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Interleukin-1, Interleukin-2 and Interleukin-10 Expression in Different Techniques of Saphenous Vein Harvesting

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ABSTRACT

Background: Levels of the cytokines Interleukin-1 (IL-1), IL-2, and IL-10 are sensitive to the traumatic effect of saphenous vein harvesting. Their levels are compared between the endoscopic and traditional open techniques of harvesting.

Methods: Samples of human saphenous veins were harvested from 90 randomly selected patients undergoing coronary artery bypass surgery (CABG), using the open or endoscopic techniques. Endothelial cells collected from the vein samples retrieved through both techniques were cultured for 72 hours. Pre and postoperative sera, in addition to the supernatants from the cultures, were analyzed for IL-1, IL-2, and IL-10 using ELISA.

Results: Mean preoperative concentrations of IL-1, Il-2, and IL-10 were 0.11± 0.04, 0.09 ± 0.04, and 0.09 ± 0.04 pg/ml, respectively. Corresponding values for postoperative sera were 0.13 ± 0.08, 0.12 ± 0.10, 0.14 ± 0.17 pg/ml, respectively. The differences between pre and postoperative means for each cytokine were not statistically significant (p= 0.13, 0.18, 0.05, respectively). Mean IL-1, IL-2, and IL-10 concentrations for endothelial cell culture supernatants did not differ significantly between the endoscopic (0.17 ± 0.11, 0.11 ± 0.05, and 0.32 ± 0.40 pg/ml, respectively) and the open method (0.19 ± 0.16, 0.11 ± 0.05, and 0.46 ± 0.80 pg/ml, respectively) (p= 0.48, 0.81, 0.30, respectively).

Conclusion: Since endoscopic and open saphenectomies are technically comparable with respect to their effects on IL-1, Il-2, and IL-10 levels, we recommend the endoscopic method for its lower morbidity and the potential for earlier hospital discharge.

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INTRODUCTION

Cytokines are low-molecular mass proteins that are produced by leukocytes and a variety of other cell types. Cytokines have a prominent role in inflammation and regulation of the immune response that serves the purpose of efficient and rapid mobilization of host defense whenever needed. At the same time, they are highly regulated with a network of anti-inflammatory cytokines that become activated in order to prevent tissue damage and systemic effects [Schwartz 1988].

Interleukin-1 (IL-1) is a protein produced mainly by macrophages. It activates a wide variety of target cells, for example T and B-lymphocytes, neutrophils, epithelial and endothelial cells. IL-2 is an "endogenous pyrogen", which acts on the hypothalamus to cause the fever associated with infections and inflammatory reaction. IL-10 mediated suppression of monokine production appears to be a direct effect on activated macrophages and monocytes, which leads to striking down regulation of numerous inflammatory monokines such as TNF-a, Interferon-g (IFN-g), IL-1, IL-6, IL-8, and colony stimulating factors (CSF). In addition to its inhibitory effect, IL-10 elevates anti-inflammatory monokines and suppresses nitric oxide (NO) production by activated macrophages [Johnson 1988].

Table 1. The groups of patients in the study, having the temperature, pressure and papaverine as variables.

	Temperature	Pressure	Papaverine
Group I	28°C	100 mmHg	No
Group II	4°C	100 mmHg	No
Group III	4°C	300 mmHg	No
Group IV	4°C	300 mmHg	No
Group V	28°C	100 mmHg	Yes
Group VI	4°C	100 mmHg	Yes
Group VII	28°C	300 mmHg	Yes
Group VIII	4°C	300 mmHg	Yes
Group IX	Control		

The levels of IL-1, IL-2 and IL-10 are very sensitive to the degree of trauma resulting from the preparation, handling, preservation, and implantation of the saphenous vein. Consequently, the inflammatory reaction mediated by these cytokines will have a major impact on the eventual health and patency of the constructed bypass conduit [Clark 1935].

The standard method of saphenous vein harvesting in coronary artery bypass graft (CABG) is the open, no-touch technique. In this technique, the greater saphenous vein is exposed and harvested through a long continuous skin incision along the medial side of the thigh and the leg. This long incision may be related to an increase in postoperative wound complications of up to 43% [Robbins 1998]. This is especially so when other risk factors such as tobacco use, female gender, diabetes and obesity are also present [Reifsnyder 1992]. Several studies have shown a decreased incidence of wound complications with a small versus continuous incision [Wipke 1996]. Therefore, there has been much interest in developing a more closed technique for saphenectomy.

Harvesting, preparation, handling, type of the storage solution, and the technique of anastomosis of the venous conduit are crucial steps in minimizing damage to the vein. Damage may include intimal disruption, deposition of platelets and leukocytes on the intima with release of various inflammatory mediators, exposure of the basement membrane, endothelial sloughing, and medial tear.

The purpose of this study was to examine the differences in the levels of IL-1, IL-2, and IL10 between the endoscopic (ESVH) and the standard open (OSVH) saphenous vein harvesting, and therefore, whether the former technique is a better alternative to OSVH for future cardiac and vascular surgery.

MATERIALS AND METHODS

Vein retrieval

The 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. Whereas in the endoscopic technique, a small incision is made 4 fingers breadth 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, fiberoptic camera and a 5mm lens were used.

We sampled two vein segments, each by using one of the two 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 Dulbecc's Modified Eagle Medium (1X), liquid high glucose (D-MEM) with 200mL penicillin-streptomycin during transport to the laboratory. In the laboratory, each vein sample was flushed and cannulated, injected with Plasma-Lyte (Baxter Healthcare Corp., Deerfield, IL) and the branches were ligated with 3.0 silk sutures.

Study groups

Veins retrieved by each technique (OSVH or ESVH) were considered a separate group. Each group (45 samples) was subdivided into nine subgroups, each containing 5 samples (see Table 1, ◉). The vein specimens in each subgroup were exposed to various temperatures, pressures, and solutions (Figure 1, ◉):

Subgroup-1: Vein segments were immersed in Plasma-Lyte solution without papaverine at 28°C and distended to 100 mmHg with the same solution for 1 hour.
Subgroup-2: The veins were immersed in Plasma-Lyte solution without papaverine at 4°C and distended to 100 mmHg with the same solution for 1 hour.
Subgroup-3: The veins were immersed in Plasma-Lyte solution without papaverine at 28°C and distended to 300 mmHg with the same solution for 1 hour.
Subgroup-4: The veins were immersed in Plasma-Lyte without papaverine at 4°C and then distended to 300 mmHg with the same solution for 1 hour.
Subgroup-5: The veins were immersed in Plasma-Lyte with papaverine at 28°C and then distended to 100 mmHg with the same solution for 1 hour.

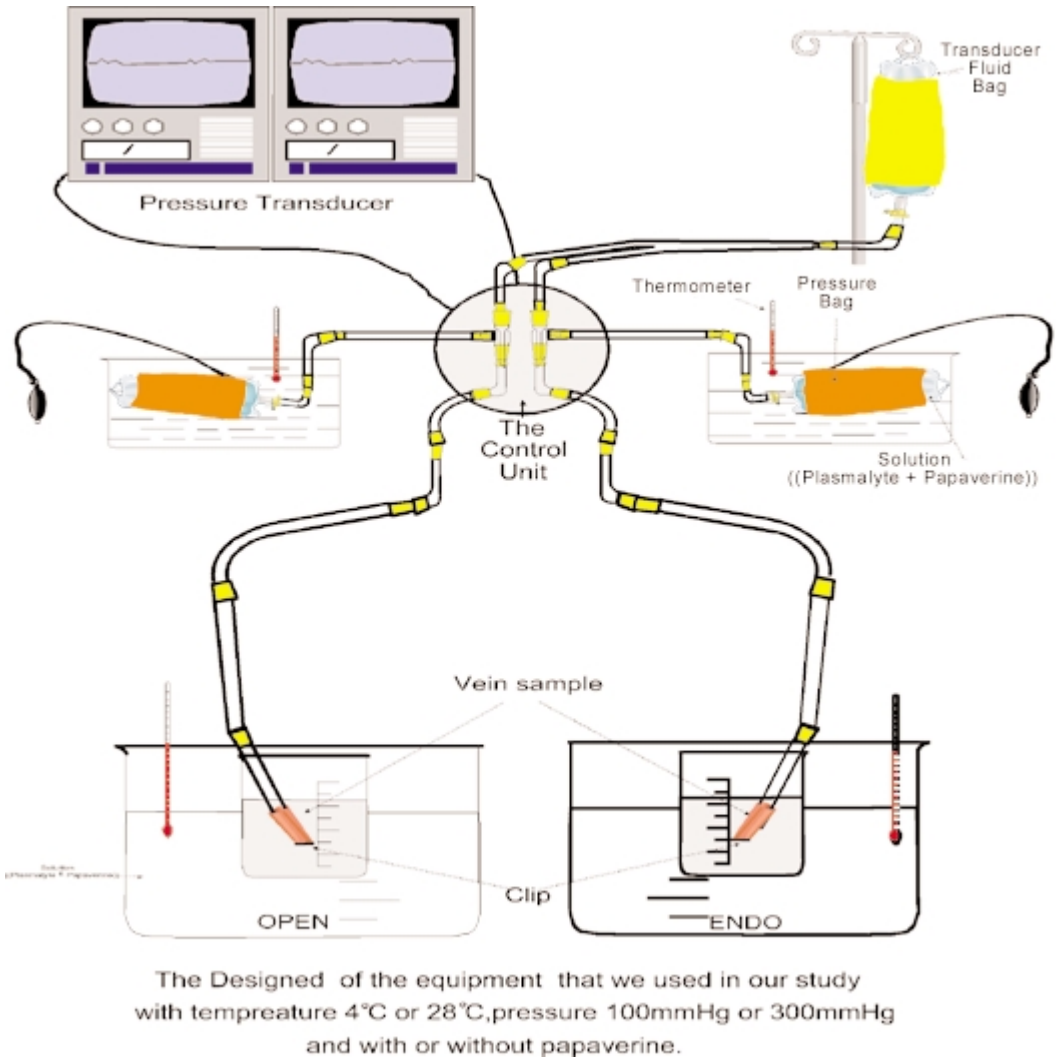

Figure 1. The design of the equipments used in the study.

Subgroup-6: The veins were immersed in Plasma-Lyte with papaverine at 4°C and then distended to 100 mmHg with the same solution for 1 hour.
Subgroup-7: The veins were immersed in Plasma-Lyte with papaverine at 28°C and then distended to 300 mmHg with the same solution for 1 hour.
Subgroup-8: The veins were immersed in Plasma-Lyte with papaverine at 4°C and then distended to 300 mmHg with the same solution for 1 hour.
Subgroup-9: veins in this subgroup were prepared as controls. Immediately after retrieval, vein segments were fixed with buffered (to pH 7.2) 3% glutaraldehyde, at room temperature, under normal pressure and without papaverine.

Solutions

The chemical characteristics of Plasma-Lyte solution was as follows: each 100 mls contained 526 mg sodium chloride USP, 502 mg sodium gluconate USP, 368 mg sodium acetate trihydrate USP, 37 mg potassium chloride USP, 30 mg magnesium chloride USP (sodium=140 mEq/L, potasium=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 (6.5 - 8.0) and the osmolarity was 294 mOsmol/L. Papaverine was added in five ml (60 mg /ml) quantities to each 500 ml of Plasma-Lyte solution.

The type and composition of the solutions, in addition to the variability of temperature and pressure 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

Table 2. Mean hIL-1, hIL-2, and hIL-10 concentrations (pg/ml) measured in pre and postoperative sera, and in the supernatants of endothelial cell cultures prepared from saphenous veins retrieved endoscopically or by the open technique.

	Preoperative	Postoperative	Endoscopic	Open
hIL-1	0.11 ± 0.03	0.12 ± 0.08	0.17 ± 0.11	0.19 ± 0.16
hIL-2	0.09 ± 0.04	0.11 ± 0.09	0.10 ± 0.04	0.11 ± 0.05
hIL-10	0.09 ± 0.04	0.14 ± 0.17	0.32 ± 0.39	0.46 ± 0.80

media (D-MEM). Then, under laminar flow hood, the pair of veins (OSVH and ESVH) was put in 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 m/mL streptomycin, 15% FBS (Fetal Bovine Serum), 30 m/mL ECGS, 130 m/mL heparin, and 2 mM L-glutamine).

Each vein was flushed with the indicated solution and divided into two pieces using a sterile scalpel (blade No.15). 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 lies flat. The luminal surface 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-hour.

Enzyme-Linked Immuno-Sorbent Assay (ELISA)

Commercially available ELISA kit (Biosource International, Camarillo, CA) was used to measure human IL-1, IL-2 and IL-10 concentrations (hIL-1, hIL-2, hIL-10). Preoperative and postoperative blood samples were immediately centrifuged at 3,000 rpm for 10 minutes at 28°C and the sera were evaluated for IL-1, IL-2 and IL-10 levels. We also measured IL-1, IL-2 and IL-10 concentrations in the supernatants of the 72 hours endothelial cell cultures.

Statistical analysis

Results for each group were expressed as mean ± standard deviation. Data were analyzed using a single factor of variance (ANOVA) program written for Lotus 1-2-3 (version 2.2). The two-tailed student t test was used to compare the mean values of IL-1, IL-2 and IL-10 concentration, with a level of significance at 0.05 or less.

RESULTS

Results are summarized in Table 2 (◉).

hIL-1

The mean concentration of IL-1 in preoperative sera was 0.11 ± 0.04 pg/ml, whereas that in postoperative sera was 0.13 ± 0.08 pg/ml. There was no statistically significant difference between the pre and postoperative means (p= 0.13). The mean concentration of IL-1 in the endothelial cell culture supernatants from the endoscopic technique was 0.17 ± 0.11 pg/ml, and that for the open technique was 0.19 ± 0.16 pg/ml, with the difference not being statistically significant (p=0.48).

hIL-2

The mean concentration of IL-2 in preoperative sera was 0.09 ± 0.04 pg/ml, whereas that in postoperative sera was 0.12 ± 0.10 pg/ml. There was no statistically significant difference between the pre and postoperative means (p= 0.18). The mean concentration of IL-2 in the endothelial cell culture supernatants from the endoscopic technique was 0.11 ± 0.05 pg/ml, and that for the open technique was 0.11 ± 0.05 pg/ml, with the difference being not statistically significant (p=0.81).

hIL-10

The mean concentration of IL-10 in preoperative sera was 0.09 ± 0.04 pg/ml, whereas that in postoperative sera was 0.14 ± 0.17 pg/ml. There was no statistically significant difference between the pre and postoperative means (p= 0.05). The mean concentration of IL-10 in the endothelial cell culture supernatants from the endoscopic technique was 0.32 ± 0.40 pg/ml, and that for the open technique was 0.46 ± 0.80 pg/ml, with the difference being not statistically significant (p=0.30).

DISCUSSION

CABG initiates a cascade of events that result in systemic cytokine release [Soeparwata 1996]. 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 [Albrightson 1986, Jimquan 1993, Krakauer 1995, Normand 1995, Tan 1995]. 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 [Nakagawa 1986].

Cytokines are proteins that are produced by leukocytes and a variety of other cells. Cytokines are peptide mediators that coordinate the interactions within the immune system. They form a family consisting of many classes, such as tumor necrosis factor (TNF), INF-g and adhesion molecules [Wittens 1994]. Activating cytokines are mainly pro-inflammatory cytokines such as IL-1 and TNF [Van Dijk 1995]. IL-2 is a growth factor for T, B and natural

killer cells. IL-2 is primarily synthesized by activated T lymphocytes and has a central role in the development of cell-mediated immunity and inflammation. It is a key factor in the induction of a complex network of cytokines that include TNF, INF-g and IL-1 [Arai 1990].

IL-10 is a recently discovered anti-inflammatory cytokine, synthesized by several cell types including monocytes and T lymphocytes. It has the ability to inhibit cytokines synthesis, T-cell proliferation, and nitric oxide formation, suggesting its immunosuppressive potential. IL-10, in addition to its immuno-inhibitory properties, is a potent recruitment signal for leukocytes migration in vivo [Meena 1996].

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. Endoscopic harvesting (ESVH) is a newly developed technique of saphenous vein harvesting that has been recently described and practiced in multiple centers in the United States and Europe. Various authors have published the results of their experience with the use of ESVH compared with the traditional OSVH, focusing on postoperative complications and hospital costs rather than the immunogenic impact of the new technique [Chester 1996].

We demonstrated in our study that there is no significant difference in the average pre and postoperative levels of IL-1, IL-2, and IL-10. In addition, either technique of saphenous vein harvesting (OSVH or ESVH) did not change their average levels. The comparability between the two methods suggests that vein manipulation and minor physical shears in operative extraction of the vein conduit would have minimal impact on the release of these cytokines, which will minimally affect the performance of the vein conduit subsequently.

One limitation of this study is that we evaluated the levels of IL-1, IL-2, and 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 cytokines 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 factors such as TNF-a and INF-g. These 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 and 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 IL-1, IL-2, IL-10 levels in both harvesting techniques of the saphenous vein indicate that the novel endoscopic technique 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.

Acknowledgment

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REVIEW AND COMMENTARY

1. Editorial Board Member GX21 writes:

Analysis of variance was used in the analysis, but the comparisons are all made using t-tests. T-tests only compare between two groups, while ANOVA allows a global, simultaneous comparison among more than two groups. It is not clear that the varying experimental conditions were accounted for in the analysis, nor the pairing between patients. Doing this could reduce the experimental variation, and thus increase the detection of differences, i.e., reduce the p-value(s). On the other hand, when there is not a significant difference, one must be careful in claiming comparability. It is better to provide confidence intervals for the differences; these would include zero, of course, if the differences was not significant, but would include an indication of the power of the test to find a difference.

Authors' Response by Sadir J. Alrawi, MD:

Statistical analysis was carefully reviewed by an expert statistician in our department and I agree with your point, but values are reviewed multiple times without much difference.

2. Editorial Board Member MY17 writes:

a) The authors used the cytokines levels to determine the safety of saphenous vein endoscopic harvesting technique, which is irrelevant.
b) The authors divided the venous samples into nine groups, but they did not compare the results among groups. This is essentially an in vitro study, and has little clinical relevance.
c) The authors distended the graft to 300mmHg, which is totally unphysiological.

Authors' Response by Sadir J. Alrawi, MD:

a) Cytokines expresses endothelial cell damage.
b) Groups of veins are according to different physiological parameters.
c) 300mmHg is used when we try to dilate the vein in preparation for CABG, and its been used in many papers.

3. Editorial Board Member SG14 writes:

a) The authors should definitely specify what the meaning of pre-postoperative is. Do they mean the CABG procedure or the vein harvesting procedure?
b) If the vein harvesting procedure is performed during sternotomy, IMA harvesting, or institution of CPB, the surgical technique used (median sternotomy or minithoracotomy), and the use of CPB or not (OPCAB vs. CABG), are of great importance. In case of using CPB, it is also very important whether normothermia was used or not. CPB time is very important if vein harvesting is performed in parallel to CPB.
c) Regardless, the use of CPB anesthesia and anticoagulations protocol are very important, as also the use of aprotinin, as they highly influence inflammatory response.

Authors' Response by Sadir J. Alrawi, MD:

a) The vein harvesting procedure was only at the time of skin incision till complete skin closure of the vein harvesting; by that time median sternotomy was started and in progress.
b) All cases of CABG were performed using CPB.
c) Anesthesia and anticoagulation were done according to the MMC protocol, the same as USA and North American protocols.