Simvastatin Reduces Graft Vessel Disease and Mortality After Heart Transplantation

A Four-Year Randomized Trial

Klaus Wenke, MD; Bruno Meiser, MD; Joachim Thiery, MD; Dorothea Nagel, PhD; Wolfgang von Scheidt, MD; Gerhard Steinbeck, MD; Dietrich Seidel, MD; Bruno Reichart, MD

Background Accelerated graft vessel disease (GVD) represents the most serious long-term complication of heart transplantation. A possible cause underlying this progressive coronary vascular disease is believed to be post-transplantation hypercholesterolemia.

Methods and Results In a 4-year prospective randomized study with heart transplant recipients, the efficacy of primary antihypercholesterolemic therapy with simvastatin was compared with that of general dietary therapy. The aim of the treatment was to maintain post-transplantation LDL-cholesterol levels at <120 mg/dL. Seventy-two heart transplant recipients receiving standard triple immunosuppression were randomly assigned to an active-treatment group (low-cholesterol diet and simvastatin, n=35) or a control group (general dietary measures, n=37). In the course of 4 years after transplantation, the simvastatin group had significantly lower LDL-cholesterol concentrations than the control group (mean±SD, 115±14 versus 156±17 mg/dL, $P=.002$), a significantly better long-term survival (88.6% versus 70.3%, $P=.05$), and a lower incidence of GVD in the coronary angiographic findings (16.6% versus 42.3%, $P=.045$). The incidence of graft rejections did not differ between the two groups, although there was a tendency toward a lower number of serious rejections in the simvastatin group (2.8% versus 13.5%, $P=.1$). Intracoronary ultrasound performed after 4 years in a subgroup of 27 patients (simvastatin, 10; control, 17) showed less intimal thickening in patients with LDL-cholesterol levels of <110 mg/dL (170±84 versus 370±171 μm, $P=.04$) and a lower intimal index (13.8±7.1% versus 27.9±12.1%, $P=.04$).

Conclusions In comparison with dietary measures alone, the combination of a low-cholesterol diet and simvastatin after heart transplantation led to a significant reduction in cholesterol levels, a significantly higher long-term survival rate, and a lower incidence of GVD. (***Circulation.* 1997;96:1398-1402.**)

Key Words • simvastatin • hypercholesterolemia • transplantation • graft vessel disease

Hypercholesterolemia—in particular, elevation of low-density lipoproteins (LDL) to >130 mg/dL—is observed in 60% to 80% of heart transplant recipients.[1,2] A number of studies have shown a correlation between hypercholesterolemia and the development of GVD.[3,4] However, other investigations have found no association between lipid levels and vessel disease in transplanted hearts.[5,6] Therefore, the importance of cholesterol levels in the pathology of graft vessel disease remains to be determined. Accelerated GVD is the most important late complication of heart transplantation, with an incidence of 5% to 10% per year.[7] The Scandinavian Simvastatin Survival Study (4S) showed that simvastatin, an HMG-CoA reductase inhibitor, significantly lowers cholesterol levels, extends overall survival, and reduces the number of serious cardiac events in nonimmunosuppressed coronary patients.[8] Even regression of existing atherosclerotic vascular wall changes has been observed after antihyperlipidemic therapy with HMG-CoA reductase inhibitors.[9-11] Moreover, in experiments in animals, simvastatin inhibited the proliferation of smooth muscle cells in cell cultures[12] and reduced the incidence of GVD after heterotopic heart transplantation.[13] Other in vitro studies have shown that the HMG-CoA reductase inhibitor lovastatin suppresses T lymphocytes (natural killer cells),[14] which may influence the development of transplantation rejection and, thus, the incidence of GVD. Furthermore, in a 12-month prospective trial, the HMG-CoA reductase inhibitor pravastatin was found to lower cholesterol levels; reduce the incidence of cardiac rejection accompanied by hemodynamic compromise, thereby improving first-year survival; delay development of GVD in the first year after cardiac transplantation; and reduce the increase of mean intimal thickness in heart transplant recipients.[15] Until now, because the concurrent use of the immunosuppressive agent cyclosporin A may lead to myolysis and rhabdomyolysis,[16,17] HMG-CoA reductase inhibitors have been used only very reluctantly for the treatment of hypercholesterolemia in heart transplant patients.[18-21]

See p 1370

In the present prospective randomized study, we set out to investigate the effects of primary long-term antihypercholesterolemic therapy with diet and simvastatin in terms of cholesterol levels, survival rate, graft rejection rate, and incidence of GVD.

Received February 28, 1997; revision received April 21, 1997; accepted April 26, 1997.

From the Department of Cardiac Surgery (K.W.), Munich-Bogenhausen; Department of Cardiac Surgery (B.M., B.R.), University Hospital, Munich-Grosshadern; Institute of Clinical Chemistry (J.T., D.N., D.S.), Medical Statistics, University Hospital, Munich-Grosshadern; and Department of Medicine I (W. von S., G.S.), University Hospital, Munich-Grosshadern, Germany.

Correspondence to Dr K. Wenke, Department of Cardiac Surgery, Munich-Bogenhausen, D-81925 Munich, Germany.

Selected Abbreviations and Acronyms

GVD = graft vessel disease
HMG-CoA = 3-hydroxy,3-methylglutaryl coenzyme A
ISLHT = International Society for Lung and Heart Transplantation
IVUS = intravascular ultrasound

Methods

Selection of Patients

After successful orthotopic heart transplantation, during the period of January 1, 1991, through December 31, 1991, 72 consecutive patients with or without hypercholesterolemia were randomly assigned to two different groups: 35 patients were treated with a low-cholesterol diet and simvastatin, and 37 patients were treated only by dietary measures. All patients received triple immunosuppression consisting of cyclosporin A (blood level >500 ng/mL), azathioprine (1 mg/kg body wt), and prednisolone (0.1 mg/kg body wt). Graft rejections were diagnosed by endomyocardial biopsy in response to clinical changes and classified according to the system of the ISLHT (mild, IA or IB; focal moderate, II; multifocal moderate, IIIA; diffuse borderline severe, IIIB).

Exclusion criteria were severe hepatic impairment (bilirubin >2 mg/dL) or renal impairment (creatinine >3 mg/dL), signs of existing myopathy, and known intolerance to HMG-CoA reductase inhibitors. The study design was accepted by the Ethics Committee of the Ludwig Maximilian University Munich.

Study Design

Patients in the active-treatment group received, in addition to a low-cholesterol diet, 5 mg/d simvastatin starting on the fourth postoperative day. The target was an LDL-cholesterol level of 110 to 120 mg/dL. In the fourth posttransplantation week, the simvastatin dose was increased to 10 mg/d, depending on the LDL-cholesterol level. After 6 weeks, the dose was again adjusted, if necessary, to a maximum of 20 mg/d simvastatin. The patients in the control group were treated by dietary measures alone. All patients received extensive dietary counseling with their partners in accordance with the guidelines for the American Heart Association stage II diet (total cholesterol intake <200 mg/d). In the first year after transplantation, laboratory tests were conducted regularly at 4-week intervals (creatinine kinase, complete blood counts, fibrinogen, lipoprotein analysis, and drug assays [simvastatin and cyclosporin A]). Clinical and echocardiographic examinations, chest radiographs, and endomyocardial biopsies were performed at the same intervals. The endomyocardial biopsies were analyzed by pathologists who had no information on the allocation of the patients to the respective treatment groups.

From the second to the fourth year after transplantation, all the aforementioned tests were performed at 3-month intervals on an outpatient basis. The study was planned to run for 4 years.

The aim of the study was to determine the efficacy of simvastatin therapy in terms of cholesterol levels, incidence of GVD, overall survival rate, and occurrence of acute graft rejections.

Coronary Angiography and IVUS

Coronary angiography was performed in the first posttransplantation month to establish the baseline coronary status and repeated at yearly intervals. GVD was defined as any angiographically demonstrated new stenosis of ≥50% or new distal obliterative changes. The angiographic findings were analyzed by two independent cardiologists who had no knowledge of the sequence of the angiographic examinations or the allocation of the patients to the treatment groups.

In a subgroup of 27 patients (simvastatin, 10; control, 17), who had given their respective consent, IVUS imaging was performed in conjunction with coronary angiography in the fourth postoperative year to detect any angiographically invisible changes of the intima. Because this technique was not available at the start of the trial, no baseline data were collected immediately after transplantation. The left anterior descending coronary artery, which served as the target vessel, was examined using a 30-MHz, 2.9-F IVUS catheter (CVIS). The images were recorded using a manual pullback from the distal LAD to the main stem of the left coronary artery. The measured data were analyzed off-line by quantitative morphometry. For quantification, the three most severely affected sites were examined and averaged. The parameters determined were the mean intimal thickness and intimal index, defined as the ratio of the area of plaque to total vessel area.

Statistical Analysis

The data from the two groups were compared with the help of the two-tailed t test and the χ^2 test. The log rank test was used to compare the Kaplan-Meier survival curves in the two groups. In all the tests used, the significance level was defined as $P=.05$.

Results

Patient Characteristics

The two treatment groups did not differ significantly in terms of the preoperative baseline data (Table 1), nor were there any differences with regard to a possible requirement for an immunosuppressive medication after transplantation. Blood cyclosporin A levels in the two groups did not differ significantly during the observation period. The average of simvastatin dose in the treatment group was 10 mg/d (5 to 15 mg/d). Infection complica-

TABLE 1. Baseline Characteristics of the Patients According to Study Group

	Simvastatin	Control
Recipient age, y*	49±11.5	46.8±14.3
Donor age, y*	30±11.1	33.9±10.4
Duration of ischemia, min*	175±62	180±52
Male/female, n	30/5	34/3
Body mass index before transplantation, kg/m^2*	22.51±3.2	23.2±2.9
Coronary heart disease before transplantation, %	28	26
Hypercholesterolemia (cholesterol >250 mg/dL) before transplantation, %	37	32
Diabetes, %	11	8
CMV-positive donor/CMV-negative recipient, %	16	18

CMV indicates cytomegalovirus. *Values are mean±SD.

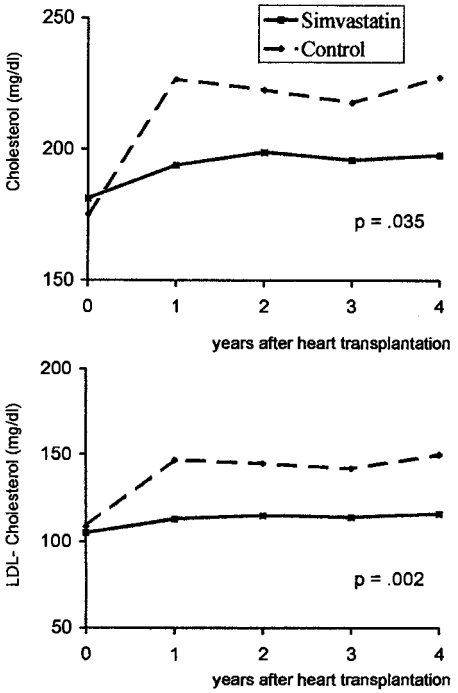

FIG 1. Mean±SD cholesterol and LDL-cholesterol levels during 4 years after heart transplantation (to convert values to mmol/L, multiply by 0.02586).

tions (6 patients in the simvastatin group versus 5 in the control group) and diagnosed cytomegalovirus infections (4 versus 3) did not differ significantly in the two groups. Hypertension requiring treatment occurred in 16 patients in the simvastatin group compared with 14 patients in the control group. All the affected patients were treated with ACE inhibitors. The mean blood pressure values were 133±13/86±8 mm Hg in the simvastatin group versus 135±14/85±11 mm Hg in the control group and did not differ significantly. Laboratory tests (creatinine kinase, complete blood counts, fibrinogen) did not reveal any significant differences between the two groups throughout the 4-year observation period.

Cholesterol Level

Cholesterol and LDL levels were analyzed repeatedly during the study (24 times per patient). The pretransplantation cholesterol levels were comparable in the two groups: 181±17 mg/dL in the simvastatin group versus 175±19 mg/dL in the control group. In the course of the study, the mean serum cholesterol level in the simvastatin group was significantly lower than that in the control group (198±18 versus 228±19 mg/dL, P=.03). The LDL levels at the time of transplantation were likewise comparable in the two groups: 105±15 mg/dL in the simvastatin group versus 109±13 mg/dL in the control group. In the long term, significantly lower LDL levels were found in the simvastatin group than in the control group (115±14 versus 156±17 mg/dL, P=.002) (Fig 1). Elevated liver enzymes and renal functional parameters were not observed. Myolysis was not observed in any patient, and creatinine kinase remained within the normal range in all the study participants.

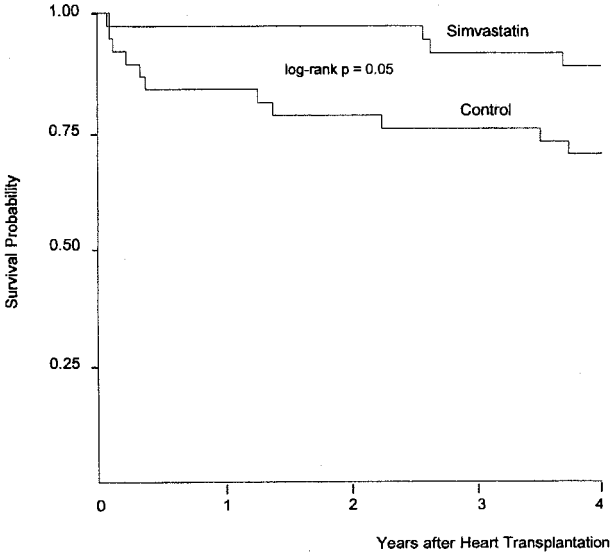

FIG 2. Kaplan-Meyer curves for survival 4 years after heart transplantation in the study patients.

Survival Rate

After 4 years of observation, the survival rate was significantly higher in the simvastatin group than in the control group. After this period, 88.6% of the patients treated with simvastatin were still alive compared with 70.3% of the control patients (P=.05) (Fig 2). The causes of death in the simvastatin group were severe graft rejection (n=1), severe pulmonary infection (n=2), and GVD (n=1); in the control group, the causes were severe graft rejection (n=5), severe pulmonary infection (n=2), multiple-organ failure (n=1), prostate cancer (n=1), and GVD (n=2).

Coronary Angiography

Of the patients in the simvastatin group, 3% showed coronary angiographic signs of GVD in the first posttransplantation year, 9.1% in the second year, 12.9% in

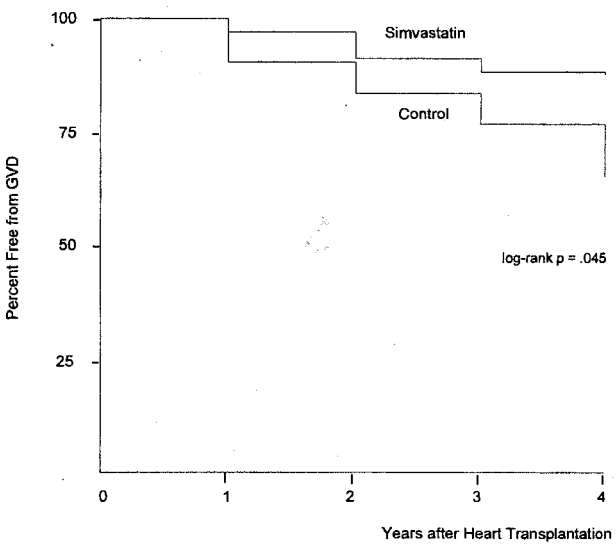

FIG 3. Kaplan-Meyer curves for freedom from graft vessel disease during 4 years after heart transplantation in the study patients.

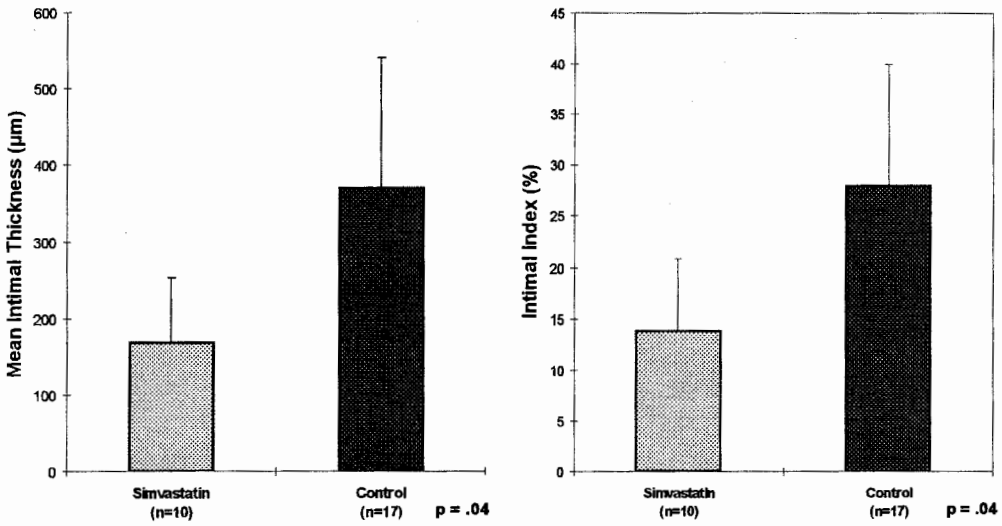

FIG 4. Compared with the low LDL-cholesterol patients (LDL <110 mg/dL), the group with LDL-cholesterol >110 mg/dL had significantly greater mean intimal thickness (P=.04) and intimal index (P=.04) 4 years after cardiac transplantation. Values are expressed as mean±SD.

the third year, and 16.6% after 4 years. The corresponding figures in the control group were 10% after the first year, 17.2% after the second year, 25.9% after the third year, and 42.3% after 4 years. The differences between the two groups were significant over the 4-year observation period (P=.045). The 3 patients who died from GVD were included in the statistical analysis (Fig 3).

Intracoronary Ultrasound

In the fourth postoperative year, 27 patients (simvastatin, 10; control, 17) were examined using an intracoronary ultrasound catheter in conjunction with coronary angiography. Patients of the treatment group with an LDL-cholesterol level of <110 mg/dL had significantly less mean intimal thickness than those of the control group, with an LDL-cholesterol level of >110 mg/dL (170±84 versus 370±171 µm, P=.04) as well as a significantly lower intimal index (13.8±7.1% versus 27.9±12.1%, P=.04) (Fig 4).

Graft Rejections

The mean incidence of mild (ISLHT Ia, Ib) and moderate (ISLHT II, IIIa) graft rejections was not significantly different between the two groups. However, the control patients showed a statistical tendency toward a higher incidence of severe graft rejections (ISLHT IIIb) accompanied by graft failure. In the simvastatin group, only 1 patient died as the result of refractory graft rejection (2.8%) compared with 5 patients in the control group (13.5%, P=.1) (Table 2).

TABLE 2. Incidence of Cardiac Rejection in the Treatment Groups

Rejection Episodes/Patient, n	Simvastatin (N=35)	Control (N=37)	*P*
Grade* IA or B	2.0±1.3	2.1±1.2	.80
Grade* II	1.6±0.9	1.2±0.8	.59
Grade* IIIA	0.6±0.5	0.7±0.6	.43
Grade* IIIB	0.3±0.5	0.6±0.7	.39
Death from rejection, %	2.8	13.5	.1

*According to the ISLHT classification system, mild cardiac rejection is classified as grade IA or IB, focal moderate rejection as grade II, multifocal moderate rejection as grade IIIA, and diffuse borderline severe rejection as grade IIIB. Values are mean±SD.

Discussion

The results of the present long-term study show that simvastatin therapy, initiated early after heart transplantation, safely and effectively reduced total and LDL cholesterol, significantly improved the 4-year survival rate, and significantly reduced the incidence of GVD. These effects could, on the one hand, result directly from the cholesterol reduction; on the other hand, cholesterol-independent effects of simvastatin on the immune system, which are still not clearly understood, are also possible. HMG-CoA reductase inhibitors have been shown to regulate DNA in cycling cells,[22] inhibit monocyte chemotaxis,[23] regulate cytotoxicity of T lymphocytes,[14,15,24] and inhibit antibody-dependent cellular cytotoxicity.[25] All the aforementioned characteristics could account for the fact that fewer severe graft rejections occurred with simvastatin. Simvastatin-induced potentiation of the immunosuppressive action of cyclosporin A is also a possibility. The immunosuppressive effect of cyclosporin A is due to blockade of interleukin-2 synthesis in activated T lymphocytes. A similar effect of the HMG-CoA reductase inhibitor lovastatin has been demonstrated in cell cultures. Lovastatin inhibited antibody-dependent cytotoxicity of T lymphocytes, an effect that was neutralized by the addition of interleukin-2 to the cell culture.[26] These observations could explain a potential synergistic immunosuppressive action of cyclosporin A and simvastatin. It should also be borne in mind that a large proportion of cyclosporin A is bound to LDL in plasma. Drug-induced reduction of LDL may therefore may lead to more free cyclosporin in the blood and, consequently, prevention of cardiac rejection.

Hypercholesterolemia is regarded as the chief risk factor for coronary heart disease.[27] Consistent treatment of hyperlipidemia brings about a marked reduction in coronary heart disease mortality and the incidence of cardiac events, as demonstrated in the 4S[8] and the Pravastatin Multinational Study.[28] According to the results of our study, patients with low cholesterol levels had a significantly lower incidence of GVD over the course of 4 years. In this respect, early postoperative initiation of simvastatin therapy appears to be important because liquid cholesterol deposits occur in the vascular wall even in the early phase.[29] GVD associated with

heterotopic heart transplantation was also significantly reduced with simvastatin in an animal model.[13] In in vitro studies, simvastatin inhibited the proliferation of smooth muscle cells,[12] a process that is believed to play a key role in atherogenesis.[30] This effect of simvastatin could explain why the IVUS-determined coronary intima is significantly less thick in patients with low cholesterol levels. In compliance with these findings, other workers[15] observed a significantly reduced increase of the intimal thickness 1 year after cardiac transplantation in patients treated with pravastatin; they also reported on a significantly better first-year survival and reduced cardiac rejection accompanied by hemodynamic compromise in these patients. However, as of today, the described effects of antihypercholesterolemic therapy on the development of GVD had not been observed in long-term studies, which are required to investigate a slow process like the development of GVD.

The baseline data showed two statistically comparable groups. The incidence of GVD, cholesterol concentrations, and the survival rate represented objective end points permitting a valid statistical analysis.

In conclusion, in heart transplant recipients, simvastatin significantly reduces cholesterol and LDL-cholesterol levels, significantly improves the long-term survival rate, lowers the incidence of GVD, and reduces graft rejections with graft failure. It therefore appears reasonable to initiate routine antihyperlipidemic therapy with simvastatin or other HMG-CoA reductase inhibitors as early as possible after heart transplantation.

Acknowledgments

We are indebted to Traute Meyer and Klaus Krischock for preparation of the manuscript.

References

1. Keogh A, Simons L, Spratt P, Esmore D, Chang V, Hickie J, Baron D. Hyperlipidemia after heart transplantation. *J Heart Transplant*. 1988;7:171-175.
2. Miller LW, Schlant RC, Kobashigawa JA, Kubo S, Renlund DG. 24th Bethesda Conference: Cardiac transplantation: Task Force 5: complications. *J Am Coll Cardiol*. 1993;22:41-54.
3. Eich D, Thompson JA, Ko DJ, Hastillo A, Lower R, Katz S, Hess ML. Hypercholesterolemia in long-term survivors of heart transplantation: an early marker of accelerated coronary artery disease. *J Heart Lung Transplant*. 1991;10:45-49.
4. Sharpless LD, Caine N, Mullins P, Scott JP, Solis E, English TAH, Large SR, Schofield PM, Wallwork J. Risk factor analysis for the major hazards following heart transplantation: rejection, infection and coronary occlusive disease. *Transplantation*. 1991;52:244-252.
5. Uretsky BF, Murali S, Reddy PS, Rabin B, Lee A, Griffith BP, Hardesty RL, Trento A, Bahnson HT. Development of coronary artery disease in cardiac transplant patients receiving immunosuppressive therapy with cyclosporine and prednisolone. *Circulation*. 1987;76:827-834.
6. Adams DH, Karnovsky MJ. Hypercholesterolemia does not exacerbate arterial intimal thickening in chronically rejecting rat cardiac allografts. *Transplant Proc*. 1989;21:437-439.
7. Gao SZ, Schroeder JS, Hunt S, Stinson SB. Retransplantation for severe accelerated coronary artery disease in heart transplant recipients. *Am J Cardiol*. 1988;62:876-881.
8. The Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary artery disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet*. 1994;344:1383-1389.
9. Brown G, Albers JJ, Fisher LD, Schaefer SM, Lin JT, Kaplan C, Zhao XQ, Bisson BD, Fitzpatrick VF, Dodge HT. Regression of coronary artery disease as a result of intensive lipid lowering therapy in men with high levels of apolipoprotein B. *N Engl J Med*. 1990;232:1289-1298.
10. Kane JP, Malloy MJ, Ports TA, Phillips NR, Diehl JC, Havel RJ. Regression of coronary atherosclerosis during treatment of familial hypercholesterolemia with combined drug regimens. *JAMA*. 1990; 264:3007-3012.
11. Blankenhorn DH, Hodis HN. Atherosclerosis: reversal with therapy. *West J Med*. 1993;159:172-179.
12. Corsini A, Raiteri M, Soma MR, Gabbiani G, Paoletti R. Simvastatin but not pravastatin has a direct inhibitory effect on rat and human myocyte proliferation. *Clin Biochem*. 1992;25:399-400.
13. Meiser BM, Wenke K, Thiery J, Wolf S, Devens CH, Seidel D, Hammer C, Billingham ME, Reichart B. Simvastatin decreases accelerated graft vessel disease after heart transplantation in an animal model. *Transplant Proc*. 1993;25:2077-2079.
14. Cutts S, Bankhurst AD. Reversal of lovastatin-mediated inhibition of natural killer cell toxicity by interleukin 2. *J Cell Physiol*. 1990; 145:244-252.
15. Kobashigawa JA, Katznelson W, Laks H, Johnson JA, Yeatman L, Wang XM, Chia D, Terasaki PI, Sabad A, Cogert GA, Trosian K, Hamilton MA, Moriguchi JD, Kawata N, Hage A, Drinkwater DC, Stevenson LW. Effects of pravastatin on outcomes after cardiac transplantation. *N Engl J Med*. 1995;333:621-627.
16. Corpier CL, Jones PH, Suki WN, Lederer ED, Quinones MA, Schmidt SW, Young JB. Rhabdomyolysis and renal injury with lovastatin use: report of two cases in cardiac transplant recipients. *JAMA*. 1988;260:239-241.
17. Kobashigawa JA, Murphy FL, Stevenson LW, Moriguchi JD, Kawata N, Kamjoo P, Brownfield E, Wilmarth J, Leonhard L, Chuck C, Drinkwater D, Laks H. Low dose lovastatin safely lowers cholesterol after cardiac transplantation. *Circulation*. 1990;82(suppl IV):IV-281-IV-283.
18. Carrier M, Pelletier GB, Genest J Jr, Cartier R, Leclerc Y, Pelletier LC. Cholesterol lowering intervention and coronary artery disease after cardiac transplantation. *Ann Thorac Surg*. 1994;57:353-356.
19. Pflugfelder PW, Huff M, Oskalus R, Rudas L, Kostuk WJ. Cholesterol lowering therapy after heart transplantation: a 12 month randomised trial. *J Heart Lung Transplant*. 1995;14:613-622.
20. Wenke K, Thiery J, Meiser B, Arndtz N, Seidel D, Reichart B. Long-term simvastatin therapy for hypercholesterolemia in heart transplant recipients. *Z Kardiol*. 1995;84:130-136.
21. Barbir M, Rose S, Kushawaha S, Akl S, Mitchell A, Yacoub M. Low dose simvastatin for treatment of hypercholesterolemia in recipients of cardiac transplantation. *Int J Cardiol*. 1991;33:214-246.
22. Doyle JW, Kandutsch AA. Requirement for mevalonate in cycling cells: quantitative and temporal effects. *J Cell Physiol*. 1988;137: 133-140.
23. Kreuzer J, Bader J, Jahn L, Hauptmann M, Kubler W, von Hodenberg E. Chemotaxis of the monocyte cell line U 937: Dependence on cholesterol and early mevalonate pathway products. *Atherosclerosis*. 1991;90:203-209.
24. Linna J, Moke M, Chen HW. Isprenoid formation and cell-mediated immunological function. In: Friedmann H, Specter S, Klein TW, eds. *Advances in Experimental Medicine and Biology: Volume 288: Drugs of Abuse, Immunity and Immunodeficiency*. New York, NY: Plenum Press; 1991:269-278.
25. Kirby J, Givan AL, Shenton BK, Talbot D, Forsythe JLR, Lennard TWJ, Proud G, Taylor RMR. Renal allograft rejection: Possible involvement of antibody-dependent cell-mediated cytotoxicity. *Transplantation*. 1990;50:225-229.
26. McPherson R, Tsoukas C, Baines MG, Vost A, Melino MR, Zupkis RV, Pross HF. Effects of lovastatin on natural killer cell function and other immunological parameters in men. *J Clin Immunol*. 1993; 13:439-444.
27. La Rosa JC, Hunninghake D, Bush D, Riqui MH, Getz GS, Gotto Jr AM, Grundy SM, Rakita L, Robertson RM. The cholesterol facts: a summary of the evidence relating dietary facts, serum cholesterol and coronary heart disease: a joint statement by the American Heart Association and the National Heart, Lung, and Blood Institute. *Circulation*. 1990;81:1721-1733.
28. The Pravastatin Multinational Study Group for Cardiac Risk Patients. Effects of pravastatin in patients with serum total cholesterol levels from 200 to 300 mg/dL plus two additional atherosclerotic risk factors. *Am J Cardiol*. 1993;73:1031-1037.
29. Lin H, Wilson JE, Kendall TJ, Radio SJ, Cornhill FJ, Herderick E, Winters GL, Costanzo MR, Porter T, Thieszen SL, McManus BM. Comparable proximal and distal severity of intimal thickening and size of epicardial coronary arteries in transplant arteriopathy of human cardiac allografts. *J Heart Lung Transplant*. 1994;13:824-833.
30. Ross R, Glomset JA. The pathogenesis of atherosclerosis. *N Engl J Med*. 1976;295:369-377.