

Effect of Different Storage Solutions on Oxidative Stress in Human Saphenous Vein Grafts

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Abstract

Background: Ischemic injury of saphenous vein grafts (SVG) during coronary artery bypass grafting surgery negatively impacts endothelial integrity and functionality and is associated with vein graft disease and failure. The aim of this study was to evaluate the level of oxidative stress in human SVG segments following ischemic storage in three intraoperative graft storage solutions: physiological saline (PS), autologous heparinized blood (HB) and DuraGraft.

Methods: 3 mm tissue rings derived from surplus SVG segments from 50 patients were stored at room temperature for 30 min in DuraGraft, PS and HB. Total oxidative status (TOS) and total antioxidant status (TAS) levels were determined and the oxidative stress index (OSI: TOS/TAS ratio) calculated. A p-value <0.017 was considered significant implementing a Bonferroni correction.

Results: TOS values were significantly lower for DuraGraft stored samples in comparison to both PS and HB; there was no difference between PS and HB (DuraGraft: 32.6 ± 1.8 , PS: 39.6 ± 2.8 and HB: 40.6 ± 2.4 $\mu\text{mol H}_2\text{O}_2$ eqv.; DuraGraft vs. PS and DuraGraft vs. HB $p < 0.0001$, PS vs. HB $p = 0.047$). TAS was higher for both DuraGraft and HB in comparison to PS (DuraGraft: 8.9 ± 0.9 , PS: 6.9 ± 1.0 and HB: 8.6 ± 0.9 mmol Trolox eqv.; DuraGraft vs PS $p < 0.0001$, DuraGraft vs. HB $p = 0.263$, PS vs. HB $p < 0.0001$). OSI differed between all groups with the lowest value for DuraGraft (DuraGraft: 3.7 ± 0.2 , PS: 5.8 ± 0.4 and HB: 4.7 ± 0.2 $\mu\text{mol H}_2\text{O}_2$ eqv./mmol Trolox eqv.; all $p < 0.0001$).

Conclusions: saphenous veins grafts stored in DuraGraft had a lower oxidative stress level, higher antioxidant level and lower oxidative stress index in comparison to saphenous vein grafts stored in physiological saline or heparinized blood.

ClinicalTrials.gov Identifier: NCT02922088

Background

Coronary artery bypass graft (CABG) surgery remains the treatment of choice for patients with multivessel and/or left main disease.¹ Other than the use of the internal mammary artery (IMA) for in-situ grafting to the left anterior descending artery (LAD), the SVG is the most frequently used conduit.² However, the durability and patency of SVGs are significantly compromised by vein graft disease (VGD). Vein graft failure (VGF) is the end-stage of VGD and can be associated with grave complications including recurrent angina, myocardial infarction (MI), the need for repeat revascularization and death.^{3,4} These complications represent hardship to the patient and an economic burden to healthcare systems. Studies have reported incidences of VGF at graft level of 15-29% at 1 year to up to 50-60% at 10 year.^{5,6}

VGD is initiated by damage that occurs to the graft intraoperatively and in particular damage to the graft's endothelial layer. While graft injury can occur from various physical stresses including over graft pressurization during flushing, and damage inflicted during endoscopic harvesting, the primary mediator of intraoperative graft damage is ischemic injury that occurs during the period between graft harvesting

and graft reperfusion.⁷ Similar to organ transplant, wherein ischemic injury followed by reperfusion injury is manifested post-transplant as delayed graft function, chronic graft dysfunction and primary non-function, VGD following CABG is the clinical manifestation of ischemia reperfusion injury (IRI) elicited by post-reperfusion responses to damaged graft endothelium.^{8,9} This process is further exacerbated by post-operative patient related inflammatory and atherosclerotic factors such as diabetes mellitus, smoking and hypertension.

Therefore, good tissue preservation that limits intra-operative ischemic injury is a major determinant of graft patency and the preservation solution plays a key role to the extent it can reduce the effects of IRI.⁸ Preservation solutions are designed to be biocompatible with human tissue and contain components that directly or indirectly interfere with the principal mechanism of ischemic injury; oxidative damage.¹⁰ Since oxidative damage is mediated through the release of reactive oxygen species (ROS) and other oxidants from endothelial and other cells in the ischemic tissue, preservation solutions must have sufficient antioxidant activity to neutralize ROS in order to prevent oxidative damage.

To date, vascular grafts are typically stored in saline- or blood-based solutions following harvesting and prior to grafting. However, these “standard-of-care solutions” have not been designed for graft preservation and therefore cannot appropriately protect vascular conduits, in particular the endothelium, from ischemic injury that occurs during the pre-grafting interval.

Clinical studies have demonstrated the negative impact of graft storage solutions on 12-month graft failure rates. Physiological saline (PS) has a non-physiological pH (pH 5.8), is not biocompatible and does not protect against IRI and was shown to cause human saphenous vein graft injury and to be associated with higher 12-month VGF rates.¹¹ This harm is partially mitigated by the use of buffered salt solutions having physiologic pH (Normosol, lactated ringers) but since these solutions don't protect against ischemic injury, 12-month failure rates were only modestly reduced compared to failure rates observed in patients whose grafts were stored in saline or autologous blood.^{9,12} Despite these findings and the importance of the prevention of VGF, PS and autologous heparinized blood (HB) are still the most frequently used solutions for intraoperative graft storage.¹³ To this end, DuraGraft has been developed as the first approved graft preservation solution. DuraGraft is a biocompatible solution containing a synergistic cocktail of potent antioxidants to prevent ischemic injury and shown to preserve graft endothelial function and integrity.

The aim of this study was to compare the overall oxidative stress levels in SVGs after storage in DuraGraft, an endothelial damage inhibitor, to PS and HB, as controls. Evaluation was performed by measuring total oxidant status, total antioxidant status and calculating oxidative stress indices in SVG samples after storage in the different graft storage solutions.

Methods

Patients

SVGs were harvested from a total of 50 patients undergoing isolated or combined CABG using the standard hospital protocol. Graft harvesting was performed by a single operator (İT) and all biochemical analysis were performed by a single analyst (SÖ). Patients were selected from the European Multicenter Registry to Assess Outcomes in CABG Patients: Treatment of Vascular Conduits With DuraGraft (ClinicalTrials.gov Identifier: NCT02922088), but this biochemistry sub-study was physician initiated and conducted independent from the sponsor.¹⁴ It was approved by the hospital ethical committee and all patients provided written informed consent.

Graft storage solutions

DuraGraft™ (Somahlution/Marizyme, Jupiter, Florida, USA) is an ionically and pH-balanced physiological salt solution containing the antioxidants, L- glutathione and L-ascorbic acid as well as L-arginine and glucose. DuraGraft is supplied as a two-container system; the two solutions are mixed at point-of use to create the preservation solutions used in the operating theatre.¹⁵ Heparin is added before use based on the standard practice of each Center. The other storage solutions were physiologic saline (PS) (0.9% sodium chloride) and autologous heparinized blood (HB).

Graft sampling and biochemical analysis

The surplus of SVG that was not used for CABG was used for the purpose of this study. From each surplus SVG, rings of 3 mm were cut and stored in the respective storage solution at regular operating room temperature for 30 min (ambient temperature). Subsequently, the samples were immediately stored at -80 °C. After defrosting, supernatants were generated by homogenizing the samples in a glass homogenizer with 300 µl phosphate buffered saline at pH 7.4. The homogenates were centrifuged at 10,000 × g for 10 min at 4 °C after which the supernatants were removed for analysis. Results were expressed as units per gram of protein in the supernatant.

Supernatant total oxidative status (TOS) and total antioxidant status (TAS) levels were determined with commercially available kits (Rel Assay Diagnostics, Gaziantep, Turkey) and Oxidative Stress Index (OSI) values were calculated as the ratio of TOS to TAS. TOS outcomes represent the level of oxidant molecules present in the sample and indicate oxidative stress levels. TAS outcomes represent the overall antioxidant status, i.e. ability to neutralize ROS and therefore prevent cellular damage caused by ROS. TOS levels were determined spectrophotometrically using a method described by Erel, and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H₂O₂ eqv.).¹⁶ TAS levels were determined using the fully automated spectrophotometric method developed by Erel.^{16,17} The results were expressed as millimolar trolox equivalent per gram (mmol Trolox eqv.).

Statistical analysis

Categorical variables are presented as frequencies and percentages. Continuous variables are displayed as means ± standard deviation. The Shapiro-Wilk test of normality was used to test for normal distribution of the biochemical test results among treatment groups (DuraGraft, HB, BS). Differences

between treatment groups for the biochemical tests were evaluated by paired t-test. If the assumption of normality was not met, the nonparametric Wilcoxon rank sum test was used to test differences between treatment groups. To account for multiple comparisons, a Bonferroni correction was applied and a p-value of 0.017 was considered significant. All statistical analysis was performed with SAS software, version 9.4 (SAS institute, Cary, NC, USA).

Results

SVG samples were obtained from 50 patients undergoing isolated or combined CABG and valve surgery and either stored in DG (n=50), HB (n=50) or PS (n=50). The majority of patient donors were male (84%). The prevalence of diabetes mellitus was 48% and hypertension and dyslipidemia in patient donors were present in 74% and 36% of the patients, respectively (table 1). Biochemical analysis were performed for all SVG samples from all patients after storage in the respective solutions. For biochemical outcomes see table 2 and figure 1. All significant differences were at a p-values <0.0001. TOS values were similar for the PS and HB group (39.6 ± 2.8 and 40.6 ± 2.4 $\mu\text{mol H}_2\text{O}_2$ eqv., $p=0.047$), whereas the TOS values were significantly lower in DuraGraft treated SVG (32.6 ± 1.8 $\mu\text{mol H}_2\text{O}_2$ eqv.) compared to both HB and PS. TAS was significantly lower in SVG segments stored in PS (6.9 ± 1.0 mmol Trolox eqv.) compared to DuraGraft (8.9 ± 0.9 mmol Trolox eqv.) and HB (8.6 ± 0.9 mmol Trolox eqv.); no difference was observed between DuraGraft and HB ($p=0.263$). The calculated OSI values were the lowest in the DuraGraft group (3.7 ± 0.2 $\mu\text{mol H}_2\text{O}_2$ eqv.)/mmol Trolox eqv.) in comparison to both PS (5.8 ± 0.4 $\mu\text{mol H}_2\text{O}_2$ eqv.)/mmol Trolox eqv.) and HB (4.7 ± 0.2 $\mu\text{mol H}_2\text{O}_2$ eqv.)/mmol Trolox eqv.).

Discussion

Rates of VGF and associated clinical events post-CABG surgery remain high despite intra-operative measures to prevent VGF include surgical techniques such as avoiding extensive handling during SVG harvesting, selecting the optimal site for the distal anastomosis to ensure a good run-off area, avoiding kinking and flattening of the graft and the no-touch harvesting technique.¹⁸ These measures are to preserve the integrity, functionality and viability of the endothelial layer, and to reduce the occurrence of early graft thrombosis and eventual clinical sequelae. However, ischemic injury and associated oxidative damage have been identified as the primary driver of intraoperative endothelial injury that leads to vein graft disease via an IRI mechanism.

IRI is initiated during ischemic episodes through damage caused by oxidative stress; oxidative damage.^{8,19} Oxidative damage is mediated by the release of ROS from endothelial and other cells in the ischemic organ or tissue and results in chemical modification of cellular and extracellular components including proteins, lipids and nucleic acids. This damage results in overall damage to the molecular integrity of a cell, tissue or organ. The net result is loss of normal cell and matrix components leading to dead, non-functional, structurally perturbed and dysfunctional tissues, cells and matrix. Subsequent reperfusion of the ischemic organ or tissue does not restore normality but instead exacerbates damage

incurred during ischemia.^{8,19} Therefore, prevention of ischemic injury also confers protection later from reperfusion injury. Similarly to allotransplantation, prevention of IRI by storage of the SV graft in a dedicated, biocompatible and protective medium that reduces ischemic injury pre-transplant is key to providing best graft and patient outcomes following transplant/grafting.⁹

The current study investigated oxidative stress indices in SVG samples stored DuraGraft, an endothelial damage inhibitor designed for the intra-operative graft preservation, versus in the standard of care solutions, PS and HB, which served as controls. Higher OSI levels predispose the graft to higher amounts of oxidative damage during ischemic storage and more oxidative damage predisposes the graft to more severe IRI- mediated VGD which contributes to poor clinical outcomes post CABG. The main finding is that SVGs stored in DuraGraft exhibit a statistically significant lower OSI compared to grafts stored in either PS or HB. Oxidative stress in the grafts reflects a higher level of ROS and other oxidants compared to the levels of available antioxidants. As ROS/oxidant levels increasingly exceed the system's antioxidant capacity or status, OSI increases and so does oxidative damage to cellular and matrix components.

The lower OSI in DuraGraft stored grafts compared to OSI in grafts stored in PS and HB is attributed to both lower TOS levels and increased TAS levels compared to those levels in grafts stored in PS and HB. The antioxidants in DuraGraft, L-glutathione and L-ascorbic acid are known to inactivate ROS/oxidants through the ability to reduce these molecules; this activity likely explains the lower TOS levels in DuraGraft stored grafts. The higher TAS levels in DuraGraft stored grafts indicate that there is a higher reserve or a surplus of antioxidants in these grafts provided by unused L-glutathione and L-ascorbic acid molecules. Once L-glutathione or L-ascorbic acid inactivates an ROS or oxidant molecule, it becomes inactive itself and levels of antioxidants will become lower as more and more ROS are neutralized. The observation that TAS levels are also higher in DuraGraft stored grafts indicate that there is a greater reserve or supply of unused or available antioxidants compared to levels in grafts stored in PS or HB meaning that the antioxidants in DuraGraft were not depleted by ROS inactivation during graft storage. Overall, the lower OSI in DuraGraft stored grafts compared to grafts stored in PS or HB is predicted to better protect grafts from ischemic damage during ex-vivo storage. Since reperfusion injury exacerbates ischemic injury, preventing or reducing ischemic injury will also reduce or prevent subsequent reperfusion injury thereby mitigating VGD.

The current findings are consistent earlier studies conducted with DuraGraft. In an in-vitro and ex-vivo study that compared heparinized DuraGraft to heparinized PS, human SV segments and isolated pig mammary veins were flushed and submerged in DuraGraft and PS for prespecified times.¹⁰ Loss of human SVG cell viability was observed as early as 15 minutes post-exposure to PS whereas viability was maintained up to 5 hours' exposure to DuraGraft. Histological analyses performed with pig mammary veins demonstrated endothelial damage in pig mammary veins stored in PS. Cytotoxicity assays demonstrated that saline-induced microscopically visible cell damage occurred within 60 minutes. DuraGraft treated cells did not show evidence of damage or reactivity.

In a human clinical study, the effect of storage solutions on SVG early anatomical changes associated with VGD was assessed using multidetector computed tomography angiography at 1, 3, and 12 months post-CABG.¹⁵ Within each patient, two SVGs were randomized to either DuraGraft or heparinized PS to exclude differences in patient characteristics as a confounding factor. DuraGraft was found to have a favorable effect on early anatomical markers of VGD such as lesser SVG wall thickness at 12 months, particularly in the proximal segment of the graft where early disease has been shown to most frequently manifest.²⁰ To further assess the performance of DuraGraft, a 3,000 patients registry including patients that underwent isolated CABG as well as combined CABG and valve surgery has been initiated.¹⁴ Enrollment has been completed end of 2019, follow-up is ongoing and the first results are eagerly awaited.

Strengths and limitations of the study

This study was conducted at a single center, all SV were harvested by the same surgeon and all biochemical analysis were performed by a single analyst. This importantly reduces the variation in SV harvesting technique and analysis methodology. Two regularly used storage solutions and one solution specifically developed for graft storage have been tested. Moreover, samples from a large number of patients representative of patients undergoing CABG have been studied. These design elements illustrate the robust design of the study and substantiate the validity of the data. A limitation is that conform the center's practice no heparin was added to PS, unlike the addition of heparin to DuraGraft and HB. It should be further acknowledged that the pathophysiological and clinical relevance of the observed statistically significant differences need further research.

Conclusion

In conclusion, SVGs intra-operatively stored in DuraGraft showed a lower oxidative stress level, higher antioxidant level and lower oxidative stress index in comparison to saphenous vein grafts stored in physiological saline or heparinized blood. This could have implications on the prevention of vein graft disease and subsequent failure and warrant further investigation. While well designed studies are needed to confirm these hypothesis generating findings, the use of dedicated graft preservation solutions with anti-oxidant characteristics are predicted to increase saphenous vein graft patency rates thereby improving long-term clinical outcomes following CABG surgery.

Abbreviations

CABG	Coronary artery bypass grafting
CI	Confidence interval
eNOS	Endothelial nitric oxide synthase
HB	Heparinized blood
IRI	Ischemia reperfusion injury
MI	Myocardial infarction
OR	Odds ratio
OSI	Oxidative stress index
PS	Physiological saline
ROS	Reactive oxygen species
SVG	Saphenous vein graft
TAS	Total antioxidant status
TOS	Total oxidative stress
VGD	Vein graft disease
VGF	Vein graft failure

Declarations

Ethics approval and consent to participate

The study was approved by the ethical committee of Antalya Medicalpark Hospital Complex, Antalya, Turkey. All patients provided written informed consent.

Consent for publication

Not applicable

Availability of data and materials

Authors confirm that all relevant data are included in the article

Competing interests

All authors declare that they have no competing interests

Funding

This was an investigator initiated study; no external funding was requested.

Author's contribution

İT is the cardiac surgeon who harvested the saphenous vein grafts. SÖ performed the biochemical analyses. All authors were involved in the design of this study and interpretation of the data. All authors read and approve the final manuscript.

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Mestan Emek, Department of Public Health, Akdeniz University Faculty of Medicine, Antalya, Turkey performed the statistical analysis. Somahlution/Marizyme prepared the figure.

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Tables

Table 1: Patient baseline and procedural characteristics

Characteristics	N=50
Age, y, mean \pm sd	64.5 \pm 8.2
Age range, y	47 - 82
Male	42 (84.0%)
Height, cm, mean \pm sd	168.2 \pm 6.5
Weight, kg, mean \pm sd	79.5 \pm 14.0
BMI, kg/m ² , mean \pm sd	28.8 \pm 4.3
Current smoker	24 (48.0%)
Diabetes mellitus	24 (48.0%)
Hypertension	37 (74.0%)
Dyslipidemia	18 (36.0%)
Renal failure	0 (0.0%)
Peripheral artery disease	5 (10.0%)
Number of distal anastomosis	
1	1 (2.0%)
2	2 (4.0%)
3	16 (32.0%)
4	24 (48.0%)
5	7 (14.0%)

Table 2: Biochemical outcomes after storage of saphenous vein grafts in DuraGraft, physiological saline and heparinized autologous blood. A p-value <0.017 was considered significant implementing a Bonferroni correction.

	DuraGraft N=50	Physiological saline (PS) N=50	Heparinized blood (HB) N=50	p-value DuraGraft vs PS	p-value DuraGraft vs HB	p-value PS vs HB
Total oxidative stress [TOS, μmol H_2O_2 eqv.]	32.6 ± 1.8	39.6 ± 2.8	40.6 ± 2.4	<0.0001	<0.0001	0.047
Total antioxidant status [TAS, mmol Trolox eqv.]	8.9 ± 0.9	6.9 ± 1.0	8.6 ± 0.9	<0.0001	0.263	<0.0001
Oxidative stress index [TOS (μmol H_2O_2 eqv.)/TAS (mmol Trolox eqv.)]	3.7 ± 0.2	5.8 ± 0.4	4.7 ± 0.2	<0.0001	<0.0001	<0.0001

Figures

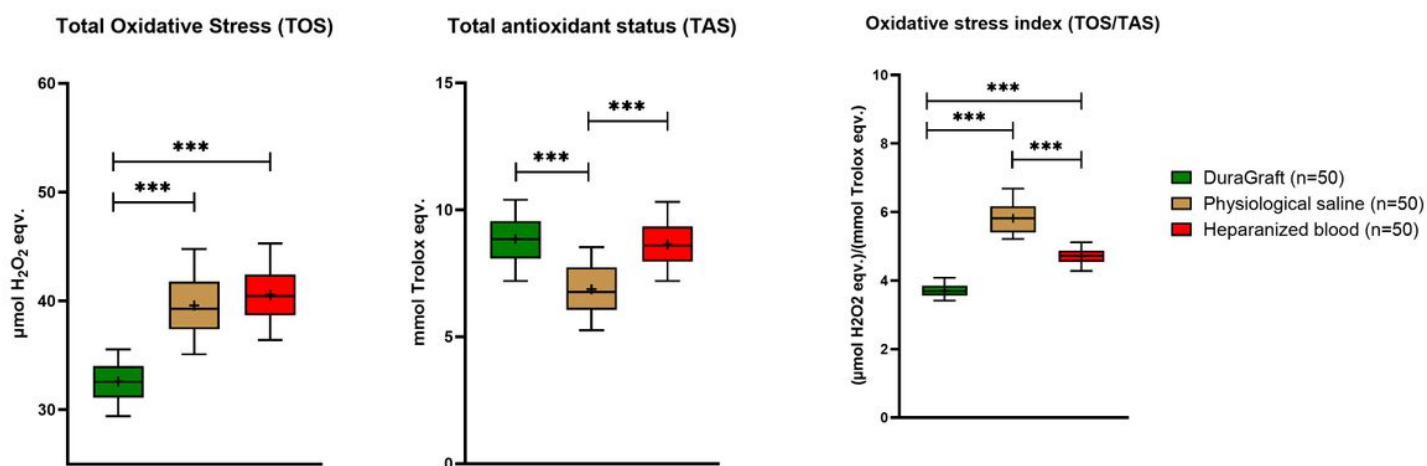


Figure 1

Box and whisker plot representing the biochemical outcomes after storage in DuraGraft, physiological saline and heparinized autologous blood with the median, mean (+), 25th and 75th percentiles, min and max values. *** indicates a p-value < 0.0001