The rupture of atherosclerotic vulnerable plaques and subsequent formation of thrombi are the main factors responsible for myocardial and cerebral infarctions. Because macrophage infiltration plays an essential role in plaque rupturing, pharmacologic therapy that reduces macrophage infiltration is required to stabilize the vulnerable plaques. The monitoring of therapeutic effect is important in assessing the therapeutic effects of drugs for individual patients. We previously reported that 18F-FDG effect is important in assessing the therapeutic effects of drugs to stabilize the vulnerable plaques. The monitoring of therapeutic response in vulnerable plaques is clinically important for risk stratification and to provide early treatment. Newly developed drugs for treating vulnerable plaques are being evaluated preclinically and clinically and for the development of new drugs that can stabilize vulnerable plaques. 18F-FDG PET is useful for evaluating the therapeutic effect of drugs clinically and for the development of new drugs that can reduce the inflammatory activity of vulnerable plaques.

Key Words: 18F-FDG PET; atherosclerosis; vulnerable plaque; therapeutic effect; probucol


The rupture of atherosclerotic plaque and ensuing thrombus formation are primarily responsible for myocardial and cerebral infarctions (1–3). Atherosclerotic plaques are classified into 2 types, stable and vulnerable, with the latter having a high risk of rupture. Thus, the detection of atherosclerotic plaques prone to rupture is clinically important for risk stratification and to provide early treatment. Inflammation plays an essential role in plaque rupturing, and macrophage infiltration is characteristic of the vulnerable plaques (4). Recently, we and another group have shown that 18F-FDG accumulates in macrophage-rich plaques, and 18F-FDG PET has been suggested to be useful for the selective detection of vulnerable plaques (5–7).

Many drugs have been tested for their ability to stabilize plaque (8), and macrophages are one of the targets of these pharmacologic therapies (9). However, because drug effects vary among individuals, monitoring the therapeutic effect is important for selecting the appropriate drug for individual patients. The present study was undertaken to investigate the usefulness of 18F-FDG PET for monitoring therapies.
that target vascular inflammation. We used probucol in Watanabe heritable hyperlipidemic rabbits with myocardial infarction (WHHLMI rabbits) (10)—a widely used animal model of atherosclerosis (11,12)—because probucol is an antioxidant known to reduce the extent of macrophage infiltration in atherosclerotic lesions in these rabbits (13). The atherosclerotic plaque of WHHLMI rabbits does not rupture but its pathologic characteristics have been reported to resemble those of the human lesion (10,14,15).

MATERIALS AND METHODS
Animals and Diets
Pure probucol powder was kindly supplied by Daiichi Pharmaceutical Co. Ltd. WHHLMI rabbits bred at Kobe University were used in this study. The animals were fed standard rabbit chow (type RC4; Oriental Yeast Co., Ltd.) until the age of 10 mo. One rabbit was killed under anesthesia with sodium pentobarbital, and the aorta was removed at the beginning of the experiment to be used as a pretreatment control to investigate atherosclerotic lesions of the aorta. Other rabbits were divided into 2 groups. Four rabbits were fed chow (130 g/d) containing 1% (w/w) probucol for 6 mo (producol group). We chose this dose according to previously published methods (16–19), although this dose is about 20 times higher than the recommended human dose. Four rabbits in the control group were given standard rabbit chow (130 g/d) for 6 mo. Water was available ad libitum for all rabbits. Before drug treatment and at 1, 3, and 6 mo after drug administration, body weight and plasma levels of total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol and triglycerides were evaluated. The Animal Care and Use Committee of the Hamamatsu University School of Medicine approved all experiments.

PET and CT
The animals were fasted for at least 6 h before receiving 18F-FDG. Before the imaging study, the individual body shape was taken with urethane hardening foam to maintain the stereotactic position. 18F-FDG PET and CT experiments were performed at 180 min after injection of 18F-FDG using an ECAT EXACT HR PET scanner (Siemens AG). We chose this position according to the method reported by Tsukada et al. using the rabbit macrophage-specific monoclonal antibody RAM-11 (Dako Corp.) (20). These slices were also costained with hematoxylin for identification of the nucleus. The number of macrophages was determined by counting the nuclei of RAM-11–positive cells in each slice. The intima and whole area were measured on an azan-Mallory–stained consecutive cross-section of RAM-11 staining.

Histology
Each arterial segment was embedded in paraffin after the radioactivity was measured. Consecutive 4-μm-thick sections were prepared from each segment and the sections were subjected to immunohistochemical staining or azan-Mallory staining. Immunohistochemistry was performed according to the method reported by Tsukada et al. using the rabbit macrophage-specific monoclonal antibody RAM-11 (Dako Corp.) (20). These slices were also costained with hematoxylin for identification of the nucleus. The number of macrophages was determined by counting the nuclei of RAM-11–positive cells in each slice. The intima and whole area were measured on an azan-Mallory–stained consecutive cross-section of RAM-11 staining.

RESULTS
Body Weight and Lipid Profile
Body weight, plasma levels of cholesterols, and plasma levels of triglycerides in the probucol and control groups are given in Table 1. During the experiments, there was no statistically significant difference in body weight between or within the groups. No significant difference was seen in the plasma concentrations of total, LDL, and HDL cholesterol or triglycerides between the probucol and control groups. Total plasma cholesterol levels decreased over the 6 mo of intervention in both groups compared with the levels at the beginning of the study, although the differences were not significant at 3 and 6 mo in the probucol group.

PET Study
Figure 1 shows the CT, PET, and superimposed images of CT and PET of the WHHLMI rabbits. The arrows indicate the position of the aorta. At the age of 10 mo, the aorta could be imaged in all rabbits. In the probucol group, the aorta was not imaged after 6 mo of treatment. In contrast, intense radioactivity was observed in control rabbits throughout the 6-mo investigation. The time course of 18F-FDG uptake over the treatment period of the thoracic aortas in individual rabbits is shown in Figure 2. The SUVs decreased in all probucol-treated rabbits after 3 mo of drug treatment,
whereas the SUVs remained constant or increased in control rabbits. SUVs for the 4 rabbits in each group are shown in Figure 3. The SUVs decreased significantly in the probucol group after 3 mo of treatment compared with the pretreatment period and the control group. The SUVs of the control group increased gradually with time.

Accumulation of 18F-FDG in Aortic Segments
DURs in the removed aortic segments after 6 mo of intervention are summarized in Figure 4. 18F-FDG uptake in the thoracic aorta was significantly lower in the probucol-treated group than that in the control group. Uptake in the abdominal aorta was also lower in the probucol group than

**TABLE 1**
Effect of Probucol Treatments on Body Weight and on Plasma Levels of Total, LDL, and HDL Cholesterol and Triglycerides in WHHLMI Rabbits

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0 mo (10 mo old)</th>
<th>1 mo</th>
<th>3 mo</th>
<th>6 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Probucol</td>
<td>Control</td>
<td>Probucol</td>
<td>Control</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>3.7 ± 0.2</td>
<td>3.8 ± 0.1</td>
<td>3.8 ± 0.2</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>965 ± 138</td>
<td>967 ± 153</td>
<td>694 ± 71*</td>
<td>741 ± 73*</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>675 ± 160</td>
<td>630 ± 167</td>
<td>509 ± 88</td>
<td>614 ± 97</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>18 ± 3</td>
<td>14 ± 3</td>
<td>16 ± 3</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>453 ± 147</td>
<td>366 ± 183</td>
<td>721 ± 241</td>
<td>447 ± 167</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. corresponding value at month 0 (paired t test).

Data are presented as mean ± SD. No significant difference was seen in all parameters between probucol and control groups (Mann-Whitney U test).

**FIGURE 1.** Typical PET, CT, and superimposed PET/CT images in sagittal (I) and coronal (II) planes for probucol-treated (A) or control (B) WHHLMI rabbits before intervention (baseline) and after 6 mo of intervention. Green arrows and pink arrowheads indicate aortas and kidneys, respectively.
that in the control group, although the difference was not significant.

Histologic Analysis

Typical images of azan-Mallory–stained and immuno-histochemically stained slices are shown in Figure 5. As expected, macrophages were already found at the beginning of the study—that is, at the age of 10 mo. Probucol treatment for 6 mo resulted in a reduction of macrophage infiltration. The number of infiltrated macrophages in the thoracic and abdominal aortas was significantly lower in the probucol-treated group than that in the control group at the end of the 6-mo investigation (Fig. 6A). Intimal thickening was affected negligibly by probucol treatment (Fig. 6B).

The relationship between 18F-FDG uptake and macrophage number in the thoracic segments is plotted in Figure 7. The segments from probucol-treated rabbits showed lower 18F-FDG uptake and macrophage infiltration than that of the control group.

DISCUSSION

Macrophage infiltration has been identified as one of the leading causes of plaque disruption. We have recently shown that 18F-FDG PET can detect macrophage-rich plaques (5). Once a vulnerable plaque is detected, a sequential follow-up of that plaque is important to ensure the effectiveness of drug therapy for the prevention of myocardial and cerebral infarctions. Lipid-lowering therapy does not always lead to stabilization of vulnerable plaques. Some statins can reduce plasma cholesterol, but they do not decrease macrophage infiltration (21,22). Thus, monitoring plasma lipids alone is not sufficient for determining the therapeutic effect of drugs.

In this study, we aimed to investigate whether 18F-FDG PET can be used to monitor the effectiveness of therapies that target vascular inflammation. The lipid-lowering effect of probucol is moderate, and probucol did not decrease plasma cholesterol in some animal studies (13,23); however, probucol can reduce macrophage-rich, fatty-streak lesions even in advanced atherosclerosis (13). Thus, probucol was selected as a model therapeutic drug, because it is known to decrease macrophage infiltration, which can be imaged by 18F-FDG PET. Although no difference in plasma cholesterol levels was shown between the probucol group and the control group in our investigation, macrophage...
infiltration was decreased in the probucol-treated rabbits, in agreement with the earlier study (13).

Ten-month-old WHHLMI rabbits, which have established atherosclerotic lesions with macrophage infiltration, were used in this study. The aorta could be imaged at the beginning of the study by 18F-FDG PET. The accumulation of radioactivity decreased over the course of probucol treatment and we were able to monitor the therapeutic outcome using 18F-FDG PET. These results were supported by the measurement of radioactivity in aortic segments with a well-type γ-counter. No difference in blood glucose level was observed between groups during the PET studies throughout the experimental period. Our previous report showed that macrophages are responsible for 18F-FDG accumulation in atherosclerotic lesions but not intimal thickening (5). Because the ratio of the intima to the whole cross-section area was unaffected by probucol treatment, the decrease in 18F-FDG uptake likely resulted from the decrease in the number of infiltrated macrophages. Thus, 18F-FDG PET enabled us to image the reduction of inflammation that is responsible for plaque rapture.

Monitoring of coronary plaques is important for the prevention of cardiovascular disease. Because coronary plaques are small and the spatial resolution of PET is low, it may be difficult to detect these small plaques. However, Johnson et al. recently demonstrated the usefulness of 99mTc-annexin V SPECT for identifying plaque apoptosis in coronary vessels of swine (24). Therefore, 18F-FDG PET would be available for evaluation of the drug effect in reducing inflammation on coronary plaque.

CONCLUSION

This study has shown the usefulness of 18F-FDG PET for monitoring the reduction of inflammation in plaque. Because the stabilization of vulnerable plaques is important for atherosclerosis therapy, clinical application of this
imaging system would be efficient for assessing the therapeutic effect of drugs. In addition, this method would also be useful for developing new drugs that can reduce inflammation in atherosclerotic plaque independently of lowering the level of cholesterol.

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