Deficiency of Tissue Factor Pathway Inhibitor Promotes Atherosclerosis and Thrombosis in Mice

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Background—Tissue factor initiates blood coagulation after atherosclerotic plaque disruption. Tissue factor pathway inhibitor (TFPI) inhibits tissue factor activity and may reduce thrombus formation in this setting. We evaluated the effect of heterozygous TFPI deficiency on the development of atherosclerosis and thrombosis in atherosclerosis-prone mice.

Methods and Results—Mice with a combined heterozygous TFPI deficiency and homozygous apolipoprotein E deficiency (TFPI+/−/apoE−/−) were generated by crossbreeding, and they were analyzed for atherosclerosis throughout the vascular tree. Compared with mice with a normal TFPI genotype (TFPI+/−/apoE−/−), mice with a TFPI deficiency exhibited a greater atherosclerotic burden involving the carotid and common iliac arteries. Staining for active tissue factor within the plaque revealed more activity in TFPI+/−/apoE−/− mice compared with TFPI+/−/apoE−/− mice. Consistent with increased plaque tissue factor activity, the time to occlusive thrombosis after photochemical carotid plaque injury was significantly decreased in TFPI+/−/apoE−/− mice.

Conclusions—These observations indicate that TFPI protects from atherosclerosis and is an important regulator of the thrombosis that occurs in the setting of atherosclerosis. (Circulation. 2001;103:3044-3046.)

Key Words: atherosclerosis ■ thrombosis ■ coagulation ■ plaque ■ carotid arteries

Thrombosis after plaque disruption is the immediate cause of most acute coronary syndromes and may contribute to the progression of atherosclerotic vascular disease.1 Atherosclerotic plaque thrombogenicity may be an important determinant of the amount of thrombus formation that occurs after plaque injury. Tissue factor, the primary initiating factor in blood coagulation, is abundant in atherosclerotic plaques, and its content seems to predict plaque thrombogenicity.2 Tissue factor–pathway inhibitor (TFPI), a Kunitz-type proteinase inhibitor that regulates extrinsic pathway initiation of coagulation by producing factor Xa–mediated feedback inhibition of the factor VIIa/tissue factor catalytic complex,3 through the inhibition of tissue factor activity, TFPI may attenuate plaque thrombogenicity. Overexpression of TFPI has been shown to reduce thrombus formation after vascular injury in animal models,4,5 whereas reduced endogenous TFPI activity may enhance thrombus formation.6

Mice genetically engineered to be completely deficient in functional TFPI demonstrate embryonic lethality, whereas mice heterozygous for TFPI deficiency appear normal, although plasma TFPI activity is reduced.7 To test the hypothesis that modest reductions in TFPI levels will promote atherosclerosis and thrombosis, mice with a combined apolipoprotein E (apoE) and TFPI deficiency were generated and analyzed for the development of atherosclerosis throughout the vascular tree and for thrombosis after plaque injury.

Methods

Mice

ApoE−/− mice on the C57BL/6J background were purchased from Jackson Laboratory, Bar Harbor, Maine. TFPI-deficient mice were generated as previously described.7 TFPI+/− mice were backcrossed to C57BL/6J mice for ≥4 generations, and apoE−/− mice were backcrossed to C57BL/6J mice for ≥10 generations before crossbreeding. Double-heterozygous (TFPI+/−/apoE−/−) mice were backcrossed to apoE−/− mice for 10 generations to produce TFPI+/−/apoE−/− mice, which were identified by polymerase chain reaction analysis of tail DNA specimens.8,9 Mice were maintained on standard chow. All animal care and experimental procedures complied with the Principles of Laboratory and Animal Care established by the National Society for Medical Research and were approved by the University of Michigan Committee on Use and Care of Animals.

Carotid Arterial Thrombosis Protocol

At 34 weeks of age, male TFPI+/−/apoE−/− mice were subjected to photochemical injury of the right carotid artery at the site of a grossly visible atherosclerotic plaque, as previously described.10 Flow in the vessel was monitored until cessation of flow occurred, at which time the experiment was terminated.

Analysis of Atherosclerotic Lesions

At 34 weeks of age, mice were perfusion-fixed with zinc formalin under intraperitoneal pentobarbital anesthesia (100 mg/kg). For quantitation of surface area occupied by atherosclerosis, the aorta and its major branches were stained with Oil Red O and then subjected to quantitative morphometry.8 For analysis of lesion thickness, the vascular tree was

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divided into multiple sections, including the right and left distal common carotid arteries, right and left subclavian arteries, ascending aorta, abdominal aorta, and aortoiliac bifurcation, and then embedded in paraffin. From each site, 10 sections at 50-μm intervals were stained with hematoxylin and eosin and then subjected to quantitative morphometry, as previously described. Staining for tissue factor was performed using digoxigenin-labeled human factor VIIa (American Diagnostica) with a digoxigenin detection kit (Roche), as previously described. Quantitation of tissue factor activity from atherosclerotic plaque homogenates was determined using the Actichrome tissue factor activity kit #846 (American Diagnostica) according to the manufacturer’s instructions.

**Statistical Analysis**
The statistical significance of differences in time to occlusion and atherosclerosis between the various groups was determined using Student’s t test. P<0.05 was considered significant.

**Results**

**Effect of TFPI Deficiency on Development of Occlusive Thrombosis After Plaque Injury**
We recently showed that photochemical vascular injury can be performed at sites of atherosclerosis and that the time to occlusion is decreased in diseased compared with nondiseased arteries, suggesting that murine plaque is thrombogenic in a manner similar to the human lesion. To examine the contribution of TFPI in this model for the acute thrombosis complicating atherosclerosis, 30-week-old TFPI+/apoE− mice were subjected to photochemical injury of the right carotid artery at the site of an atherosclerotic plaque. The mean time to occlusion in these mice (n=6) was 28±4 minutes, which is significantly shorter than our previously reported occlusion time of 44±5 minutes in 30-week-old TFPI+/apoE− mice on a C57BL/6J genetic background (n=9; P<0.05) performed under identical experimental conditions.

No difference in the time to occlusive thrombosis was observed in 11-week-old TFPI+/ (52±7 minutes, n=4) and TFPI+/− (56±9 minutes, n=4) mice on a wild-type apoE background, indicating the particularly relevant effect of TFPI on thrombosis in the setting of an atherosclerotic plaque.

**Effect of TFPI Deficiency on Development of Atherosclerosis Throughout the Vasculature**
To determine the effect of TFPI on the development of atherosclerosis, 34-week-old TFPI+/apoE− and TFPI+/−/apoE− male mice that were maintained on a normal chow diet were euthanized. Then, the aorta with its major branches was dissected free of connective tissue and stained for lipid with Oil Red O. Significantly more atherosclerosis by surface area staining was present at the carotid and common iliac bifurcations in TFPI+/apoE− mice than in TFPI+/−/apoE− mice (Figures 1A through 1C). Quantitation of intimal lesion thickness by hematoxylin and eosin analysis of cross-sections at several predefined sites, including the carotid artery bifurcation, proximal subclavian arteries, ascending aorta, abdominal aorta, and iliac bifurcation, demonstrated significantly increased lesion thickness at only the carotid bifurcation in TFPI+/apoE− mice (Figure 1D).

**Tissue Factor Analysis**
No significant differences in atherosclerotic lesion composition or morphology were evident among the different groups of mice on routine histological analysis. To determine whether increased tissue factor activity in the atherosclerotic plaque was detectable in TFPI+/apoE− mice and thus contributed to the propensity to develop atherosclerosis and thrombosis, carotid sections were stained with labeled factor VIIa, which binds to active tissue factor. An increase in active tissue factor staining was noted in the TFPI+/apoE− mice compared with TFPI+/−/apoE− mice. Quantitation of atherosclerosis surface area throughout the vascular tree in 34-week-old TFPI+/apoE− mice (open bar; n=7) and TFPI+/−/apoE− mice (solid bar; n=6) on normal chow. *P<0.05. D, Cross-sectional intimal area of atherosclerotic lesions in 34-week-old TFPI+/apoE− mice (open bar; n=7) and TFPI+/−/apoE− mice (solid bar; n=6). *P<0.01. C indicates carotid artery bifurcation; S, proximal subclavian arteries; Ao, ascending aorta; Ab, abdominal aorta; and I, iliac bifurcation.
that alterations in fibrinolysis affect atherosclerosis in a site-specific manner, with protection from atherosclerosis noted at the carotid bifurcation in mice with enhanced fibrinolysis.8 By regulating tissue factor–mediated factor Xa and thrombin generation, TFPI may similarly influence atherosclerosis at sites of turbulent flow.

These findings suggest that reduced TFPI expression may be a risk factor for the development of atherothrombotic complications and that therapy targeted at reducing tissue factor activity might be beneficial.

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References
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