Significance of Interferon-γ in Coronary Artery Bypass Surgery

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ABSTRACT

Background: Gamma interferon (IFN-γ) is produced by activated natural killer and T cells under pathologic circumstances. The objective of our study was to compare the level of IFN-γ in open and endoscopic methods of vein harvesting for coronary artery bypass surgery (CABG).

Method: Ninety samples of human saphenous veins harvested from patients prepared for CABG. Pre- and postprocedure sera of the patients, in addition to supernatants of 3-day endothelial cell culture, were analyzed for IFN-γ.

Results: The mean preoperative IFN-γ level (0.09 ± 0.03 pg/mL) and that for postoperative sera (0.08 ± 0.02 pg/mL) were not significantly different (P = 0.2). The mean IFN-γ level in endothelial cell culture from the endoscopic (0.18 ± 0.21 pg/mL) and the open method (0.19 ± 0.39 pg/mL) were not significant (P = 0.89).

Conclusion: We recommend the endoscopic method of vein harvesting because of its lower morbidity and earlier hospital discharge.

Key Words: IFN-γ, CABG, Endoscopic and open saphenectomies.

INTRODUCTION

Vascular endothelial cells constitute the interface between the bloodstream and tissues.[1,2] In this strategic function, endothelial cells (EC) play an active role in all phases of immunologic and nonimmunologic inflammation through cytokine networks.[3-10]

EC produce various cytokines, such as interleukin 1 (IL-1), interleukin 6 (IL-6), colony stimulating factors (CSF), and chemotactic factors, and express adhesion molecules.[11-14] These data on the function of the endothelial cells in the immune response were obtained from EC culture experiments. These experiments found that EC can also be modulated by interleukins, interferons, and tumor necrosis factors.[15-19]

Interferons of all 3 types (α, β, and γ) interact with specific receptors on virtually all cells of the body, 1 receptor for α and β and another for γ.[20] Gamma interferon (IFN-γ) is a 45-KD homodimeric glycosylated protein detectably produced only under pathologic circumstances (trauma, infection, cancer, and autoimmunity) by particular lymphocyte populations, namely, activated natural killer (NK) cells and T cells. IFN-γ suppresses cultured human vascular endothelial cell proliferation,[21] induces these cells to express MHC class II antigen,[22] and causes them to change shape and cell organization.[23,24]

The impact of trauma on IFN-γ production is our major concern in saphenous vein preparation for coronary artery bypass graft (CABG) surgery. The present study was undertaken to evaluate and compare the levels of IFN-γ in the endoscopic technique of vein harvesting with the standard open method, as the new technique is preferred now in CABG, due to the lower incidence of postoperative complications.

MATERIALS AND METHODS

The protocols used in this project were reviewed and approved by the Institutional Review Board of the Maimonides Medical Center. Patients undergoing coronary artery bypass graft (CABG) surgery provided informed consent before enrolling as subjects in this research protocol. A total of 45 patients were enrolled in the project.

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Saphenous Vein Harvest

Saphenous vein harvest (SVH) was performed using both the open and endoscopic techniques on each patient undergoing elective CABG surgery. Therefore, both an open technique sample and an endoscopic technique sample were obtained from each patient enrolled in the study. Standard endoscopic instrumentation, consisting of a subcutaneous tissue dissector, a retractor, and a modified vein stripper, was used for the endoscopic SVH (Endopath, Ethicon Endosurgery Inc., Cincinnati, OH). In addition, standard endoscopic equipment including a television monitor, light source, and fiberoptic camera with a 5-mm lens were used.

Prior to the initial skin incision, a preoperative blood sample was obtained to determine baseline systemic cytokine levels. The blood sample was immediately centrifuged at 3,000 rpm for 10 minutes at 28°C, and the supernatants were collected.

The patient was placed in the "frog-leg" position, and a 3- to 4-cm longitudinal incision was made approximately 4-finger breadth posterior to the patella along the medial genicular region. The greater saphenous vein (SV) was identified with the sharp dissecting technique. The portion of the SV dissected below the skin incision was considered the open technique sample. This segment remained in place until after adjacent segments were harvested with the endoscopic method. Next, a 5-cm sample was retrieved by dissecting cranially with the endoscopic technique. This portion of the SV was clipped distally to identify the direction of blood flow. An additional 5-cm endoscopic technique sample was obtained by dissecting below the knee joint. Finally, the portion of the SV remaining at the site of the open incision was removed and labeled "open-technique sample." Significant segments of the SV remained after collecting samples, and these were used as the grafts in the CABG surgery. At the conclusion of SV harvesting, during skin closure, a second blood sample was drawn. Vein samples were harvested and handled under sterile conditions according to the operating room protocol at Maimonides Medical Center.

Vein Preparation

Vein samples were incubated in 10-mL endothelial cell culture medium (ECCM) consisting of Iscove's Modified Dullbecco's Medium (IMDM, Fischer Scientific, Cincinnati, OH) with 200 mL penicillin-streptomycin solution (Fischer Scientific, Cincinnati, OH) during transport to the laboratory. In the laboratory, each vein sample was flushed and cannulated. They were then injected with plasmalyte solution (Baxter Scientific, St. Louis, MO) containing 60-mg/mL papaverine (Fischer Scientific, Cincinnati, OH), and the branches were ligated with 3.0 silk sutures. Plasmalyte solution contained sodium 140 mEq/L, potassium 5 mEq/L, magnesium 3 mEq/L, chloride 98 mEq/L, acetate 27 mEq/L, and gluconate 23 mEq/L. The pH was adjusted to 7.4 by addition of sodium hydroxide, and the final osmolarity was 294 mOsm/Kg.

Endothelial Cell Culture

Endothelial cell cultures were prepared as described previously. Vein samples were placed into a culture dish (Fischer Scientific, Cincinnati, OH) containing 5 mL of ECCM with the addition of 130 U/mL heparin and 2 mM L-glutamine. Vein samples were slit open and placed flat with the luminal surface facing up. The luminal surface was gently scraped with a sterile scalpel blade, with light single strokes, covering each area only once. The cells, which collected on the scalpel blade, were shaken off into the ECCM in the culture dish. Next, the vein was rinsed with an aliquot of ECCM, and the rinsing fluid was added to the culture dish. The ECCM and scraped endothelial cells in the culture dish was collected and transferred to a 25 mL culture flask (Fischer Scientific, Cincinnati, OH). The cells were incubated at 37°C in humidified room air with 5% CO_2 for the next 72 hours.

IFN-γ Measurement

A commercially available enzyme-linked immuno-sorbent assay (ELISA) was used to measure the presence of human IFN-γ (Biosource International, California). IFN-γ levels were determined in samples of sera and ECCM. Blood sera were diluted 1:10 in plasmalyte solution before IFN-γ levels were determined. The ECCM was aspirated from the cell cultures after 72 hours and diluted 1:50 in plasmalyte solution. The IFN-γ level was then determined in the diluted samples.

Statistical Analysis

Results for each group are expressed as mean ± standard deviation of the quantity of IFN-γ measured in the samples. Data were analyzed with the two-tailed Student

t test. A *P*-value of less than 0.05 was designated as a statistically significant difference between groups.

RESULTS

The mean level of IFN-γ for preoperative sera in the 45 patients was 0.096 ± 0.032 pg/mL (mean ± standard deviation) with a confidence interval (CI) = 0.087-0.105, and that for postoperative sera was 0.089 ± 0.019 pg/mL, with a confidence interval (CI) = 0.083-0.095. The means were not statistically significant (*P* = 0.2).

The mean level of IFN-γ measured in endothelial cell culture from the endoscopic technique was 0.185 ± 0.215 with CI = 0.122-0.248, whereas that for the open method was 0.194 ± 0.39 with CI =0.079-0.308. The difference between the means of IFN-γ for the 2 techniques was not statistically significant (*P* = 0.9) **(Figure 1)**.

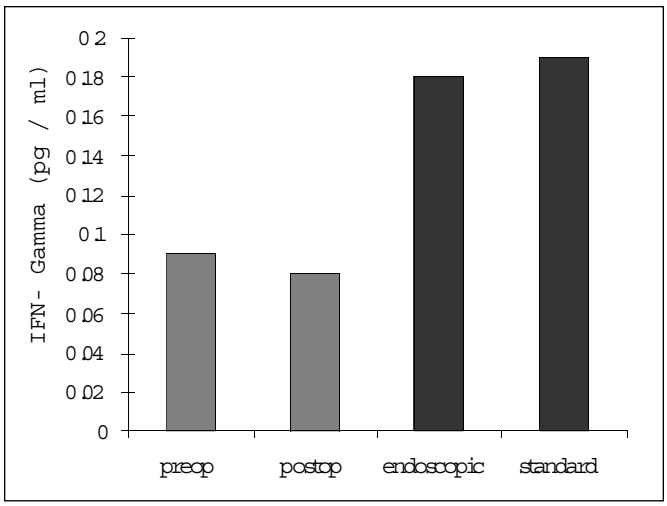

Figure 1. Graphic presentation of the level of interferon-γ in preoperative, postoperative, endoscopic, and open techniques of saphenous vein harvesting.

DISCUSSION

The autologous greater saphenous vein is considered the best material for coronary artery revascularization. However, the morbidity associated with saphenous vein harvesting is significant with reported wound complications as high as 43%.[25-30]

Historically, the greater saphenous vein is exposed and harvested through a long continuous incision. This incision may be related to an increase in wound problems; therefore, there has been much interest in developing a more closed technique for saphenectomy.[27-29] More recently, a newly developed endoscopic technique for saphenous vein harvesting has been described with encouraging results.[31-34]

Studies of cytokines, leukocytes, and endothelial cell interaction, and the ensuing biologic events, have received much attention in recent years.[35,36] These studies found that vascular endothelium can produce several biologically active compounds, including nitric oxide, prostacyclin, and various cytokines.[30] IFN-γ is a cytokine, produced by natural killer cells, that plays an important role in bringing about acute inflammation, mainly because of the activating effect of IFN-γ on adhesive properties of endothelial cells and on mediator production by mononuclear phagocytes.[37]

Impairment of the immune response by surgery is suggested by clinical observations of the high rate of infection seen in postoperative patients. The principal immunological deficit after trauma and major surgery is decreased cell-mediated immunity from an impaired natural killer (NK) cell response and T helper (Th) 1 lymphocyte development. Cell-mediated immunity has been shown to be depressed for 3 to 10 days postoperatively in patients who have undergone major surgery.[38] In addition, many studies have shown that IFN-γ is reduced in the early postoperative phase, reflecting impaired function of (Th) 1 cells and therefore of cell-mediated immune response.[39]

In contrast, our study showed that no significant difference exists in the level of IFN-γ pre- and postoperatively. We also demonstrated that no significant difference exists in the level of IFN-γ using both techniques of saphenous vein harvesting (StdSVH and EndoSVH). However, the number of patients in most of the other studies was small, and the measured levels of IFN-γ varied widely. Therefore, conclusions about perioperative changes of INF-γ are tentative at best.

The comparable expressions of IFN-γ in the endoscopic and the traditional technique of vein harvesting suggests that vein manipulation and minor physical shears would have a minimal impact on the levels of IFN-γ that may subsequently affect the performance of the vein conduit.

The limitation of our study is that open saphenectomy was not performed as a standard procedure on our

patients undergoing CABG, so we were unable to devise 2 separate groups of patients undergoing each technique alone.

The method of endothelial cell harvesting and culture might have falsely affected the level of IFN-γ. Furthermore, in our study, we stored plasma at -2°C, various investigators had used different temperatures for plasma storage in evaluating IFN-γ expression. At the same time, we have used supernatants of cell culture to compare endoscopic with the open technique. Other studies used peripheral blood mononuclear cells to measure IFN-γ level,[40] which might affect our results. Also, measurement of IFN-γ in blood samples taken 3 to 10 days postoperatively would have incorporated the effect of CABG itself as a major surgery, and the individual role of vein harvesting could not be clarified.

In conclusion, our findings indicate that endoscopic and open saphenectomies are comparable for IFN-γ expression in CABG patients. However, we still recommend the endoscopic method of vein harvesting due to fewer postoperative leg wound complications compared with traditional open saphenectomy, which ultimately results in less postoperative pain, reduced hospital stay, and reduction in health care costs.[41]

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