Eur J Vasc Endovasc Surg (2018) ■, 1–8

Targeting Phosphodiesterase-5 by Vardenafil Improves Vascular Graft Function

Gábor Veres [a,*], Martin Hagenhoff [a], Harald Schmidt [a], Tamás Radovits [b], Sivakkanan Loganathan [a], Yang Bai [a], Sevil Korkmaz-Icöz [a], Paige Brlecic [a], Alex Ali Sayour [b], Matthias Karck [a], Gábor Szabó [a]

[a] Department of Cardiac Surgery, University of Heidelberg, Heidelberg, Germany
[b] Heart Centre, Semmelweis University, Budapest, Hungary

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 ($n = 6–7$/group) were divided into Group 1, control; Group 2, donor rats received intravenous saline; Group 3, received intravenous vardenafil (30 μg/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 ± 2%; saline 22 ± 2% vs. vardenafil 39 ± 4%, $p < .001$). Vardenafil pre-treatment significantly reduced DNA fragmentation (control 9 ± 1%, saline 66 ± 8% vs. vardenafil 13 ± 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 (vardenafil 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 ± 4%, saline 22 ± 2% vs. vardenafil 40 ± 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.

Article history: Received 21 August 2017, Accepted 24 March 2018, Available online XXX

Keywords: Vardenafil, Endothelial dysfunction, Graft, Ischaemia reperfusion injury, cGMP

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

* Corresponding author. Laboratory of Cardiac Surgery, Department of Cardiac Surgery, University of Heidelberg, INF 326, 69120, Heidelberg, Germany.

E-mail address: gaborveres@yahoo.com (Gábor Veres).

1078-5884/

https://doi.org/10.1016/j.ejvs.2018.03.025

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 modelling 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 μg/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 μg/kg iv bolus, a dose similar to that of previous cardiovascular studies[16] 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 anticoagulation was performed in the recipient rat and the

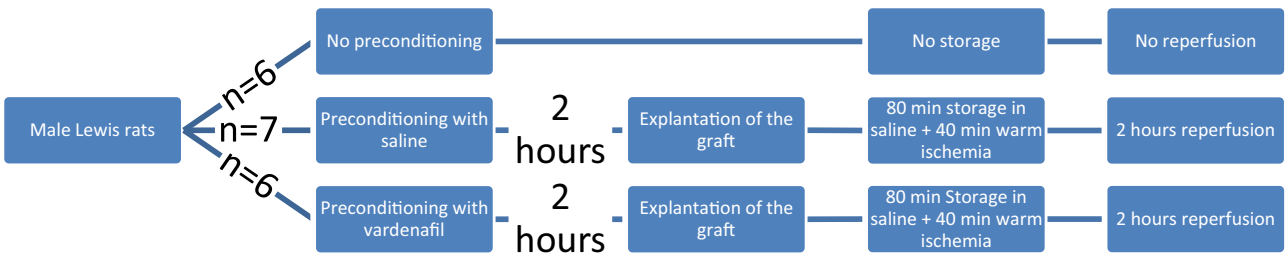

Figure 1. Groups and treatments.

Please cite this article in press as: Veres G, et al., Targeting Phosphodiesterase-5 by Vardenafil Improves Vascular Graft Function, European Journal of Vascular and Endovascular Surgery (2018), https://doi.org/10.1016/j.ejvs.2018.03.025