Specific Graft Treatment Solution Enhances Vascular Endothelial Function

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Abstract

Background: Saline is still the most widely used storage and rinsing solution for vessel grafts during cardiac surgery despite knowing evidence of its negative influence on the human endothelial cell function. Aim of this study was to assess the effect of DuraGraft©, an intraoperative graft treatment solution, on human saphenous vein segments and further elaborate the vasoprotective effect on rat aortic segments in comparison to saline. Methods: Human Saphenous vein (HSV) graft segments from patients undergoing aorto-coronary bypass surgery (n = 15), were randomized to DuraGraft© (n = 15) or saline (n = 15) solution before intraoperative storage. Each segment was divided into two subsegmental parts for evaluation. These segments as well as rat aortic segments stored in DuraGraft© underwent assessment of vascular function in a multichamber isometric myograph system in comparison to Krebs-Henseleit solution (KHS), a physiologic organ buffer solution. Results: Potassium-Chloride (KCL)-induced contraction depicted a tendency towards increase when treated with DuraGraft© compared to saline preservation of HSV segments (23.02 ± 14.77 vs. 14.44 ± 9.13 mN, p = 0.0571). Vein segments preserved with DuraGraft© showed a significant improvement of endothelium-dependent vasorelaxation in response to cumulative concentrations of bradykinin compared to saline treated segments (p < 0.05). Rat aortic segments stored in saline showed significantly impaired vasoconstriction (3.59 ± 4.20, p < 0.0001) and vasorelaxation when compared to KHS and DuraGraft© (p < 0.0001). Conclusions: DuraGraft© demonstrated a favorable effect on graft relaxation and contraction indicating preservation of vascular endothelial function. Clinical Trial Registration Number: NCT04614077.

Keywords: vein graft; preservation solution; endothelium; coronary artery bypass grafting; myograph

1. Introduction

Coronary artery bypass grafting (CABG) continues to be the “gold standard” for patients with complex multivessel coronary artery disease because of the superior long-term outcome. In the modern era of arterial revascularization, saphenous vein grafts (SVGs) still remain the most often used conduits for CABG worldwide [1,2]. As widely known the graft patency is influenced by multiple factors. One of these is intimal hyperplasia progressing to vein-graft disease and graft failure [3]. Various aspects and procedures have been studied and published with fundamental improvements like pedicled harvesting technique, no touch methods and of course external stenting of the vein graft [4,5]. However, one topic was neglected for a long period but currently gains more and more attention [6]. The type of graft storage, flushing and rinsing solution that is intraoperatively used to store the conduits can largely influence endothelial integrity and vessel function [7]. Since the moment of grafting is the last time when the surgeon can influence the “auto-transplanted” vessel, every measure and care should be taken to ensure the best possible long-term outcome. This includes the intraoperative storage and flushing solution. The benefits of meticulous surgical handling, training, experience and various precautions during harvesting are redundant if the procedure is interfered by using the incorrect storage solution. In line that, a subsection of the PREVENT IV trial demonstrated that using a buffered solution resulted in lower vein graft failure rates when studied by angiography at 12 to 18 months after surgery and showed generally a better clinical outcome compared to non-buffered acidic saline (pH value of 5.5) or autologous whole blood solutions (AWB) [8]. Since AWB is only beneficial as long as inside the intact circulation and harmful once outside e.g. in the operative setting, due to an alkalotic pH (above 8) as a result of CO₂ loss we did not include AWB in the current experiments. The results for AWB in the literature are well reported and are undoubtedly not beneficial for the endothelium [9,10]. Still heparinized saline and even AWB are in use in the clinical setting, not only in coronary procedures but also for peripheral vascular surgery where the same principles apply. Therefore, novel
approaches should limit the early endothelial dysfunction or preserve endothelial integrity to boost graft patency which has become a highly demanded clinical goal these days.

The current study compared the impact of intraoperative preservation of SVGs from patients undergoing CABG in a specific ionically balanced storage solution (DuraGraft®, Maryzyme Inc, Jupiter, FL, USA) versus saline solution in the isolated organ bath testing system. DuraGraft®, is also a pH-balanced physiological salt solution containing L-glutathione, L-ascorbic acid, L-arginine and other additives that protect the graft from the damaging effects of ischemia and handling during CABG. DuraGraft® is a CE marked intra-operative graft storage solution and currently approved in Europe and several other global health care systems.

Since this is currently the only specific storage solution DuraGraft® was the target solution in this study to be compared to the still most widely used solution saline. In addition further characterization of the impact of saline on vascular endothelial function in rat aortic segments and the direct influence on human umbilical vein endothelial cells (HUVECs) was undertaken to specify initial findings.

2. Patients and Methods

The study was approved by the local Ethics Committee Nr. EK-20-219-1020 of the City of Vienna/Austria and registered as observational study by ClinicalTrials.gov under the number NCT04614077.

Saphenous vein segment remnants were collected from 15 CABG patients after their informed consent. Patient’s details are presented in Table 1. Within 15 patients undergoing aortocoronary bypass surgery, saphenous vein graft segments were randomized to DuraGraft© (n = 15) or heparinized saline (B Braun AG, Melsungen, Germany) (n = 15) solution before intraoperative storage. Each 2 cm long segment of human saphenous vein (HSV) was divided into two 1 cm long parts for twofold evaluation. In total n = 28 HSV segments were collected and n = 23 segments/conditions were used for endothelial-dependent and endothelial-independent vasorelaxation assessment. Special care was taken to exclude patients with concomitant diseases or medical treatment that could interfere with the outcome, testing methods with special focus on vessel wall reactivity and further pathophysiological vascular conditions.

The following inclusion criteria were applied:
Age between 18–80 years.
Planned CABG operation.
Suitable vein grafts with the absence of blow outs, varicose veins or previous stripping.
Exclusion criteria:
Age <18 or >80 years.
Emergency CABG.
Preoperative myocardial infarction <48 h.
Re-operation.

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<th>Table 1. Descriptive patients characteristics preoperative.</th>
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<td>Patients characteristics and cardiovascular risk factors</td>
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<td>Age, median (years)</td>
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n, numbers of patients; % indicates percentage.

Prior PCI <48 h.
Ejection fraction (EF) <30%.
Severe organ dysfunction (any malignancy, sepsis <5 days, life expectancy <3 years).
Pregnant women were not included.
Any disease of the lower veins.
Any vasculitis.
Hemoglobin A1c (HbA1c) levels >6.5% (mmol/mol).

The segments of human saphenous vein were harvested in open technique. All patients underwent preoperative ultrasound scanning of the vein segments and varicose veins (outer diameter above 3.5 mm) were excluded. Special care was taken not to stretch by brisk handling or to touch the vessel frankly during the harvest procedure, therefore vein grafts were only harvested by experienced surgeons.

2.1 Assessing Vasoactivity in Human Saphenous Vein Segments Using Wire Myograph

Human Saphenous vein segments were cut out in 20 mm pieces, carefully pressure controlled flushed with 10 mL (mL, NaCl or DuraGraft®) at room temperature and placed in the solution they were assigned to (NaCl or Duragraft), any contact with any other substance was fully avoided. There was no mixture of the substances. Flushing,
To further test the potential vascular protective effects of DuraGraft®, segments of the abdominal aorta were used from Sprague-Dawley rats. Male adult Sprague-Dawley rats (12–14 weeks old, body weight of 350–380 gram; Department for Laboratory Animal Science and Genetics, Himberg, Austria) were used. The experimental protocol was approved by the Ethics Committee for Laboratory Animal Experiments at the Medical University of Vienna and the Austrian Ministry of Science and Research (BMWF-66.009/0023-WF/V/3b/2016) and conforms with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) [12,13]. Briefly, rats were anaesthetized by intraperitoneal injection of a mixture of Xylazine (4 mg/kg; Bayer, Leverksen, Germany) and Ketamine (100 mg/kg; Dr. E. Gräub AG, Switzerland), heparin was injected (iv. femoral artery) and the abdominal aorta was collected as described previously [12].

After cleaning, the segment was cut into 2 mm sections and mounted onto a multi-chamber isometric myograph system (Model 620M, Danish Myo Technology, Aarhus, Denmark). The chambers were filled with one the following solution: (1) KHS (gold standard, positive control), (2) DuraGraft® and (3) physiological saline (0.9%). Each individual chamber was further heated and bubbled during the whole procedure. To determine the resting tension the AD Instruments’ LabChart® DMT Normalization Module to mimic physiological conditions was used with a target pressure of 100 mmHg. Segments were allowed to equilibrate for 45 minutes and resting tension was continuously adjusted during this period as described previously [11,14]. Reference contractions were elicited by hyperkalaemic (124 mM, KCl) solution. Precontraction of the aorta segments was achieved by Phenylephrine (PE, 1 nM–1 µM, Sigma Aldrich). Endothelial dependent and independent relaxation was tested by the cumulative dosage of Acetylcholine (ACh, 1 nM–10 µM, a nitric oxide-independent vasodilator, Sigma Aldrich) and sodium nitroprusside (SNP, 0.1 nM–1 µM, a nitric oxide-independent vasodilator Merck), respectively.
2.5 Data Analysis

The contractile response was defined by the stress, which was calculated using the force generated by the vein rings. Vascular relaxation to Bradykinin and ACh was expressed as percentage of contraction to NE or PE, respectively. Differences in concentration-dependent relaxations induced by Bradykinine and ACh were analysed using two-way ANOVA followed by Bonferroni’s test when appropriate. Differences between multiple groups were analysed using one-way ANOVA followed by Bonferroni’s test. The number of experimental observations (n) refers to the number of vascular segments in respective experiments.

To demonstrate the good comparability of all cohorts, statistical testing for differences in baseline, procedural, and follow-up data has been performed. Depending on the variable’s distribution continuous data are either expressed as means and standard deviation (+/- SD) or median and were analyzed with one-way analysis of variance (ANOVA). Categorical variables are expressed in absolute numbers and percentages.

All data were expressed as mean ± SD and were calculated using GraphPad Prism (Version 7.03; GraphPad Software Inc., San Diego, CA, USA).

Statistical significance was accepted when p < 0.05.

3. Results

We took maintenance of pH was vital to physiologic function and cellular viability of SVGs as given. In addition, it is important to state therefore that SVG segments after 30–60 minutes incubation with saline or DuraGraft© were transferred into Krebs solution and subsequently all measurements were performed in Krebs solution in this study.

3.1 Effect of Preservation Solution on Contractile Responses in SVGs

Maximal contraction in response to KCl (124 mmol) in Krebs solution, showed a tendency towards increase in contraction in DuraGraft© when compared to normal saline preservation HSV (23.02 ± 14.77 vs 14.44 ± 9.13 mN, p = 0.0571; segments/group, Fig. 1A). Next, when the resting tension reached a stable baseline, no attempt was made to adjust the HSV tension. After the equilibrium, the HSV segments were primed by the addition of norepinephrine (1 × 10⁻⁶ M) to the organ bath in order to measure the maximal contraction in response to α₁ adrenoceptor activation. There was no difference in maximal contraction (% of KCl contraction) achieved by NE between the groups (Saline 85.31 ± 33.9% and DuraGraft© 72.66 ± 33.13%; p = 0.167, Fig. 1B).

3.2 Effect of Preservation Solution on Endothelial-Dependent and Independent Vasorelaxation in HSV

To investigate the potential protective efficacy of DuraGraft© on the vascular endothelium, we assessed the vascular reactivity of HSV segments in patients planned for elective CABG. The HSV segments were stored in DuraGraft© showed a significantly preserved endothelium-dependent vasorelaxation in response to cumulative dosage of Bradykinin in comparison to saline stored segments (Fig. 2A, p < 0.05). Endothelium-independent vasorelaxation was assessed by the response to cumulative dosage of SNP, and there was no difference between the two storage conditions (Fig. 2B).

3.3 Vasoconstriction and Endothelial-Dependent Relaxation in Rat Aortae

In the next step, rat aorta segments were used as a model of normal vascular tissue to further characterize and compare the vascular protective effects of DuraGraft© solution. The myograph chambers were filled with KHS, saline and DuraGraft© and the aorta segments from rat were mounted. Aorta segments that were kept in DuraGraft© showed comparable response to KCl (contraction; 20.97 ± 3.37 vs 22.87 ± 2.57 mN, Fig. 3A) and ACh (endothelium dependent relaxation; Fig. 3B) as KHS solution. In contrast, the aorta segments were kept in physiological saline showed significant impairment in response to both vasoconstriction (3.59 ± 4.20, p < 0.0001, Fig. 3A) and vasorelaxation when compared to Krebs Solution as well as DuraGraft© preservation (Fig. 3B, p < 0.0001), respectively.

3.4 Cell Viability in HUVECs

Fig. 4C displays the morphology of HUVECs cultured in control medium (M200 Medium). The cells were aliquoted and kept in one of the following conditions: (1) control group (M200 Medium), (2) KHS, (3) physiologically saline and (4) DuraGraft© solution for 30 minutes or 60 minutes. Then the cell viability was evaluated by XTT assay. Saline treatment for 30 or 60 minutes markedly inhibited cell viability (Fig. 4A,B compared to control, KHS and DuraGraft©, respectively (p < 0.0001). Of importance, cell viability was similar between the control and DuraGraft© group after 30 or 60 minutes incubation (Fig. 4C), suggesting DuraGraft© maintains endothelial cells viability and metabolism, indicating the fact that it is an optimal solution for preserving endothelial cells viability and function for at least 60 minutes. In addition, HUVECs were cultured under M200 Medium, KHS or DuraGraft© presented a confluent elongated shape, while those that were subjected to saline became spherical and non-confluence.
Fig. 1. Effect of preservation solution on contractile responses in HSV. (A) In response to the high K⁺ (KCl) Krebs solution, the HSV segments showed tendency toward increase when stored in DuraGraft© compared to normal saline preservation (23.02 ± 14.77 vs 14.44 ± 9.13 mN, p = 0.0571). (B) In response to NE, HSV segments showed no difference between the storage conditions (Saline 85.31 ± 33.9% and DuraGraft© 72.66 ± 33.13%; p = 0.167). The NE response is expressed as percentage of KCl contraction. n = 15 patients and n = 23–25 segments/condition.

Fig. 2. Endothelial dependent and independent vasorelaxation-saphenous vein grafts. Effects of NaCl (black) and DuraGraft® (red) on vascular reactivity in the saphenous vein grafts. (A) Vein rings were precontracted with NE and relaxed with the cumulative dosages of Bradykinin. The Bradykinin response is expressed as percentage of the maximum NE response and baseline tension. (B) Saphenous vein were precontracted with NE and relaxed with the cumulative dosages of sodium nitroprusside (SNP). The SNP response is expressed as percentage of the maximum NE response and baseline tension. Data are expressed as mean ± SD, n = 15 patients n = 23 segments/condition.

4. Discussion

Previous studies have already suggested that preservation in physiologic saline may harm vascular conduits and can accelerate the development of neointimal hyperplasia formation [3,5–8]. Still saline is one of the most or the most widely used solution for intraoperative graft storage or graft flushing besides AWB preparations or individual mixtures. Saline if non buffered has an acidic pH value of 5.5, the physiological pH of circulating blood is 7.32 pH. AWB becomes alkalotic once outside the human circulation as described above. As Veres et al. [15] stated in 2015 storage with physiological saline and heparinized blood solutions is unable to protect the endothelium against cold ischaemia and warm reperfusion injury. Already in 2014, Harskamp et al. [8] examined the influence of the preservation solutions on vein graft failure using data from the PREVENT IV
Fig. 3. Contractile response and endothelial dependent vasorelaxation-rat aorta. (A) Aorta segments that were kept in DuraGraft© or Krebs-Henseleit solution (KHS) showed comparable response to KCl (contraction; 20.97 ± 3.37 mN vs 22.87 ± 2.57 mN) until saline stored segments showed significant impairment in vasoconstriction (3.59 ± 4.20 mN, p < 0.0001). (B) Rat aortic rings were precontracted with PE and relaxed with the cumulative dosage of ACh.Physiological treated aorta segments had a significantly impaired endothelial function compared to both DuraGraft© or KHS treated ones (***p < 0.0001 KHS vs saline; ###p < 0.0001 DuraGraft© vs saline). Data are mean ± SD, n = 3 rats and 4 segments/rat.

Fig. 4. Human Umbilical Vein Endothelial Cells-Viability. Effects of standard medium, NaCl, DuraGraft® and Krebs-Henseleit solution (KHS) on HUVECs viability after (A) 30 minutes and (B) 1 h incubation with the respective conditions. (C) Representative images of the cell morphology under the respective conditions after 1 h. Data are mean ± SD, n = 7–16 replicates/condition ***p < 0.0001 vs saline (NaCl).

trial. Grafts were randomized to different groups of preservation solution consisting of saline, buffered saline and autologous whole blood. Grafts stored in buffered saline had significantly lower one-year vein graft failure rates compared to the other two groups, and were associated with a lower risk of five-year death, myocardial infarction and secondary revascularization, suggesting that intraoperative graft preservation is one of the key procedures in order to reduce graft failure risk. Despite these important clinical findings, heparinized normal saline is still widely used in
coronary artery bypass grafting. Interestingly the first representative study was by O’Connell et al. [16] in 1974, conducted on the intima of arterial and not venous grafts. This study clearly demonstrated negative effects of normal saline (NS) on vascular endothelium and graft patency in a rabbit model. The topic of a specific graft storage or even treatment solution was neglected in cardiovascular research but gains in recent years more and more interest [6,7,17]. The data from the current study showed a clear positive effect for DuraGraft© as a representative for a specific solution. As presented in the results the HSV segments that were preserved with DuraGraft© showed significantly preserved endothelium-dependent vasorelaxation in response to cumulative dosage of Bradykinin when compared to saline preservation. The solution itself is buffered and upholding the cell metabolism due to preserving glucose levels but also reducing oxidative stress and amino acid (L-Arginine) related vasodilatation [17]. The product is a relatively novel solution against endothelial-damage developed to efficiently protect the structural and functional integrity of the vascular endothelium. DuraGraft© is described as structural and functional endothelial stabilizer in aortocoronary bypass surgery, antioxidative, radical-scavenging, nitric oxide (NO)-synthetize-supporting, anticoagulant, isotonic solution against endothelial-damaged developed to effectively preserve the endothelial cell metabolism. DuraGraft© representing a specific storage solution for preserving endothelial cell viability and function in vascular procedures. Maintaining endothelial cell integrity may be also important to reduce the risk of graft occlusion, myocardial infarction and repeat-revascularisation due to early endothelial affection and later graft failure. In this context, recent clinical studies demonstrated that intraoperative graft treatment with DuraGraft showed a favorable effect on saphenous vein graft wall thickness compared to saline treatment at 12 months follow up period [12,15,18,24,25]. Current literature shows additionally that not only saphenous vein grafts but also arterial grafts benefit from a specific treatment solution. A recent study by Aschacher et al. [14] depicted lower levels of reactive oxygen species (ROS) after the treatment. Interestingly in this study an increased expression of transforming growth factor β (TGFβ), platelet-derived growth factor α/β (PDGFα/β), and heme oxygenase-1 (HO-1) which are indicative for vas-
cular protective function was reported after Duragraft exposure. Once more, as summarized in the literature and detected in this triple approach, saline is clearly not beneficial for the human endothelium whilst Duragraft® being a representative for a specific storage and treatment solution demonstrated a significantly positive, at least superior to non-buffered saline solution effect. This study and the current results call for the stop of saline as vascular storage and graft flushing solution and for the use of a specific agent instead [7,12,14,15,17–19,21–27].

5. Limitations

The study was limited by its single center design and patient’s numbers in terms of the HSV samples. Although specific care was taken to avoid any influence on the vessel and endothelium in terms of preparation or transport some undetectable risk factors or patient’s details might be present but not obvious at the time of hospitalization. In addition, animal study experiments confirm that Duragraft® is as efficient as KHS in respect of vascular reactivity, and superior than saline. However, we have not stored the segments of aorta (rat) either in saline or that Duragraft® prior to performing vascular reactivity assessment and performed on isolated aortic not on venous segments. However, we do not anticipate a difference between the artery and the venous segment in respect to vascular protection by Duragraft®. This study represented a momentum snapshot of the influence and further longterm data is urgently needed to confirm the protective effects. The author WB is participating in the European Multicenter Trial VASC as national PI. Nevertheless, the protective effect of Duragraft® was demonstrated on human specimens in this study.

6. Conclusions

Saline is still the most widely used storage and flushing solution for vessel grafts during cardiac surgery. Saline is clearly not beneficial for the human endothelium whilst Duragraft® being a representative for a specific storage and treatment solution demonstrated a positive effect. This study and the current results call for the stop of saline as vascular storage and graft flushing solution and for the use of a specific agent instead.

Author Contributions

AK—Conceptualization; Data curation; Formal Analysis; Investigation; Methodology; Project administration; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing. PLS—Conceptualization; Investigation; Methodology; Project administration; Supervision; Validation; Writing – review & editing. CD—Conceptualization; Writing – review & editing. ZA—Conceptualization; Writing – review & editing. DG—Conceptualization; Writing – review & editing. IC—Conceptualization; Writing – review & editing. MG—Conceptualization; Writing – review & editing. BKP—Conceptualization; Investigation; Methodology; Project administration; Supervision; Validation; Writing – review & editing. BW—Conceptualization; Investigation; Methodology; Project administration; Supervision; Validation; Writing – review & editing.

Ethics Approval and Consent to Participate

The study was approved by the local Ethics Committee Nr. EK-20-219-1020 of the City of Vienna/Austria and registered as observational study by ClinicalTrials.gov. under the number NCT04614077.

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Conflict of Interest

The authors declare no conflict of interest. Attila Kiss is serving as one of the Guest editors of this journal. We declare that Attila Kiss had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Dragan M. Djuric and Tero Inoue.

References


