

Storage of saphenous vein grafts prior to coronary artery bypass grafting: is autologous whole blood more effective than saline in preserving graft function?

Maria Tsakok^{a,*}, Sarah Montgomery-Taylor^b and Teresa Tsakok^c

^a Green Templeton College, University of Oxford, Oxford, UK

^b Christ Church, University of Oxford, Oxford, UK

^c Academic Foundation Programme, Guy's and St Thomas' NHS Foundation Trust, London, UK

* Corresponding author. Tel: +44-7903543252; e-mail: maria.tsakok@gtc.ox.ac.uk (M. Tsakok).

Received 8 March 2012; received in revised form 8 May 2012; accepted 16 May 2012

Abstract

A best evidence topic in thoracic surgery was written according to a structured protocol. The question addressed was: 'Is storage of saphenous vein grafts in autologous whole blood prior to coronary artery bypass grafting (CABG) more effective than storage in saline in preserving graft function?' Altogether more than 580 papers were found using the reported search, of which, 10 represented the best evidence to answer the clinical question. The authors, journal, date, country of publication, patient group studied, study type, relevant outcomes and results of these papers are tabulated. Preservation of the vein graft endothelium during graft preparation is of well-recognized importance in forestalling graft occlusion and saphenous vein graft disease following CABG, however, the different preservation capabilities of saline vs autologous whole blood are not well validated. Although there is a complete lack of randomized clinical trials addressing this issue, some studies using basic *in vitro* techniques and animal models can be extrapolated to answer the clinical question in hand. All are consistent in demonstrating the detrimental effects of saline on vascular endothelium and therefore graft patency, but there is some disagreement in the literature as to whether autologous whole blood is superior as a storage medium. Though three well-designed studies suggest preserved endothelial function when saphenous vein grafts are stored in saline compared with storage in autologous whole blood, data from other studies are unimpressive, with two studies showing no difference. Furthermore, two elegant experiments that seek to mimic *in vivo* conditions by comparing outcomes postarterialization show no benefit of prior storage in autologous whole blood, despite the initial better-preserved endothelium. Instead, some notice should be taken of alternative storage solutions such as the University of Wisconsin solution, as some early studies suggest that it may be advantageous over both blood and crystalloid solution.

Keywords: Saline • Autologous blood • Coronary artery bypass grafting • Storage

INTRODUCTION

A best evidence topic was constructed according to a structured protocol. This is fully described in the ICVTS [1].

THREE-PART QUESTION

In [patients undergoing coronary artery bypass grafting] is [flushing and storage of the saphenous vein graft (SVG) in autologous whole blood] superior to [saline solution] in [preserving graft function]?

CLINICAL SCENARIO

While assisting in theatre you notice that intraoperative storage of the venous graft takes place in heparinized autologous whole blood (AWB), not normal saline. The consultant explains that heparinized AWB is more likely than saline to preserve graft

patency as it more closely imitates the *in vivo* environment, preventing de-endothelialization and thrombogenicity. He also informs you that despite the evidence, saline is still the solution used most frequently worldwide for intraoperative graft storage. Baffled as to why such logical evidence has not changed practice worldwide, you decide to evaluate the literature for yourself.

SEARCH STRATEGY

Medline from 1948 to April week 1 2012 using the OVIDSP interface (exp Saphenous Vein/ or vein graft\$.mp. OR Saphenous vein\$.mp) AND (preservation.mp. or exp Tissue Preservation/ or storage.mp).

SEARCH OUTCOME

Five hundred and eighty papers were found using this reported search. From these, 10 papers were identified that provided the

Table 1: Best evidence papers

| Author, journal, country, Study type (level of evidence) | Patient group | Study endpoints | Key results | Comments |
|--|--|---|--|---|
| O'Connell et al. [2], J Surg Res, USA and UK Controlled <i>in vivo</i> (animal model) studies (level IIa) | Seventeen rabbits: anaesthetized and carotid arteries exposed and a section clamped. Autologous serum or normal physiological saline were infused into either side for varying lengths of time with or without removing the clamps after periods of time | Morphological changes and intimal thickening | Arteries infused with saline showed marked disorientation of arterial morphology increasing with time exposed to saline; 3+, 4+ for 3 h saline compared with 1+, 1+ for 3 h of autologous serum All arteries demonstrated some thickening, but the extent of this was significantly increased when the infusion time increased. This xenogenic antibody damage was also further potentiated by saline infusion; 4+ thickening was seen in two rabbits with the saline compared with 0 rabbits with the autologous serum | This study demonstrates that the process of saline infusion appears to cause significant damage to the structure of the artery and thickening of the intima in a time-dependent manner |
| Wilbring et al. [3], Eur J Cardiothorac Surg, Germany Controlled <i>in vitro</i> study (human patients) (level IIa) | Nineteen patients: all suffered arterial hypertension, hyperlipidaemia and three-vessel coronary artery disease. Harvested SVGs were stored in two parts: one in PSS and the other in Ti Protec for on average 1.5 h before examination | Vessel wall tension and constriction kinetics Endothelial-dependent vasodilation Endothelial-independent vasodilation | PSS: mean tension 3.08 mN mm ⁻¹ , Ti Protec: 8.85 mN mm ⁻¹ (P = 0.01) Constriction kinetics delayed by 100 ms in PSS compared with Ti Protec (P = 0.02) Bradykinin response at maximum concentration was 15.2 vs 32.5% for PSS vs Ti Protec (P = 0.048) Response curves of SNP did not differ significantly: 77.4% in PSS vs 90.2% in Ti Protec (P = 0.12) | This study shows the detrimental effect of saline on several specific features of arterial function: wall tension and endothelium-derived vasodilation. This suggests that Ti Protec is a superior storage solution to saline |
| Gundry et al. [4], Surgery, USA Controlled <i>in vitro</i> study (human patients) (level Ib) | Thirty patients: effects of blood and saline immersion at cold (4°) and warm (28°) temperatures, and two distension pressures (100 and 300 mmHg) | Scanning electron microscopy to examine for damage | Warm saline: massive endothelial cell loss Warm blood: moderate damage Cold blood: fully preserved Cold saline: mural oedema Distension to 300 mmHg: severe endothelial damage and oedema | The authors concluded that optimal harvesting techniques comprise immersing veins in cold blood and avoiding distension above 100 mmHg. |
| Bush et al. [5] J Vasc Surg, USA Controlled <i>in vivo</i> and <i>in vitro</i> studies (animal model) (level Ib) | Seventy-five mongrel dogs: bilateral external jugular veins were dissected and flushed with either tissue culture medium 199 at 37° (Group I), heparinized AWB and stored at 37° (Group II) or heparinized normal saline at | Luminal PGI release | Although there were some early differences, one arterialization became a factor there was a progressive decline in all three experimental groups of 6-keto PGF compared with the prearterialization | This study highlights that although there are some clear advantages to AWB in the early stages of vein harvesting, this difference does not |

Continued

Table 1: Continued

| Author, journal, country, Study type (level of evidence) | Patient group | Study endpoints | Key results | Comments |
|---|---|--|---|--|
| | 4° and 200 mmHg (Group III). Vein grafts were constructed in the carotid circulation of each dog and then studied for biochemical and morphological features before or after arterialization | Luminal TxB2 release | levels ($P < 0.05$) Prearterialization: Groups I and III both had low levels while Group II was significantly elevated ($P < 0.05$) One week after arterialization all three groups demonstrated a rise, although this was greatest in Group II ($P < 0.01$), but by 6–12 weeks all groups had fallen in the levels of TxB2 to similar amounts | persist postarterialization |
| | | Luminal morphology with scanning electron microscopy | Group III treated vein segments showed significant acute injury compared with Groups I and II After arterialization, Group I developed abnormal surfaced by 24 h. Group II showed significant damage in 24 h greater than that of Group I. The healing process was about 4 weeks in Group I and 6 weeks in Group II. Despite the abnormal surface before arterialization in Group III, the morphological changes and repair process were similar to that of Group II | |
| Lawrie <i>et al.</i> [6] J Thorac Cardiovasc Surg, USA Controlled <i>in vitro</i> study (human patients) (level Ib) | Eighty-five patients undergoing coronary bypass grafting: 139 open ring preparations of SVG. The grafts were stored in either room temperature saline or room temperature blood. Effects of pressure and temperature changes were also measured | EDRF was measured | Room temperature saline and pressurization to 400 mmHg: EDRF relaxation 10.6 vs 32.4% for control segments ($P < 0.05$) Room temperature saline alone: 17.4 vs control of 29.6% ($P < 0.05$) Room temperature heparinized blood: EDRF relaxation 31.4 vs 34.1% ($P < 0.05$) Plasma-lyte solution: 28.4 vs 30.1% ($P > 0.05$) Stored at 2–4°: 18.2 vs 34.0% ($P < 0.05$) Pressurization to 400 mmHg: 20 vs 34% ($P < 0.05$) | There are various factors that are investigated in this study, but it is evident that saline is inferior to blood and plasma-lyte. High pressures and low temperatures also appear to be detrimental to graft function |

Continued

Table 1: Continued

| Author, journal, country, Study type (level of evidence) | Patient group | Study endpoints | Key results | Comments |
|---|--|---|--|--|
| Chester et al. [7] Eur J Cardiothorac Surg, UK Controlled <i>in vitro</i> study (human patients) (level IIa) | Twenty-four patients undergoing coronary artery bypass surgery: 210 ring segments of SVG removed and placed into either: (i) heparinized AWB, (ii) heparinized saline, (iii) 199-TC solution, (iv) St Thomas' cardioplegic solution, (v) plasma-lyte solution. All solutions at room temperature for 1 h | Contractile action of noradrenaline, 5-hydroxytryptamine, dopamine, histamine and ACh | Tension generated: heparinized blood 37.8 mN; heparinized saline 38.4 mN; 199-TC solution 47.1 mN; St Thomas' cardioplegic solution 56.5 mN; plasma-lyte solution 28.9 mN The responses following storage in plasma-lyte were reduced compared with cardioplegic ($P < 0.001$), 199-TC ($P < 0.001$) and heparinized saline ($P < 0.05$). The response seen after storage in cardioplegic was significantly greater than those seen in either blood ($P < 0.005$) or heparinized saline ($P < 0.005$) | These results show that storage in AWB neither enhances nor depresses vascular reactivity. However, the study also does not show a significant difference in the use of saline and, therefore, the potentiating effect is not shown to be due to depression of vasodilator mechanisms |
| Santoli et al. [8] Eur J Cardiothorac Surg, Italy Controlled <i>in vitro</i> study (human patients) (level IIa) | Fifteen patients undergoing CABG. Portions of distal saphenous vein were then either immediately fixed (control), immersed in AWB, UWS or HSSP | Relaxant action of either ACh or SNP was assessed after precontraction with histamine Electron microscopy of endothelial structure | There was no difference in the response of each substance in the different storage solutions Controls: no pathological changes AWB: extensive damage was visible including endothelial cell loss, medial oedema and necrosis HSSP: well preserved endothelium in 12 cases and partial detachment and oedema in 3 cases after 30 min, but only very few endothelial cells surviving after 5 h of immersion UWS: after 30 min no severe alteration in the cells and partial oedema after 5 h | This study demonstrates that the autologous blood is not without its problems and identifies that it can cause significant damage to the endothelium Development of newer solutions such as UWS may be more effective due to components such as adenosine that may delay degradation of adenine nucleotides to persevere human organs |
| Weiss et al. [9] Int J Clin Exp Med, Germany Controlled <i>in vitro</i> study (human patients) (level IIa) | Two hundred and ninety-three patients undergoing CABG. The grafts were stored in either saline, saline +5% albumin, HTK solution or plasma preparation (PP) freed of isoagglutinins and coagulation factors | Morphology on scanning electron microscopy | Saline: endothelium disintegrates almost immediately and after 2 h >40% cells are dead Saline +5% albumin: slightly slower process of decline but still significant numbers dead after 2–5 h HTK solution: tissue failed to main integrity PP: long-term stability and survival were observed | This study highlights that only a very specific PP appears to be suitable to preserving function of vein grafts. It also points to the conclusion that AWB is just as likely to induce a prothrombotic state as saline storage |

Table 1: Continued

| Author, journal, country, Study type (level of evidence) | Patient group | Study endpoints | Key results | Comments |
|---|--|---|--|---|
| | | Transendothelial filtration (hydrodynamic conductivity) | Only when kept in PP was the endothelium able to maintain its normal barrier function | |
| | | Adhesion of platelets to the surface of vein grafts | After exposure to all solutions the numbers of adhering platelets were equally high except for those stored in PP solution ($P < 0.001$) | |
| Dumanski <i>et al.</i> [10] In Vivo, Poland Controlled <i>in vitro</i> study (human patients) (level IIa) | Forty-eight patients undergoing coronary artery surgery. Preparation fluid used either heparinized blood or saline | Expression of surface adhesion factors | VCAM1 (40.23 and 42.71% for blood and saline, respectively vs 11.51% control) ICAM1 (48.42 and 50.63% for blood and saline, respectively vs 12.60% control) | This study suggests that damage occurs to the veins regardless of the preparation used simply as a result of the pressure required to flush the vein grafts |
| Cavallari <i>et al.</i> [11] J Surg Res, USA and Italy Controlled <i>in vitro</i> study (animal model) (level Ib) | Canine external jugular and common femoral vein segments were preserved in UWS or AWB or NS at 4°C for 45 min and 24 h | Scanning electron microscopy Isometric tension studies | Marked neutrophil migration and separation of endothelial cells were noted for veins stored in AWB and NS compared with UWS Maximum contractile responses were significantly reduced ($P < 0.05$) after storage in AWB (0.09 g/mm ²) or NS (0.12 g/mm ²) but not in UWS (0.36 g/mm ²) | This study shows that there is little difference between the use of NS and AWB in terms of structural and functional damage to the endothelium. UWS is shown to be superior in both these aspects |

CABG: coronary artery bypass grafting; SVG: saphenous vein graft; AWB: autologous whole blood; PGI: prostacyclin; PGF: prostaglandin F; HTK: histidine-tryptophan-ketoglutarate; EDRF: endothelium-dependent relaxation factor; VCAM1: vascular cell adhesion molecule 1; ICAM1: intercellular adhesion molecule 1; PSS: physiologic saline solution; ACh: acetylcholine; SNP: sodium nitroprusside; UWS: University of Wisconsin solution; HSSP: heparinized saline solution with papaverine; NS: normal saline.

most applicable evidence to answer the question. However, no double-blinded, randomized controlled trials were identified, and as such these papers do not represent the best possible evidence. These are presented in Table 1.

RESULTS

O'Connell *et al.*'s [2] seminal study demonstrated that the negative effects of saline on vascular endothelium and graft patency were the first to challenge the universally practiced use of saline for the intraoperative storage of SVGs. The authors used scanning microscopy to reveal a significant topographical alteration of the rabbit vessel wall following saline infusion. They also found that, when examined 1 month after saline infusion, the vessels had moderate to severe intimal thickening compared with autologous serum infusion. More recently, Wilbring *et al.* [3] showed that even short-term storage (90 min) in saline impairs graft endothelial vascular function, significantly reducing maximum vessel wall tension and endothelium-derived vasodilatory

function. This study was limited, however, by the small number of examined segments ($n = 19$).

Gundry *et al.*'s [4] elegant study later compared the effects of different temperatures of AWB and saline for SVG storage, using a scanning electron microscope. They show that while saphenous veins immersed in warm saline solution showed massive endothelial cell loss, corroborating the findings of O'Connell *et al.*, those immersed in warm blood showed only moderate endothelial damage. Meanwhile, at 4°C, although both cold blood and cold saline immersion fully preserve endothelium, cold saline immersion produced mural oedema. However, one criticism of this study is that it does not go on to investigate any differences following arterialization of the vein grafts.

The study by Bush *et al.* [5] first substantiates the above finding by demonstrating that whole blood preserved biochemical function better than saline solution, as assessed by the continued production of a prostacyclin (PGI) metabolite, and subsequently sought to clarify whether the superior endothelial preservation observed in saphenous veins stored in whole blood compared with saline persisted after arterialization. Surprisingly,

AWB and saline-preserved veins did not significantly vary in their capacity to release the PGI metabolite or in time taken to repair endothelial damage.

A different marker of endothelial integrity was used in a study by Lawrie *et al.* [6]: endothelium-dependent relaxation factor (EDRF). In vein graft preparations stored in saline, EDRF response was reduced to a greater extent than when compared with storage in heparinized AWB. Moreover, the EDRF response was not statistically significantly different from the control when stored in plasma-lyte solution, suggesting that this is a far better storage medium. Another study suggesting AWB's superiority as a storage medium [7] used the extent of vasoconstriction to determine endothelial graft damage. They showed that storage in saline gave the largest response to vasoconstrictors, suggesting altered vascular reactivity as a result of deterioration in endothelium morphology. The results showed that storage in patients' own heparinized blood neither enhanced nor depressed vascular reactivity, but such differences compared with saline were not statistically significant.

Nevertheless, an appealing study by Santoli *et al.* [8] strongly attests to the detrimental effect of blood on the endothelium when blood is taken out of its natural environment and used as a storage solution. Endothelial integrity following storage in different solutions was assessed using electron microscopy. All specimens stored in AWB were extensively and severely damaged, with fragmentation and detachment of the endothelium, and supplemental necrosis after 5 h immersion. Specimens stored in the other two solutions were far superiorly preserved. Though we cannot use these results to favour heparinized saline (in this study heparin was added), we can conclude that preservation with AWB was indeed damaging to the graft.

In Weiss *et al.*'s [9] well-constructed study, the authors attempted to gain a deeper insight into the reasons for graft failure. A finding worth highlighting was that vein grafts stored in heparinized AWB were equally prothrombotic as saline-stored grafts. This finding is significant, as the study environment closely mimics that which occurs during a CABG procedure. Though the scanning electron microscopic evidence is qualitative, such results are consistent with Bush *et al.*'s findings postarterialization.

Further studies have also failed to find any superiority of AWB to saline in preserving graft function. A study by Dumanski *et al.* [10] investigating the expression of adhesion molecules following flush with heparinized blood or saline found similarities in endothelial surface loss, and expression of VCAM1, ICAM1 and P-selectin. The authors conclude that damage may occur irrespective of fluid used as a result of pressure on the conduit's walls during the flushing process. A flaw in this study, however, is that all fragments were initially flushed with saline to remove residual blood, and therefore, a degree of damage from saline flushing may already have occurred. Nevertheless, the study is to be commended in using a novel, quantitative method of establishing graft susceptibility to occlusion. Cavallari *et al.* [11] also demonstrates that there is no benefit to autologous whole blood compared with normal saline in a study using scanning microscopy on canine veins stored in different media at 4°C for 45 min and 24 h. Instead, University of Wisconsin is shown to be a far superior storage medium when compared with autologous whole blood and normal saline.

CLINICAL BOTTOM LINE

Preservation of the vein graft endothelium during graft preparation is of well-recognized importance in forestalling graft occlusion and SVG disease following CABG, however, the different preservation capabilities of saline vs AWB are not well validated. Although there is a complete lack of randomized clinical trials addressing this issue, some studies using basic *in vitro* techniques and animal models can be extrapolated to answer the clinical question in hand. All are consistent in demonstrating the detrimental effects of saline on vascular endothelium and therefore graft patency, but there is some disagreement in the literature as to whether AWB is superior as a storage medium. Though three well-designed studies suggest preserved endothelial function when SVGs are stored in saline compared with storage in AWB, data from other studies are unimpressive, with two studies showing no difference. Furthermore, two elegant experiments that seek to mimic *in vivo* conditions by comparing outcomes postarterialization show no benefit of prior storage in AWB, despite the initial better-preserved endothelium. Instead, some notice should be taken of alternative storage solutions such as University of Wisconsin solution, as some early studies suggest that it may be advantageous over both blood and crystalloid solution.

Conflict of interest: none declared.

REFERENCES

- [1] Dunning J, Prendergast B, Mackway-Jones K. Towards evidence-based medicine in cardiothoracic surgery: best BETS. *Interact CardioVasc Thorac Surg* 2003;2:405-9.
- [2] O'Connell TX, Sanchez M, Mowbray JF, Fonkalsrud EW. Effects on arterial intima of saline infusions. *J Surg Res* 1974;16:197-203.
- [3] Wilbring M, Tugtekin SM, Zatschler B, Ebner A, Reichensperner H, Matschke K *et al.* Even short-time storage in physiological saline solution impairs endothelial vascular function of saphenous vein grafts. *Eur J Cardiothorac Surg* 2011;40:811-5.
- [4] Gundry SR, Jones M, Ishihara T, Ferrans VJ. Optimal preparation techniques for human saphenous-vein grafts. *Surgery* 1980;88:785-94.
- [5] Bush HL, Jakubowski JA, Curl GR, Deykin D, Nabseth DC. The natural-history of endothelial structure and function in arterialized vein grafts. *J Vasc Surg* 1986;3:204-15.
- [6] Lawrie GM, Weilbacher DE, Henry PD. Endothelium-dependent relaxation in human saphenous-vein grafts - effects of preparation and clinicopathological correlations. *J Thorac CardioVasc Surg* 1990;100:612-20.
- [7] Chester AH, O'Neil GS, Tadjakirimi S, Borland JA, Yacoub MH. Effect of perioperative storage solution on the vascular reactivity of the human saphenous-vein. *Eur J Cardiothorac Surg* 1993;7:399-404.
- [8] Santoli E, Di Mattia D, 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* 1993;7:548-52.
- [9] Weiss DR, Juchem G, Kemkes BM, Gansera B, Nees S. Extensive deendothelialization and thrombogenicity in routinely prepared vein grafts for coronary bypass operations: facts and remedy. *Int J Clin Exp Med* 2009;2:95-113.
- [10] Dumanski A, Sopol M, Pelczar M, Kustrzycki W, Zabel M. Influence of pressure on the endothelium of the saphenous vein coronary artery bypass graft. *In Vivo* 2007;21:785-9.
- [11] Cavallari N, Abebe W, Mingoli A, Hunter WJ, Agrawal DK, Sapienza P *et al.* Functional and morphological evaluation of canine veins following preservation in different storage media. *J Surg Res* 1997;68:106-15.