Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 346 (2006) 102–107
BBRC
www.elsevier.com/locate/ybbrc

Insulin resistance increases PAI-1 in the heart

Burton E. Sobel *, David J. Schneider, Yong-Ho Lee, Richard E. Pratley

University of Vermont, Cardiovascular Research Institute Burlington, VT, USA

Received 27 April 2006
Available online 22 May 2006

Abstract

To determine whether insulin resistance increases expression of plasminogen activator inhibitor type-1 (PAI-1) in the heart, studies were performed in 22 mice with and 38 without myocardial infarction. Insulin resistance in transgenic animals genetically rendered insulin resistant was confirmed with the use of intraperitoneal glucose tolerance tests. Myocardial infarction was induced by coronary ligation, verified echocardiographically, and quantified by assay of depletion of creatine kinase (CK) from the left ventricle 2 weeks later. PAI-1 increased markedly in zones of infarction to 10.4 ± 2.1 (SF) and significantly more to 27.3 ± 3.6 in normal and insulin resistant mice compared with 0.45 ± 0.04 and 0.50 ± 0.03 in normal myocardium. Thus, insulin resistance induced accumulation of PAI-1 in the heart, particularly in zones of infarction. Such increases may contribute to fibrosis and diastolic dysfunction typical late after infarction in patients with insulin resistance.

Keywords: Cardiomyopathy; Fibrosis; Heart failure; Infarction; Insulin resistance

This study was designed to determine whether insulin resistance increases expression of plasminogen activator inhibitor type-1 (PAI-1) in the heart subjected to acute myocardial infarction (MI). Insulin resistance is accompanied by increased expression of PAI-1 in the vasculature [reviewed in 1] and in diverse organs and tissues including liver [2] and adipose tissue [3]; however, to the best of our knowledge, its impact on cardiac expression of PAI-1 has not yet been elucidated. Increased PAI-1 expression in the heart has been associated with altered ventricular remodeling [4], heart failure [5], and both increased [6,7] and decreased [8] cardiac fibrosis depending on the extent of concomitant activation of macrophages [8].

Materials and methods

Use of animals. Transgenic mice were used in conformity with a protocol approved by the University of Vermont Institutional Animal Care and Use Committee and with adherence to the NIH Principles of Animal Care. Apolipoprotein E knockout (ApoE−/−) mice congenic with respect to the C57BL6 background were purchased from Taconic Corporation (Germantown, NY). To render mice insulin resistant insulin receptor substrate (IRS) 2 heterozygous, deficient mice (IRS2+/−) were rendered congenic on the C57BL6 background by backcrossing 10× and were used for breeding [9]. They were crossed with the ApoE−/− mice to generate insulin resistant IRS2+/− ApoE−/− mice [10,11]. C57BL6 mice were used as controls. All mice were fed normal chow from weaning unless otherwise noted in which case a high fat diet was used (20% fat, 1.5% cholesterol; Teklad, Harlon Laboratories, Madison, WI).

Genotyping. The mice were genotyped [9] by analysis of a 0.5–1 cm tail clip snipped at weaning. DNA extraction and PCR amplification were performed with the use of a Sigma RED Extract-N-Amp Tissue PCR Kit (product code: XNAT, Sigma Chemical Co., St. Louis, MO) consistent with instructions provided by the manufacturer. Primers were obtained from Invitrogen, Corp., Carlsbad, CA. For IRS2−/−, the primers were: CTTGGCTACCATGTTATTGTC (5′); AGCTCTGGATTACTTTCCT AG (3′ wild type); and GCTACCCGTGATATTGCTGAAGAG (3′ encoding knockout/neomycin region). For ApoE−/−, the corresponding primers were: TGTCTTCCACTATTGGCTCG; CAGCTCTTTCACC CTCGGCA; and GTATCCATCATGGCTGATGC.

Metabolic studies. To verify the presence of insulin resistance, we performed intraperitoneal (i.p.) glucose tolerance tests (GTT). We used a one compartment model since our objective was simply to verify the presence of insulin resistance. Absorption of glucose following i.p. injection is far from instantaneous and, accordingly, assumptions used in multi-compartmental models applied to intravenous GTTs are violated. The GTTs were performed by administerting 1.5 g/kg body weight of

* Corresponding author. Fax: +1 802 656 8957.
E-mail address: burt.sobel@vtmednet.org (B.E. Sobel).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.05.078

glucose i.p. followed by acquisition of tail nick samples for assay of plasma glucose at 30 min intervals. Disappearance of glucose from blood during the interval of 60–120 min after injection was found to constitute the best fit mono-exponential portion of the curve of the log transformed data with linearity reflected by r values exceeding 0.98 in every case. The time required for 50% disappearance of glucose from the peak value to the trough or to 80 mg/dl, whichever was lower, was calculated as follows:

$$[G]_{t_{1/2}} = [G]_p \, e^{-kt_{1/2}}$$

$$1/2 = e^{-kt_{1/2}}$$

$$k^{t_{1/2}} = \ln 2$$

$$k'_{1/2} = 0.693$$

$$k = 0.693/t_{1/2}$$

in which $[G]$ = concentration of glucose, $[G]_p$ = peak concentration of glucose, $[G]_{t1/2}$ = 50% of $[G]_p$, k = disappearance rate (min^{-1}), $t_{1/2}$ = time at which 50% of decline of concentration of glucose from $[G]_p$ has occurred.

Induction of myocardial infarction. Myocardial infarction was induced in mice at 10 weeks of age following anesthesia initiated with 4% and maintained with 2% isoflurane, intubation, thoracotomy, resection of the pericardium, and occlusion of the left anterior descending coronary artery with an 8.0 suture on a tapered needled. The hearts were harvested 2 weeks later because at that time zones of infarction are clearly demarcated and readily apparent by gross visualization.

Ultrasonic imaging of mouse hearts in vivo (Echocardiography). Anesthetized animals (4% followed by 2% isoflurane) were weighed and placed supine onto a 37 °C imaging platform. A VisualSonics (Toronto, Canada) Vivo 770 high-resolution ultrasound imaging system was used with a 45 MHz transducer. Imaging was performed through warmed ultrasound gel that had been centrifuged to remove air bubbles. Representative images are shown in Fig. 1. Image equality obtained with this system permitted reproducible assessment of segmented wall thickening and fractional shortening with virtually identical results consistently obtained by two blinded observers.

Echocardiographic images were obtained in parasternal long and short axis views. The parasternal long axis view was used to assess function of the apex. In the short axis view the heart was interrogated at 3 sites roughly dividing left ventricular chamber into thirds: basal (the junction between the chordae tendinae and the papillary muscle), mid (maximal papillary muscle mass), and apical.

Traditional echocardiographic M-mode measurements were performed at the short axis mid papillary muscle level for determination of anteroseptal and posterior wall thickness as well as internal dimensions during systole and diastole. Fractional shortening ([diastolic–systolic]/diastolic) was derived from the chamber dimensions.

A functional assessment of 13 segments patterned after the American Society of Echocardiography 17 segment model was performed as follows: each segment was scored based on wall thickening (1 = normal, >25%; 2 = hypokinetic, 10–25%; 3 = akinetic, <10%). Normal function of all segments yielded a score of 13, each quarter of the short axis view providing 4 segments for analysis and the apex providing the 13th segment. Echocardiography scores were determined independently by two observers, each masked with respect to estimates of infarct size and genotypes of the animals imaged.

Assessment of the extent of infarction. We assessed the extent of infarction with the use of a biochemical marker so that PAI-1 could be assayed biochemically in the same samples and to avoid potential pitfalls that can occur with or histological or histochemical determinations because of sampling bias, temporal-dependent changes in the evolution of histological and histochemical criteria, and factors influencing the activity of the dehydrogenases that interact with the dye among others. Assessment of the extent of infarction with the use of a biochemical marker of viable cells in homogenates of the whole left ventricle facilitates quantitative comparisons of hearts from different groups of animals. Accordingly, we assessed infarct size by assaying residual left ventricular (LV) CK, measured directly in homogenates [12]. Loss of CK from myocardium correlates closely with extent of infarction measured with a variety of independent methods [13,14]. The relationship underlies estimates of infarct size based on measurement of CK released into blood [15] but is much more direct. The latter, in turn, correlates with scintigraphic [16] and autopsy assessments [17] in patients. Residual left ventricular content of other macromolecular markers such as troponin or lactate dehydrogenase (LDH) may not correlate directly with infarct size because of antibody reactions with proteolytic fragments in the case of troponin and red cell contributions to total activity in the case of LDH [18,19].

Left ventricular myocardial CK content was determined 2 weeks after the animals had been subjected to acute myocardial infarction as follows:

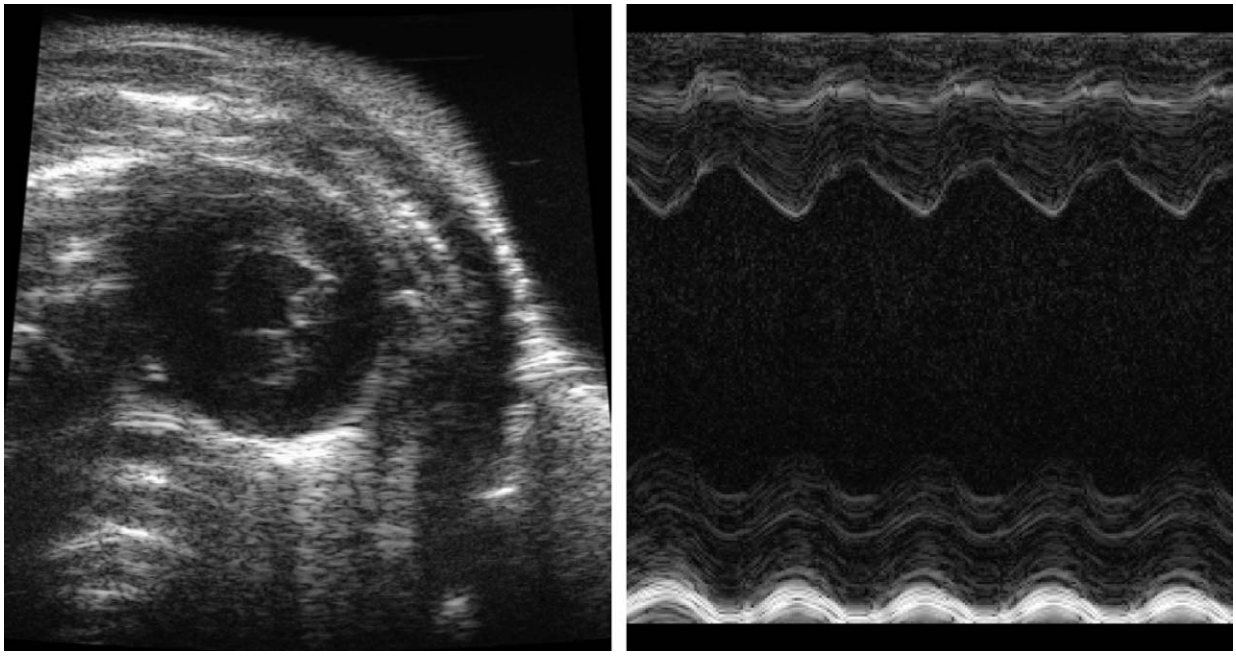

Fig. 1. Representative echocardiographic images from a normal C57BL6 mouse. Left, a short axis view at the mid-papillary muscle level; right, an M-mode image demonstrating wall thickening with systole.

the mice were killed humanely under isoflurane anesthesia by exsanguination. The hearts were excised, the atria and right ventricle trimmed and removed, and the left ventricle frozen in isotonic saline and stored at −80 °C. For analysis tissue from the center of homogeneous grossly evident infarction manifesting wall thinning and obvious pallor was homogenized as was normal left ventricular myocardium not supplied by the ligated left anterior descending coronary artery. Whole left ventricular content of analytes was calculated by pooling results from both homogenates. Tissue was homogenized after thawing in 5 mL polypropylene tubes in 50 mM Tris, 0.606 g/100 ml, 5 mM dithiothreitol (DTT), 0.077 g/100 ml chilled to 4 °C with the pH adjusted to 7.6 with HCl. Three bursts of a Polytron (Ultra Turrax T25) probe were applied at 1/2 maximum speed with a 15 s interval between bursts. After centrifugation in a microcentrifuge at maximum speed and 0–4 °C for 20 min, the supernatant fluid was aspirated and transferred to another tube for analysis.

CK assays were performed on the same day as homogenization [12] to avoid the potential impact of oxidants present in the tissue on CK activity, as were protein [20] and PAI-1 assays. The extent of infarction (percent) was calculated based on observed total LV CK (IU/mg protein) CK in normal hearts that was found to average 8.1 ± 0.6 (SD) IU/mg. Maximum CK depletion in homogeneous zones of infarction was found to yield residual CK averaging 1.4 ± 0.1 IU/mg. Thus, percent infarction was calculated as = [8.1 − measured LV CK (IU/mg protein)]/6.7 × 100.

Assay of PAI-1 protein. Assays of PAI-1 in homogenates of normal left ventricle and zones of infarction as well as in plasma were performed at room temperature with the use of a murine PAI-1 ELISA kit with monoclonal antibodies and reagents from Innovative Research, Inc., Southfield, MI [21]. TMB, a reagent containing both hydrogen peroxide and tetramethylbenzidine in an aqueous buffer, was added to elicit development of color at 450 nm read with a microplate reader. Standard curves were performed with the use of serial dilution of standards. Reactions were terminated with the addition of 50 μl of 1 N sulfuric acid. The amount of color developed reflected the concentration of total (free, latent, and complexed) PAI-1 in the sample.

Assay of plasminogen activator (PA) activity. To determine whether changes in PAI-1 protein were paralleled by changes in PA activity in homogenates of whole left ventricles, normal myocardium, and zones of infarction, we assayed PA activity with a chromogenic kinetic assay [22] with substrate S2251 from Diapharma (West Chester, OH) and Glu-plasminogen from Enzyme Research Laboratories (South Bend, IN). Fibrin fragments were prepared from fibrinogen obtained from Enzyme Research Laboratories, and tissue-type plasminogen activator was obtained from Genentech, Inc. (South San Francisco, CA).

Statistics. Results were expressed as means ± standard deviations unless noted otherwise. Comparisons of continuous data were made with the use of Student's *t*-tests with the Bonferroni correction for avoidance of a type 1 error. *P* values $\leqslant 0.05$ were considered to be significant.

Results

Confirmation of insulin resistance

As shown in Table 1, the IRS2+/− ApoE−/− mice were verified to be insulin resistant. Thus, the calculated disappearance rate of glucose from blood was markedly and significantly less than that in C57BL6 mice.

Table 1
Estimated disappearance rates of glucose in blood

Strain	*n*	*k* (min^{-1})
C57BL6	4	0.029 ± 0.002
IRS2+/− ApoE−/−	4	0.007 ± 0.006[a]

Results are means ± SEs.
[a] $P < 0.05$ compared with the *k* value observed in C57BL6 mice.

Myocardial and plasma PAI-1 in animals without infarction

As shown in Table 2, compared with controls undergoing no procedure, sham-operated animals exhibited low and similar myocardial PAI-1 content. The same was true of PAI-1 concentrations in plasma (Table 3).

Results in animals subjected to myocardial infarction (MI)

Infarct size averaged 13 ± 10 (SD) and 26 ± 12 in C57BL6 ($n = 13$) and IRS2+/− ApoE−/− ($n = 9$) mice (Table 4). Only 1 of 10 IRS2+/− ApoE−/− and 2 of 15 C57BL6 failed to survive for 2 weeks. Rupture was seen in only 1 mouse in each of these two groups.

CK content in the grossly normal, unblanched, thick-walled left ventricular myocardium that was not supplied by the ligated coronary artery in C57BL6 and IRS2+/− ApoE−/− mice was similar averaging 8.1 ± 0.6 IU/mg protein and 8.8 ± 0.5 in IRS2+/− ApoE−/− mice. These values were not significantly different from those in grossly normal zones in hearts harboring MI in the two strains (8.1 ± 0.8 and 7.7 ± 0.6, respectively).

Echocardiographic score reflecting left ventricular dysfunction (Table 4) correlated with infarct size ($r = 0.81$) calculated based on myocardial CK depletion 2 weeks after infarction in the 13 C57BL6 mice and 9 IRS2+/− ApoE−/− mice studied echocardiographically (Fig. 2). Fractional shortening correlated much less closely with infarct size than did score ($r = 0.50$). This is not

Table 2
PAI-1 content in the heart

Strain	Tissue	Procedure	*n*	Myocardial PAI-1 (ng/mg protein)
C57BL6	Whole LV	Non-operated	21	0.45 ± 0.04
C57BL6	Whole LV	Sham-operated	9	0.42 ± 0.02
IRS2+/− ApoE−/−	Whole LV	Non-operated	8	0.50 ± 0.03
C57BL6	Grossly normal myocardium	Coronary ligation	13	0.72 ± 0.05[a]
IRS2+/− ApoE−/−	Grossly normal myocardium	Coronary ligation	9	0.83 ± 0.04[a]

Results are means ± SEs.
[a] Significantly increased compared with myocardial PAI-1 in sham-operated or non-operated animals of the same genotype.

Table 3
PAI-1 in plasma

Strain	Procedure	*n*	PAI-1 in plasma (ng/mg protein)	Diet
C57BL6	Non-operated	12	1.70 ± 0.02	Normal chow
C57BL6	Sham-operated	5	1.60 ± 0.3	Normal chow
C57BL6	Coronary ligation	5	1.40 ± 0.3	Normal chow
IRS2+/− ApoE−/−	Coronary ligation	2	2.31 ± 0.85	Normal chow
IRS2+/− ApoE−/−	Non-operated	8	2.70 ± 1.3	Normal chow

Results are means ± SDs.

Table 4
Echocardiographic data in mice subjected to myocardial infarction

Animal genotype	LVEDD (mm)	LVESD (mm)	FS (%)	DWT Ant (mm)	SWT Ant (mm)	DWT Post (mm)	SWT Post (mm)	Score	% infarct (CK)	HR
IRS2+/− ApoE−/−	3.5	2.4	33	1.2	1.4	1.1	1.3	19	12	369
IRS2+/− ApoE−/−	3.5	2.6	26	0.6	0.7	0.7	0.8	20	14	466
IRS2+/− ApoE−/−	5.0	4.0	20	0.3	0.6	0.9	0.9	25	30	432
IRS2+/− ApoE−/−	3.4	1.9	43	0.4	0.7	1.0	1.3	18	15	387
IRS2+/− ApoE−/−	4.6	3.8	18	0.6	0.7	0.7	0.6	25	30	455
IRS2+/− ApoE−/−	5.1	3.57	29	0.70	0.99	0.61	1.02	21	37	495
IRS2+/− ApoE−/−	3.4	1.93	44	0.79	1.14	0.89	1.16	13	16	449
IRS2+/− ApoE−/−	5.6	4.97	11	0.61	0.64	0.92	1.07	28	42	485
IRS2+/− ApoE−/−	4.9	4.16	15	0.68	0.9	0.22	0.41	28	37	442
Mean	4.3	3.3	26	0.7	0.9	0.8	0.9	22	26	442
SD	0.9	1.1	12	0.3	0.3	0.3	0.3	5	12	42
C57/BI6	4.4	3.0	32	0.8	1.3	0.8	3.0	20	17	435
C57/BI6	3.7	2.8	23	0.7	0.9	1.0	0.8	21	17	441
C57/BI6	4.8	4.0	17	0.9	0.9	0.8	0.8	19	20	456
C57/BI6	4.4	3.3	25	0.7	1.0	0.7	0.8	23	18	519
C57/BI6	4.1	2.9	29	0.6	0.9	0.8	0.9	15	6	436
C57/BI6	3.9	2.8	28	0.8	1.0	0.6	0.7	13	0	495
C57/BI6	3.4	2.3	32	0.9	1.0	0.7	0.8	13	0	536
C57/BI6	5.3	4.8	9	0.7	0.4	0.5	0.5	24	30	459
C57/BI6	3.8	2.5	28	0.8	1.0	0.8	0.9	13	1	464
C57/BI6	4.2	3.5	17	0.5	0.3	1.1	1.5	25	19	478
C57/BI6	4.2	3.1	27	0.7	1.0	0.7	0.7	16	6	462
C57/BI6	5.4	4.8	11	0.3	0.2	0.7	0.7	21	13	492
C57/BI6	5.7	5.6	3	0.3	0.3	0.7	0.7	31	23	500
Mean	4.4	3.5	22	0.7	0.8	0.8	1.0	20	13	475
SD	0.7	1.0	9	0.2	0.3	0.2	0.6	5	10	32

Abbreviations: LVEDD and SD, left ventricular end diastolic and end systolic dimensions; FS, fractional shortening; DWT and SWT, diastolic and systolic wall thickness; Ant and Post, anterior and posterior; % infarct, based on residual CK; HR, heart rate.

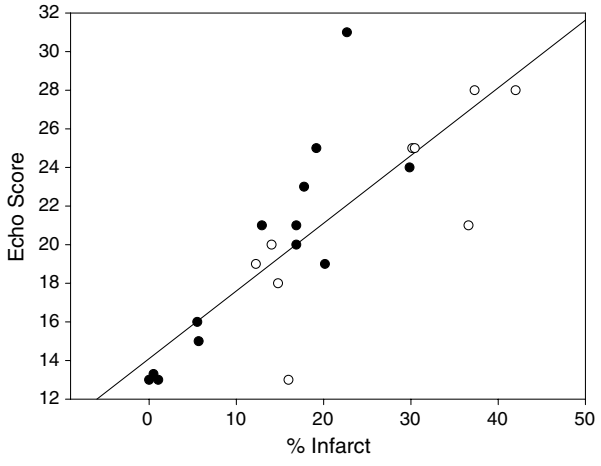

Fig. 2. The correlation (least squares regression line) between the echocardiographic score obtained as described in the text and percent infarct determined by assay of left ventricular CK in 13 C57BL6 mice (closed circles) and 9 IRS2+/− ApoE−/− mice (open circles) studied 2 weeks after induction of infarction ($r = 0.81$).

surprising because the extent to which the mid-ventricular region is affected by the induction of infarction will have a major influence on fractional shortening measured echocardiographically at the mid-papillary muscle region as will angulation of the echocardiographic beam at the time of interrogation.

Myocardial content of PAI-1 in hearts from mice with myocardial infarction

Two of 15 C57BL6 and 1 of 10 IRS2+/− ApoE−/− did not survive for the 2-week interval preceding planned echocardiography and tissue harvest. Their hearts were not included in the analyses. As can be seen in Fig. 3, in zones of infarction in C57BL6 control mice myocardial PAI-1 content was approximately 14-fold and significantly greater than that in grossly normal myocardium not supplied by the ligated coronary artery. Directionally similar results were seen in IRS2+/− ApoE−/− mice subjected to myocardial infarction (Fig. 3). Regions of myocardial infarction in the IRS2+/− ApoE−/− mice exhibited marked increases in PAI-1. Compared with results in C57BL6 control mice, the regions of infarction in the IRS2+/− ApoE−/− mice exhibited a 2.7-fold increase in PAI-1. Compared with PAI-1 content in grossly normal regions of the hearts from the same IRS2+/− ApoE−/− animals, the increase in PAI-1 was approximately 32-fold.

No PA activity was detected in homogenates of zones of infarction or in normal zones in mice of either strain consistent with the predominance of inhibition of PA activity attributable to PAI-1. The specific activity of plasminogen activator in tissues exceeds 200,000 IU/mg protein. The sensitivity of the assay used was such that as little as 10^{-6} IU of PA activity/mg protein would have been detectable.

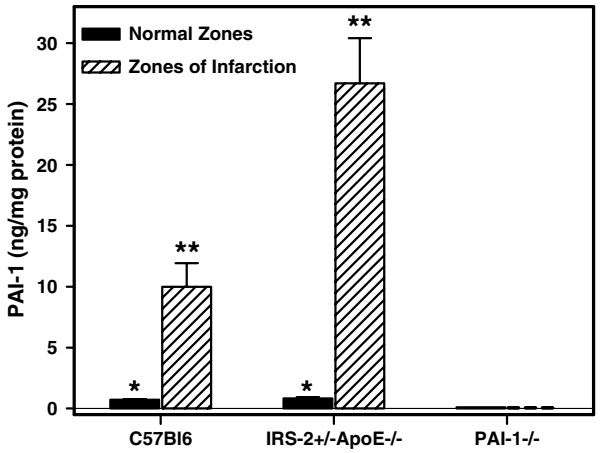

Fig. 3. PAI-1 in left ventricular myocardium from three strains of mice subjected to infarction. Results are means ± SEs. Thirteen C57BL6, 9 IRS2+/− ApoE−/−, and 2 PAI-1 knockout mice were studied. *Significantly greater than PAI-1 in hearts of normal C57BL6 mice (0.45 ± 0.04, $n = 21$) and that in hearts from sham-operated animals (0.42 ± 0.02, $n = 9$) and significantly greater as well than PAI-1 in hearts of normal IRS2+/− ApoE−/− mice (0.50 ± 0.03, $n = 8$). **Significantly greater than PAI-1 in grossly normal zones of the same hearts. There was no detectable PAI-1 in the hearts of the two PAI-1 knockout mice.

Discussion

Results obtained in this study indicate that PAI-1 increases markedly in infarct zones in both insulin sensitive and insulin resistant mice and that insulin resistance in IRS2+/− ApoE−/− transgenic mice leads to markedly greater increases in PAI-1 in zones of infarction in comparison with the increases in controls that are not insulin resistant.

We have previously shown that PAI-1 co-localizes with fibrosis and that attenuation of the increased expression of PAI-1 by inhibition of angiotensin converting enzyme or angiotensin 2, known to mediate increased PAI-1 expression, diminishes coronary perivascular fibrosis in genetically obese mice [6,7]. Others have implicated increased PAI-1 expression in cardiac myocytes as a profibrotic determinant after infarction [23]. Increased PAI-1 expression occurs in activated macrophages [24]. Most information available indicates that increased expression of PAI-1 predisposes to fibrosis by inhibiting degradation of extravascular fibrin that serves as a scaffold for developing fibrosis. However, some have found that deficiency of PAI-1 can cause cardiac fibrosis [8] by reducing inhibition of urokinase-mediated macrophage infiltration that is profibrotic. Thus, effects of PAI-1 on fibrosis may depend on the company it keeps. When macrophage activation is prominent, increased PAI-1 may be anti-fibrotic. Under other conditions in which extravascular fibrin is prominent or another factor such as insulin resistance is present, it may be profibrotic.

Heart failure and insulin resistance

Type 2 diabetes is known to be strongly associated with insulin resistance, implicated as an etiologic factor. It is also a powerful risk factor for and probable determinant of heart failure. An apparent diabetic cardiomyopathy acting synergistically with hypertension and coronary artery disease results in a high incidence of heart failure [25–29]. Diastolic dysfunction, an early clinical manifestation of diabetic cardiomyopathy, is seen frequently. Increased ultrasonic backscatter indicative of changes consistent with deposition of collagen, accumulation of advanced glycation end products (AGEs), or interstitial edema is evident [30–32]. Several studies have demonstrated fibrosis in hearts of insulin resistant animals [33–35]. Of particular note, heart failure is emerging as a leading cause of death in patients with type 2 diabetes. We performed the present study to test the hypothesis that insulin resistance increases expression of PAI-1 in the heart, a potentially profibrotic phenomenon that could contribute to heart failure.

Increased PAI-1 in blood is seen with insulin resistance in human subjects [36,37]. However, contributions of increased PAI-1 in blood were found in the infarcts to the PAI-1 we found in the infarct and peri-infarct zones in the IRS2+/− ApoE−/− mice are likely to have been trivial in view of the actual concentrations seen in blood and the low regional myocardial blood volume in mice (11.8 ± 0.8 volume percent [38]).

Our results show that insulin resistance markedly increases accumulation of PAI-1 in zones of infarction. Such changes may predispose to cardiac impairment typically seen late after infarction in patients with type 2 diabetes and insulin resistance.

Acknowledgments

We appreciate the technical assistance of Keara McElroy-Yaggy, Dagnija Neimane, and Patricia Q. Baumann and the preparation of the manuscript by Lori Dales. The work was supported in part by a research grant from the American Diabetes Association. This work was supported in part by an American Diabetes Association research award grant (B.E.S., PI; ADA Award No: 1-04-RA-105).

References

[1] B.E. Sobel, D.J. Taatjes, D.J. Schneider, Intramural plasminogen activator inhibitor type-1 and coronary atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 23 (2003) 1979–1989.
[2] M.C. Alessi, D. Bastelica, A. Mavri, P. Morange, B. Berthet, M. Grino, I. Juhan-Vague, Plasma PAI-1 levels are more strongly related to liver steatosis than to adipose tissue accumulation, Arterioscler. Thromb. Vasc. Biol. 23 (2003) 1262–1268.
[3] H.R. Lijnen, M.C. Alessi, B. Van Hoef, D. Collen, I. Juhan-Vague, On the role of plasminogen activator inhibitor-1 in adipose tissue development and insulin resistance in mice, J. Thromb. Haemost. 3 (2005) 1172–1173.
[4] A.T. Askari, M.-L. Brennan, X. Zhou, J. Drinko, A. Morehead, J.D. Thomas, E.J. Topol, S.L. Hazen, M.S. Penn, Myeloperoxidase and plasminogen activator inhibitor 1 play a central role in ventricular remodeling after myocardial infarction, J. Exp. Med. 197 (2003) 615–624.
[5] S. Heymans, A. Luttun, D. Nuyens, G. Theilmeier, E. Creemers, L. Moons, G.D. Dyspersin, J.P. Cleutjens, M. Shipley, A. Angellilo, M. Levi, O. Nube, A. Baker, E. Keshet, F. Lupu, J.M. Herbert, J.F.

Smits, S.D. Shapiro, M. Baes, M. Borgers, D. Collen, M.J. Daemen, P. Carmeliet, Inhibition of plasminogen activators or matrix metalloproteinases prevents cardiac rupture but impairs therapeutic angiogenesis and causes cardiac failure, Nat. Med. 5 (1999) 1135–1142.
[6] A.K. Zaman, S. Fujii, H. Sawa, D. Goto, N. Ishimori, K. Watano, T. Kaneko, T. Furumoto, T. Sugawara, I. Sakuma, A. Kitabatake, B.E. Sobel, Angiotensin-converting enzyme inhibition attenuates hypofibrinolysis and reduces cardiac perivascular fibrosis in genetically obese diabetic mice, Circulation 103 (2001) 3123–3128.
[7] A.K. Zaman, S. Fujii, D. Goto, T. Furumoto, T. Mishima, Y. Nakai, J. Dong, S. Imagawa, B.E. Sobel, A. Kitabatake, Salutary effects of attenuation of angiotensin II on coronary perivascular fibrosis associated with insulin resistance and obesity, J. Mol. Cell. Cardiol. 37 (2004) 525–535.
[8] H. Moriwaki, A. Stempien-Otero, M. Kremen, A. Cozen, D.A. Dichek, Overexpression of urokinase by macrophages or deficiency of plasminogen activator inhibitor type 1 causes cardiac fibrosis in mice, Circ. Res. 95 (2004) 637–644.
[9] D.J. Withers, D.J. Burks, H.H. Towery, S.L. Altamuro, C.L. Flint, M.F. White, Irs-2 coordinates Igf-1 receptor-mediated β-cell development and peripheral insulin signaling, Nat. Genet. 23 (1999) 32–40.
[10] M.H. Clough, D.J. Schneider, B.E. Sobel, M.F. White, M.P. Wadsworth, D.J. Taatjes, Attenuation of accumulation of neointimal lipid by pioglitazone in mice genetically deficient in insulin receptor substrate-2 and apolipoprotein E, J. Histochem. Cytochem. 53 (2005) 603–610.
[11] W.T. Cefalu, Z.Q. Wang, D.J. Schneider, P.M. Absher, L.C. Baldor, D.J. Taatjes, B.E. Sobel, Effects of insulin sensitizers on plaque vulnerability associated with elevated lipid content in atheroma in ApoE-knockout mice, Acta Diabetol. 41 (2004) 25–31.
[12] J.K. Kjekshus, B.E. Sobel, Depressed myocardial creatine phosphokinase activity following experimental myocardial infarction in rabbit, Circ. Res. 27 (1970) 403–414.
[13] P.R. Maroko, J.K. Kjekshus, B.E. Sobel, T. Watanabe, J.W. Covell, J. Ross Jr., E. Braunwald, Factors influencing infarct size following experimental coronary artery occlusions, Circulation 43 (1971) 67–82.
[14] E.S. Weiss, S.A. Ahmed, M.J. Welch, J.R. Williamson, M.M. Ter-Pogossian, B.E. Sobel, Quantification of infarction in cross sections of canine myocardium in vivo with positron emission transaxial tomography and 11C-palmitate, Circulation 55 (1977) 66–73.
[15] A.S. Binbrek, N.S. Rao, D. Neimane, E. Hatou, S. Abdulali, B.E. Sobel, Comparison of rapidity of coronary recanalization in men with tenecteplase—vs—alteplase in acute myocardial infarction, Am. J. Cardiol. 93 (2004) 1465–1468.
[16] C.H. Croft, R.E. Rude, S.E. Lewis, R.W. Parkey, W.K. Poole, C. Parker, N. Fox, R. Roberts, H.W. Strauss, L.J. Thomas, D.S. Raabe, B.E. Sobel, H.K. Gold, P.H. Stone, E. Braunwald, J.T. Willerson for the MILIS study group, comparison of left ventricular function and infarct size in patients with and without persistently positive technetium-99 m pyrophosphate myocardial scintigrams after myocardial infarction, Am. J. Cardiol. 53 (1984) 421–428.
[17] D.B. Hackel, K.A. Reimer, R.E. Ideker, E.M. Mikat, T.D. Hartwell, C.B. Parker, E.B. Braunwald, H.K. Gold, D.S. Raabe, R.E. Rude, B.E. Sobel, P.H. Stone, M. Buja, J.E. Muller, R. Roberts for the MILIS study group, comparison of enzymatic and anatomic estimates of myocardial infarct size in man, Circulation 70 (1984) 824–835.
[18] B.E. Sobel, M.M. LeWinter, Ingenuous interpretation of elevated blood levels of macromolecular markers of myocardial injury: a recipe for confusion, J. Am. Coll. Cardiol. 35 (2000) 1355–1358.
[19] B.E. Sobel, Acute myocardial infarction, in: J.C. Bennett, F. Plum (Eds.), Cecil Textbook of Medicine, 20th ed., W.B. Saunders, Philadelphia, 1996, pp. 301–316.
[20] M. Bradford, A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding, Anal. Biochem. 72 (1976) 248–254.
[21] P.J. Declerck, M. Verstreken, D. Collen, Immunoassay of murine t-PA, u-PA, and PAI-1 using monoclonal antibodies raised in gene-inactivated mice, Thromb. Haemost. 74 (1995) 1305–1309.
[22] J. Chmielewska, B. Wiman, Determination of tissue plasminogen activator and its "fast" inhibitor in plasma, Clin. Chem. 32 (1986) 482–485.
[23] K. Takeshita, M. Hayashi, S. Iino, T. Kondo, Y. Inden, M. Iwase, T. Kojima, M. Hirai, M. Ito, D.J. Loskutoff, H. Saito, T. Murohara, K. Yamamoto, Increased expression of plasminogen activator inhibitor-1 in cardiomyocytes contributes to cardiac fibrosis after myocardial infarction, Am. J. Pathol. 164 (2004) 449–456.
[24] H.A. Chapman, X.L. Yang, L.Z. Sailor, D.J. Sugarbarker, Developmental expression of plasminogen activator inhibitor type 1 by human alveolar macrophages. Possible role in lung injury, J. Immunol. 145 (1990) 3398–3405.
[25] P.T. Donnan, D.I. Boyle, J. Broomhall, K. Hunter, T.M. MacDonald, R.W. Newton, A.D. Morris, Prognosis following first acute myocardial infarction in type 2 diabetes: a comparative population study, Diabetic Med. 9 (2002) 448–455.
[26] I. Gustaffson, B. Brendorp, M. Seibaek, H. Burchardt, P. Hildebrandt, L. Kober, C. Torp-Pedersen, Danish Investigatord of Arrhythmia and mortality on Dofetilde study group, Influence of diabetes and diabetes-gender interaction on the risk of death in patients hospitalized with congestive heart failure, J. Am. Coll. Cardiol. 43 (2004) 771–777.
[27] B.M. Fisher, G. Gillen, G.B. Lindop, H.J. Dargie, B.M. Frier, Cardiac function and coronary arteriography in asymptomatic type 1 (insulin-dependent) diabetic patients: Evidence for a specific diabetic heart disease, Diabetologia 29 (1986) 706–712.
[28] S.A. Hayat, B. Patel, R.S. Khattar, R.A. Malik, Diabetic cardiomyopathy: mechanisms, diagnosis and treatment, Clin. Sci. (Lond.) 107 (2004) 539–557.
[29] Z.Y. Fang, J.B. Prins, T.H. Marwick, Diabetic cardiomyopathy: evidence, mechanisms, and therapeutic implications, Endocr. Rev. 25 (2004) 543–567.
[30] J.E. Perez, J.B. McGill, J.V. Santiago, K.B. Schechtman, A.D. Waggoner, J.G. Miller, B.E. Sobel, Abnormal myocardial acoustic properties in diabetic patients and their correlation with the severity of disease, J. Am. Coll. Cardiol. 19 (1992) 1154–1162.
[31] V. Di Bello, O. Giampietro, E. Matteucci, L. Talarico, D. Giorgi, A. Bertini, M.T. Caputo, F. Piazza, M. Paterni, C. Giusti, Ultrasonic videodensitometric analysis in type 1 diabetic myocardium, Coron. Artery Dis. 7 (1996) 895–901.
[32] V. Di Bello, L. Talarico, E. Picano, C. DiMuro, L. Landini, M. Paterni, E. Matteucci, C. Giusti, O. Giampietro, Increased echodensity of myocardial wall in the diabetic heart: an ultrasound tissue characterization study, J. Am. Coll. Cardiol. 25 (1995) 1408–1415.
[33] K. Mizushige, L. Yao, T. Noma, H. Kiyomoto, Y. Yu, N. Hosomi, K. Ohmori, H. Matsuo, Alteration in left ventricular diastolic filling and accumulation of myocardial collagen at insulin-resistant prediabetic stage of a type II diabetic rat model, Circulation 101 (2000) 899–907.
[34] M. Shimizu, K. Umeda, N. Sugihara, H. Yoshio, H. Ino, R. Takeda, Y. Okada, I. Nakanishi, Collagen remodelling in myocardium of patients with diabetes, J. Clin. Pathol. 46 (1993) 32–36.
[35] B. Haider, C.K. Yeh, G. Thomas, H.A. Oldewurtel, M.M. Lyons, T.J. Regan, Altered myocardial function and collagen in diabetic rhesus monkeys on atherogenic diet, Trans. Assoc. Am. Phys. 91 (1978) 197–203.
[36] J.B. McGill, D.J. Schneider, C.L. Arfken, C.L. Lucore, B.E. Sobel, Factors responsible for imparied fibrinolysis in obese subjects and NIDDM patients, Diabetes 43 (1994) 104–109.
[37] Y. Kruszynska, J.G. Yu, B.E. Sobel, J.M. Olefsky, Effects of troglitazone on blood concentrations of plasminogen activator inhibitor 2 in patients with type 2 diabetes mellitus and in lean and obese normal subjects, Diabetes 49 (2000) 633–639.
[38] J.U.G. Streif, M. Nahrendorf, K.-H. Hiller, C. Waller, F. Wiesmann, E. Rommel, A. Haase, W.R. Bauer, In vivo assessment of absolute perfusion and intracapillary blood volume in the murine myocardium by spin labeling magnetic resonance imaging, Magn. Reson. Med. 53 (2005) 584–592.