Modulation of Adipose Tissue Development by Pharmacological Inhibition of PAI-1

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Objective—The effect of a novel small molecule plasminogen activator inhibitor (PAI-1) inhibitor on adipose tissue physiology was investigated.

Methods and Results—In human preadipocyte cultures, PAI-039 inhibited both basal and glucose-stimulated increases in active PAI-1 antigen, yet had no effect on PAI-1 mRNA, suggesting a direct inactivation of PAI-1. Differentiation of human preadipocytes to adipocytes was associated with leptin synthesis, which was significantly reduced in the presence of PAI-039, together with an atypical adipocyte morphology characterized by a reduction in the size and number of lipid containing vesicles. In a model of diet-induced obesity, pair-fed C57 Bl/6 mice administered PAI-039 in a high-fat diet exhibited a dose-dependent reduction in body weight, epididymal adipose tissue weight, adipocyte volume, and circulating plasma active PAI-1. Plasma glucose, triglycerides, and leptin were also significantly reduced in drug-treated mice, and concentrations of PAI-039 associated with these physiological effects were near the in vitro IC50 for the inhibition of PAI-1.

Conclusions—Our results indicate that a small molecule inactivator of PAI-1 can neutralize glucose-stimulated increases in PAI-1 in human preadipocyte cultures, reduce adipocyte differentiation, and prevent the development of diet-induced obesity. These data suggest the pharmacological inhibition of PAI-1 could be beneficial in diseases associated with expansion of adipose tissue mass. (Arterioscler Thromb Vasc Biol. 2006;26:2209-2215.)

Key Words: adipocyte • diabetes • obesity • PAI-1

Type 2 diabetes accounts for >90% of diabetes globally and continues to increase at epidemic proportions. Cardiovascular morbidity is a major burden in patients with type 2 diabetes, and their risk of death from cardiovascular disease is increased significantly over nondiabetic subjects. Aggressive treatment of both diabetes and cardiovascular disease in this population through a combination of diet, exercise, and pharmacological therapy has recently been reported to result in a 20% reduction in cardiovascular events, whereas treatment of hyperglycemia alone produces less benefit. Taken together, a significant number of diabetics continue to exhibit vascular disease that is refractory to current therapy.

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Plasminogen activator inhibitor-1 (PAI-1) is the principal inhibitor of both tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA), and is physiologically involved in both thrombosis and atherosclerosis. PAI-1 is found in the circulation of healthy individuals at low concentrations, and although it has a potential regulatory role in tumorigenesis, inflammation, and thrombosis, PAI-1 is also elevated in the plasma of type 2 diabetic patients and is an important predictor of the onset of the disease. The discovery that PAI-1 is synthesized by adipose tissue suggested that it represents a possible causal link between obesity and the increased atherothrombosis observed in diabetics. This hypothesis is further supported by additional studies establishing that plasma PAI-1 is elevated with obesity, is reduced with weight loss, and correlates with the amount of visceral adipose tissue. Although PAI-1 has not been established in this disease caused in part by the absence of an agent that specifically normalizes elevated plasma PAI-1.

Our laboratory has recently identified a small molecule inhibitor of PAI-1 using a pharmacology-based approach of drug screening and medicinal chemical synthesis. The goal of the present research was to determine its effect on PAI-1 inactivation in human adipose tissue cultures and investigate the impact of pharmacological inhibition of PAI-1 on adipose tissue differentiation both in cell culture and in a murine model of diet-induced obesity. Ultimately, these data may be used to determine the role of adipose tissue PAI-1 neutralization on adipose tissue growth, development, and metabolism.

Methods

Preadipocyte Cell Culture

Human preadipocytes (#SP-F; Zen-Bio Inc, Research Triangle Park, NC) were grown to confluence using preadipocyte medium contain-
Effect of PAI-039 on PAI-1 Secretion by Preadipocytes: Basal and Glucose-Stimulated Conditions

Preliminary experiments were performed to determine whether PAI-039 had any cytotoxic or mitogenic effects. PAI-039 was added at different concentrations to preadipocyte cultures (up to 50 μmol/L), and cell number was determined over a 3-day period. Cells were plated at 5000 cells per well on day 1, and by day 3 cell number was equivalent between treatments with and without PAI-039, resulting in an average of 8159±104 cells, indicating that concentrations of PAI-039 up to 50 μmol/L had no effect on preadipocyte viability and was not mitogenic. PAI-1 was measured using a commercially available antibody capture technique that detects active and latent PAI-1 as antigen. Preliminary experiments indicated that active PAI-1 inhibited by PAI-039 resulted in a reduction in PAI-1 antigen. These data confirm that the inactivation of PAI-1 in the presence of inhibitor results in a reduction in active PAI-1 antigen without affecting mass.

We next determined the effect of PAI-039 on basal levels of PAI-1 antigen in preadipocyte conditioned medium. Over a 6-hour period, preadipocytes released 25.4±3.1 ng/mL per 1000 cells of PAI-1 antigen under control conditions, whereas this value was reduced to 18.1±1.3 ng/mL per 1000 cells in drug-treated cultures (20 μmol/L of PAI-039; P<0.05), indicating that PAI-039 could reduce the basal levels of PAI-1 after release by preadipocytes. In the presence of 24 mmol/L glucose, a 2.5-fold increase in PAI-1 (P<0.05) was observed compared with control values, which was decreased in a concentration-dependent pattern by the addition of PAI-039, reaching statistical significance at 20 μmol/L of PAI-039 (P<0.05) (Figure 1). Cell number was determined in the same wells, and again, was not affected by PAI-039, indicating that the reduction in PAI-1 antigen was caused by a direct action of the compound, and not variability in cell number as a result of the culture conditions.

To confirm that compensatory effects on PAI-1 mRNA do not account for the PAI-039-induced reduction in PAI-1 antigen, gene expression studies were performed. An initial control time course analysis of the effect of 24 mmol/L glucose on the expression of PAI-1 by human preadipocytes indicated a 2-fold increase after 2 hours of stimulation, which returned to basal levels at 6 and 24 hours. In separate experiments, both glucose and PAI-039 were added to preadipocytes, and gene expression determined. When compared...
with the effect of 24 mmol/L glucose treatment alone, no change in PAI-1 gene expression was observed in cells treated with PAI-039 (5 to 50 \(\mu\)mol/L). When comparing values between glucose-stimulated cells to those treated with 20 \(\mu\)mol/L PAI-039, PAI-1 mRNA expression normalized to cyclophilin mRNA was similar between treatments, again indicating that the treatment of the cells with the compound did not affect PAI-1 gene expression (data not shown).

**Effect of PAI-039 on Adipocyte Differentiation**

Because differentiation uses a serum-free defined medium differing in composition from the preadipocyte medium, an initial PAI-039 dose-response was performed in differentiating adipocytes. As shown in Figure 2, a dose-dependent reduction in PAI-1 antigen was associated with PAI-039 treatment. In the serum-free differentiation medium, the compound also exhibited increased potency, as concentrations from 1 to 5 \(\mu\)mol/L reduced PAI-1 antigen at each time point assayed between 4 and 8 days (\(P<0.01\)). The effect of PAI-039 on adipocyte differentiation was next determined by assaying leptin in the conditioned medium, and through microscopic imaging of the differentiating adipocytes. Addition of 5 \(\mu\)mol/L PAI-039 was associated with suppression of the elevated leptin observed in control cells that readily differentiated into adipocytes (Figure 2). In addition, a 90% decrease in leptin mRNA was observed concurrently in the presence of 5 \(\mu\)mol/L PAI-039 (Figure 2). Peroxisome proliferator–activated receptor (PPAR\(\gamma\)) mRNA was also reduced (supplemental Figure I, available online at http://atvb.ahajournals.org). Image analysis indicated that PAI-039 treatment resulted in the maintenance of many cells as preadipocytes, as well as a reduction in both the size and number of lipid vesicles within differentiating adipocytes (Figure 3). Finally, addition of inactive protein complexes of PAI-1 and tPA dose-dependently (500 nM and 50 nM) inhibited leptin production (supplemental Figure II).

**In Vivo Studies**

Using a pair-feeding regimen, the highest dose of PAI-039 resulted in a significant reduction in body weight gain and final body weight compared with control mice (Figure 4; Table 1). The mean body weight of each group was equal at the beginning of the study, but significant changes in gain were observed throughout the 4 weeks of study, with those animals receiving the highest concentration of the drug exhibiting the lowest body weights. Although liver weight was unaffected by the drug treatments, epididymal adipose tissue weight and adipocyte volume was reduced (Table 1). Statistically significant reductions in adipose tissue weight and cell volume were observed at the highest dose, and the

![Figure 1.](image1.png)

**Figure 1.** Effect of PAI-039 on glucose-stimulated active PAI-1 antigen levels in conditioned medium of human preadipocytes (\(* P<0.05\) vs glucose alone). Black bars, cell number; white bars, PAI-1 antigen.

![Figure 2.](image2.png)

**Figure 2.** A, PAI-1 antigen secretion during adipocyte differentiation without (○; solid line) or with 1(△), 2.5 (●), 5 (●) \(\mu\)M PAI-039. Supernatants were harvested every 48 hours. Values are mean±SEM of 6 different wells. B, Leptin secretion during adipocyte differentiation without (○; solid line) or with (●) 5 \(\mu\)mol/L PAI-039. Supernatants were harvested every 48 hours. Values are mean±SEM of 5 wells. Insert corresponds to leptin mRNA from cells differentiated during 8 days in absence (−) or presence (+) of 5 \(\mu\)mol/L PAI-039.

![Figure 3.](image3.png)

**Figure 3.** Morphology of cultured human adipocytes treated with 5 \(\mu\)mol/L PAI-039. Adipocytes were imaged at day 8 after the analysis of leptin concentration in the conditioned medium. A=Control; B=PAI-039–treated.
percentage decrease compared with controls was approximately equivalent for each value. The contribution of the preadipocyte population was not directly determined in these analyses, because only lipid-containing mature adipocytes were counted. Circulating levels of active PAI-1 was also reduced with drug treatment, with the greatest impact seen in the 5-mg PAI-039 group (P<0.05). PAI-039 treatment was associated with significant reductions in plasma triglycerides, glucose and leptin (Table 2). In addition, a significant (P<0.01) decrease in adipose tissue leptin mRNA was observed in the 5 mg PAI-039 group (supplemental Figure III). Plasma cholesterol and insulin were unchanged. HOMA-IR (mmol/L*mU/L) was 21.9±3.0 for the control, 21.9±6.2 for the low-dose and 13.4±2.5 for the high-dose PAI-039–treated group (P<0.05 versus control).

Pharmacokinetic studies indicated that PAI-039 had a low volume of distribution, poor tissue penetration, high protein binding, and was not extensively metabolized. PAI-039 was excreted predominantly unchanged in the bile and feces, suggesting that after inactivation of PAI-1, it would facilitate clearance of the protein. Plasma concentrations of PAI-039 increased dose-proportionally, with 1.6±0.26 µg/mL for the low-dose group, and 8.3±1.3 µg/mL for the high-dose group (3.6±0.6 and 18.8±3.0 µmol/L, respectively).

Table 1. Effect of High-Fat Diet With or Without PAI-039 on Body Weight, Epididymal Adipose Tissue Morphology, and Plasma PAI-1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PAI-039 (1 mg)</th>
<th>PAI-039 (5 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>28.7±0.6</td>
<td>27.6±1.0</td>
<td>24.3±0.8*†</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>1.2±0.0</td>
<td>1.3±0.1</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>Epididymal adipose weight, g</td>
<td>1.2±0.1</td>
<td>1.1±0.1</td>
<td>0.6±0.1†</td>
</tr>
<tr>
<td>Percent decrease in epididymal adipose weight, %§</td>
<td>NA</td>
<td>7.7±6.7</td>
<td>47.6±4.2†</td>
</tr>
<tr>
<td>Epididymal adipocyte cell diameter, µm</td>
<td>65.3±4.5</td>
<td>55.2±4.1</td>
<td>51.9±2.8</td>
</tr>
<tr>
<td>Percent decrease in adipocyte cell diameter, %§</td>
<td>NA</td>
<td>15.4±6.3*</td>
<td>20.6±4.3*</td>
</tr>
<tr>
<td>Epididymal adipocyte cell volume, pl</td>
<td>218.1±43.8</td>
<td>133.1±29.0</td>
<td>95.2±13.2*</td>
</tr>
<tr>
<td>Percent decrease in adipocyte cell volume, %§</td>
<td>NA</td>
<td>39.0±13.3*</td>
<td>56.4±6.1*</td>
</tr>
<tr>
<td>Active PAI-1, ng/mL</td>
<td>0.81±0.10</td>
<td>0.57±0.06</td>
<td>0.47±0.07*</td>
</tr>
<tr>
<td>PAI-1 antigen, ng/mL</td>
<td>2.73±0.3</td>
<td>2.29±0.2</td>
<td>2.08±0.2</td>
</tr>
</tbody>
</table>

Data expressed as mean±SEM. 
*P<0.05 as compared to control. 
†P<0.05 as compared to PAI-039 (1 mg). 
§Percent decrease as compared to control. 
n=6 mice per group. 
NA indicates not applicable.

Discussion

There is an established relationship between human obesity and plasma PAI-1, with increased body mass index directly correlated to circulating levels of the serpin. Investigations using human adipose tissue explants and primary cell culture have shown that human adipose tissue is a rich source of PAI-1 protein that directly contributes to increased plasma concentrations in obesity, and is synthesized by both preadipocytes and adipocytes. The health outcomes impact associated with increased plasma PAI-1 is significant, with data from the Insulin Resistance Atherosclerosis Study (IRAS) study supporting a role for elevated plasma PAI-1 in the development of type 2 diabetes independent of insulin resistance. PAI-1 deficiency also retards the development of diabetic nephropathy in streptozotocin-induced diabetes. In addition, PAI-1 synthesis is stimulated by a variety of other factors, including cytokines, lipids, and growth factors, and its production by and action on the vasculature can directly impact the etiology of atherosclerosis. Because PAI-039 is an orally active direct inhibitor of PAI-1 that exhibits efficacy in models of acute thrombosis and accelerated atherosclerosis, we decided to evaluate its effects on adipose tissue development and diet-induced obesity.
PAI-039 dose-dependently reduced both basal and glucose-stimulated active PAI-1 antigen in human preadipocytes without affecting PAI-1 gene expression, indicating that the inhibition of PAI-1 was through direct interaction with the target protein. PAI-039 treatment also retarded the differentiation of preadipocytes to adipocytes when assessed by leptin synthesis and cellular morphology. Because inhibition of adipocyte differentiation by small molecule PPARγ antagonists has been reported, the effect of PAI-039 on PPARs was determined as part of the development of this compound. In a cofactor recruitment assay, PAI-039 had no effect on PPARα or PPARδ, and exhibited partial agonist characteristics for PPARγ, but at concentrations several fold greater than those associated with inhibition of adipogenesis. We next tested PAI-039 in vivo both to further explore the various hypotheses on the role of PAI-1 in obesity and to extend our own cell culture findings. We initially observed an inverse correlation between plasma active PAI-1 and plasma concentrations of PAI-039, which importantly were below the no toxic effect level of the drug. Oral administration of PAI-039 in a high-fat diet also resulted in a dose-dependent reduction in body weight and epididymal adipose tissue mass. PAI-039 treatment was also associated with an improved metabolic profile, evidenced by a reduction in plasma glucose, triglyceride, leptin, and HOMA-IR, all of which could have been contributed to by the significantly smaller adipocytes in the drug-treated mice. Our in vivo observations are therefore consistent with recently published findings in which adipose tissue weight was reduced in PAI-1 null mice on a high-fat diet. Lijnen et al have also shown that administration of PAI-039 for 4 weeks improved insulin sensitivity in wild-type mice on a standard chow diet.

The mechanistic basis for the effect of PAI-039 on prevention of diet-induced obesity could be multifaceted, including regulation of cell migration, angiogenesis and lipid metabolism. Active migration of human preadipocytes is stimulated by latent PAI-1, and inactivation of PAI-1 by PAI-039 would produce similar results, suggesting that increased potential for migration could reduce adipose tissue differentiation. This hypothesis is supported by reduced leptin after PAI-1 inhibition in developing adipocytes in culture, and histological studies indicating that preadipocytes require attachment and cessation of migration before differentiation into mature adipocytes. Glucose-stimulated increases in PAI-1 are seen in arterial endothelial cells and vascular smooth muscle cells, and the Sp1 binding site in the PAI-1 promoter has been characterized as critical for this response. Specific drugs known to be beneficial to diabetic patients have also been used to attenuate PAI-1 mRNA and protein in cultured cells, and some comparison to PAI-039 is warranted. Troglitazone reduces PAI-1 antigen in cultured human adipocytes predominantly through regulation at the transcriptional level, simvastatin reduces PAI-1 gene expression in human vascular smooth muscle and endothelial cells, and niacin attenuates the expression of PAI-1 mRNA and the rate of PAI-1 protein synthesis in human liver cells. The concentration of each drug required for an inhibitory effect was similar to PAI-039, yet the studies differ mechanistically, because PAI-039 directly inactivated PAI-1 without affecting gene expression. These data
would suggest that combinations of PAI-039 with molecules from the statin or glitazone class could further reduce PAI-1.

The search for novel therapies for the treatment of cardiovascular and metabolic disease continues as the incidence of obesity and type 2 diabetes increases, while remaining largely refractory to current therapies. We have identified a small molecule inhibitor of PAI-1 that directly impacts adipose tissue differentiation in vitro and in vivo. Although additional studies are required to determine the potentially multiple mechanisms resulting in these effects, PAI-039 represents an important tool in furthering the understanding of PAI-1 as either a risk factor or risk marker in this population.

Acknowledgments

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Disclosures

None.

References

11. Eriksson P, Reynisdottir S, Kruskmanurthy G, Stenby E, Hamsten, Arner P. PAI-039 represents an important tool in furthering the understanding of PAI-1 as either a risk factor or risk marker in this population.
35. Morange PE, Lijnen HR, Alessi MC, Kopp F, Colen D, Juhan-Vague I. Influence of PAI-1 on adipose tissue growth and metabolic parameters in...


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Preadipocyte RNA Preparation and Gene Expression Analysis

For determination of the effect of PAI-039 on PAI-1 gene expression, preadipocytes were plated at 50,000 cells/well in a 24 well tissue culture plate. Triplicate wells were treated with 24mM glucose and PAI-039 for different time periods, the plate washed twice with 1X PBS, followed by addition of 0.5ml TRIzol (Invitrogen, Carlsbad, CA; #15596-018) and 10μg of glycogen (Ambion, Austin, TX; #9510) to each well. Total RNA was isolated according to the manufacturer’s recommendations, and quantitated using the RiboGreen RNA Quantitation kit from Molecular Probes (R-11490).

Gene-specific mRNA quantitation for PAI-1 was performed by real-time quantitative PCR on an ABI Prism 7900HT Sequence detection system (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. Samples (50-100 ng) of total RNA were assayed in triplicate in 50 μl reactions using one-step RT-PCR and the standard curve method to estimate specific mRNA concentrations. The gene-specific primer and probe sets were designed with Primer Express Software (Applied Biosystems, Foster City, CA). RT and PCR reactions were performed according to PE Applied Biosystem’s protocol for Taqman Gold RT-PCR. The primer sequences for human PAI-1 (Genebank Accession # M16006) were 5’-CGCCAGAGCAGGACGA-3’and 5’-GGAGACATCTGCATCCTGAAGTT-3’and probe, 6FAM-CGCCAATCGCAAGGCACCTCTG-TAMRA.

Human leptin and PPARγ were measured by real-time quantitative PCR on ABI Prism 7700 system using the SYBR green method. Amplification was performed with SYBR Green Universal PCR Master Mix (Applied Biosystems, Foster City, CA) in a 25μl volume containing 200 to 400 nM of primers. Reverse transcription and denaturation for 2 min at 50°C and 10 min at 95°C, were followed by forty cycles of two-step PCR amplification employing denaturation at 95°C for 15 s and an annealing / extension step at 60°C for 60 s. The primers for leptin were 5’-GTG CGG ATT CTT GTG GTG TT-3’ and 5’-GGA ATG AAG TCC AAA CCG GTG-3’ (GenBank accession no. NM 000230). The primers for PPARγ1 were 5’-GTG GCC GCA GAA ATG ACC-3’and 5’-CCA CGG AGC TGA TCC CAA-3’. The primers for 18S rRNA were 5’-CTA CCA CAT CCA
AGG AAG GCA-3' and 5'-TTT TTC GTC ACT ACC TCC CC-3' (GenBank accession no. X03205). RNA levels were calculated according to the comparative Ct method described in user bulletin #2 (Applied Biosystems, Foster City, CA). Results were normalized to 18S rRNA and mRNA levels expressed as the % difference relative to basal state (without the drug).

For determination of the effect of PAI-039 on leptin gene expression in adipose tissue from mice treated with the drug, total RNA was extracted using RNeasy Lipid Tissue Midi Kit (Qiagen, Valencia, CA) according to manufacturer's instructions. Leptin expression was determined by real-time quantitative RT-PCR analysis on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Samples (25ng) of total RNA were assayed in duplicate in 50 µl reactions using two-step RT-PCR. cDNA was prepared using TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA). PCR was performed using the TaqMan PCR Core Reagent Kit (Applied Biosystems, Foster City, CA), with 200 nM concentration of each primer and 400nM concentration of the probe, according to manufacturer's instructions. Leptin-specific primer and probe sequences were designed with Primer Express Software (Applied Biosystems, Foster City, CA). Primer sequences for mouse leptin (Genebank Accession# NM_008493) were 5’-CATCTGCTGGCCTTCTCCAA-3’ and 5’-TCCAGGCTCTCTGGCTTCTG-3’, probe sequence was 5’-AGCTGCTCCCTGCCTCAGACCAGTG-3’. Leptin gene expression levels were calculated using comparative Ct method using 18S rRNA gene expression for normalization.

Figure Legends
Figure I: Effect of 5µM PAI-039 on PPARγ1 mRNA expression after 10 days of differentiation. Human preadipocytes were differentiated as described under Methods. PPARγ1 mRNA was determined by real time PCR. mRNA data were normalized to 18S mRNA and expressed in % of control value (without PAI-039). Values are mean ± SEM of 5 different wells. *p<0.05
Figure II: Effect of serpin-serine protease complex on leptin synthesis by differentiating human adipocytes. Equimolar concentrations of PAI-1 were complexed with tPA for 30 minutes at room temperature, then added to the adipocyte medium. Fresh complexes and medium were added to the cultures every 3 days, and on Day 10, the medium was harvested and assayed for leptin (R&D Systems, Minneapolis, MN). H = 500 nM tPA and PAI-1, L = 50 nM tPA and PAI-1. **p<0.01 compared to Control values of n = 4 separate experiments.

Figure III: Effect of PAI-039 on adipose tissue leptin mRNA. Mice were provided PAI-039 in the diet at either 1 or 5 mg per gram of food, pair-fed for 4 weeks, and epididymal adipose tissue was used for preparation of mRNA. Leptin mRNA was determined by real time PCR, and normalized to 18S mRNA and expressed in % of control value from those mice receiving normal chow diet. Values are mean ± SEM from 6 different animals. **p<0.01
Figure I

mRNA PPARγ1 (% of Control)

PAI-039 (5 µM) - +

*
Figure II
Figure III