

Targeting Phosphodiesterase-5 by Vardenafil Improves Vascular Graft Function

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WHAT THIS PAPER ADDS

Failure of vascular grafts after bypass surgery occurs at a substantial rate and has a large impact on two main endpoints, survival and quality of life. During bypass surgery, preservation of vascular grafts causes endothelial dysfunction, leading to an enhanced rate of unfavourable early and late outcome (e.g., early graft thrombosis, vasospasm, restenosis, and occlusion). Pre-treatment with vardenafil was proven to efficiently improve the endothelial function of vascular graft and therefore it could be a novel approach in vascular and cardiac surgery.

Objectives: Ischaemia reperfusion (IR) injury occurs during vascular graft harvesting and implantation during vascular/cardiac surgery. Elevated intracellular cyclic guanosine monophosphate (cGMP) levels contribute to an effective endothelial protection in different pathophysiological conditions. The hypothesis that the phosphodiesterase-5 inhibitor vardenafil would protect vascular grafts against IR injury by upregulating the nitric oxide–cGMP pathway in the vessel wall of the bypass graft was investigated.

Methods: Lewis rats ($n = 6\text{--}7/\text{group}$) were divided into Group 1, control; Group 2, donor rats received intravenous saline; Group 3, received intravenous vardenafil ($30\text{ }\mu\text{g/kg}$) 2 h before explantation. Whereas aortic arches of Group 1 were immediately mounted in an organ bath, aortic segments of Groups 2 and 3 were stored for 2 h in saline and transplanted into the abdominal aorta of the recipient. Two hours after transplantation, the implanted grafts were harvested. Endothelium dependent and independent vasorelaxations were investigated. TUNEL, CD-31, ICAM-1, VCAM-1, α -SMA, nitrotyrosine, dihydroethidium and cGMP immunochemistry were also performed.

Results: Compared with the control, the saline group showed significantly attenuated endothelium dependent maximal relaxation (R_{max}) 2 h after reperfusion, which was significantly improved by vardenafil supplementation (R_{max} control, $91 \pm 2\%$; saline $22 \pm 2\%$ vs. vardenafil $39 \pm 4\%$, $p < .001$). Vardenafil pre-treatment significantly reduced DNA fragmentation (control $9 \pm 1\%$, saline $66 \pm 8\%$ vs. vardenafil $13 \pm 1\%$, $p < .001$), nitro-oxidative stress (control 0.8 ± 0.3 , saline 7.6 ± 1.3 vs. vardenafil 3.8 ± 1 , $p = .036$), reactive oxygen species level (vardeafil 36 ± 4 , control 34 ± 2 vs. saline 43 ± 2 , $p = .049$), prevented vascular smooth muscle cell damage (control 8.5 ± 0.7 , saline 4.3 ± 0.6 vs. vardenafil 6.7 ± 0.6 , $p = .013$), decreased ICAM-1 (control 4.1 ± 0.5 , saline 7.0 ± 0.9 vs. vardenafil 4.4 ± 0.6 , $p = .031$), and VCAM-1 score (control 4.4 ± 0.4 , saline 7.3 ± 1.0 vs. vardenafil 5.2 ± 0.4 , $p = .046$) and increased cGMP score in the aortic wall (control 11.2 ± 0.8 , saline 6.5 ± 0.8 vs. vardenafil 8.9 ± 0.6 , $p = .016$). The marker for endothelial integrity (CD-31) was also higher in the vardenafil group (control $74 \pm 4\%$, saline $22 \pm 2\%$ vs. vardenafil $40 \pm 3\%$, $p = .008$).

Conclusions: The results support the view that impairment of intracellular cGMP signalling plays a role in the pathogenesis of the endothelial dysfunction of an arterial graft after bypass surgery, which can effectively be prevented by vardenafil. Its clinical use as preconditioning drug could be a novel approach in vascular/cardiac surgery.

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INTRODUCTION

Failure of venous/arterial grafts after bypass surgery occurs at a substantial rate and has a large impact on two main endpoints, survival and quality of life.¹

During vascular/cardiac surgery, arterial and venous grafts are often stored in saline solution. Some studies

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have indicated that application of the routinely used physiological saline preservation solution for vascular grafts is incapable of reducing the endothelial damage and has a weak preservation effect on endothelium.^{2,3} In a vascular graft, during reperfusion, the activated leukocytes produce reactive oxygen species (ROS), which cause DNA strand breakage, mitochondrial function disruption, and consequently endothelial dysfunction.⁴ Mitochondrial ROS production was also found to be a critical early driver of Ischaemia reperfusion (IR) injury to cardiomyocytes;⁵ however, its specific role on endothelial cells has not yet been elucidated. There is evidence that endothelial dysfunction is an important cause of acute and intermediate vein graft failure.⁶ It has also been proven that ROS play an important role in neointimal hyperplasia, which leads to restenosis.⁷ The nitrogen oxide (NO) cyclic guanosine monophosphate (cGMP) cGMP dependent protein kinase (PKG) pathway was shown to have a considerable role in vascular and cardioprotection.^{8,9} Intracellular cGMP accumulation was also proven to reduce tissue injury in conditions associated with increased free radical release and oxidative stress.^{10,11}

Preconditioning with vardenafil, which is a selective inhibitor of phosphodiesterase-5 (PDE-5), an enzyme that catalyzes the breakdown of cGMP, an essential second messenger involved in smooth muscle relaxation, was shown to have beneficial effects against myocardial IR injury and an advantageous protective effect on vascular endothelium.^{8,9} Previously vardenafil has also been demonstrated to have a protective effect when it was added to a preservation solution, as seen by restored impaired endothelial function of vascular grafts following in vitro induced re-oxygenation.¹² Additionally, in a clinically relevant large animal model of cardiopulmonary bypass with cardiac arrest, this group showed that the application of vardenafil improves endothelial functions of the coronary artery.¹³ However, in vitro IR models are not adequate for modeling oxidative stress, only in vivo reperfusion models involve the full scale of damaging effects by restored arterial flow after ischaemia.^{14,15}

Based upon the positive effect of intracellular cGMP accumulation induced by vardenafil, the study was designed to test the hypothesis that vardenafil preconditioning could provide better protection of vascular grafts against reperfusion injury after in vivo cold ischaemia and warm reperfusion in a rat bypass model.

METHODS

Animals

Young male Lewis Rats (weight 250–350 g; Charles River Sulzfeld, Germany) ($n = 6$ in the control and vardenafil, $n = 7$ in the saline group) were used for the experiment. Procedures concerning animals conformed to the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication no. 86–23, revised 1996). The investigations were reviewed and approved by the local Ethics Committee for Animal Experimentation.

Donor animals of the IR groups received either saline or vardenafil (30 $\mu\text{g}/\text{kg}$) intravenously 2 h before explantation. In both groups, the ischaemic period was standardised to 2 h. The third group was a non-IR control. Recipients in all groups did not receive any treatment (Fig. 1).

Heterotopic aortic transplantation

The aortic rings of the control group were prepared from freshly isolated native aortic arches without ischaemic incubation and reperfusion. Control group aortic arches were cut into rings and freshly mounted in the organ bath. After the rats were randomly assigned to the experimental groups, donor animals of the IR group ($n = 6$ in the control and vardenafil groups, $n = 7$ in the saline group) received either saline or vardenafil (30 $\mu\text{g}/\text{kg}$ iv bolus, a dose similar to that of previous cardiovascular studies¹⁶ before explantation of the aortic arch. 0.014 mg/kg intravenous vardenafil is equivalent to the clinical dose of 1 mg administered to a 70 kg patient. The simple randomisation method was used as previously reported.¹⁷ To inject the solution (saline solution or vardenafil solution) in the tail vein, the donor rats were anaesthetised with isoflurane (3% to initiate anaesthesia, 1.75–2.5% to maintain anaesthesia). The depth of anaesthesia was checked using the toe pinch method.

For explantation of the aortic arch, the recipient rats were anaesthetised again with isoflurane. They were placed on controlled heating pads. The core temperature of the rats was measured at 37 °C. The donor aortic arch was harvested and flushed using cold physiological saline solution (4 °C) and stored for 80 min in cold solutions (Fig. 1). After 80 min of cold ischaemia, systemic anti-coagulation was performed in the recipient rat and the

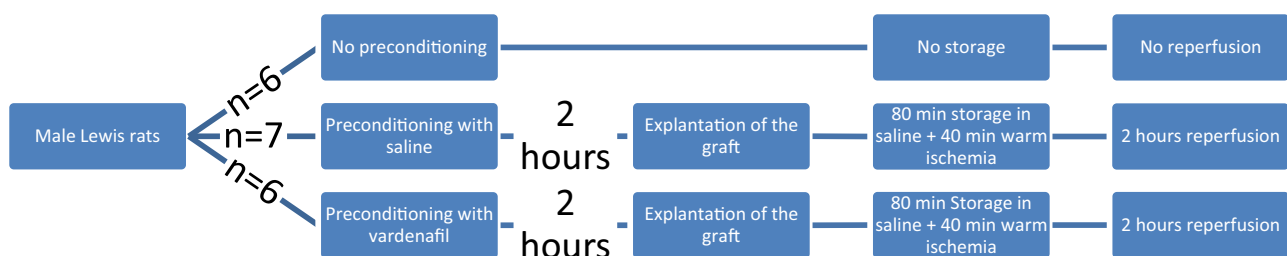


Figure 1. Groups and treatments.

aortic arch was heterotopically transplanted by two end to end anastomoses into the abdominal aorta of the isogenic recipient (~40 min surgical procedure; warm ischaemia). The total ischaemic time was 120 min altogether. Two hours after transplantation, recipient animals were killed and the implanted graft harvested. The graft segment was cut transversely into 4 mm wide rings. Isolated aortic rings were mounted on hooks in individual organ baths (Radnoti Glass Technology, Monrovia, CA, USA). The control rat also underwent anaesthesia of 2 h with isoflurane inhalation.

Additional aortic arch segments from the recipient from each experimental group were fixed in buffered paraformaldehyde solution (4%) and embedded in paraffin. Then, 4 µm thick sections were placed on adhesive slides.

In vitro organ bath experiments

Isolated aortic rings were used for functional isometric tension measurements as previously described.⁴ The aortic rings (2 aortic rings/animal in each group) were placed under a resting tension of 2 g (found optimal in preliminary experiments^{4,18}) and equilibrated for 60 min. During this period, tension was periodically adjusted to the desired level and the Krebs–Henseleit solution was changed every 30 min. At the beginning of each experiment, maximal contraction forces to potassium chloride (KCl, 80 mM) were determined and aortic rings were washed until the resting tension was again obtained. Aortic preparations were pre-constricted with an α adrenergic receptor agonist, phenylephrine (PE, 10^{-6} M) until a stable plateau was reached, and relaxation responses were examined by adding cumulative concentrations of endothelium dependent dilator acetylcholine (ACh, 10^{-9} – 10^{-4} M). For testing the relaxing response of smooth muscle cells, a direct NO donor, sodium nitroprusside (SNP, 10^{-10} – 10^{-5} M) was used. Half maximal response (EC_{50}) values were obtained from individual concentration response curves by fitting experimental data to a sigmoidal equation using Origin 7.0 (Microcal Software, Northampton, MA, USA). Contractile responses to PE are expressed as a percentage of the maximal contraction induced by KCl. The sensitivity to vasorelaxants was assessed using $pD_2 = -\log EC_{50}$ (M), vasorelaxation (and its maximum, R_{max}) is expressed as a percentage of the contraction induced by PE (10^{-6} M). Only one datum (the mean value of 2 aortic rings/animal) was used for the statistical analysis.

TUNEL reaction and dihydroethidium staining

Terminal deoxynucleotidyl transferase mediated dUTP nick end labelling (TUNEL) staining was performed to detect DNA strand breaks as previously described.⁹ Semi-quantitative histomorphological assessment was performed on all of the stained specimens using a fluorescence microscope. Four different sections from six different animals of each group were photographed with a digital camera at 200 × magnification. TUNEL -positive cell nuclei were counted by two blinded observers and, thus, the mean

value of the counted TUNEL positive cell nuclei was used for the statistical analysis.

In situ detection of ROS was performed using the oxidative fluorescent dye dihydroethidium (DHE; Sigma–Aldrich, St. Louis, MO, USA).¹⁹

Immunohistochemical staining (CD-31, nitrotyrosine, cGMP, α -SMA, ICAM-1, VCAM-1)

To detect the loss of endothelial cells in the lumen of the vascular grafts, following the manufacturer's protocol CD-31 staining was performed (anti-CD31 mouse IgG, Santa Cruz Biotechnology Inc, Heidelberg, Germany). The endothelium covered area was measured by Cell-A imaging software (Olympus, Hamburg, Germany). Expression of CD-31 was assessed microscopically, as a percentage of the endothelial surface showing a positive immunocytochemical reaction. According to the previously described methods, immunohistochemical staining was performed on aortic rings for α -SMA and ICAM-1, VCAM-1.

According to the previously described methods,⁹ immunohistochemical staining on aortic rings was performed for nitrotyrosine, a marker of peroxynitrite-mediated damage.

cGMP immunohistochemical staining was performed for identification of intracellular cGMP content as previously described.⁹

The evaluation of the immunohistochemical staining was conducted by an investigator blinded to the experimental groups. One aortic ring per animal in each group was used in the immunohistological analysis.

Chemical reagents

Vardenafil was provided by Bayer HealthCare (Wuppertal, Germany). Sodium nitroprusside was produced by Rottapharm S.L. (Barcelona, Spain). PE, ACh, and SNP were obtained from Sigma–Aldrich (Taufkirchen, Germany).

Statistical analysis

Data were tested for normal distribution (Shapiro–Wilk) and where they met the requirements for parametric analysis; means were tested by one way analysis of variance followed by a Student *t* test with Bonferroni correction for multiple comparisons. For the analysis of DHE, SMA, ICAM-1, VCAM-1, nitrotyrosine and TUNEL results, the Kruskal–Wallis test for multiple comparison was used. Effective size value (η^2) was also measured. A *p* value <.05 was considered statistically significant.

RESULTS

Vasomotor function

In aortic rings pre-contracted with PE, ACh induced a concentration dependent relaxation (Fig. 2). After 2 h of ischaemia and 2 h of warm reperfusion, a significant impairment of the endothelial function (endothelium dependent vaso-relaxation to ACh) of vascular rings was demonstrated in saline and vardenafil pretreatment groups compared with the control (*p* < .001). However, vardenafil

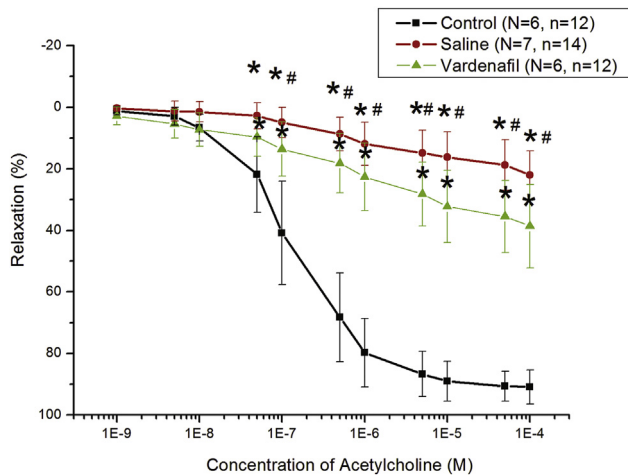


Figure 2. The effect of vardenafil on vasomotor function of rat vascular rings. (A) acetylcholine induced endothelium dependent vaso-relaxation (B) sodium nitroprusside induced endothelium independent vaso-relaxation are shown in control, saline and vardenafil groups. Values represent mean $\pm$ SD; * $p < .05$ versus control; # $p < .05$ versus vardenafil.

pretreatment improves endothelium dependent function of vascular rings when compared with the saline group (Fig. 2A). The endothelial dysfunction was indicated by the reduced maximal relaxation of vascular rings to ACh and the rightward shift of the concentration response curve compared with control (Fig. 2). There was no significant difference in $R_{\max}$ for endothelium independent vaso-relaxation of the vascular rings to SNP between the experimental groups (Table 1). Contractile responses of vascular rings to PE (10^{-6} M) are shown in Table 1. The preservation of vascular segments in saline solution increased PE induced maximum contraction in the saline and vardenafil groups compared with the control group.

Vardenafil decreases nitro-oxidative stress and DNA strand breaks in vascular grafts

To evaluate the levels of oxidative and nitrosative stress in the vascular wall after IR injury, nitrotyrosine immunoreactivity was assessed. There was a large increase in intensity of nitrotyrosine staining in the saline group compared with control segments which was reduced after vardenafil pretreatment, as evidenced by decreased brown staining ($p = .036$, $\eta^2 = 0.162$).

Increased density of TUNEL positive nuclei was observed in the wall of aortic rings in the saline group after in vivo reperfusion injury indicating DNA fragmentation (Fig. 3). However, vardenafil pretreatment significantly decreased DNA strand breaks when compared with the saline group ($p < .001$, $\eta^2 = 0.55$).

The red fluorescence signal intensity of DHE stained vascular sections was markedly increased after IR injury in the saline group suggesting a generation of ROS (Fig. 3; area intensity score vardenafil 36 ± 4 , control 34 ± 2 vs. saline 43 ± 2 , $p = .049$, $\eta^2 = 0.073$).

Vardenafil increases cGMP level, CD-31 score and decreases ICAM-1, VCAM-1 score in vascular grafts

An increased score indicated a high grade cGMP level in the aortic wall of vascular rings in the control group. In the saline group, significantly lower cGMP immunoreactivity was detected than in the control group. However, in the vardenafil group after in vivo reperfusion, a significantly higher score of cGMP staining was observed when compared with the saline group ($p = .016$, $\eta^2 = 0.499$, Fig. 4).

The inner walls of all aortic segments (control, saline, and vardenafil) were covered with endothelium, and CD-31 was detected. In the control group, the endothelial surface showing positive reaction for the CD-31 antigen was estimated as $74 \pm 4\%$ (Fig. 5). A significant increased CD-31 reaction was shown in the vardenafil group when compared with the saline group ($p = .008$, $\eta^2 = 0.435$).

Immunohistochemical staining revealed a high expression of ICAM-1 and VCAM-1 in the saline and vardenafil groups (Fig. 5). Control grafts barely expressed ICAM-1. ICAM-1 and VCAM-1 scores were significantly lower in the vardenafil group than in the saline group ($p = .031$, $\eta^2 = 0.122$, and $p = .046$, $\eta^2 = 0.114$, respectively).

Expression of smooth muscle α -actin

Semi-quantitative analysis showed a remarkable difference in the media of the grafts in the saline and vardenafil groups after 2 h of reperfusion when compared with the control group (Fig. 4). However, the α -SMA presence in medial layers of the vessels was significantly lower in the saline group than in the control and vardenafil groups ($p = .013$, $\eta^2 = 0.159$).

Table 1. Values of maximal relaxation ($R_{\max}$, %) and pD_2 to acetylcholine, to sodium nitroprusside (SNP) and contraction forces induced by phenylephrine (10^{-6} M) in control, saline, or vardenafil bypass graft rings.

	Control (n = 6 rats)	Saline (n = 7 rats)	Vardenafil (n = 6 rats)
$R_{\max}$ to acetylcholine (%)	90.9 $\pm$ 5.5; 91.9	21.9 $\pm$ 7.8; 21.9 ^a	38.6 $\pm$ 13.6; 33.3 ^{a,b}
pD_2 to acetylcholine	6.8 $\pm$ 0.3; 6.8	5.9 $\pm$ 1.4; 5.9 ^a	6.1 $\pm$ 1.4; 6.1 ^a
$R_{\max}$ to SNP (%)	100.5 $\pm$ 1.0; 100.7	106.5 $\pm$ 8.3; 103.2	103.9 $\pm$ 6.4; 103.3
pD_2 to SNP	8.1 $\pm$ 0.4; 8.1	7.6 $\pm$ 0.6; 7.5	7.8 $\pm$ 0.5; 7.8
Phenylephrine (% of KCl)	95.5 $\pm$ 20.6; 91.9	147.0 $\pm$ 45.8; 133.9 ^a	150.1 $\pm$ 45.2; 145.4 ^a

Values represent mean $\pm$ SD; median.

^a $p < .05$ versus control.

^b $p < .05$ versus saline.

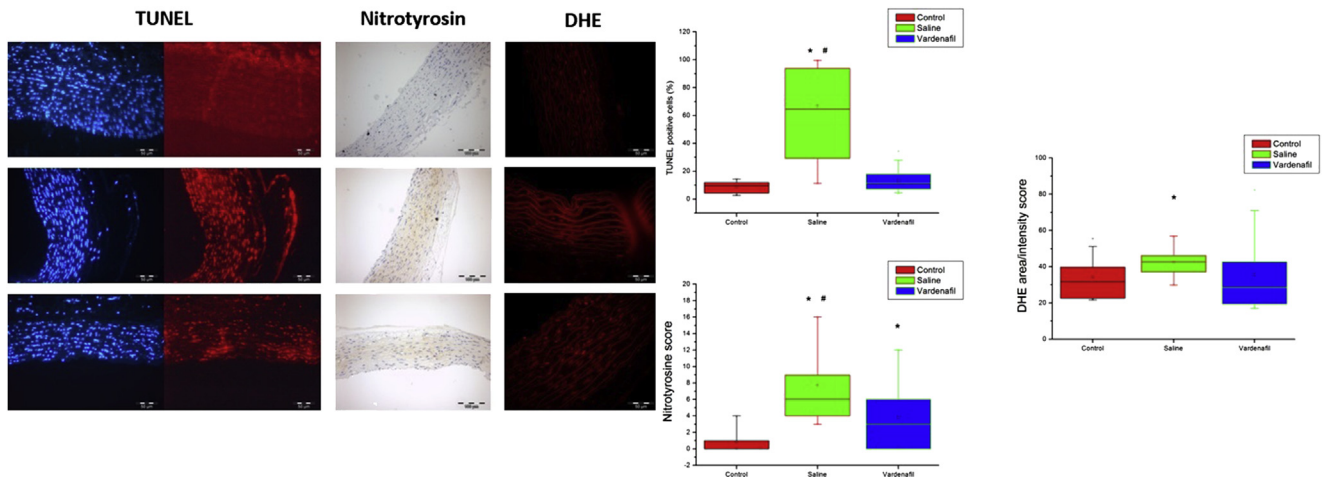


Figure 3. Effects of vardenafil on DNA strand breaks, ROS levels and nitro-oxidative stress in vascular graft. Groups: A, Control ($n = 6$ rats); B, Saline ($n = 7$ rats); C, Vardenafil ($n = 6$ rats). Individual points in each group overlay box plot. The box defines the 25th to 75th interquartile range and both median (line) and mean (empty square) values are shown within the box. * $p < .05$ versus control; # $p < .05$ versus vardenafil.

DISCUSSION

The major finding of this study is that vardenafil pretreatment provides significantly better endothelial protection of the vascular graft against IR injury with respect to the

function and structure of the endothelium. The protective effect of vardenafil was demonstrated by improved vasodilatory capacity, lower grade DNA strand breaks, reduced nitro-oxidative stress, maintained CD-31, and decreased

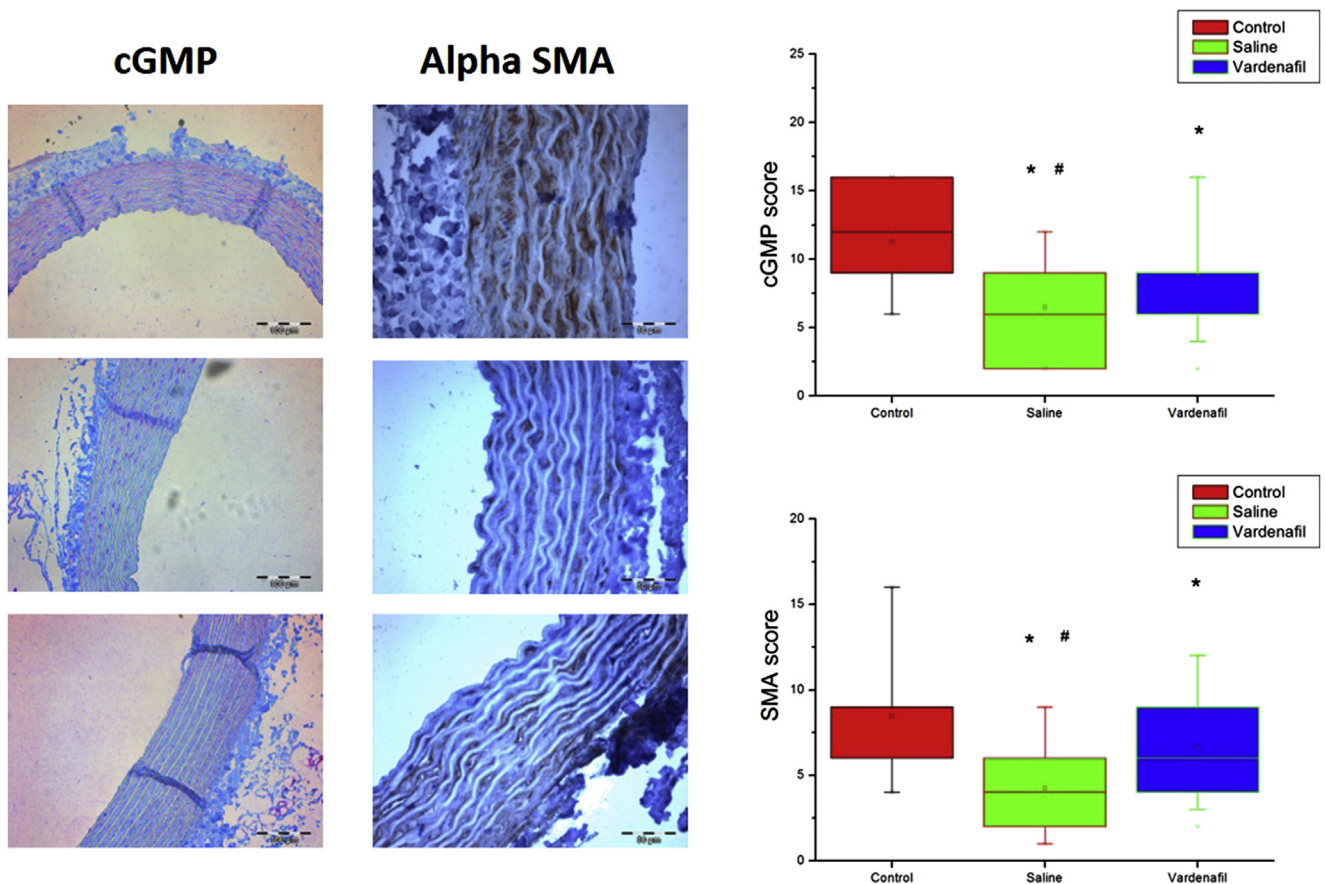


Figure 4. Representative photomicrographs and semi-quantitative scoring of immunohistochemical staining for cGMP and α -SMA. Groups: A, Control ($n = 6$ rats); B, Saline ($n = 7$ rats); C, Vardenafil ($n = 6$ rats). Individual points in each group overlay box plot. The box defines the 25th to 75th interquartile range and both median (line) and mean (empty square) values are shown within the box. * $p < .05$ versus control; # $p < .05$ versus vardenafil.

ICAM-1/VCAM-1 expression of the vascular wall and more intracellular cGMP expression in the smooth muscle cells.

Endothelial dysfunction occurs within a few minutes after reperfusion during bypass surgery, characterised by decreased release of NO, increased ROS production, neutrophil activation, and adhesion molecule upregulation.²⁰ Indeed, in the present study, a severely impaired vasodilatory capacity of the vascular graft and a significant upregulation of the adhesion molecule ICAM-1 and VCAM-1 were found after short-term reperfusion injury. Furthermore, reperfusion resulted in an increased nitro-oxidative stress and a marked formation of DNA strand breakages in the vessel wall as evidenced by nitrotyrosine, DHE, and TUNEL staining. In addition, the structural integrity of the endothelial cells was severely damaged, the number of endothelial cells was dramatically reduced after short-term reperfusion, as evidenced by the CD-31 immunohistochemical staining. Furthermore, the cGMP level was significantly lower than the control group. For testing relaxing responses of vascular smooth muscle, a NO donor, SNP was used in this experiment. In the current study, IR injury did not significantly affect $R_{\max}$ of vascular grafts to SNP. Given these results, the impaired endothelium dependent vaso-relaxation could be explained by a reduced number of endothelial cells of the surface of vascular graft and a reduced ability of the vascular endothelium to generate NO and not due to a reduced ability of the vascular smooth muscle to relax in response to NO, since the relaxant effect of the NO donor SNP remained unaltered. Consistent with these data, previous works on vascular graft storage have reported similar results.

Furthermore, a significant increase in contractile forces induced by PE in the in vivo IR groups (saline and vardenafil) was also found. This might be due to leukocyte induced impairment of basal NO release of the endothelium. This hypothesis is also supported by previous findings showing

no difference regarding contraction forces in rat aortic rings without endothelium after peroxynitrite exposure.⁴

The NO—cGMP—sGC pathway plays an important role in cardiovascular regulation of the endothelium and the smooth muscle cells.²¹ Previous experimental studies have demonstrated powerful myocardial and endothelial protection by pharmacological PDE-5 inhibition,²² by sGC activation,⁹ and by supplementation of an NO donor, L-arginine.²³

It has not been previously tested whether the selective inhibition of PDE-5 would also be capable of preventing IR injury of vascular grafts after bypass surgery. For the first time, a significantly improved endothelial function of bypass grafts and a preserved integrity of smooth muscle cells by pharmacological preconditioning with the PDE-5 inhibitor vardenafil has been reported.

Apart from inhibiting PDE-5, vardenafil has a potent antioxidant activity. It has been demonstrated that oxidative stress increased PDE-5 expression in the cardiomyocytes in heart failure²⁴ and PDE-5 inhibitors attenuated oxidative stress by inhibiting free radical formation and by supporting antioxidant redox systems.²⁵

In the current study, as a direct result of the treatment with vardenafil an elevated expression of cGMP was found in the smooth muscle layer. Intracellular cGMP accumulation has previously been shown to reduce reperfusion injury in conditions associated with increased free radical release and oxidative stress.⁹ The cytoprotective effect of vardenafil was indicated in several models of regional and global IR injury.^{13,26} Speranza et al.²⁷ treated human monocytes with PDE-5 inhibitors and found a lower concentration of ROS. In addition, vardenafil pretreatment reduced hypochlorite induced vascular injury in a rat model.²⁸ Furthermore, other experimental studies showed that PDE inhibition improved the vascular function of human umbilical vein endothelial cells in diabetes.^{29,30}

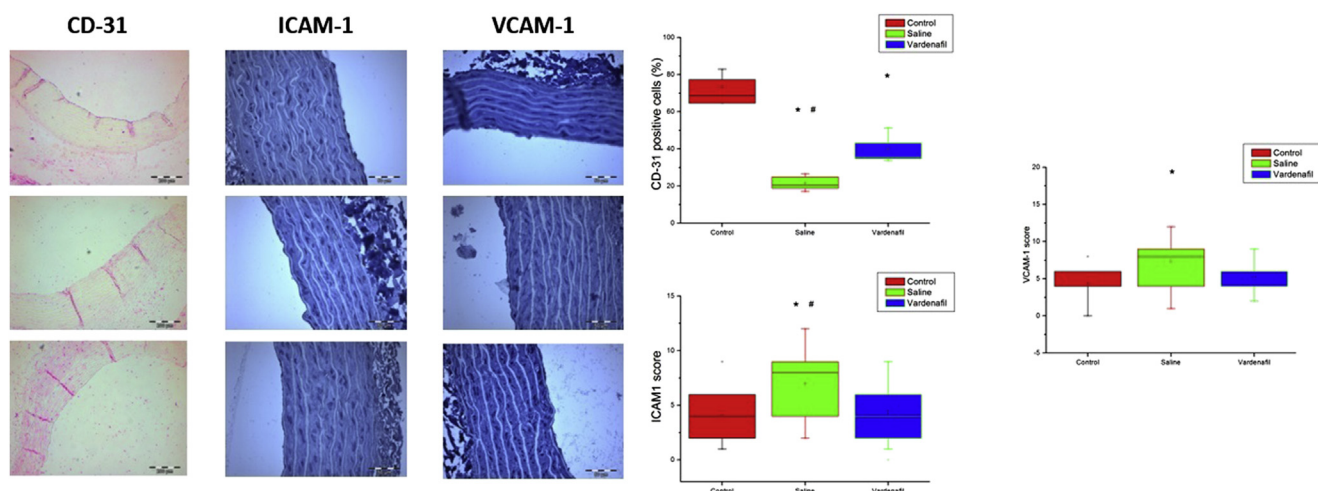


Figure 5. Representative photomicrographs and semi-quantitative scoring of immunohistochemical staining for CD-31, ICAM-1 and VCAM-1. Groups: A, Control ($n = 6$ rats); B, Saline ($n = 7$ rats); C, Vardenafil ($n = 6$ rats). Individual points in each group overlay box plot. The box defines the 25th to 75th interquartile range and both median (line) and mean (empty square) values are shown within the box. * $p < .05$ versus control; # $p < .05$ versus vardenafil.

In accordance with the literature, it was proven that DNA strand breaks as an indicator for ROS induced injury could be effectively prevented by vardenafil pretreatment as evidenced by TUNEL. Furthermore, the cytoprotective effect of vardenafil could be also documented by the nitrotyrosine immunohistochemistry results. As a consequence of lower oxidative stress, the endothelial structures (CD-31 expression) remain intact in the vardenafil group. The possible molecular mechanism for this endothelial protection may be because of the increase in intracellular cGMP levels through application of vardenafil, which induces increased PKA or/and PKG activation and more smooth muscle relaxation. The PKG dependent cytoprotective mechanism of vardenafil also involves the opening of mitochondrial K⁺ ATP channels as demonstrated by other studies.^{16,31}

Study limitations

The PDE-5 inhibitor vardenafil may have therapeutic potential to lower oxidative stress with the aim of improving the clinical outcome of IR injury in vascular grafts after bypass surgery. However, a limitation of this study is that rat aortic tissue differs from arterial/venous grafts for bypass surgery in the structure of the vessel wall, which limits the transferability of the results. Furthermore, direct correlation between graft patency and early vascular impairment was not investigated in this study. Moreover, additional studies would be interesting to investigate the effect of vardenafil on venous grafts.

CONCLUSIONS

In conclusion, it has been reported for the first time that preconditioning with the PDE-5 inhibitor vardenafil restored impaired endothelial function of vascular graft after short-term cold storage followed by warm reperfusion. This work supports the view that PDE-5 inhibition might be advantageous in the treatment of endothelial dysfunction of vascular grafts.

CONFLICT OF INTEREST

None.

FUNDING

None.

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