Regulation of Plasma PAI-1 Concentrations in HAART-Associated Lipodystrophy During Rosiglitazone Therapy

Hannele Yki-Järvinen, Jussi Sutinen, Angela Silveira, Elena Korsheninnikova, Rachel M. Fisher, Katja Kannisto, Ewa Ehrenborg, Per Eriksson, Anders Hamsten

Objective—Patients with highly active antiretroviral therapy–associated lipodystrophy (HAART+LD+) have high plasminogen activator inhibitor-1 (PAI-1) concentrations for unknown reasons. We determined whether (1) plasma PAI-1 antigen concentrations are related to liver fat content (LFAT) independently of the size of other fat depots and (2) rosiglitazone decreases PAI-1 and LFAT in these patients.

Methods and Results—In the cross-sectional study, 3 groups were investigated: 30 HIV-positive patients with HAART+LD+, 13 HIV-positive patients without lipodystrophy (HAART+LD−), and 15 HIV-negative subjects (HIV−). In the treatment study, the HAART+LD+ group received either rosiglitazone (8 mg, n=15) or placebo (n=15) for 24 weeks. Plasma PAI-1 was increased in HAART+LD+ (28±2 ng/mL) compared with the HAART+LD− (18±3, P<0.02) and HIV− (10±3, P<0.001) groups. LFAT was higher in HAART+LD+ (7.6±1.7%) than in the HAART+LD− (2.1±1.1%, P<0.001) and HIV− (3.6±1.2%, P<0.05) groups. Within the HAART+LD+ group, plasma PAI-1 was correlated with LFAT (r=0.49, P<0.01) but not with subcutaneous or intra-abdominal fat or serum insulin or triglycerides. In subcutaneous adipose tissue, PAI-1 mRNA was 2- to 3-fold higher in the HAART+LD+ group than in either the HAART+LD− or HIV− group. Rosiglitazone decreased LFAT, serum insulin, and plasma PAI-1 and increased serum triglycerides but had no effect on intra-abdominal or subcutaneous fat mass or PAI-1 mRNA.

Conclusions—Plasma PAI-1 concentrations are increased in direct proportion to LFAT in HAART+LD+ patients. Rosiglitazone decreases LFAT, serum insulin, and plasma PAI-1 without changing the size of other fat depots or PAI-1 mRNA in subcutaneous fat. These data suggest that liver fat contributes to plasma PAI-1 concentrations in these patients. (Arterioscler Thromb Vasc Biol. 2003;23:688–694.)

Key Words: fibrinolysis ▪ tissue plasminogen activator ▪ steatosis ▪ insulin ▪ triglycerides

Highly active antiretroviral therapy (HAART)-associated lipodystrophy (LD) has rapidly become the most common form of human LD affecting roughly half of all patients receiving HAART for >12 to 18 months. This LD is characterized by loss of subcutaneous fat, an increase in visceral fat, marked hypertriglyceridemia, and hyperinsulinemia.1 These adverse metabolic effects might compromise the improved prognosis conferred by HAART by increasing the risk for cardiovascular disease.

Several mouse models of LD have documented that neither subcutaneous nor visceral fat is necessary for insulin resistance to develop.2,3 Indeed, in mice, treatment of LD with subcutaneous fat transplantation normalizes insulin resistance.4 In the LD mouse models, the best correlate of insulin resistance seems to be fat accumulation in insulin-sensitive tissues, such as the liver and skeletal muscles.5 Plasma plasminogen activator inhibitor-1 (PAI-1) concentrations are increased in conditions characterized by increased visceral or subcutaneous fat, hypertriglyceridemia, and hyperinsulinemia.5 However, limited information is available regarding the tissue and cellular origin of plasma PAI-1. Both visceral and subcutaneous adipose tissue produce PAI-1, but data are controversial as to whether visceral adipose tissue produces more, similar amounts of, or less8 PAI-1 than subcutaneous adipose tissue. PAI-1 has also been suggested to originate from stromal cells in adipose tissue rather than from adipocytes.6 Plasma concentrations of PAI-1 have been found to be correlated positively with the amount of PAI-1 produced by subcutaneous adipose tissue in vitro.9 In obese subjects, PAI-1 gene expression and secretion from subcutaneous...
Adipose tissue are increased and could therefore contribute to increased circulating plasma PAI-1 concentrations. However, in patients with HAART-associated lipodystrophy and increased PAI-1 concentrations, metformin, which mainly acts in the liver, lowers PAI-1 concentrations. These data raise the possibility that increased plasma PAI-1 could originate from the liver. Consistent with such a hypothesis, plasma PAI-1 concentrations are increased and could therefore contribute to circulating plasma PAI-1 during PPARγ agonist therapy is a consequence of the decrease in LFAT (or the accompanying insulin concentration) by rosiglitazone. To gain insight into the regulation of plasma PAI-1 concentrations in patients with HAART-associated LD, we treated these patients with rosiglitazone or placebo for 24 weeks.

**Methods**

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**Results**

**Physical and Biochemical Characteristics of the Study Groups**

Clinical and biochemical characteristics of the study groups are given in Table 1. The total amount of fat in the abdominal region (by magnetic resonance imaging) was comparable between the groups, but its distribution was different (Table 1). The HAART+LD+ group had significantly more intra-abdominal fat and less subcutaneous fat than the HAART+LD− or the HIV− group (Table 1). Serum insulin and triglyceride concentrations were significantly increased in the HAART+LD+ compared with the other groups (Table 1).

**Liver Fat**

LFAT in the HAART+LD+ patients (7.6±1.7%) was 262% higher than in the HAART+LD− group (2.1±1.1%, P<0.001) and 111% higher than in the HIV− group (3.6±1.2%, P<0.05); LFAT did not differ between the HAART+LD− and HIV−

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<thead>
<tr>
<th>TABLE 1. Characteristics of the Study Groups, Cross-Sectional Study</th>
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<td>Men/women</td>
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<td>Body mass index, kg/m²</td>
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<td>Total fat, cm³ (MRI)</td>
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<td>Subcutaneous fat, cm³ (MRI)</td>
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<td>Intra-abdominal fat, cm³ (MRI)</td>
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<td>Plasma glucose, mmol/L</td>
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<td>Serum HDL cholesterol, mmol/L</td>
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<td>Waist, cm</td>
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<td>Waist-to-hip ratio</td>
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<td>S-alanine aminotransferase, U/L</td>
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<td>Alcohol consumption, g/wk</td>
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<td>Plasma PAI-1 antigen, ng/mL</td>
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<td>Plasma tPA antigen, ng/mL</td>
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Data are shown as mean±SEM.

*P<0.05, **P<0.01, and ***P<0.001 for comparisons between HAART+LD+ and HIV−.

†P<0.05, ††P<0.01, and †††P<0.001 for comparisons between HAART+LD+ and HAART+LD−.

**Yki-Järvinen et al. PAI-1 and Liver Fat in Lipodystrophy**

Given that hepatocytes in the fatty liver may overexpress PAI-1, one can hypothesize that the decrease in plasma PAI-1 during PPARγ agonist therapy is a consequence of the decrease in LFAT (or the accompanying insulin concentration) by rosiglitazone. To gain insight into the regulation of plasma PAI-1 concentrations in patients with HAART-associated LD, we treated these patients with rosiglitazone or placebo for 24 weeks.
groups. Within the HAART + LD+ group, LFAT was correlated closely with fasting serum insulin (r=0.49, P<0.01).

**Circulating PAI-1, tPA, and Cytokine Concentrations**

Plasma PAI-1 antigen concentrations were significantly increased in the HAART + LD+ compared with the HAART + LD− and the HIV− groups (Table 1). Plasma tissue plasminogen activator (tPA) concentrations were similarly altered (Table 1). These differences persisted even after age was included as a covariate in the ANOVA.

Serum tumor necrosis factor-α (TNFα) concentrations were comparable between the groups (1.6±0.1 vs 1.5±0.2 vs 2.0±0.8 pg/mL, HAART+LD+ vs HAART+LD− vs HIV−, respectively). Serum interleukin-6 (IL-6) concentrations, on the other hand, were increased in both HIV+ groups relative to the HIV− group (2.2±0.2 vs 1.9±0.6 vs 0.7±0.2 pg/mL, P<0.05 for HIV+ groups vs HIV−).

**PAI-1 Expression in Subcutaneous Adipose Tissue**

In subcutaneous adipose tissue, PAI-1 mRNA concentration relative to β2-microglobulin averaged 0.019±0.003 in the HAART+LD+ group, which was higher than in the HAART+LD− (0.007±0.002, P<0.005) and the HIV− (0.006±0.001, P<0.005) groups (Figure 1). Fat cell diameter was greater in the HIV− (94±3 μm) than in the HAART+LD+ (74±3 μm, P<0.01) or the HAART+LD− (80±4 μm, P<0.05) group.

**PPARγ and Leptin Expression in Adipose Tissue**

The concentrations of the mRNAs of both PPARγ (0.0132±0.0021 vs 0.0283±0.0057 vs 0.0214±0.0041 for LD−, LD+, and HIV−, respectively), leptin (0.06±0.02 vs 0.17±0.03 vs 0.28±0.06, respectively; P<0.005 for HAART+LD+ vs HAART+LD− and P<0.05 for HAART+LD− vs HIV−) relative to the housekeeping gene were decreased in the HAART+LD+ group compared with the 2 other groups.

**Relations Between PAI-1 and Physical and Biochemical Characteristics Before Rosiglitazone Therapy**

Figure 2 depicts the relations of plasma PAI-1, and Figure 3, those of tPA antigen concentrations to LFAT and subcutaneous and intra-abdominal fat in all study groups. Within the HAART+LD+ group, plasma PAI-1 antigen concentration was correlated with LFAT before rosiglitazone therapy (r=0.49, P<0.01, Figure 2) but not with serum TNFα (r=−0.03, NS) or IL-6 (r=0.18, NS), subcutaneous fat (r=−0.05, NS, Figure 2), intra-abdominal fat (r=0.33, NS, Figure 2), serum C-peptide (r=0.33, NS), insulin (r=0.26, NS) or body weight (r=0.26, NS). Within the HAART+LD+ group, the correlation coefficient between PAI-1 mRNA concentrations in subcutaneous adipose tissue...
and plasma PAI-1 concentrations before rosiglitazone therapy was 0.39 (P<0.05).

Effects of Rosiglitazone Treatment

Compared with baseline, PAI-1 mRNA expression in adipose tissue decreased slightly in the placebo group (P<0.05 for before vs after) but not in the rosiglitazone group (P>0.1); the changes between the groups were not statistically significant (Figure 1). Rosiglitazone decreased plasma PAI-1 from 30±4 to 23±2 ng/mL (P<0.05; Figure 1). Plasma PAI-1 remained unchanged in the placebo group (27±3 vs 26±3 ng/mL, before vs after; NS; Figure 1). During rosiglitazone treatment, serum triglycerides increased significantly, by 66%, from 3.5±0.5 to 5.8±2.0 mmol/L, but remained unchanged in the placebo group. Rosiglitazone also decreased serum insulin concentrations and LFAT (P<0.05 for the change between groups; Table 2). Serum alanine aminotransferase activity decreased in the rosiglitazone group but remained unchanged in the placebo group. Rosiglitazone had no effect on body weight or body mass index, the waist-to-hip ratio, or the intra-abdominal to total fat ratio, nor on the size of intra-abdominal or subcutaneous fat depots (Table 2). Mean fat cell size or the distribution of fat cell sizes were not changed by placebo or rosiglitazone treatment (data not shown). Serum IL-6 concentration did not change with rosiglitazone treatment (from 2.0±0.3 to 1.8±0.3 pg/mL) when compared with placebo (from 2.4±0.4 to 2.1±0.4 pg/mL). Plasma tPA decreased from 11.3±0.8 to 10.5±0.8 ng/mL (P<0.05) in the rosiglitazone group but remained unchanged in the placebo group (10.1±0.7 vs 9.8±0.7 ng/mL; NS). Serum TNFα increased almost significantly in the placebo group (1.5±0.2 vs 1.8±0.3 pg/mL; P=0.06) but remained unchanged in the rosiglitazone group (1.7±0.2 vs 1.7±0.2 pg/mL; NS). The changes in serum TNFα concentrations between the groups did not differ significantly.

During rosiglitazone therapy, the PPARγ mRNA concentration tended to increase (from 0.0125±0.0033 to 0.0133±0.0024) and tended to decrease in the placebo group (0.0139±0.0026 vs 0.0120±0.0024, before vs after). The change between the groups in PPARγ expression was significant (P<0.05). Leptin expression remained unchanged in both the rosiglitazone (0.078±0.025 vs 0.061±0.023, before vs after) and the placebo (0.052±0.20 vs 0.046±0.019) groups.

The change in plasma PAI-1 within the HAART+LD+ group was significantly correlated with the change in serum fasting insulin (r=0.42, P<0.05), which in turn was significantly correlated with the change in LFAT (r=0.49, P<0.01). The correlation coefficient between the change in plasma PAI-1 and the change in LFAT was 0.37 (P<0.05). Except for the latter correlation, there were no significant correlations between the change in plasma PAI-1 and changes in body composition, nor were there any significant correlations between change in plasma PAI-1 and changes in serum TNFα and IL-6 concentrations (data not shown). The change in PAI-1 were not correlated with the change in PAI-1 mRNA in subcutaneous fat (r=−0.04; NS).

Discussion

The present study shows that the increase in plasma PAI-1 concentrations in HIV-infected patients with LD and insulin resistance is related to an increase in LFAT. Rosiglitazone treatment, despite having no effect on subcutaneous and intra-abdominal fat depots and increasing plasma triglycerides, decreased plasma PAI-1 concentrations and LFAT. The changes in LFAT were correlated with those of plasma PAI-1. These results suggest that the increase in plasma PAI-1 in HAART-associated LD could, at least in part, be due to LFAT or the associated insulin resistance.

PAI-1 Expression in Adipose Tissue

Previous data on PAI-1 gene expression in human adipose tissue indicate an approximate 2-fold increase in obese subjects and a correlation between plasma PAI-1 and PAI-1 mRNA in subcutaneous adipose tissue. Given that the obese subjects studied had mean body mass indexes of 42.6 kg/m² and 35.6 kg/m², adipose tissue mass was markedly increased, and thus, subcutaneous adipose tissue probably contributed substantially to the increase in plasma PAI-1 concentrations. We found a 2- to 3-fold increase in PAI-1 gene expression in subcutaneous adipose tissue in the LD compared with the non-LD patients and the normal subjects. However, subcutaneous adipose tissue was almost 2-fold
TABLE 2. Effects of Rosiglitazone and Placebo in Patients With HAART-Associated Lipodystrophy as Compared With HIV—Subjects

<table>
<thead>
<tr>
<th></th>
<th>HAART-Associated Lipodystrophy</th>
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<tbody>
<tr>
<td></td>
<td>Rosiglitazone (n=15)</td>
<td>Placebo (n=15)</td>
<td>HIV— (n=15)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 Weeks</td>
<td>24 Weeks</td>
<td>0 Weeks</td>
<td>24 Weeks</td>
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<tr>
<td>Age, y</td>
<td>44.3±3.4**</td>
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<td>33.3±3.3</td>
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<tr>
<td>Weight, kg</td>
<td>72.9±2.5</td>
<td>73.7±2.5</td>
<td>73.3±3.4</td>
<td>74.4±3.6</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.7±0.6</td>
<td>23.3±0.8</td>
<td>23.6±0.6</td>
<td>23.9±0.9</td>
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<tr>
<td>Waist-to-hip ratio</td>
<td>0.98±0.02***</td>
<td>0.98±0.02***</td>
<td>0.99±0.02***</td>
<td>0.99±0.02***</td>
</tr>
<tr>
<td>Intra-abdominal fat, cm³ (MRI)</td>
<td>2100±2250**</td>
<td>2030±260**</td>
<td>1830±330**</td>
<td>1780±320**</td>
</tr>
<tr>
<td>Subcutaneous fat, cm³ (MRI)</td>
<td>980±200*</td>
<td>1040±230*</td>
<td>1300±250</td>
<td>1330±250</td>
</tr>
<tr>
<td>Intra-abdominal to total fat ratio (MRI)</td>
<td>0.68±0.06***</td>
<td>0.68±0.06***</td>
<td>0.59±0.05***</td>
<td>0.57±0.05***</td>
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<td>Fasting serum insulin, mU/L</td>
<td>12.6±1.6***</td>
<td>9.3±0.6***†</td>
<td>9.6±1.8**</td>
<td>16.3±5.7*</td>
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<td>Liver fat, %</td>
<td>7.3±1.6**</td>
<td>6.2±1.3†</td>
<td>8.0±3.1†</td>
<td>10.1±3.3*</td>
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<tr>
<td>S-alanine aminotransferase, U/L</td>
<td>46.7±7</td>
<td>32.4±4†</td>
<td>45.6±6†</td>
<td>42.5±5*</td>
</tr>
</tbody>
</table>

Data are shown as mean±SEM.

*P<0.05, **P<0.01, and ***P<0.001 for comparisons between patients with HAART-associated lipodystrophy and HIV—subjects.
†P<0.05 for comparisons between baseline and 24 weeks.
‡P<0.05 for comparisons of changes during the treatment in the rosiglitazone vs the placebo group.

reduced in the abdominal region, and similar decreases were observed at 6 other locations, based on skinfold measurements (data not shown). This implies that even though the concentration of PAI-1 mRNA was increased by 2- to 3-fold in subcutaneous fat, this increase, in the face of a 2-fold reduction in total subcutaneous adipose tissue mass, is unlikely to explain the >2-fold increase in plasma PAI-1 antigen concentration. Another factor worth considering is that PAI-1 has been suggested to originate from stromal cells rather than adipocytes in adipose tissue.6 In LD patients, the fraction of inflammatory or other cells could be increased while the adipocytes are undergoing apoptosis. The lack of a correlation between the size of the subcutaneous adipose tissue depot and plasma PAI-1 could therefore be due to altered composition of adipose tissue. Again, even here, opinions are divided, as some authors have suggested that subcutaneous fat tissue does not contribute to circulating plasma PAI-1 concentrations.24

Significant associations between the amount of visceral rather than subcutaneous adipose tissue and plasma PAI-1 concentrations have been found.25 Visceral fat may produce more PAI-1 than subcutaneous fat.6,25 In the present study, within the LD group, which had a 2-fold increase in intra-abdominal fat, no association was found between the amount of intra-abdominal fat and plasma PAI-1.

Significance of LFAT
A striking finding of the present study was the demonstration that plasma PAI-1 concentrations were closely associated with LFAT within the HAART+LD+ and HAART+LD—groups. Thus, judging from the correlation analyses, one would conclude that plasma PAI-1 concentration is likely to be regulated directly (through changes in production or uptake of PAI-1 by the liver) or indirectly (through alterations in serum insulin concentrations due to changes in hepatic insulin sensitivity14,26 by the liver). This interpretation is also supported by the intervention study (vide infra).

In the liver, PAI-1 mRNA has been localized to endothelial cells but not to normal human hepatocytes.27 On the other hand, PAI-1 synthesis in hepatocytes is induced by specific mediators and under certain pathological conditions. These include mediators of the acute-phase response (IL-1 alone and in combination with IL-6), which stimulate PAI-1 gene transcription in hepatocytes.28,29 Insulin can increase PAI-1 production in cultured hepatocytes and HEPG2 cells.30,31 Hepatosteatosis is associated with increased synthesis of a variety of proteins, including hepatic enzymes and coagulation factors.32 The latter include PAI-1 and tPA, which are increased in men with steatosis, independent of obesity.12 Liver enzymes are also correlated with plasma PAI-1 concentrations, independent of serum triglycerides in hypertriglyceridemic subjects33 and independent of triglycerides, insulin, and obesity in asymptomatic hyperlipidemic men.34 Recently, increases in PAI-1 mRNA were found in hepatocytes of rabbits with a fatty liver after high-fat feeding.13 These studies support the notion that the liver may be a source of circulating PAI-1 in subjects with a fatty liver. Whether it is the simultaneous increase in serum triglycerides or insulin, or perhaps changes in circulating free fatty acids or some other factor that increases PAI-1 under such conditions, remains unclear. Of note, because PAI-1 is cleared by the liver,35 fat accumulation in the liver could increase PAI-1 concentration by impairing its clearance.

Effects of Rosiglitazone
In the present study, 24 weeks of rosiglitazone treatment had, disappointingly, no effect on either intra-abdominal or subcutaneous fat or any other measure of body size. This is in contrast to studies in patients with type 2 diabetes, in which 8 mg rosiglitazone increased fat mass by 3 to 4 kg in 2 independent studies in just 12 weeks.21,36 Also unexpectedly,
serum triglycerides, which in other studies remained unchanged during rosiglitazone therapy. Increased markedly in this study. Despite the lack of effect of rosiglitazone on fat mass or PAI-1 mRNA concentrations in subcutaneous adipose tissue and despite induction of hypertriglyceridemia, plasma PAI-1 concentrations decreased significantly during rosiglitazone therapy. The decrease in plasma PAI-1 concentrations is similar to that reported with troglitazone in studies of non–HIV-infected non-LD subjects. We did observe a small decrease in PAI-1 expression in adipose tissue in the placebo group and no change in the rosiglitazone group. The changes between the groups did not differ and could therefore not explain the decrease of PAI-1 in plasma. However, the cause of the decrease in PAI-1 expression in the placebo group is unclear. Of all clinical and biochemical parameters, the only significant correlates of the decrease in plasma PAI-1 concentrations were the decreases in serum insulin and LFAT. The decrease in LFAT by rosiglitazone, although following the same pattern as insulin (Table 2), was significant only when the change in LFAT in the rosiglitazone group was compared with the change in the placebo group. Similar trends for worsening of metabolic abnormalities were also observed in other parameters in the placebo group: fasting serum insulin tended to increase (Table 2), serum TNFα concentrations increased, and also PPARγ expression decreased. These changes in the placebo group may reflect worsening of HAART-associated metabolic abnormalities over time. Because these changes were observed in the absence of any changes in subcutaneous or intra-abdominal fat mass, they could be the consequence of progressive deposition of fat in the liver in a situation where adipose tissue is unable to normally store fat.

In conclusion, patients with HAART-associated LD had elevated plasma PAI-1 concentrations, which were correlated closely with increased LFAT but not the size of other fat depots. Rosiglitazone decreased plasma PAI-1, insulin, and LFAT, despite having no effect on other fat depots and despite inducing marked hypertriglyceridemia. The data thus suggest that the fatty liver may significantly contribute to plasma PAI-1 concentrations by affecting either the synthesis or the clearance of PAI-1.

Acknowledgments
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References


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