The effect of lidocaine on the endothelium damage in rabbit vein grafts

Yusuf Unal, MD, Oncul Sema, MD, Demirel C. Bekir, MD, Yilmazer Demet, MD, Arslan Mustafa, MD, Kavutcu Mustafa, PhD, Alper Murat, MD, Ilvan Gizem, MD.

ABSTRACT

الأهداف: التحقق من آثار حقن عقار ليدوكين داخل الأوردة وفي محيط الأوعية وذلك على تركيب البطانة الوريدية بعد سحب الأوردة واستعمالها في تطعيم الشرايين الإكليلية.

الطريقة: أُجريت هذه الدراسة في جامعة غازي لأبحاث الحيوانات، أنقرة، تركيا وذلك خلال الفترة من 16 مارس إلى 20 مارس في العام 2009م، وشملت هذه الدراسة 6 أرانب حيث تم استخدام 3 أوردة أذنية في كل أرنب. لقد تم تقسيم الحيوانات إلى المجموعات التالية: مجموعة التحكم (مجموعة C العدد=6 أوعية)، وتم قطع جزء الوريد من نهاية القاصية. ومجموعة الأوعية المحيطية (مجموعة p العدد=6)، وتم حقن 2 مل من ليدوكين (1%) في محيط الأوعية بالوريد الثاني من نفس الأذن، ومن ثم تم قطع جزء الوريد. ومجموعة الأوعية الداخلية (مجموعة I العدد=6)، وتم استخدام الملقاط للأوردة في الأذن الثانية، ومن ثم تم حقن 2 مل من ليدوكين (1%) داخل الأوعية، وبعد مرور 5 دقائق تم قطع 2 سم من جزء الوريد. لقد تم تحليل مستويات كلاً من حامض الثيوباربيوتريك، وسينثاز أكسيد النيتريك، في حين تم فحص الأنسجة مجهرياً من أجل تقييم ميكانيكية عمل عقار ليدوكين والآثار المترتبة من استخدامه.

النتائج: أشارت الدراسة إلى تشابه نتائج تشريح الأنسجة في المجموعات C، وP، وI. ولقد كانت مستويات حامض الثيوباربيوتريك أقل في المجموعتين P (p=0.041)، وI (p=0.024) بالمقارنة مع المجموعة C، غير أن مستويات سينثاز أكسيد النيتريك في المجموعتين P (p =0.037)، وI (p=0.026) كانت أعلى من المجموعة C.

خاتمة: أثبتت الدراسة أن كلاً من ارتشاح محيط الأوعية وحقن العقار داخل الأوعية قد يحميان من عواقب تضرر بطانة الأوعية وذلك بعد سحب الأوردة واستعمالها في تطعيم الشرايين الإكليلية.

Objectives: To investigate the effects of perivascular and intravenous application of lidocaine on venous endothelial morphology of harvested rabbit vein graft.

Methods: This study was conducted in Gazi University Animal Research Laboratory, Ankara, Turkey in March 2009. Three ear veins of each rabbits (n=6) were used. Control group (Group C, n=6 vessels), vein segment was excised from the distal end. Perivascular group (Group P, n=6); 2 ml lidocaine 1% was injected in the perivascular area of the second vein of the same ear, and the vein segment was excised. Intravascular group (Group I, n=6); the vein in the second ear was clamped and 2 ml of lidocaine 1% was administered intravascular, and 5 minutes later, a 2 cm vein segment was excised. Nitric oxide synthase and thiobarbutiric acid reactive substance levels were measured, and histopathologic examination was performed to assess the effects of lidocaine administration.

Results: Histopathological findings in groups C, P and I were similar. There were lower thiobarbituric reactive substances level in groups I (p=0.024) and P (p=0.041) than the control group. Nitric oxide synthase activity was higher in groups I (p=0.026) and P (p=0.037) when compared to group C.

Conclusion: Our results showed both perivascular infiltration and intravascular administration could prevent the consequences of endothelium damage that occurs during the harvesting of veins.

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From the Departments of Anesthesiology and Reanimation (Unal, Sema, Bekir, Mustafa A, Gizem), Biochemistry (Mustafa K), Faculty of Medicine, Gazi University, and the Department of Pathology (Demet, Murat), Diskapi Yildirim Beyazit Education and Research Hospital, Ankara, Turkey.

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Address correspondence and reprint request to: Dr. Yusuf Unal, Department of Anesthesiology and Reanimation, Faculty of Medicine, Gazi University, Ankara, Turkey. Tel. +90 (312) 2024166. Fax. +90 (312) 2447833. E-mail: yunal71@yahoo.com/yunal@gazi.edu.tr

Autologous saphenous vein is the most commonly used conduit for coronary artery bypass surgery (CABG), however it has high occlusion rates.[1] The patency rate at one year was 78%, 5 years was 65%, and 10 years was 57%.[1] One important cause of this high failure rate may be the vascular trauma occurring during vein harvesting and graft insertion. Manipulation of the outer layer of the vein during the surgery induces vasospasm and causes severe damage to the vasa vasorum. Damage of the adventitia and concomitant disruption of the vasa vasorum are associated with graft occlusion, owing to neointimal hyperplasia and subsequent reduction in lumen diameter. These events lead to the final stages of graft failure due to a further increase in neointima formation and superimposed atherosclerosis.[2] Although the exact mechanism of vein's reaction (spasm) to harvesting is not clear, stimulation of the perivascular sympathetic nerves during harvesting can be a major factor.[3] Specific changes occur in the endothelium during ischemia-reperfusion (I/R) injury. Malondialdehyde (MDA) is currently estimated by measurement of thiobarbituric acid reactive substances (TBARS). These are end products of lipid peroxidation, which reflect inflammatory response, and production of toxic oxygen metabolites.[4] The preservation of nitric oxide (NO) pathway and nitric oxide synthase (NOS) activity is another factor reflecting the endothelial function.[5,6] Standard surgical handling of vein grafts induces cytokines-driven inflammation in the vessel wall and impairs vascular function. This may potentially contribute to both early and late graft occlusion.[7] Local anesthetics have been shown to reduce cytokines production in different inflammatory models.[8] Lidocaine is still largely used as a short-acting local anesthetic and antiarrhythmic agent. Lan et al[9] demonstrated that increased in vitro neutrophil and endothelial cell adhesion molecule expression on exposure to plasma obtained during the early reperfusion phase is diminished by lidocaine. The protective effects of lidocaine, when used as adjunctive to preservative solution, on saphenous vein endothelium was shown in our previous study.[10] Yokoyama et al[11] also showed that topical lidocaine administration prevents vasospasm after microsurgery. The effects of perivascular or intravascular administration of lidocaine in venous graft before harvesting may prevent vein graft failure, and to test this hypothesis, we investigated NOS, TBARS levels, and histological changes in vein graft treated with or without lidocaine.

Methods. This study was conducted in Gazi University Animal Research Laboratory, Ankara, Turkey in March 2009. The Animal Research Committee, Gazi University, Ankara, Turkey approved the experimental protocol. All animals were maintained in accordance with the recommendations of the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Six male adult New Zealand Rabbits weighing 2-3 kgs were included in this study. The rabbits were anesthetized by intramuscular injection of 35 mg/kg ketamine and 5 mg/kg xylazine before vein harvesting. The same surgeon dissected 3 ear veins in each rabbits using a classical surgical technique for purposes of harvesting. The harvested veins were divided into 3 groups: 1) Control group (Group C); after dissection, a vein segment of 2 cm was excised from the distal end. Half of this 2 cm segment was placed in 10% formalin solution for histologic evaluation and the other half was stored in -80°C for biochemical analysis. 2) Perivascular group (Group P); 2 ml of lidocaine 1% was injected with a 24 G needle in the perivascular area of the second vein of the same ear. Two centimeter of vein segment were excised from the distal end, 5 minutes after injection. Half of this one cm segment was placed in 10% formalin solution for histologic evaluation and the other half was stored in -80°C for biochemical analysis. 3) Intravascular group (Group I); Proximal end of the vein in the second ear was clamped, and 2 ml of lidocaine 1% was administered intravascular with a 24 G needle. The vein was kept clamped during the waiting period of 5 minutes. Subsequently, 2 cm of the vein segment was excised from the distal end, and divided into 2 parts of one cm each. The first half was placed in 10% formalin solution for histologic evaluation and the other half was stored in -80°C for biochemical analysis.

Histologic evaluation. Vein grafts were fixed in 10% buffered neutral formalin, dehydrated in increasing degrees of ethanol, and then stained with hematoxylin and eosin. Histologic specimens were evaluated according to previously defined criteria,[10] and scored. Histologic evaluation criteria and scores were: 0 = no injury; 1 = minimal injury, minimal endothelial desquamation and minimal basal laminar separation; 2 = medium injury, medium endothelial desquamation and edema in media and intima; 3 = severe injury, extended endothelial desquamation and extended edema formation in media and intima.

Biochemical analysis. Vascular tissues were washed with deionized cold water to discard blood, and tissues were homogenized in 4 volumes of ice-cold Tris-HCl buffer (50 mM, pH: 7.4) for 2 min at 5000 rpm using a homogenizer (Diax 900, Heidolf Instruments GMBH & Co Kg, Germany). The homogenate was centrifuged at 5000 g for 60 min to remove debris, and clear upper supernatant fluid was taken. Thiobarbituric acid (TBA) reactive substances were analyzed at this stage. The supernatant was extracted with an equal volume

of an ethanol/chloroform mixture (5/3, v/v). After centrifugation at 5000 g for 30 min, the clear upper layer was taken, and used in the NOS activities. The NOS activities were measured as described below.

Nitric oxide synthase activity method was based on the diazotization of sulfanilic acid by nitric oxide at acid pH and subsequent coupling to N-(1-napthyl-ethylene diamine), which is a modification of a previous study.[12] The analysis scheme of the NOS activity measurement method has been described in a previous study.[12] Measurement of the NO pool (mainly consisting of NO^- +$NO2^-$) is also based on the same chemical reaction, in which to a greater extent nitric oxide (NO^-), and to a lesser extent nitrite anion ($NO2^-$), but not nitrate anion ($NO3^-$), give a diazotization reaction with sulfanilic acid. The absorbance of complexone formed with N-(1-napthyl-ethylene diamine) reflects the sum of NO^- and $NO2^-$ levels in the reaction medium. In this method, sodium nitroprusside is used as the chemical standard, and the reaction scheme given for the NOS activity measurement, except for the incubation of the sample with arginine, is followed. The thiobarbutiric acid-reactive substances levels were determined in the same supernatant fraction by using the thiobarbituric acid method of Durak et al,[12] and were expressed in nmol/mg protein.

Statistical analysis. The statistical analyses were performed using the Statistical Package for Social Sciences version 12.0 software program (SPSS Inc, Chicago, IL, USA). A $p<0.05$ was considered the statistically significant level. The findings were expressed as mean ± standard deviation. The data were evaluated by using Kruskal-Wallis variance analysis. The variables with significance were evaluated with Bonferroni corrected Mann-Whitney U test.

Results. Thiobarbituric acid reactive substances are end products of lipid peroxidation, which reflect inflammatory response, and the TBARS level in the intravascular (p=0.024) and perivascular lidocaine groups (p=0.041) were lower than in the control group (**Table 1**). Higher NOS activity reflects the viability of endothelial function and the NOS activity in the intravascular (p=0.026) and perivascular lidocaine groups (p=0.037) were higher than the control group (**Table 1**). The same surgeon with classical surgical technique harvested 3 ear veins of each rabbit, and there was no significant difference among the histological scores within the groups (**Table 1**). Histologic evaluation results were: **Figure 1** = minimal injury; minimal endothelial desquamation and minimal basal laminar separation. **Figure 2** = medium injury; medium endothelial desquamation and edema in media and intima. **Figure 3** = severe injury; extended endothelial

Table 1 - Vascular nitric oxide synthases (NOS) activities (mIU/mg protein), thiobarbutiric acid-reactive substances (TBARS) levels (nmol/mg protein), and histological scores of the groups.

Parameters	Control (n=6)	Intravascular (n=6)	Perivascular (n=6)
TBARS	13.49 ± 8.80	4.08 ± 1.86*	5.18 ± 1.98*
NOS	2.83 ± 2.21	7.62 ± 2.42*	6.74 ± 3.56*
Histological scores	2.17 ± 0.75	1.33 ± 0.52	1.83 ± 0.41

Values are expressed as mean ± SD.
*$p<0.05$ compared with the control group

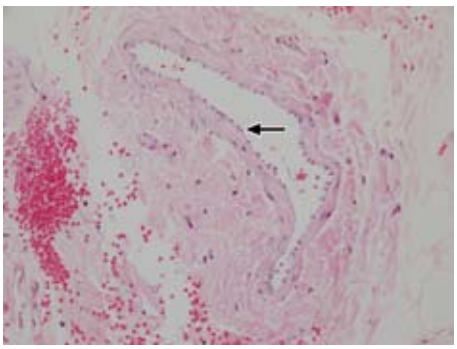

Figure 1 - Grade 1: minimal injury, minimal endothelial desquamation and minimal basal laminar separation (hematoxylin & eosin x200).

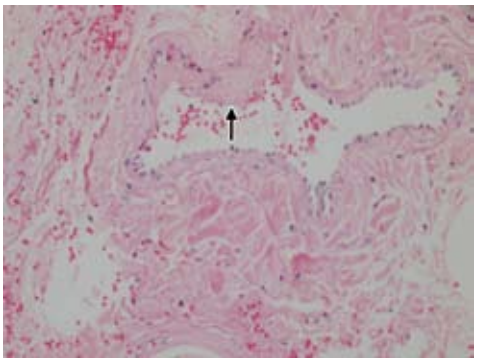

Figure 2 - Grade 2: medium injury, medium endothelial desquamation and edema in media and intima (hematoxylin & eosin x200).

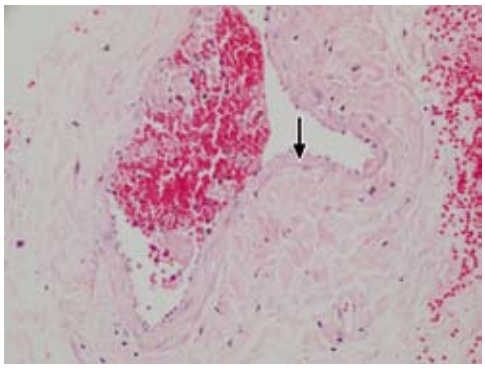

Figure 3 - Grade 3: severe injury, extended endothelial desquamation and extended edema formation in media and intima (hematoxylin & eosin x200).

desquamation and extended edema formation in the media and intima.

Discussion. The findings of this study suggest that both intravascular administered and perivascular injected lidocaine have protective effects on vein endothelium by reducing TBARS levels, and increasing NOS activity. The saphenous vein (SV) is the most commonly used conduit in patients undergoing CABG. However, a high proportion of vein grafts occlude within the first year, and >50% of patients require further grafting within 10 years. Using conventional harvesting techniques, the saphenous vein is damaged due to considerable surgical and mechanical trauma, a situation that affects graft patency.[13] Vasospasm and vasa vasorum damage are induced by the manipulation of the vein during harvesting. Generally, high pressure distension technique is used to overcome vasospasm, but this procedure causes denudation of the luminal endothelium, and may lead to platelet aggregation, thrombus formation, and early graft occlusion.[2] The mechanism of the vein's reaction (spasm) to harvesting procedures is still unknown. The perivascular sympathetic nerves can be a major contributor to the SV spasm.[3] Loesch and Dashwood[3] demonstrated that the contractility of SV arise due to transmural nerve stimulation, which is induced by noradrenaline and N-type calcium channels. The sympathetic and parasympathetic nerve fibers that were arranged as plexi at the adventitial/medial border of the human SV, and provide the presynaptic/postsynaptic control of this vessel. We though that vein spasm could be prevented by local anesthetic exposure, which are blocking the nerve fibers before harvesting. Lidocaine is still largely used as a short-acting local anesthetic and anti-arrhythmic agent. Vasodilator effect of lidocaine is partly due to attenuation of the sympathetic adrenergic neurotransmission in the vascular wall.[14] Lidocaine has been shown to inhibit both high potassium ion (K^+) and noradrenaline-induced contractions, but rather potentiate the caffeine-induced contraction in the vascular smooth muscle of rabbit aorta.[14] Lidocaine was also found to induce relaxations in human radial artery rings,[15] and in human internal mammary artery rings.[16] Additionally, Yokoyama et al[11] topically applied 0.2 mL lidocaine (4%) every 15 minutes for the first 3 hours after surgery to the region of anastomosis, and to the recipient artery and its surrounding tissue for preventing post-surgical vasospasm. They showed that this method was effective in the prevention of postoperative vasospasm occurring after reconstructive surgery of the extremities.[11] Boccara et al[17] showed that intravenous or intraperitoneal administration of lidocaine inhibited arterial vasoconstriction induced by vasoactive hormones on carbon dioxide pneumoperitoneum in pigs.[17] In this study, we did not evaluate spasm degree, but NOS activity was higher in lidocaine groups than in the control. Higher NOS activity documented in both lidocaine groups reflect the viability of endothelial function. We concluded that lidocaine acts as a blocker for sympathetic activation, and therefore, this proposed method is useful for prevention of vasospasm, which occurs following sympathetic activation.

Nitric oxide synthase is associated with lumenal endothelial cells and a strong marker of NO existence. During surgery and in the immediate postoperative period, venospasm is often encountered, and NO donors have been shown to reduce this via their vasodilator action.[18] Preservation of adequate endogenous NO with careful harvesting is an important issue that may provide protection against venospasm. In addition to preoperative technical measures, lidocaine administration can be regarded as a protective mechanism, which may reduce any effects of endogenous vasoconstrictors to which the vein graft is highly sensitive.[18] Dashwood et al[19] showed that there was a reduction in endothelial nitric oxide synthase and nitric oxide release in saphenous veins harvested by conventional surgical methods compared with those prepared atraumatically. These observations may influence graft performance.[5] Dashwood et al[19] in another study show that surgical trauma caused by the removal of perivascular tissue and high-pressure distension using conventional harvesting techniques damages the integrity of the endothelium, and the adventitia in SVs used for CABG surgery, leading to a reduction in endothelial nitric oxide synthase (eNOS) protein and eNOS activity.[19] Several recent studies supported the view that reduced expression of eNOS, and the consequent lower NO production, may be caused by the endothelial loss during the harvesting process.[20,21] The inhibition of leukocyte metabolism, and superoxide anion formation by local anesthetics had been well documented in several studies.[22,23] These direct scavenging effects of local anesthetics have been attributed to various mechanisms. There are evidences suggesting that local anesthetics penetrate into the cell membranes, they interact with membrane lipids and proteins to quench oxygen and NO free radical formation.[22] Lipid peroxidation reflects the production of toxic oxygen metabolites and it is a product of inflammatory response. Thiobarbituric acid reactive substances are end products of lipid peroxidation. There was a significant difference between TBARS values of control group and lidocaine administrated groups in our study. Decreasing TBARS levels in lidocaine groups are parallel to the increase in NOS activity.

Our study had some limitations. The first of these was the small number of animals in each group. With higher number of animals, statistical significance

would be clinically more important, and provide more concrete evidence on the vascular effects of lidocaine in 2 different administration. Furthermore, the study was conducted on animals during harvesting, however, this vascular graft harvesting was not evaluated in long term use.

In conclusion, NOS sources are maintained by both intravascular administration and perivascular infiltration of lidocaine before vein harvesting. The preservation of the NO-producing capacity of veins harvested with this method is likely to represent an important mechanism contributing to the improved early patency rate reported with this technique. The contribution of this mechanism to long-term graft patency remains to be established with ongoing clinical follow-up studies.

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