Effect of Leptin on Arterial Thrombosis Following Vascular Injury in Mice

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Context Complications of atherosclerosis are the leading cause of morbidity and mortality in industrialized societies. Obesity has emerged as an independent risk factor for complications of atherosclerotic vascular disease. Leptin, a hormone produced by the adipocyte, increases with obesity and appears to modulate energy balance and food intake. In addition, other actions of leptin have been proposed, including an in vitro effect on platelet aggregation. Thus, the elevated plasma leptin levels in obese individuals may promote vascular thrombosis.

Objective To test the hypothesis that leptin contributes to in vivo thrombosis via the leptin receptor.

Design and Materials Between September 2000 and September 2001, a vascular thrombosis model was used to test male 10- to 12-week-old mice completely deficient in leptin or the leptin receptor and mice with platelet leptin-receptor deficiency.

Main Outcome Measure Time to formation of an occlusive thrombus in the common carotid artery following experimentally induced endothelial injury.

Results Following onset of vascular injury, wild-type mice (n=8) formed occlusive thrombosis in a mean (SD) of 42.2 (4.6) minutes, whereas leptin-deficient (n=9) and leptin receptor–deficient mice (n=7) formed occlusive thrombosis in 75.2 (10.1) and 68.6 (10.3) minutes, respectively (leptin deficient vs wild-type mice, P=0.008; leptin receptor–deficient vs wild-type, P=0.03). When recombinant murine leptin was administered to leptin-deficient mice (n=4), the time to occlusion was reduced to 41.8 (6.6) minutes (P=0.035 vs vehicle control). Following bone marrow transplantation from leptin receptor–deficient (donor) mice to wild-type (recipient) mice, the time to occlusion was prolonged from 22.3 (2.8) minutes in wild-type mice receiving wild-type marrow (n=3) to 56.8 (5.0) minutes in wild-type mice receiving leptin receptor–deficient bone marrow (n=5) (P=0.003).

Conclusions Leptin contributes to arterial thrombosis following vascular injury in vivo and these prothrombotic effects appear to be mediated through the platelet leptin receptor.

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cured in the supine position and placed under a dissecting microscope (Nikon SMZ-2T, Mager Scientific Inc, Dexter, Mich). After a midline cervical incision, the right common carotid artery was isolated, and a Doppler flow probe (model 0.5 VB, Transonic Systems, Ithaca, NY) was applied. The probe was connected to a flow meter (Transonic model T106, Transonic Systems) and interpreted with a computerized data acquisition program (Windaq, DATAQ Instruments, Akron, Ohio). The photochemical rose bengal (Fisher Scientific, Fair Lawn, NJ) was diluted to 10 mg/mL in phosphate-buffered saline and then injected into the tail vein in a volume of 0.12 mL at 50 mg/kg by use of a 27-gauge Precision Glide needle (Becton Dickinson and Co, Franklin Lakes, NJ). To activate the rose bengal, a 1.5-mW green light laser (540 nm, Melles Griot, Carlsbad, Calif) was applied to the mid common carotid artery from a distance of 6 cm. Flow in the vessel was monitored continuously from the time of the injection until the end of the experiment. The end point for these studies was determined by 2 criteria: the cessation of flow in the carotid artery as measured by the Doppler flow probe and the visualization of an occlusive thrombus via a dissecting microscope by an observer blinded to the treatment group.

**Leptin Administration**

Recombinant murine leptin (Research Diagnostics Inc, Flanders, NJ) was reconstituted according to the manufacturer's instructions and kept frozen until immediately before administration. Recombinant leptin (9 µg per injection) was administered to leptin-deficient and leptin receptor–deficient mice via intraperitoneal injection 10 minutes before and 10 minutes after the intravenous injection of the rose bengal. With the same protocol, vehicle control injections were given to leptin-deficient mice. A commercially available assay (Crystal Chem Inc, Chicago, Ill) was used according to the manufacturer's instructions to determine plasma leptin levels in duplicate from platelet-poor plasma.

**Bone Marrow Transplantation**

Bone marrow transplantation experiments were performed with methods similar to those previously described. Briefly, 8-week-old male wild-type mice were used as recipients for bone marrow transplantation from wild-type and leptin receptor–deficient mice. Bone marrow was harvested from the donor mice by flushing their femurs and tibias with RPMI 1640 medium containing 2% fetal bovine serum. Cells were then centrifuged at 300 g and resuspended in phosphate-buffered saline before injection. Each recipient mouse was irradiated (2 × 650 rad [0.02 × 6.5 Gy]) and injected with 4 × 10⁸ bone marrow cells via the tail vein. Four weeks after transplantation, blood was withdrawn from the retro-orbital sinus, and platelet counts were determined by using platelet-rich plasma in Coulter Counter Z2 (Beckman Coulter, Miami, Fla) according to the manufacturer's instructions. The arterial thrombosis protocol was performed the following day.

**Histologic Analyses**

For some mice, 3 wild-type and 3 leptin-deficient, the arterial vasculature was perfused with formalin immediately after the thrombosis protocol was completed, and the common carotid artery at the site of the occlusive thrombus was excised, sectioned, and subjected to hematoxylin-eosin staining.

**Statistical Analysis**

The analysis of differences in time to occlusion was determined with the t test; P <.05 was considered statistically significant (SigmaStat version 2.03, SPSS Science, Chicago, Ill).

**RESULTS**

To examine the influence of leptin and the leptin receptor on in vivo thrombosis, we examined mice deficient in leptin (n = 5) or the leptin receptor (n = 7) in a photochemical injury model of arterial thrombosis. Compared with wild-type mice (n = 8), leptin-deficient mice had a prolonged time to occlusion following photochemical injury (Figure 1) and Figure 2, as did leptin receptor–deficient mice (Figure 2).

To determine whether the phenotype of the leptin-deficient mice was due to the lack of leptin, we administered recombinant murine leptin during the photochemical injury. In leptin-deficient mice (n = 4), this treatment resulted in a significant reduction in the time to occlusive thrombosis, which was similar to the occlusion times observed in wild-type mice (Figure 2). Occlusion times in mice lacking the leptin receptor (n = 4) were not affected by recombinant murine leptin (Figure 2) (P = .90). Blood samples obtained immediately after the arterial thrombosis experiments revealed plasma leptin levels between 50 and 750 ng/mL, with a mean plasma leptin concentration of 299 ng/mL. Normal physiological leptin levels in mice range from 1 to 170 ng/mL. To examine whether the leptin receptor–deficient phenotype was due to the lack of the receptor on the endothelial cells or the platelet, we performed bone marrow transplantations from leptin receptor–deficient mice to wild-type mice. With this procedure, mice that underwent successful transplantations would be deficient for the leptin receptor only in bone marrow–derived cells, allowing specific assessment of address the platelet leptin receptor. Four weeks after the bone marrow transplantation, the mean platelet counts between mice that received either wild-type (n = 3; 390 × 10⁸/µL) or leptin receptor–deficient bone marrow (n = 5; 321 × 10⁸/µL) were similar. In addition, mean (SD) plasma leptin levels were not significantly different between control (1.3 [0.1] ng/mL) and leptin receptor–deficient (1.6 [0.5] ng/mL) bone marrow recipients (P = .65). Mice that had received leptin receptor–deficient bone marrow (n = 5) had a significantly longer mean (SD) time to occlusive thrombosis (56.8 [5.0] minutes) compared with wild-type mice (n = 3) that had received wild-type bone marrow (22.3 [2.8] minutes; P = .003).
A control mouse that did not receive bone marrow following irradiation died 9 days later, demonstrating effective bone marrow ablation following the irradiation.

COMMENT

Thrombotic complications of vascular disease are the leading cause of morbidity and mortality in industrialized societies. Although obesity is now considered an independent risk factor for cardiovascular disease, the mechanisms that link obesity to cardiovascular disease risk are unclear.

Studies have demonstrated associations of obesity with enhanced expression of factors that may favor thrombosis, including plasminogen activator inhibitor type 1, tissue factor, and tumor necrosis factor α. However, in vivo data demonstrating a causal relationship with obesity-related gene expression changes and thrombosis have not been established.

The recent identification of a long-form functional leptin receptor (OB-Rb) on the human platelet suggests there might be signaling between the adipocyte and the platelet. This interaction between leptin and the platelet was supported by the dose-dependent increase in in vitro platelet aggregation in the presence of a platelet agonist. The platelet-stimulating effect was shown to be closely linked with the phosphorylation of platelet protein tyrosine residues. The tyrosine phosphorylation and platelet aggregation were demonstrated at relatively high levels of leptin, suggesting that the interaction between leptin and the platelet would be most relevant in obese and diabetic individuals in whom leptin levels are elevated.

Although leptin has been demonstrated to affect food intake and metabolism, it may also affect many other processes. In this report, by using an experimental model that is sensitive to changes in coagulation and fibrinolytic factors, we have demonstrated that the absence of leptin or the leptin receptor has an important influence on in vivo thrombosis. Furthermore, we provide evidence that the short-term addition of leptin reverses the antithrombotic phenotype of the leptin-deficient mice but not the leptin receptor–deficient mice, demonstrating the receptor-dependent effect of leptin. Moreover, these findings suggest that the absence of the platelet leptin receptor following the bone marrow transplantation resulted in prolongation of the time to occlusion in wild-type mice with relatively low plasma leptin levels. Although
we cannot exclude an effect from other bone marrow-derived cells, the platelet-rich nature of the thrombus in this model suggests that the interaction between circulating leptin and the platelet-leptin receptor is highly sensitive and physiologically important even at low leptin levels. Therefore, the interaction between leptin and the leptin receptor may affect platelet aggregation and thrombosis in both lean and obese individuals. In addition, after our study was conducted, Konstantinides et al. published describing a different thrombosis model with a similar leptin effect.

These studies provide a potential link between obesity and cardiovascular disease risk. Wallace et al. demonstrated that leptin is an independent risk factor for coronary events. In their case-control study of hypercholesterolemic men, a 1-SD increase in leptin was associated with a 20% increase in risk for coronary events. Although further clinical studies are necessary to determine the magnitude of the cardiovascular risk associated with elevated leptin levels, these findings raise the possibility that therapeutic strategies aimed at reducing plasma leptin levels may reduce cardiovascular events. However, the potential beneficial effects must be weighed against the adverse effects that may arise with drug-induced leptin reduction, such as weight gain and its associated comorbidities. Since leptin levels correlate well with adiposity, strategies aimed at weight reduction should remain first-line therapies. Nevertheless, a strategy specifically targeting the leptin receptor on the platelet could provide benefit independent of changes in leptin.

In conclusion, we have demonstrated that leptin and the leptin receptor affect arterial thrombosis following endothelial injury and that these effects appear to be mediated by an interaction between leptin and the platelet-leptin receptor. These findings provide a potential link between increasing adiposity and thrombotic risk.

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REFERENCES

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