered into rats.

On days 1, 3, 5 and 7 after DXR injection, the animals were sacrificed by cervical dislocation one hour after injection of 18FDG at the dose of 20 μCi / 0.3 ml, which was supplied by Cyclotron and Radioisotope Center, Tohoku University.

The distribution of 18FDG in the heart was measured by auto-gamma counter and expressed by % injected dose / g tissue. The uptake value of 18FDG in the heart of DXR-administered rat was compared to that of control normal rat without DXR administration and
the uptake-ratio was defined as follows: Uptake-ratio = [\% injected dose / g tissue of the heart (DXR-administered)] / [mean of \% injected dose / g tissue of the heart (not DXR-administered)]

3. Examination of ECG

Firstly, ECG was examined in each group of 6 rats on days 1, 3, 5 and 7 after injection of DXR. Secondly, on a specific rat ECG was examined likewise after DXR injection. Rats were anesthetized with pentobarbital sodium (30 mg / kg, i.p.) and limb lead ECG were examined in a prone position after 20-30 min. of anesthetic injection.

4. Histological examination

Specimens in the transverse section of both ventricles of the rat heart were fixed in 10% formalin solution and stained with hematoxylin and eosin, which were examined by light microscopy. The rest of the specimens were fixed in 2% glutar aldehyde and 1% osmium tetroxide fixative, embedded in EPON 812, and stained with uranyl acetate and sodium citrate. These specimens were offered for examination by electron microscopy.

Results

1. Uptake-ratio of $^{18}$FDG in the heart shown in Fig. 1

The uptake-ratio after administration of DXR at the dose of 4.25mg / kg was the lowest (0.35) on day 3 and recovered to the normal range on days 5 and 7.

2. ECG findings shown in Tables 1 and 2

Parameters on ECG of 6 animals on days 1, 3, 5 and 7 after DXR administration were described below. Heart rate, PR interval, QRS complex, ST interval, QT interval and T wave height did not change any day. Tendency of QRS height decrease was observed on day 3, but these value changes were not significant (Table 1). ECG examination on a specific rat was also performed in course of time (Table 2). QRS height on day 5 was comparatively low, but no great change was observed on other ECG parameters.

3. Histological findings

No change was observed by light microscopy any day. By electron microscopy, on day 1 a slight swelling of the mitochondria was observed but no change in cristae. The most swelling like aneurysma in the mitochondria was observed on day 3 but this degree of change could recover to normal. On day 5, a slight round formation of the mitochondria was found. On day 7, swelling of the mitochondria was hardly observed. Electron-microscopical examination showed no histological changes in small organoids and cell membrane any day.

Discussion
Cardiotoxicity caused by anticancer drugs is acute and chronic clinically. The anthracyclines, in particular Doxorubicin (DXR), are the most active agents in cancer chemotherapy. The clinical use of these agents, however, is limited by its potential to induce myocardial damage leading to congestive heart failure \(^2\)\(^-\)\(^5\). Various monitoring methods have been advocated for the early detection of the myocardial dysfunction induced with these agents, such as ECG, echocardiography, cardiac biopsy and radionuclide ventriculography. In recent years, endomyocardial biopsy is the most reliable means to evaluate the degree of cardiotoxicity caused by these anticancer agents \(^6\). In Japan, however, this biopsy examination does not seem to be popular due to its risk of the method.

We have experimentally studied acute and chronic cardiotoxicity induced with anthracyclines using a positron-emitting radiopharmaceutical, \(^{18}\)FDG. The decrease of \(^{18}\)FDG uptake in the heart of rat after administration of DXR was observed in the experiments concerning acute cardiotoxicity and chronic one, and the myocardial dysfunction may be clinically predicted in terms of changes of myocardial images by positron emission tomography (PET) \(^1\)\(^,\)\(^7\).

This report deals with our experimental studies on glucose metabolic dysfunction of the myocardium by DXR comparing to ECG findings and histological ones.

Prolongation of QRS interval on ECG was already reported concerning rats which were repeatedly administered DXR \(^8\), but total dose in their studies was as four-fold as ours. ECG changes were not observed in our experiments.

By light microscopy, formation of vacuoles and fibrillation in the myocardium after DXR administration were reported \(^9\), but in our experiment no changes were observed at one-third of their dose.

Degeneration of the mitochondria and porosis of the myofibril in the myocyte of myocardium were reported by electron microscopical observation after DXR administration\(^9\). On our experiment at one-third dose of DXR in their experiment, swelling changes of the mitochondria which is the area producing energy were slight on day 1, reached the peak on day 3, gradually recovered on day 5, and were hardly observed on day 7, that is, the mitochondria was similar to that of the control animal on day 7. The uptake-ratio of \(^{18}\)FDG after DXR administration was the smallest (0.35) on day 3, and recovered to normal on days 5 and 7 (Fig.1). Therefore, the course of changes of \(^{18}\)FDG uptake of the heart and histological findings of the mitochondria corresponded apparently. Namely, the sensitivity to detect changes on glucose metabolism in the myocardium using \(^{18}\)FDG is similar to, or higher than that of histological changes by electron microscopy.

**Conclusion**

Myocardial uptake of \(^{18}\)FDG in rat decreased, and the uptake-ratio showed the nadir on day 3 and recovered to the normal range on day 7 after DXR administration.
Sensitivity to detect $^{18}$FDG uptake changes was higher than those on ECG and histological examination by light microscopy. Change pattern of $^{18}$FDG uptake in course of time after DXR administration corresponded to that of the mitochondria by electron microscopy which was currently the most reliable method.

By utilizing a short-lived positron emitter, $^{18}$FDG, DXR-induced cardiotoxicity may be noninvasively predicted in terms of change of myocardial images by PET.

Acknowledgments

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References

Table 1. Changes on ECG of rats injected DXR (4.25 mg/kg, i.v.). No significant change was observed on ECG parameters.

<table>
<thead>
<tr>
<th>Days after injection (n=6)</th>
<th>ECG</th>
<th>Heart Rate (/min.)</th>
<th>PR interval (sec.)</th>
<th>QRS complex (sec.)</th>
<th>ST interval (sec.)</th>
<th>QT interval (sec.)</th>
<th>T wave height (mV)</th>
<th>QRS wave height (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>263.3 ± 10.3</td>
<td>0.050 ± 0.000</td>
<td>0.033 ± 0.005</td>
<td>0.025 ± 0.005</td>
<td>0.058 ± 0.004</td>
<td>0.058 ± 0.012</td>
<td>0.471 ± 0.083</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>273.3 ± 13.7</td>
<td>0.052 ± 0.004</td>
<td>0.030 ± 0.000</td>
<td>0.027 ± 0.005</td>
<td>0.057 ± 0.005</td>
<td>0.050 ± 0.000</td>
<td>0.333 ± 0.129</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>268.3 ±9.8</td>
<td>0.052 ± 0.004</td>
<td>0.030 ± 0.000</td>
<td>0.028 ± 0.004</td>
<td>0.058 ± 0.004</td>
<td>0.054 ± 0.010</td>
<td>0.571 ± 0.058</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>271.7 ± 9.8</td>
<td>0.048 ± 0.004</td>
<td>0.030 ± 0.000</td>
<td>0.030 ± 0.000</td>
<td>0.060 ± 0.000</td>
<td>0.050 ± 0.000</td>
<td>0.487 ± 0.130</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>270.0 ± 8.9</td>
<td>0.048 ± 0.007</td>
<td>0.032 ± 0.004</td>
<td>0.027 ± 0.005</td>
<td>0.058 ± 0.004</td>
<td>0.054 ± 0.010</td>
<td>0.517 ± 0.072</td>
</tr>
</tbody>
</table>

Mean ± standard deviation

Table 2. Changes on ECG of a specific rat injected DXR (4.25 mg/kg, i.v.). No significant change was observed in course of time on ECG parameters.

<table>
<thead>
<tr>
<th>ECG</th>
<th>Heart Rate (/min.)</th>
<th>PR interval (sec.)</th>
<th>QRS complex (sec.)</th>
<th>ST interval (sec.)</th>
<th>QT interval (sec.)</th>
<th>T wave height (mV)</th>
<th>QRS wave height (mV)</th>
</tr>
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<tr>
<td>0</td>
<td>332</td>
<td>0.05</td>
<td>0.04</td>
<td>0.02</td>
<td>0.03</td>
<td>0.03</td>
<td>0.35</td>
</tr>
<tr>
<td>1</td>
<td>330</td>
<td>0.05</td>
<td>0.03</td>
<td>0.02</td>
<td>0.03</td>
<td>0.03</td>
<td>0.37</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>0.05</td>
<td>0.04</td>
<td>0.02</td>
<td>0.03</td>
<td>0.03</td>
<td>0.31</td>
</tr>
<tr>
<td>5</td>
<td>340</td>
<td>0.05</td>
<td>0.03</td>
<td>0.02</td>
<td>0.03</td>
<td>0.03</td>
<td>0.23</td>
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<tr>
<td>7</td>
<td>330</td>
<td>0.05</td>
<td>0.03</td>
<td>0.02</td>
<td>0.03</td>
<td>0.05</td>
<td>0.34</td>
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</table>
Fig. 1. Uptake-ratio of $^{18}$FDG in the heart of rats administered DXR. The uptake-ratio of $^{18}$FDG revealed the nadir 3 days after administration of Doxorubicin (DXR) at the dose of 4.25 mg/kg.

LD50: 8.4 mg/kg  LD10: 8.1 mg/kg

Fig. 2. Histological changes by electron microscopy in the myocardium of rats administered DXR (4.25 mg/kg, i.v.). The most swelling of the mitochondria revealed the peak on day 3, and the change recovered to normal on day 7.