Preservation solution impacts physiologic function and cellular viability of human saphenous vein graft

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Introduction. *Recent clinical data suggest intraoperative preservation of human saphenous vein (HSV) in normal saline is associated with vein graft failure. We evaluated the influence of several preservation media on acute physiologic function and cellular viability of HSV conduit.*
Methods. *Unprepared (UP) HSV obtained from coronary artery bypass graft patients was characterized on a muscle bath after 2-hour storage in 6 solutions: Plasma-Lyte A, 0.9% NaCl (normal saline), University of Wisconsin solution, Celsior solution, autologous whole blood, or glutathione-ascorbic acid L-arginine (GALA) solution. Vascular smooth muscle contractility was assessed after exposure to depolarizing KCl and phenylephrine. The relaxation of phenylephrine-precontracted HSV to sodium nitroprusside and carbachol (endothelial-independent and -dependent relaxation, respectively) was also assessed. Cellular viability was determined via the methyl thiazolyl tetrazolium (MTT) assay. Rat aortae were used to assess the effect of pH during graft preservation on endothelial-dependent relaxation.*
Results. *Preservation of HSV in normal saline and autologous whole blood impaired contractile responses to KCl relative to UP tissues, whereas preservation in University of Wisconsin solution and Celsior solution enhanced contractile responses ($P < .05$). Relative to UP tissues, responses to phenylephrine were decreased with preservation in normal saline, whereas preservation in University of Wisconsin solution, Celsior solution, and GALA all potentiated these responses ($P < .05$). Only preservation in normal saline impaired endothelial-independent relaxation ($P = .005$). Preservation in Plasma-Lyte A ($P = .02$), normal saline ($P = .002$), and University of Wisconsin solution ($P = .02$) impaired endothelial-dependent relaxation. Normal saline preservation decreased MTT viability index relative to UP tissues (0.02 ± 0.002 $mg^{-1}0.5$ mL^{-1} vs 0.033 ± 0.005 $mg^{-1}0.5$ mL^{-1}; $P = .03$). Endothelial function was impaired by acidic pH in rat aorta.*
Conclusion. *Preservation of HSV in normal saline causes graft injury leading to impaired physiologic function and decreased viability of the HSV. This harm is mitigated by the use of buffered salt solutions as preservation media. (Surgery 2015;158:537-46.)*

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THE HUMAN SAPHENOUS VEIN (HSV) is the most widely used conduit in aortocoronary and peripheral bypasses.[1] During typical intraoperative preparation, the HSV is subject to a series of "back-table" manipulations. One such manipulation is preservation in a storage medium before implantation. Previous studies have suggested preservation in 0.9% NaCl (normal saline) may harm vascular conduits and promote neointimal hyperplasia.[2,3] In 2014, Harskamp et al retrospectively examined the influence of the preservation solution failure of coronary artery vein graft using the large multi-center patient cohort from the Project of Ex-vivo Vein Graft Engineering via Transfection (PREVENT) IV trial.[4,5] Grafts were stratified by preservation solution into normal saline, buffered saline, and autologous whole blood groups. Balanced, buffered electrolyte solution-preserved grafts had 1-year vein graft failure rates significantly less than other 2 groups, and were associated with a lesser risk of 5-year death, myocardial infarction, and secondary revascularization.[5]

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Despite these findings, heparinized normal saline is still used widely in coronary artery bypass grafting, and it is unclear what comprises an optimal HSV preservation solution.[5,6]

Impaired physiologic responses as measured in a muscle bath and decreased cellular viability represent acute tissue injury and have been shown to correlate with accelerated intimal growth.[6,7] In our study, we assessed the influence of preservation solutions, including Plasma-Lyte A, normal saline, University of Wisconsin solution, Celsior solution, autologous whole blood, and glutathione-ascorbic acid L-arginine solution (GALA)[8] on cellular physiology and viability. We hypothesized that normal saline preservation would be detrimental to vein graft physiologic function and viability, and this may be owing to its acidity. These data may also provide insights into identifying the components of an HSV storage medium that enhance graft function.

METHODS

Materials and reagents. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified. University of Wisconsin solution, Celsior solution, and GALA were prepared in the laboratory and sterile filtered before use.[8-10] The compositions of the various solutions are outlined in Table I. Preservation solutions for all experiments contained 10 U/mL unfractionated heparin; GALA contained 40 U/mL.[8]

Procurement of HSV. Unprepared (UP) HSV segments were collected from coronary artery bypass grafting patients after informed consent in accordance with Institutional Review Boards of the Vanderbilt University Medical Center and the VA Tennessee Valley Healthcare System, Nashville, Tennessee, immediately after surgical dissection. Patient confidentiality was maintained in compliance with the Health Insurance Portability and Accountability Act. The segments were placed into heparinized (10 U/mL, Hospira) Plasma-Lyte A and taken to the laboratory promptly for testing. Approximately 15–30 minutes elapsed from procurement to placement in the appropriate solution. HSV preservation in Plasma-Lyte A over this duration of warm ischemia time has been shown not to influence acute functional responses.[6] Arterial, autologous whole blood was collected from select patients for use as a preservation solution.

Procurement of rat aortae. Rat aortae were collected from female Sprague–Dawley rats ($n = 13$). Animal procedures followed study protocols approved by the Vanderbilt Institutional Animal Care and Use Committee and adhered to National Institute of Health guidelines for care and use of laboratory animals. Rats were sacrificed by carbon dioxide (5%) euthanasia. Immediately after killing the rats, the descending thoracic and upper abdominal aorta was isolated via median sternotomy and dissection, placed in cold heparinized University of Wisconsin solution, and transported to the laboratory for immediate testing.

Physiologic measurements of HSV smooth muscle and endothelial function. The segments were carefully dissected free of fat and connective tissue and cut into 1- to 2-mm rings. Two rings were hung immediately (UP control), and remaining rings were placed in the preservation solutions of normal saline, Plasma-Lyte A, University of Wisconsin solution, Celsior solution, GALA, or autologous whole blood for 2 hours at room temperature before suspension in the muscle bath.

HSV rings from CABG patients, both male and female, were suspended in a muscle bath containing a bicarbonate buffer (120 mmol/L NaCl, 4.7 mmol/L potassium chloride [KCl], 1.0 mmol/L $MgSO_4$, 1.0 mmol/L NaH_2PO_4, 10 mmol/L glucose, 1.5 mmol/L $CaCl_2$, and 25 mmol/L Na_2HCO_3, pH 7.4) bubbled with 95% O_2 and 5% CO_2 at 37°C. The rings were stretched to 4 g of tension, maintained at a resting tension of 1 g, and equilibrated for 2 hours.[7,11] Force measurements were obtained using a Radnoti Glass Technology (Monrovia, CA) force transducer (159901A) interfaced with a PowerLab data acquisition system and Chart software (AD Instruments, Colorado Springs, CO). The rings were treated first with 110 mmol/L KCl (with equimolar replacement of NaCl in bicarbonate buffer) to depolarize and contract functionally viable smooth muscle cells, and the force generated was measured. Rings generating $\leq 0.025 \times 10^5$ Newtons (N)/m^2 were considered nonviable and were not used for further testing.[12] Viable tissues were allowed to reequilibrate in bicarbonate buffer for 30 minutes after KCl challenge, after which contractile response of HSV rings to a physiologic agonist, phenylephrine (1–5 μmol/L, dosed uniquely to each individual patient to obtain a contraction 50–80% that of KCl on UP tissue) was tested. Endothelial-dependent relaxation of HSV was measured by addition of carbachol (0.5 μmol/L), a cholinomimetic, and endothelial-independent relaxation, with sodium nitroprusside (0.1 μmol/L) to phenylephrine-precontracted tissues. For each experimental condition, all rings were suspended in duplicate.

Determination of pH sensitivity of endothelial function in rat aortae. Rat aortae were dissected