Simvastatin Decreases Myocardial Tumor Necrosis Factor α Content in Heart Transplant Recipients

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Background: Statins improve patient survival and decrease rejection episodes in heart transplant recipients. We studied the effects of simvastatin treatment on myocardial tumor necrosis factor α (TNF-α) expression; TNF-α is a potent pro-inflammatory cytokine associated with hypertrophy and fibrosis in heart transplant recipients.

Methods: We randomized 10 consecutive heart transplant recipients to receive either 20 mg/day simvastatin ($n = 5$) or placebo ($n = 5$) for 6 months after cardiac transplantation. Routine surveillance endomyocardial biopsy specimens were obtained from all patients. We analyzed tissues for myocardial TNF-α content, total collagen content, and myocyte size using semiquantitative immunohistochemistry.

Results: Myocyte size and total collagen content of placebo and simvastatin groups did not show a statistically significant difference at any biopsy time point. Myocardium TNF-α content (% tissue area stained) at 1 week after transplantation was similar in the simvastatin and placebo groups. At the 24th week after transplantation, when compared with Week 1 values, we found a significant decrease in myocardium TNF-α content in the simvastatin group (15.0% ± 2.3% vs 5.8% ± 2.4%, $p = 0.02$) that was not observed in the placebo group (15.0% ± 1.5% vs 12.0% ± 2.6%, $p =$ not significant).

Conclusion: Simvastatin treatment in heart transplant recipients decreased myocardium TNF-α expression. This decrease did not translate into a difference in the markers of hypertrophy. However, decreased myocardial TNF-α may be a marker of a general statin-mediated decrease in inflammation in the transplanted heart that leads to improved graft and patient survival. J Heart Lung Transplant 2005; 24:46–51. Copyright © 2005 by the International Society for Heart and Lung Transplantation.

Treatment with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) decreases plasma low-density lipoprotein (LDL) while increasing high-density lipoprotein (HDL). The use of statins decreases mortality and rejection episodes in cardiac transplant recipients;[1–3] however, the mechanisms responsible for the beneficial effects in heart transplant recipients are not known. An increasing number of reports indicate that the effects of statins are achieved not only through direct decrease in atherosclerosis secondary to LDL cholesterol decrease, but also through modulation of the immune response through down-regulation of intermediate products of the cholesterol biosynthetic pathway. For example, mevalonate, the product of HMG-CoA reduction, is necessary for lymphocyte proliferation;[4,5] in vitro and in vivo evidence suggests that statins consequently may inhibit lymphocyte proliferation and functionality.[6,7] Consistent with the hypothesis that statin therapy results in down-regulation of the inflammatory response, Calabresi et al[8] have shown that increased HDL concentration may exert a direct cardioprotective effect through inflammatory modulation as manifested by dose-dependent decreases in cardiac tumor necrosis α (TNF-α).[8] Studies of carotid plaques in patients treated with simvastatin showed significantly fewer macrophages, T lymphocytes, and HLA-DR+ cells than in untreated controls.[9] In heart transplant recipients, treatment with pravastatin is associated with a decrease in natural-killer cell cytotoxicity,[1] and a decrease in circulating concentrations of TNF-α, a decrease that reversed when pravastatin therapy was discontinued.[10]

Myocardial TNF-α is a pro-inflammatory cytokine that, when overexpressed, may lead to cardiomyopa-

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thy, left ventricular hypertrophy, and decreased cardiac contractility.[11] Tumor necrosis factor α is not present in normal myocardium; however, in failing myocardium, TNF-α expression increases and the receptors for TNF-α are down-regulated.[12] Furthermore, circulating concentrations of TNF-α also are increased in patients with heart failure, and the degree of increase correlates with worsening heart failure.[13] Together, these data indicate that TNF-α may play an important pathogenetic role in conditions of cardiac TNF-α overexpression.

In transplanted hearts, myocardial TNF-α concentrations increase rapidly after surgery and remain persistently increased. Interestingly, in association with neoexpression of TNF-α, accelerated cardiac hypertrophy also occurs, a condition that may explain post-transplant cardiomyopathy and diastolic dysfunction.[14] Thus, we suggest that TNF-α, by virtue of its ability to induce cardiac hypertrophy may contribute to long-term cardiac dysfunction after heart transplantation. Because HMG-CoA reductase inhibitors improve outcomes in heart transplant recipients and exert immunomodulatory effects, we investigated whether the use of simvastatin, an HMG-CoA reductase inhibitor, decreases myocardial TNF-α content in heart transplant recipients.

METHODS

Patient Cohort

We conducted the studies after written informed consent was obtained from each patient and with approval of the institutional review board of the Methodist Hospital and Baylor College of Medicine. The cohort was comprised of 10 consecutive patients who underwent cardiac transplantation at the Methodist Hospital/Baylor College of Medicine Multi-Organ Transplant Center. We conducted these studies before the clinically accepted practice of routine statin use in our program (1997).

Source of Human Myocardium

Heart transplant recipients were observed with routine rejection surveillance and clinically indicated endomyocardial biopsies. According to our institution protocol, serial endomyocardial biopsy specimens were obtained from all transplant recipients every week for the 1st month, every other week for the 2nd and 3rd months, and then once a month through the 6th month. Myocardial biopsy specimens (size, 0.5–1 mg; 4 per patient) were obtained with a bioptome under fluoroscopic guidance from the right ventricular side of the septum.

Myocardial Biopsies

Endomyocardial biopsy samples were immediately immersed in 2% paraformaldehyde for 45 minutes, followed by 75% alcohol, then dehydrated into increasing concentrations of alcohols, then cleared through xylene, and subsequently embedded in paraffin.

Total Collagen Content

Human myocardial tissue samples were sectioned at 5 μm and stained for 1 hour in picrosirius red solution (0.1% solution Sirius Red F3B in saturated aqueous picric acid, Direct Red 80 obtained from Sigma-Aldrich Chemical Company; Milwaukee, WI), as previously described.[15–17] The stained sections were then dehydrated rapidly in 3 changes of 95% and 100% alcohols, cleared in xylene, and mounted in xylene-based mounting medium. Total collagen content was the sum of all areas stained within the slide including interstitial, perivascular, and microscopic scars.

Myocyte Size

Endomyocardial biopsy samples were sectioned at 5 μm and stained with hematoxylin–eosin to measure myocyte size. At 40× magnification, a point-to-point perpendicular line was drawn across the cross-sectional area of the myocytes at the level of the nucleus, and computer-imaging software (Image-Pro® Plus Version 4.1, Media Cybernetics; Silver Spring, MD) then measured this diameter length. We excluded transverse- or oblique-sectioned myocytes. We measured 50 myocytes per slide from each tissue specimen and expressed results as mean and standard error measured.

Myocardial TNF-α Concentrations

We performed immunohistochemistry using a standard immunoperoxidase technique on 5-μm human tissue sections.[18] Endomyocardial biopsy samples were immediately immersed in 2% paraformaldehyde for 45 minutes followed by 75% alcohol and then dehydrated into increasing concentrations of alcohols, cleared through xylene, and subsequently embedded in paraffin. To detect TNF-α, deparaffinized sections were blocked for endogenous peroxidase activity and quenched by preincubating slides in 0.3% H_2O_2 in methanol for 20 minutes in a humidity chamber. Next, we flooded the slides with −20°C acetone for 3 minutes. After washing in phosphate buffer saline (PBS), we incubated the slides for 30 minutes in 1% blocking solution (1 g 99% albumin, bovine fraction V and 10 ml PBS).

A mouse monoclonal anti–TNF-α immunoglobulin G_1(IgG_1) antibody (dilution 1:10, Santa Cruz Biotechnology; Santa Cruz, CA) was applied before incubation for 2 hours in a humidity chamber. The tissues were then washed in PBS and incubated with biotinylated anti-mouse IgG, (dilution, 1:200; Vector Laboratories; Burlingame, CA) for 30 to 60 minutes. After another PBS washing, we treated tissue sections with streptavidin conjugated to horseradish peroxidase (Vector Laboratories; Burlingame, CA) for 30 minutes. After washes in