

Nitric Oxide Expression by Interleukin-10 in the Endoscopic and Open Methods of Vein Harvesting in Coronary Artery Bypass Surgery

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ABSTRACT

Objective: Interleukin-10 (IL-10) is an anti-inflammatory cytokine that suppresses lymphocyte functions, regulates production of proinflammatory cytokines, and suppresses nitric oxide production by activated macrophages. We examined IL-10 expression and its value as a surrogate index for nitric oxide (NO) production in endothelial cultures obtained from saphenous vein samples.

Methods: Using 2 different techniques (the open and endoscopic), we harvested samples of human saphenous veins from 90 randomly selected patients undergoing coronary artery bypass surgery (CABG). Endothelial cells collected from the vein samples retrieved through both techniques were cultured for 72 hours. Using a solid phase enzyme linked-immuno-sorbent assay (ELISA), we analyzed pre- and postoperative sera, in addition to the supernatants from the cultures, for IL-10.

Results: Mean preoperative levels of IL-10 (0.09 ± 0.04 pg/mL) did not differ significantly from that for postoperative sera (0.14 ± 0.17 pg/mL) ($P = 0.54$). Mean IL-10 levels for endothelial cell culture supernatants did not differ significantly between the endoscopic (0.32 ± 0.39 pg/mL) and the open method (0.46 ± 0.80 pg/mL) ($P = 0.30$).

Conclusion: Our findings indicate that endoscopic and open saphenectomies are technically comparable with respect to their effects on IL-10 release during saphenous vein harvesting for CABG. We recommend the endoscopic method for its low morbidity and earlier hospital discharge.

Key Words: Interleukin-10, Endoscopic saphenous vein harvesting, Coronary artery bypass grafting.

INTRODUCTION

Cytokines play an important role in the recruitment of leukocytes from blood to sites of tissue injury and inflammation.¹⁻⁶ IL-10, a pleiotropic mediator, inhibits the production of cytokines by macrophages and downregulates their antigen-presenting function. By identification of the cytokine networks and their mechanism of action, we have a better understanding of the regulatory role of IL-10 and other cytokines on endothelial cells leading to inflammatory and allergic reactions.⁷

IL-10 was originally characterized as a cytokine that inhibits certain immune responses and was described as a "cytokine synthesis inhibitory factor."⁸ It was recently discovered that IL-10 has the ability to inhibit synthesis of other cytokines, T-cell proliferation, and nitric oxide (NO) formation suggesting its immunosuppressive potential.^{9,10} IL-10 is synthesized by T helper-2 cells, CD-8 cells, monocytes,¹¹ keratinocytes,¹² activated B cells and lymphocytes.¹³ IL-10 also has a direct stimulatory effect on B-cells and promotes antibody production. IL-10 downregulates endothelial cell adhesion molecules resulting in decreased leukocyte adhesion, a process that is probably mediated through NO release. NO seems to be an important mediator of many biological functions including vascular dilatation, microbicidal activity, and platelet aggregation. IL-10 has important and highly significant inhibitory activities on immune and vascular functions. These actions seem to be derived in part from its ability to diminish various immune and endothelial functions by suppression of cellular activity. IL-10 levels measured at 6 hours after cardiopulmonary bypass surgery are higher than levels found during surgery. This postsurgical increase in IL-10 levels may be an immunoregulatory attempt to downregulate the diffuse inflammation associated with the trauma of surgery.^{14,15}

The purpose of this study was to examine the expression of IL-10 and its value as a surrogate index for the production of NO in endothelial cultures that are obtained from saphenous vein samples. These samples were obtained by 2 methods of vein harvesting performed during coronary artery bypass grafting (CABG), a new endoscopic saphenous vein harvesting method (ESVH), and a conventional open vein harvesting method (OSVH).

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METHODS

Vein Retrieval

The study was conducted at 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. Whereas in the endoscopic technique, a small incision is made 4-finger breadths posterior to the proximal margin of the patella, the greater saphenous vein is identified, dissected both cranially and distally under endoscopic visualization through the incision and 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 2 vein segments, each by using 1 of the 2 harvesting techniques, from the same leg of each study patient. This was done by excising a 5-cm segment of the thigh portion of the saphenous vein cranially dissected and retrieved endoscopically as the endoscopic sample. The sample was clipped distally for orientation of blood flow direction. At the same time, 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 Dulbecco's Modified Eagle Medium (1X), liquid high glucose (D-MEM) with 200 mL penicillin-streptomycin during transport to the laboratory. In the laboratory, each vein sample was flushed and cannulated, injected with plasmalyte, and the branches were ligated with 3.0 silk sutures.

Endothelial Cell Culture

At the conclusion of the previous procedure, vein samples were collected and transported using the same transport

medium (D-MEM). Then, under a laminar flow hood, the pair of veins (OSVH and ESVH) was put into a Petri dish containing 5 mL of endothelial cell culture medium [IMDM with 25 mM HEPES, 3.024 g/L NaHCO₃, 100 U/mL penicillin, 100 µ/mL streptomycin, 15% FBS (fetal bovine serum), 30 µ/mL ECGS, 130 µ/mL heparin, and 2 mM L-glutamine].

Each vein was flushed with the indicated solution and divided into 2 pieces with a sterile scalpel (No. 15 blade). The proximal piece was used for culture (see below), and the distal one for microscopic analysis (light and electron microscopy, both scanning and transmission). 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 in the culture medium, and the supernatant-containing endothelial cells were then preserved in 25 mL culture flasks. These flasks were then incubated at 37°C and 5% CO₂ with humidifier for the next 72 hours.

IL-10 Measurements

The Bio-Source Cytoscreen™ human IL-10 (h IL-10), a solid phase sandwich enzyme linked-immuno-sorbent assay (ELISA) was used for quantitative determination of human IL-10 (hIL-10). This is a sensitive ELISA with an antibody specific for hIL-10 coated on to the wells of the micro-titer strips.

Statistics

Data are presented as mean ± standard deviation. The 2-tailed Student *t* test was used to compare the mean expression values of hIL-10 with a level of significance at *P* = 0.05 or less.

RESULTS

The mean concentration of IL-10 in preoperative sera was 0.09 pg/mL ± 0.04, and that in postoperative sera was 0.14 pg/mL ± 0.17, the difference not being statistically significant (*P* = 0.54). Similarly, no significant difference (*P* = 0.30) existed in the mean concentration of IL-10 in endothelial cell culture supernatants obtained by OSVH (0.46 pg/mL ± 0.80) and the ESVH (0.32 pg/mL ± 0.39) (**Table 1**).

DISCUSSION

Coronary artery bypass grafting (CABG) initiates a cascade of events that results in systemic cytokine release.¹⁶ Cytokine release depends on the strength of trauma inflicted on vascular endothelial cells. Numerous reports have documented the presence and changes in cytokine levels during surgical trauma.

Vascular endothelial cells constitute the interface between the blood stream and the tissues. Endothelial cells might perform several key roles in the development of inflammatory responses including adhesion to inflammatory leukocytes and control of vascular permeability.¹⁷⁻²¹ However, the vascular endothelium is unlikely to be exposed to the local action of a single cytokine in vivo. The concerted action of a range of leukocyte-derived signals, acting sequentially or in concert, is responsible for the eventual outcome of potentially inflammatory events.²²

IL-10 was initially described as an inhibitor of cytokine production and antigenic-specific proliferation of CD4+ T helper cells in mice.²³ IL-10 is a cytokine produced by activated macrophages and some lymphocytes. IL-10 is now known to have multiple biological activities. Many studies have demonstrated that IL-10 inhibits macrophage synthesis of the inflammatory cytokine IL-1 and tumor necrosis factor- α (TNF- α). On the other hand, IL-10 is produced as a result of IL-1 and TNF- α stimulation from the very cell that secretes the proinflammatory cytokine in an attempt to downregulate their excitatory actions.²⁴⁻²⁶ It was demonstrated that IL-10 stimulates the release of NO from human saphenous vein endothelium. The NO release can be antagonized by an antibody to IL-10 and by nitric oxide synthases (NOS). Removal of the endothelium also results in a lack of response to IL-10 suggesting that IL-10 exerts its action through endothelial NO. Furthermore, IL-10-coupled NO release is of functional significance in that the adherence of human granulocytes and monocytes to vein endothelium can be diminished after IL-10 exposure.

Presumably, this occurs because of the presence and action of NO on the immunocytes or endothelium or both.

Recently, a newly developed technique of saphenous vein harvesting, endoscopic harvesting, has been described and practiced in the United States. It was well 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. In our present study, we demonstrated that no significant difference exists in the level of IL-10 in pre- and postoperative sera in either the OSVH or ESVH technique.

Our study demonstrates that no significant difference exists in the average pre- and postoperative levels of IL-10. In addition, neither saphenous vein harvesting (OSVH or ESVH) technique changed average IL-10 levels. The comparability between the 2 methods suggests that vein manipulation and minor physical shears in operative extraction of the vein conduit would have minimal impact on IL-10 and therefore NO release from endothelial cells, which will subsequently affect the performance of the vein conduit.

One limitation of this study is that we evaluated the levels of IL-10 in the supernatants of the endothelial cell cultures for 72 hours only. Follow-up that requires culture for a longer period, and additionally, daily measurement of the cytokine 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 IL-1, IL-2, TNF- α , and INF- γ . These cytokines and other released factors in the blood can cause upregulation of adhesion molecules and thus affect future performance of bypass conduits. The pathway and the complexity of up- and downregulation of various adhesion molecules and cytokines including endothelial cell culture and viability have been reported previously, showing comparable results for both techniques.²⁶⁻²⁷

The light and the electron microscopic study of both harvesting techniques showed comparable results comparing variable parameters of vein injury.²⁸

Longer follow-up of the patency rate of saphenous vein grafts in patients who had CABG with the endoscopic technique for vein harvesting and comparing them to ran-

Table 1.

Mean IL-10 concentrations (pg/mL) pre- and post-CABG, and in endothelial cell culture supernatants from saphenous veins harvested with the endoscopic or open techniques.

Preoperative	Postoperative	Endoscopic	Open
0.09 ± 0.04	0.14 ± 0.17	0.32 ± 0.39	0.46 ± 0.80

domly selected patients with grafts harvested by the traditional technique will be required to further validate our results.

CONCLUSION

The comparable results of IL-10 release in both harvesting techniques of the saphenous vein indicate that the novel endoscopic technique is equivalent to the open technique. This endoscopic technique is safe and unique 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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