
Application of ^{18}F -FDG PET for Monitoring the Therapeutic Effect of Antiinflammatory Drugs on Stabilization of Vulnerable Atherosclerotic Plaques

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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 ^{18}F -FDG accumulates in macrophage-rich plaques. The present study was undertaken to investigate the usefulness of ^{18}F -FDG PET for monitoring therapies that target vascular inflammation. **Methods:** Myocardial infarction-prone Watanabe heritable hyperlipidemic rabbits were used in this study. The antioxidant probucol was included in the diet fed to 4 rabbits starting at 10 mo of age (probuco group). In a control study, 4 rabbits received standard rabbit chow (control group). ^{18}F -FDG PET experiments were performed on both groups before the study and at 1, 3, and 6 mo after treatment. After the last imaging session, the rabbits were sacrificed at 3 h after injection of ^{18}F -FDG, and the aortas were removed. The accumulated radioactivity was then measured, and the number of macrophages was determined by examination of stained sections. **Results:** At the age of 10 mo, before the treatment, the aorta could be imaged by ^{18}F -FDG PET in all rabbits. The aorta could not be imaged after 6 mo of probucol treatment, whereas intense radioactivity was observed in the control rabbits throughout the investigation. The standardized uptake values (SUVs) of the aorta were decreased significantly in the probucol group after 3 mo of intervention as compared with the pretreatment period. The SUVs of the control group were increased gradually at 6 mo. Radioactivity in the aorta was significantly lower in the probucol group than that in the control group. Macrophages were already present at the be-

ginning of the study, and probucol treatment for 6 mo resulted in a significant reduction of macrophage infiltration. **Conclusion:** ^{18}F -FDG PET was able to image the reduction of inflammation by probucol. ^{18}F -FDG PET should be useful for evaluating the therapeutic effect of drugs clinically and for the development of new drugs that can stabilize vulnerable plaques. ^{18}F -FDG PET should be useful for evaluating the therapeutic effect of drugs clinically and for the development of new drugs that can reduce inflammation of vulnerable plaques.

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

J Nucl Med 2006; 47:1845–1850

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 ^{18}F -FDG accumulates in macrophage-rich plaques, and ^{18}F -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 ^{18}F -FDG PET for monitoring therapies

Received May 30, 2006; revision accepted Jul. 28, 2006.
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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 (probucol 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 ^{18}F -FDG. Before the imaging study, the individual body shape was taken with urethane hardening foam to maintain the stereotactic position. ^{18}F -FDG PET and CT experiments were performed at 10 mo of age and after 1, 3, and 6 mo of intervention in both groups according to a previously reported method with slight modification (5). Briefly, the rabbits were anesthetized and ^{18}F -FDG (37–185 MBq) was injected into a marginal ear vein. PET was performed for 15 min at 180 min after injection of ^{18}F -FDG using an ECAT EXACT HR PET scanner (Siemens AG). We chose this time point according to the method of Tawakol et al. (6). After the PET study, a CT angiogram was acquired with an X-force CT scanner (Toshiba Medical Corp.) using iohexol as a contrast agent. PET images were calibrated to the injected dose of ^{18}F -FDG. For measurement of radioactivity in the thoracic aorta, 4 circular regions of interests (ROIs) (3 mm² for each ROI) were drawn on the aorta. The analysis was undertaken with no knowledge of the animal group. The standardized uptake value (SUV) was calculated by dividing the tissue concentration (kBq/mL) by the injected dose per gram of body weight (kBq/g).

^{18}F -FDG Biodistribution Study

The biodistribution study was performed on the day after the CT and PET study, 6 mo after probucol administration. The rabbits were anesthetized and ^{18}F -FDG (370 MBq) was injected into a marginal ear vein. Three hours after the ^{18}F -FDG injection, the

animals were sacrificed with an overdose of sodium pentobarbital. Perfusion fixation was then performed using PLP solution (75 mmol/L L-(+)-lysine hydrochloride and 4% paraformaldehyde in 37.5 mmol/L phosphate buffer, pH 7.4). The thoracic and abdominal aortas were subsequently removed, cut into 10-mm segments, and weighed. The segments were immediately postfixed in PLP solution, and radioactivity was measured with a well-type γ -counter (COBRA; Packard Co. Ltd.). The results are expressed as the differential uptake ratio (DUR):

$$\text{DUR} = \frac{\text{tissue activity (Bq)/tissue weight (kg)}}{\text{injected RI activity (Bq)/animal body weight (kg)}}$$

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.

Statistical Analysis

Data are presented as the mean \pm SD. Statistical analysis was performed using the Mann-Whitney *U* test or paired *t* test for comparisons between or within groups, respectively. Statistical significance was established at $P < 0.05$.

RESULTS

Body Weight and Lipid Profile

Body weight, plasma levels of cholesterol, 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 ^{18}F -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,

TABLE 1

Effect of Probucol Treatments on Body Weight and on Plasma Levels of Total, LDL, and HDL Cholesterol and Triglycerides in WHHLM1 Rabbits

Parameter	0 mo (10 mo old)		1 mo		3 mo		6 mo	
	Probucol	Control	Probucol	Control	Probucol	Control	Probucol	Control
Body weight (kg)	3.7 ± 0.2	3.8 ± 0.1	3.8 ± 0.2	3.9 ± 0.2	4.0 ± 0.2	4.0 ± 0.3	3.9 ± 0.2	4.0 ± 0.3
Total cholesterol (mg/dL)	965 ± 138	967 ± 153	694 ± 71*	741 ± 73*	781 ± 48	716 ± 98*	685 ± 220	660 ± 36*
LDL (mg/dL)	675 ± 160	630 ± 167	509 ± 88	614 ± 97	587 ± 67	529 ± 85	483 ± 185*	488 ± 50
HDL (mg/dL)	18 ± 3	14 ± 3	16 ± 3	14 ± 3	13 ± 2	14 ± 6	11 ± 3*	12 ± 2
Triglycerides (mg/dL)	453 ± 147	366 ± 183	721 ± 241	447 ± 167	629 ± 154	526 ± 160*	602 ± 216	572 ± 401

**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).

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

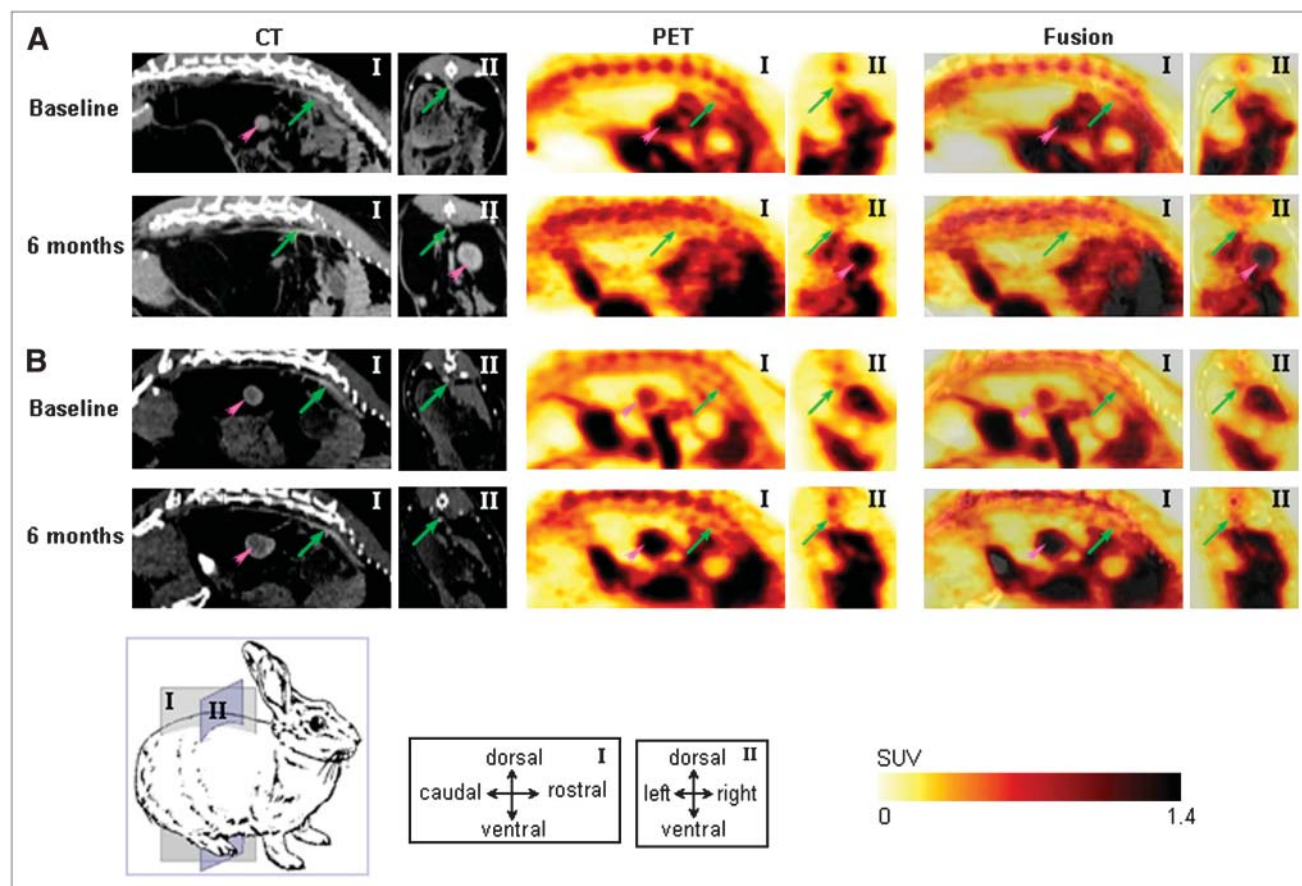


FIGURE 1. Typical PET, CT, and superimposed PET/CT images in sagittal (I) and coronal (II) planes for probucol-treated (A) or control (B) WHHLM1 rabbits before intervention (baseline) and after 6 mo of intervention. Green arrows and pink arrowheads indicate aortas and kidneys, respectively.

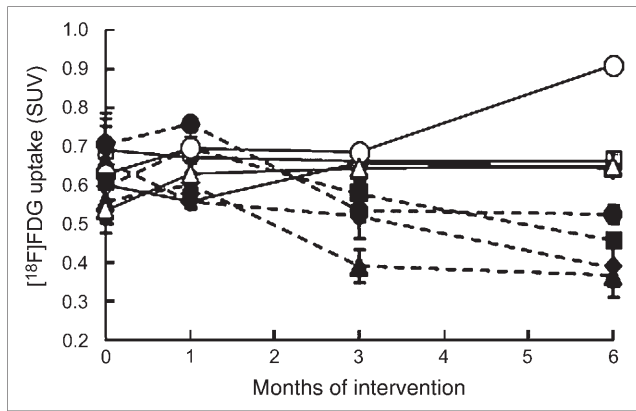


FIGURE 2. Course of ^{18}F -FDG uptake (SUV) over treatment period in WHHLM rabbit thoracic aorta determined by PET. Solid lines and open symbols represent data from each control rabbit ($n = 4$); dashed lines and closed symbols represent data from each probucol-treated rabbit ($n = 4$).

that in the control group, although the difference was not significant.

Histologic Analysis

Typical images of azan-Mallory–stained and immunohistochemically 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

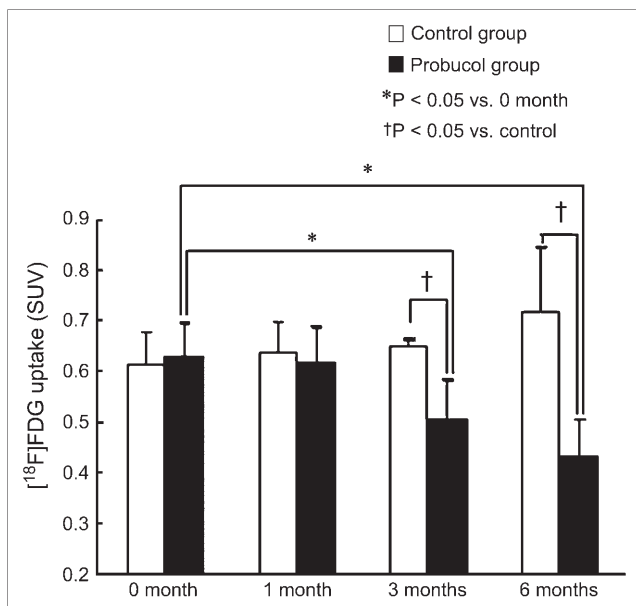


FIGURE 3. Sequential effect of probucol on ^{18}F -FDG uptake in WHHLM rabbit thoracic aorta. Data were obtained using PET. Each bar represents summed results for 4 rabbits per group.

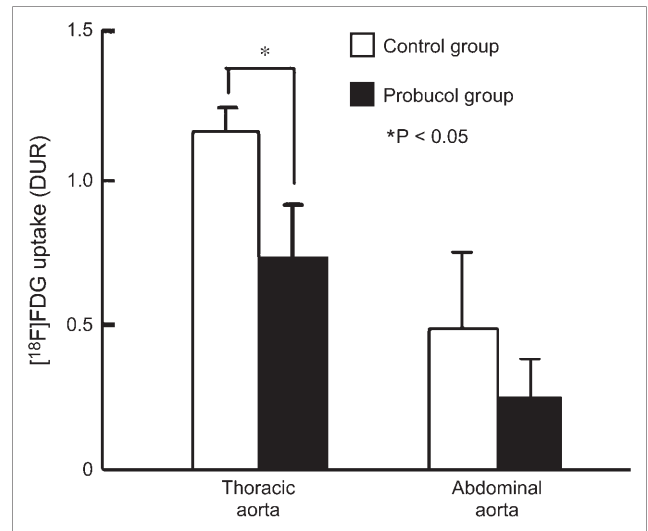


FIGURE 4. ^{18}F -FDG uptake by aortic segments from WHHLM rabbits in each group after 6 mo of intervention. ^{18}F -FDG uptake was measured using a γ -counter.

6-mo investigation (Fig. 6A). Intimal thickening was affected negligibly by probucol treatment (Fig. 6B).

The relationship between ^{18}F -FDG uptake and macrophage number in the thoracic segments is plotted in Figure 7. The segments from probucol-treated rabbits showed lower ^{18}F -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 ^{18}F -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 ^{18}F -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 ^{18}F -FDG PET. Although no difference in plasma cholesterol levels was shown between the probucol group and the control group in our investigation, macrophage

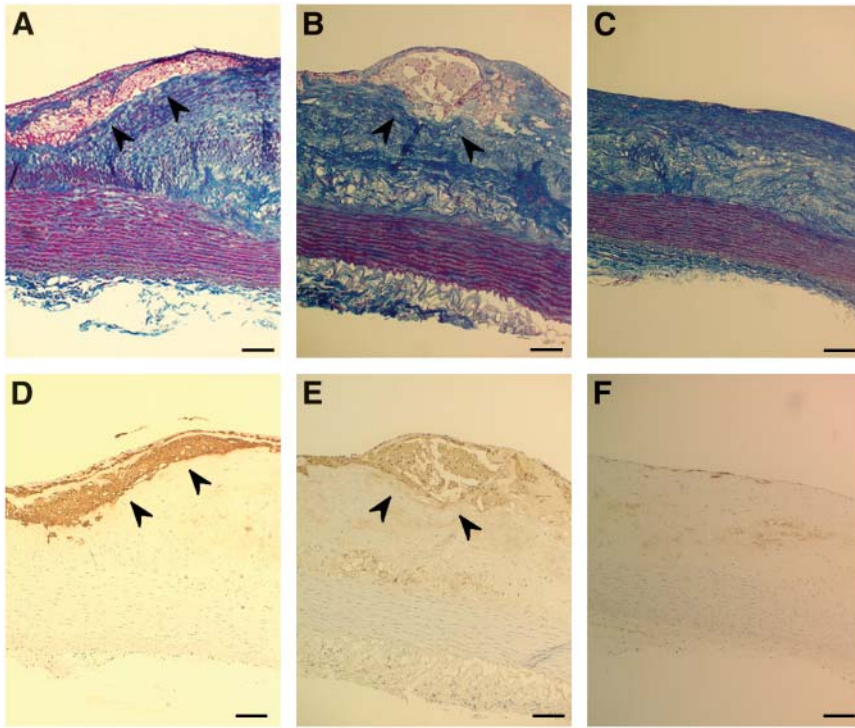


FIGURE 5. Photomicrographs of atherosclerotic lesions in thoracic aortas of pretreatment control 10-mo-old WHHLM rabbits (A and D), control WHHLM rabbits (B and E), and probucol-treated WHHLM rabbits (C and F) after 6 mo of intervention. Azan-Mallory staining (A–C) and immunohistochemical staining with antimacrophage monoclonal antibody RAM-11 (D–F) are shown. Macrophages can be seen (arrowheads) in each stained serial section from pretreatment control rabbits (A and D) and control rabbits (B and E). Bars = 100 μ m.

infiltration was decreased in the probucol-treated rabbits, in agreement with the earlier study (13).

Ten-month-old WHHLM 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 ^{18}F -FDG PET. The accumulation of radioactivity decreased over the course of probucol treatment and we were able to monitor the therapeutic outcome using ^{18}F -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 ^{18}F -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 ^{18}F -FDG uptake likely resulted from the decrease in the

number of infiltrated macrophages. Thus, ^{18}F -FDG PET enabled us to image the reduction of inflammation that is responsible for plaque rupture.

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 $^{99\text{m}}\text{Tc}$ -annexin V SPECT for identifying plaque apoptosis in coronary vessels of swine (24). Therefore, ^{18}F -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 ^{18}F -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

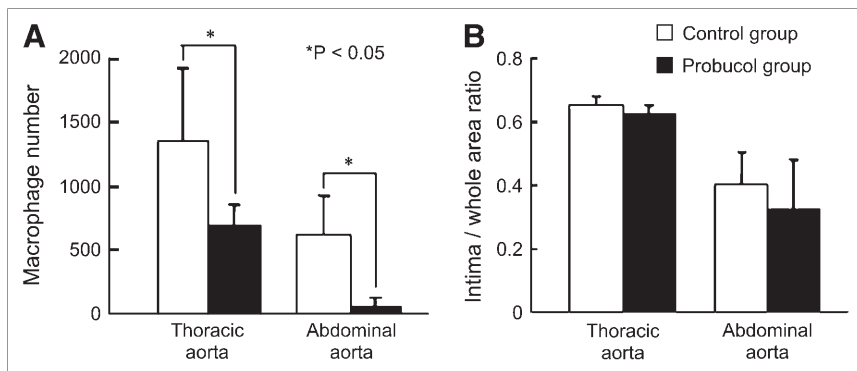


FIGURE 6. Effect of 6-mo probucol treatment on macrophage number (A) and ratio of intima to whole cross-section area (B).

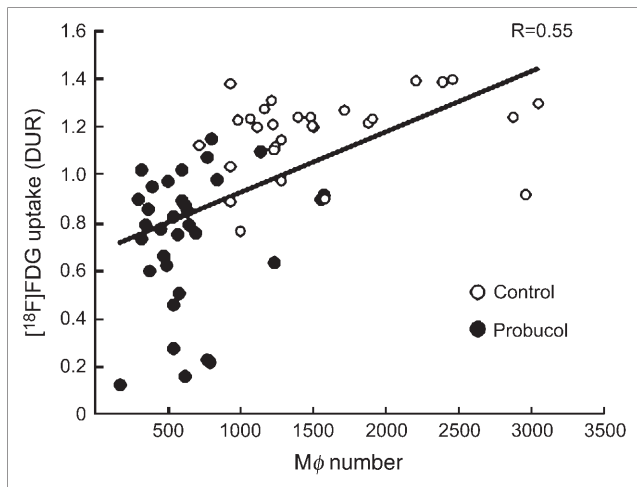


FIGURE 7. Correlation between ^{18}F -FDG uptake and macrophage ($\text{M}\phi$) number in thoracic aortic segments from WHHLM rabbits.

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.

ACKNOWLEDGMENTS

The authors thank Eri Mikata (Hamamatsu University School of Medicine) and Kumiko Yoshimura (National Center for Geriatrics and Gerontology) for technical assistance. We also thank Junichiro Abe (National Center for Geriatrics and Gerontology) for cyclotron operation and ^{18}F -FDG synthesis. This study was supported by Health and Labor Sciences Research Grants, Research on Advanced Medical Technology. This study was also supported in part by a Grant in Aid for the Center of Excellence at Hamamatsu University School of Medicine (Medical Photonics) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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