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Intercellular and Vascular Cell Adhesion Molecule Levels in Endoscopic and Open Saphenous Vein Harvesting for Coronary Artery Bypass Surgery

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ABSTRACT

Background: Numbers of intercellular and vascular cell adhesion molecules (ICAM and VCAM) and major ligands on endothelial cells for adherence of activated polymorphonuclear leukocytes, macrophages, and lymphoid cells increase in many inflammatory disorders and after trauma to different tissues.

Methods: Samples of human saphenous veins were harvested from 90 randomly selected patients who underwent coronary artery bypass graft (CABG) surgery, utilizing two different techniques (open and endoscopic). Endothelial cells were collected from the vein samples and cultured for 72 hours. Pre- and postoperative sera, in addition to the supernatants from the cultures, were analyzed for ICAM-1 and VCAM-1 using enzyme-linked immunosorbent assay.

Results: Mean preoperative levels of ICAM-1 and VCAM-1 (0.95 ± 0.58 ng/mL and 1.81 ± 1.03 ng/mL, respectively) did not differ significantly from that of postoperative sera (0.98 ± 0.451 ng/mL and 1.74 ± 1.05 ng/mL, respectively) (p = 0.77 and p = 0.73, respectively). Mean ICAM-1 and VCAM-1 levels in endothelial cell culture supernatants did not differ significantly between the endoscopic (0.16 ± 0.05 ng/mL and 0.23 ± 0.10 ng/mL, respectively) and the open method (0.18 ± 0.08 ng/mL and 0.30 ± 0.27 ng/mL, respectively) (p = 0.19 and 0.13, respectively).

Conclusion: Our findings indicate that endoscopic and open saphenectomies are technically comparable in their effects on ICAM-1 and VCAM-1 synthesis during saphenous vein harvesting for CABG. We recommend the endoscopic method for its low morbidity and earlier hospital discharge.

INTRODUCTION

Meticulous preservation of the endothelial lining of vein grafts harvested during coronary artery bypass graft (CABG) surgery is undoubtedly an important factor in determining patency rates following bypass procedures. Intact, confluent endothelium serves as an electrical, mechanical and physiological barrier between flowing blood and the subendothelium. Destruction of the endothelial lining of the vein graft prior to graft implantation results in a more thrombogenic graft, occlusive medial smooth muscle cell proliferation and intimal migration [Cunningham 1981].

Among the most important factors that affect the integrity of the endothelial lining of the harvested vein is the technique of harvesting. Historically, the saphenous vein is exposed and harvested through a long continuous incision. It has been suggested that the long skin incision may be related to an increase in wound complications by as much as 43% [Johnson 1988, Schwartz 1988, Wengrovitz 1990, Reifsnyder 1992, Wipke-Tevis 1996, Robbins 1998], that include cellulitis, hematoma, seroma, edema, saphe-

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nous neuropathy/neuralgia, and ischemic sequelae. This is especially true for patients with risk factors such as female gender, obesity, smoking, hypertension, diabetes mellitus, and peripheral vascular disease [Utly 1989, Cable 1998].

Endoscopic saphenous vein harvesting (ESVH) is a new technique in which the greater saphenous vein is retrieved through a small incision at the knee, instead of through the long continuous incision along its course that is made during the open saphenous vein harvesting (OSVH). The motivation behind the endoscopic method is to decrease wound complication and postoperative pain, and ultimately to decrease the length of hospital stay and cost, but the structural and morphological effect of this technique on the vein is not clear [Allen 1998].

Intercellular adhesion molecule (ICAM)-1 and vascular adhesion molecule (VCAM)-1, two major cytokines that belong to the immunoglobulin supergene family, play a prominent role in modulating the inflammatory reaction secondary to the trauma of vein harvesting. They are expressed or induced on vascular endothelium and are able to regulate the adhesion of leukocytes to the endothelial cell surface [Bevilacqua 1993, Kanda 1998]. Vascular endothelial cells are therefore important participants in normal leukocyte trafficking and the development of inflammatory reactions [Heraldsen 1996], and CAM-mediated leukocyte trafficking is an important feature in the initiation and progression of the inflammatory state involving microvasculature [Granger 1994]. Assays of ICAM-1 and VCAM-1 in sera and culture supernatants are becoming an important part of clinical laboratory practice. Enzyme-linked immunosorbent assay (ELISA) is the most widely used assay for this analysis.

The purpose of this study was to evaluate the difference in levels of these two adhesion molecules in the new endoscopic and the classic open techniques of saphenous vein harvesting for CABG.

MATERIALS AND METHODS

Vein Retrieval

This study was done in Maimonides Medical Center (MMC) from November 1998 to May 1999. Saphenous veins were harvested from 45 patients prepared for CABG. In the standard technique of open (no-touch) vein harvesting, the greater saphenous vein is exposed and harvested under direct vision through a long continuous skin incision, with the patient's leg in a "frog-leg" position. In the endoscopic technique, a small incision is made 4 fingers in breadth posterior to the proximal margin of the patella; the greater saphenous vein is identified and dissected both cranially and distally under endoscopic visualization through the incision, then stripped and retrieved. The standard instrumentation used in the procedure is commercially known as the Endo-Path (Ethicon Endo-Surgery, Inc, Cincinnati, OH). It comprises a subcutaneous dissector, retractor, and a modified vein stripper. In addition, standard endoscopic equipment including a television monitor, light source, fiber optic camera and a 5-mm lens were used.

We sampled two vein segments by using each of the two harvesting techniques on the same leg of each study patient. This was done by excising a 5-cm segment of the thigh portion of the saphenous vein, which was cranially dissected and retrieved endoscopically, as the endoscopic sample; the sample was clipped distally for orientation of blood flow direction. In the same procedure, the vein segment remaining at the site of the incision, about 10 cm in length, was excised as the direct or open sample.

The veins were harvested and handled under sterile conditions according to the operating room protocol at MMC.

Vein Preparation

Vein samples were incubated in 10 mL Iscove's Modified Dulbecco's Medium (IMDM) with 200 µL penicillin-streptomycin during transport to the laboratory. In the laboratory, each vein sample was flushed and cannulated, injected with plasma-lyte, and the branches were ligated with 3.0 silk sutures.

Solutions

The chemical characteristics of the plasma-lyte solution was as follows: each 100 mL contained 526 mg sodium chloride USP, 502 mg sodium gluconate USP, 368 mg sodium acetate trihydrate USP, 37 mg potassium chloride USP, and 30 mg magnesium chloride USP (sodium = 140 mEq/L, potassium = 5 mEq/L, magnesium = 3 mEq/L, chloride = 98 mEq/L, acetate = 27 mEq/L, gluconate = 23 mEq/L). The pH of the solution was adjusted with sodium hydroxide to 7.4 (pH 6.5–pH 8.0) and the osmolarity was 294 mosmol/L. The type and composition of the solutions in our experiments were chosen based upon MMC cardiac surgical practice recommendations and recent surveys of North America Cardiac Surgery Centers, as well as upon recipes suggested in the current literature.

Endothelial Cell Culture

At the conclusion of the previous procedure, vein samples were collected and transported using the same transport medium (IMDM). Then, under a laminar flow hood, the pair of veins (OSVH and ESVH) were placed in petri dishes each containing 5 mL of endothelial cell culture medium (IMDM with 25 mM HEPES [hydroxy ethyl piperazine ethane sulfonic acid], 3.024 g/L $NaHCO_3$, 100 U/mL penicillin, 100 µg/mL streptomycin, 15% FBS [fetal bovine serum], 130 U/mL heparin, and 2 mmol/L L-glutamine).

Each vein was flushed with the indicated solution and divided into two pieces using a sterile scalpel (no. 15 blade). The proximal piece was used for culture and the distal piece for microscopic analysis (unpublished data). The proximal piece of the vein was slit open so that it lay flat. The luminal surface was scraped with a sterile scalpel (no. 11 blade) using light, single strokes, covering each area only once. Cells that built up on the scalpel blade were shaken off into the endothelial culture medium. The vein thereafter was rinsed using the culture medium, and the supernatant containing endothelial cells was then preserved in 25-mL culture flasks. These flasks were then incubated at 37°C and 5% CO_2 with humidifier for the next 72 hours.

Table 1. Mean human ICAM concentrations (ng/mL), pre- and post-CABG, and in endothelial cell culture supernatants from saphenous veins harvested using the endoscopic or open techniques*

Preoperative	Postoperative	Endoscopic	Open
0.95 ± 0.58	0.98 ± 0.45	0.16 ± 0.05	0.18 ± 0.08

*ICAM indicates intercellular adhesion molecule; CABG, coronary artery bypass grafting.

Table 2. Mean human VCAM concentrations (ng/mL), pre- and post-CABG, and in endothelial cell culture supernatants from saphenous veins harvested using the endoscopic or open techniques*

Preoperative	Postoperative	Endoscopic	Open
1.81 ± 1.03	1.74 ± 1.05	0.23 ± 0.10	0.30 ± 0.27

*VCAM indicates vascular cell adhesion molecule; CABG, coronary artery bypass grafting.

Enzyme-Linked Immunosorbent Assay

Preoperative and postoperative blood samples were immediately centrifuged at 3000g for 10 minutes at 28°C. Sera from the blood samples and the supernatants of the 72-hour endothelial cell cultures were evaluated for ICAM-1 and VCAM-1 levels by ELISA. A commercially available ELISA kit (Biosource International, CA, USA.) was used.

Well plates were pre-coated with an antibody specific for human (h)-ICAM and h-VCAM. Samples were diluted 1:10 and 1:50 with standard diluent buffer. The standard samples and biotinylated secondary antibody were incubated for one hour at room temperature on microtiter strips. Wells were washed four times with washing buffer; streptovidin-peroxidase (enzyme) was added, then incubated for 30 minutes at room temperature.

Subsequently, the wells were washed four times with the same buffer; a substrate solution, chromogen, was added. The wells were then incubated for 30 minutes at room temperature in a dark place. Finally, a stop solution was added. The optical density of the resulting color was read at 450 nm with a microplate reader.

Statistical Analysis

Results were expressed as mean ± standard deviation. Data were analyzed using a single analysis of variance program written for Lotus 1-2-3 (version 2.2; Lotus Development Corporation) (Table 1, ◎). The two-tailed Student *t* test was used to compare the mean values of h-ICAM and h-VCAM levels, with a level of significance (p value) of 0.05 or less.

RESULTS

Human ICAM

The mean concentration of ICAM-1 in preoperative sera was 0.95 ± 0.58 ng/mL and for postoperative sera was 0.98 ± 0.45 ng/mL. The difference between the means was not statistically significant (p = 0.77). The mean concentration of h-ICAM in the 72-hour endothelial cell culture supernatants from ESVH was 0.16 ± 0.05 ng/mL, and for the OSVH was 0.18 ± 0.08 ng/mL. The difference between these means was also not statistically significant (p = 0.19).

Human VCAM

The mean concentration of VCAM-1 in preoperative sera was 1.81 ± 1.03 ng/mL and that for postoperative sera was 1.74 ± 1.05 ng/mL (Table 2, ◎). The difference between the means was not statistically significant (p = 0.73). The mean concentration of h-VCAM in the 72-hour endothelial cell culture supernatants from ESVH was 0.23 ± 0.10 ng/mL and from OSVH was 0.30 ± 0.27 ng/mL. The difference was not statistically significant (p = 0.13).

DISCUSSION

The success of the saphenous vein conduit used as a CABG has been linked to a number of specific properties of the vessel wall [Luscher 1991, Chester 1996]. Endothelial adhesion molecules are classified according to their molecular structure. ICAM and VCAM are molecules that belong to the immunoglobulin supergene family, are 95–130 kDa in weight, comprise 90–100 amino acids arranged in a sandwich of two sheets of anti-parallel β strands, and are stabilized by a central disulfide bridge [Alzari 1988, Springer 1990, William 1998]. The immunoglobulin molecule ICAM-1 binds to its ligand lymphocyte function–associated antigen (LFA)-1, which is present on all leukocytes [Marlin 1987]. LFA-1 is primarily responsible for regulating the avidity of all cell interactions [Springer 1990]. VCAM-1 binds to its co-receptor, the very late antigen (VLA)-4 which is present on monocytes and lymphocytes but not on neutrophils [Elices 1990]. It is believed that recruitment of cells by these adhesion molecules plays a role in atherogenesis [Grimbrone 1990, Wood 1993].

The up-regulation of cell adhesion molecules on the surface of vascular endothelium cells by inflammatory cytokines facilitates the adhesion and extravasation of peripheral leukocytes. Not only is leukocyte trafficking important in the microcirculation where its pathophysiologic significance has been well characterized [Shanley 1995, Kanda 1998], but cell adhesion molecule–mediated leukocyte adhesion and extravasation are also consequential events in the development of large-vessel vasculopathies [Cybulsky 1991]. In addition, it has been clearly shown that antibodies against ICAM-1 are capable of preventing reperfusion injury induced by polymorphonuclear leukocytes [Ma X-I 1992], supporting the hypothesis considering the significance of adhesion molecules in vein-conduit patency.

Although earlier studies did not extensively examine the underlying physiologic and biochemical mechanisms

[Cunningham 1981], it was understood that steps involved in the preparation and handling of the saphenous vein were critically important and had a major impact on the eventual health and patency of the constructed bypass conduit [Etchberger 1991, Quist 1992]. The assumption was that a vein graft with normal morphology at the time of reimplantation had the greatest likelihood of adapting to the arterial flow without subsequent lumen reduction due to thrombus or intimal fibromuscular lesion formation [Cunningham 1981].

Much of the focus in the literature has been on the importance of vein handling, i.e., preservation relevant to the open, no-touch technique of vein harvesting. In 1985, Meldrum-Hanna et al. described a new technique of saphenous vein harvesting which was a version of the standard well-known open method at that time, using multiple small incisions and a Mayo "stripper" rather than a single long cut, thus minimizing major wound complications associated with it [Wyatt 1966]. They concluded at the end of their study that vein harvesting by their method was easy, with good results both morphologically and functionally. In addition, patients had minimal wound complications and superior cosmetic results. Later in 1996, Allen et al. described a further development with the introduction of the endoscopic technique, focusing on the lower wound complication rates compared with the traditional method and the minimal impact on vein morphology and structure [Allen 1998]. In contrast to older beliefs, ESVH has revolutionized surgical intervention with a uniquely safe vein conduit, both in structure and morphology [David 1990, Santoli 1993].

We demonstrated in our study that there was no significant difference between the pre- and postoperative levels of ICAM and VCAM and that neither technique of saphenous vein harvesting (OSVH or ESVH) changed the mean concentration levels of these adhesion molecules. The comparability of the two methods suggests that vein manipulation and minor physical shears in operative extraction of the vein conduit would have minimal impact on adhesion molecule release from the endothelial cells, which will subsequently affect the performance of the vein conduit.

One limitation of this study is that we evaluated the levels of both adhesion molecules in the supernatants of the endothelial cell cultures for 72 hours only. Follow-up that requires culturing for a longer period and daily measurement of the adhesion molecules would be more accurate than single measurements.

The other disturbing limitation was the effect of cardiopulmonary bypass that causes activation of leukocytes, especially neutrophils with the release of a number of cytokines such as interleukin (IL)-1, IL-2, IL-10, tumor necrosis factor α, and interferon γ [Finn 1992, Gu 1992, Gillinov 1993, Deng 1995]. These cytokines and other released factors in the blood can cause up-regulation of adhesion molecules and thus affect future performance of bypass conduits.

Longer follow-up of the patency rate of saphenous vein grafts in patients who had CABG using the endoscopic technique for vein harvesting, then comparing them to randomly selected patients with grafts harvested by the old traditional technique, will be required to further validate our results.

CONCLUSION

The comparable results of adhesion molecule expression in both techniques indicates that the novel endoscopic technique of vein harvesting could be a safe and unique method for vein preparation during CABG surgery, keeping in mind its low morbidity rates. Thus, this method is a reasonable alternative to the standard open method and has several potential advantages.

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REFERENCES

1. Allen KB, Griffith GL, Heimansohn DA, Robison RJ, Matheny RG, Schier JJ, et al. Endoscopic versus traditional saphenous vein harvesting: a prospective, randomized trial. Ann Thorac Surg 66:26–32, 1998.
2. Alzari PM, Lascombe MB. Poljak RJ. Three-dimentional structure of antibodies. Annu Rev Immunol 6:555–80, 1988.
3. Bevilacqua MP. Endothelial-leukocyte adhesion molecules. Annu Rev Immunol 11:767–804, 1993.
4. Cable DG, Dearani JA, Pfeifer EA, Daly RC, Schaff HV. Minimally invasive saphenous vein harvesting: endothelium integrity and early clinical results. Ann Thorac Surg 66: 139–43, 1998.
5. Chester AH, Borland JA, Taylor PM, Rose ML, Yacoub MH. Vascular adhesion molecules and immunogenicity in blood vessels used as coronary artery bypass grafts. Eur J Cardiothorac Surg 10:676–83, 1996.
6. Cunningham JN, Spencer FC. Vein contraction and smooth muscle cell extension as causes of endothelial damage during graft preparation. Ann Surg 194:2, 1981.
7. Cybulsky MI, Gimbrone MA Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science 257:788–91, 1991.
8. David D, Mohammed SF, Woodward SC, Nelson RM. The influence of technique on endothelial preservation in saphenous veins. J Surg Res 52:219–25, 1990.
9. Deng MC, Wiedner M, Erren M, Mollhoff T, Assmann G, Scheld HH. Arterial and venous cytokine response to cardiopulmonary bypass for low risk CABG and relation to hemodynamics. Eur J Cardiothorac Surg 9:22–9, 1995.
10. Elices MJ, Osborn L, Takada Y, Crouse C, Luhowskyj S, Hemler ME, et al. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. Cell 60:577–84, 1990.
11. Etchberger KJ, Rodewald AJ, Raper BA, Kevorkian MA, Herring MB. Storage condition affect endothelial cell morphology and yields from adult human veins. In Vitro Cell Dev Biol 27:352–4, 1991.
12. Finn A, Rebuck N, Moat N. Neutrophil activation during car-

diopulmonary bypass. J Thorac Cardiovasc Surg 6:1746–8, 1992.
13. Gillinov AM, Bator JM, Zehr KJ, Redmond JM, Burch JM, Ko C, et al. Neutrophil adhesion molecule expression during cardiopulmonary bypass with bubble and membrane oxygenators. Ann Thorac Surg 56:847–53, 1993.
14. Granger ND, Kubes P. The microcirculation and inflammation; modulation of leukocyte-endothelial cell adhesion. J Leukoc Biol 55:662–75, 1994.
15. Grimbrone MA, Bevilacqua MP, Cybulsky MI. Endothelial-dependent mechanism of leukocyte adhesion in inflammation and atherosclerosis. Ann N Y Acad Sci 598:77–85, 1990.
16. Gu YJ, Van Oeveren W, Boostra PW, De Hann J, Wildevuur CRH. Leukocyte activation with increased expression of CR3 receptors during cardiopulmonary bypass. Ann Thorac Surg 53:839–43, 1992.
17. Haraldsen G, Kvale D, Lien B, Farstad IN, Brandtze P. Cytokine-regulated expression of E-selectin, intercellular adhesion molecule-1 (ICAM), and vascular cell adhesion molecule-1 (VCAM-1) in human intestinal microvascular endothelial cells. J Immunol 156:2558–65, 1996.
18. Johnson JA, Cogbill TH, Strutt PJ, Gunderson AL. Wound complications after infrainguinal bypass. Arch Surg 123: 859–62, 1988.
19. Kanda KG, Hayman GT, Silverman MD, Lelkes PI. Comparison of ICAM-1 and VCAM-1 expression in various human endothelial cell types and smooth muscle cells. Endothelium 6:33–44, 1998.
20. Luscher TF. Vascular biology of coronary bypass grafts. Curr Opinions Cardiol 6:826–31, 1991.
21. Ma X-I, Lefer DJ, Lefer AM, Rothlein R. Coronary endothelial and cardiac protective effects of a monoclonal antibody to intracellular adhesion molecule-1 in myocardial ischemia and reperfusion. Circulation 86:937–47, 1992.
22. Marlin SD, Springer TA. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antgen-1(LFA-1). Cell 52:813–19, 1987.
23. Quist WC, Haundenschild CC, LoGerfo FW. Qualitative microscopy of implanted vein grafts: effects of graft integrity on morphologic fate. J Thorac Cardiovasc Surg 103:671–7, 1992.
24. Reifsnyder T, Bandyk D, Seabrook G, et al. Wound complications of the in situ saphenous vein bypass technique. J Vasc Surg 15:843–50, 1992.
25. Robbins MR, Hutchinson SA, Helmer SD. Endoscopic saphenous vein harvest in infrainguinal bypass surgery. Am J Surg 176:908–14, 1998.
26. Santoli E, Mattia DD , Boldorini R, Mingoli A, Tosoni A, Santoli C. University of Wisconsin solution and human saphenous vein graft preservation: preliminary anatomic report. Eur J Cardiothorac Surg 7:548–52, 1993.
27. Schwartz ME, Harrington EB, Schanzer H. Wound complication after in situ bypass. J Vasc Surg 7:802–7, 1988.
28. Shanley TP, Warner RL, Ward PA. The role of ctyokines and adhesion molecules in the development of inflammatory injury. Mol Med Today 195:40–5, 1995.
29. Springer TA. Adhesion receptors of the immune system. Nature 346:425–34, 1990.
30. Utly JR, Thomanson ME, Wallace DJ, et al.. Postoperative correlates of impaired wound healing after saphenous vein excision. J Thorac Cardiovasc Surg 98:147–9, 1989.
31. Wengrovitz M, Atnip RG, Girrord RRM, et al.. Wound complications of autogenous subcutaneous infrainginal arterial bypass surgery; predisposing factors and management. J Vasc Surg 11:156–63, 1990.
32. William AF, Barclay AN. The immunoglobulin superfamily-domains for cell surface recognition. Annu Rev Immunol 6:381–405, 1988.
33. Wipke-Tevis DD, Stotts NA, Skov P, Carrieri-Kohlman V. Frequency, manifestations, and correlates of impaired healing of saphenous vein harvest incisions. Heart Lung 25:108–16, 1996.
34. Wood KM, Cadogan MD, Ramshaw AL, Parums DV. The distribution of adhesion molecules in human atherosclerosis. Histopathology 22:437–44, 1993.
35. Wyatt AP, Taylor GW. Vein grafts: changes in the endothelium of 15 autogenous free vein grafts used as arterial replacements. Br J Surg 53:943–7, 1966.