ORIGINAL PAPER

doi: 10.5455/medarh.2017.71.188-192

MED ARCH. 2017 JUN; 71(3): 188-192

RECEIVED: APR 15, 2017 | ACCEPTED: JUN 15, 2017

Comparing the Effects of Krebs Plus Verapamil Solution on Endothelial Function of Harvested Human Greater Saphenous Vein with Heparinized Blood, an Invitro Study

Mitra Shokri [1], Reza Moradpour [2], Majid Malekzade Shafaroudi [1], Nourollah Rezaei [1], Shervin Ziabakhsh Tabary [3]

[1]Department of Anatomy and Cell Biology, Cell and Molecoular Research Center (CMRC), Medical Faculty, Mazandaran University of Medical Sciences, Sari, Iran

[2]Rouzbahan Institute of Higher Education, Sari, Iran

[3]Department of Cardiac Surgery, Mazandaran Heart Center, Mazandaran University of Medical Sciences, Sari, Iran

Correspondent author: Shervin Ziabakahsh Tabary, Department of Cardiac Surgery, Mazandaran University of Medical Sciences, Sari, Iran. ORCID ID: http://www.orcid.org/0000-0002-6857-7995. E-mail: Shervin.zia@gmail.com,

ABSTRACT

Introduction: Integrity of the great saphenous vein (GSV) endothelium is the most important key element for long-term patency rate of grafts in coronary artery bypass graft (CABG). Storage solutions play an important role in maintaining viability of vein endothelium. Diminished nitric oxide (NO) because of endothelial dysfunction may facilitate vascular inflammation and formation of atherosclerotic plaque. **Aim:** So, we decided to find a reasonable alternative preservative solution instead of heparinized blood (HB) by measuring NO concentration with Griess assay. **Material and Method:** SVG samples were obtained from 54 patients undergoing elective CABG. 3 mm rings were stored in solutions: heparinized blood (HB), Krebs (K), Krebs + Propranolol (K+P) 6.66 g/l, Krebs + Adrenaline (K+A) 200 µl/l, and Krebs + Verapamil (K+V) 200 µl/l for 30, 45, 60 and 90 min. Nitrite concentration was measured by Griess assay at 540 nm. H&E staining was performed for histologic test. Statistical analysis was performed using SPSS (V16). Results were expressed as (Means ± SE) followed by One-Way ANOVA for finding best preservative solution. Repeated measurement test was used to investigate best time. In all analysis, ($P<0.05$) was considered significant. **Results:** Average concentration of NO in (K+V) compare with HB (1st control), K (2nd control), (K+A) and (K+P) showed higher rate in all times from 30 to 90 min (**16.55±1.85**) and in (K+A, K+P) compare with (HB) and (K) there was no statistically significant difference in the same times. Comparing the average concentration of (NO) between (HB) and (K) showed no significant difference (K+V>HB=K=K+A=K+P). Also, our investigations showed that NO concentration in (K+V) has the highest rate in time 90 min (**10.07±0.56, p=0.002**). More than 50 percent of endothelial cells stay normal in (K+V) compare with other solutions. **Conclusion**: It seems that (K+V) is the best solution for the maintenance of normal physiology of SVGs endothelial cells. The most appropriate SVGs endothelial function is within 90 minutes after harvesting.

Keywords: Endothelium, Saphenous vein, Heparinized blood, Verapamil.

1. INTRODUCTION

Atherosclerosis is the main cause of coronary artery disease (CAD) (1). This disease affects millions of people around the world (2) and causes thousands of deaths yearly because obstructed vessels cannot supply blood flow to the myocardial tissue (3, 4). more than 30% of deaths annually allocated to Cardiovascular diseases (5). Coronary artery bypass graft (CABG) that was developed in the 1960s and 1970s, is the most common procedure in cardiovascular operations and is used for the revascularization of obstructed coronary arteries to relieve ischemia resistant to medical treatment, prevent myocardial infarction and also to increase a productive life span (6). Autologous saphenous vein grafts (SVGs) are routinely used in this surgery and other vascular reconstructive procedures (7). Patency rates of these grafts are low, because of surgical manipulation, uncontrolled hydrostatic distension during SVG harvesting and inappropriate preservation solutions that are used for preserving them during surgery in order to maintain their viability prior to anastomosis (8). These cause considerable damage not only to the endothelium, but also to the media and adventitia. Ten year patency rate for them is reported to be 61% to

© 2017 Mitra Shokri, Reza Moradpour, Majid Malekzade Shafaroudi, Nourollah Rezaei, Shervin Ziabakhsh Tabary

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

65% (9). 20 years after CABG, only 25% of SVGs provide sufficient blood flow (10, 11). Intact vascular endothelium plays an essential role in the regulation of vascular tone and contributing to maintain the balance between vasodilatation – vasoconstriction (12) by endothelium-derived relaxing factor (EDRF) production, that is identified as nitric oxide (NO) (13) and is important for inhibition of smooth muscle cell proliferation (14), adhesion of platelets (15) and leukocytes to the endothelium (16). Intact endothelium serves as an electrical, mechanical and physiological barrier between flowing blood and the sub-endothelium (17). Disruption of endothelial cells results in oxidation of lipoproteins, extracellular matrix deposition, accumulation of lipid-rich material, platelet activation, medial smooth muscle cell proliferation (18, 19). All of these consequences of endothelial dysfunction may contribute to thrombus formation, developing the expression of atherosclerosis and early SVG occlusion (20). The consequence is a non-patent SVG that is the major cause of recurrent angina after CABG (21). Decreased NO production is closely associated with endothelial dysfunction (22). Measurement of (NO) in biological fluids is hard because of its low physiological concentration (1nM) and its short half-life of 2–30 s (22). So, it's stable metabolites nitrite and nitrate will be used for measuring(1) because it reacts rapidly with molecular oxygen and form nitrite (22, 23).

Aim of this study was to compare the protective effects of different storing solutions (Krebs, Krebs plus Adrenaline, Krebs plus Propranolol, Krebs plus Verapamil) on the endothelium of saphenous vein grafts with heparinized blood (HB) that is typically used for preserving grafts during CABG in heart surgery operation rooms by measuring NO concentration that was released from the endothelium in these solutions.

2. MATERIAL AND METHODS

Total amount of 54 patients who underwent elective CABG surgery in Mazandaran Heart Center, Sari, Iran from January 2014 to September 2016 were included in the study. Greater saphenous veins were obtained from classic incision in their legs then vessels cut into 3 mm rings and stored in preserving solutions (23). There were 4 different solutions: Heparinized blood (HB: first control group), Krebs (K: second control group), Krebs plus Propranolol (K+P), Krebs plus Adrenaline (K+A), and Krebs plus Verapamil (K+V) at a rate of 5 ml (volume: 5 ml) for 30, 45, 60 and 90 minutes after harvesting. All solutions continuously perfused with 95% O2 and 5% CO2 at a constant rate of 5 mL/min and maintained at (37 ° C). The composition of Krebs' solution was :NaCl (6.95 g), KCl (0.19 g), NaH2PO4 (0.14 g), CaCl2·2H2O (0.37 g), MgCl2·6H2O (0.26 g), D-glucose (1.98 g) in one ml distilled water. plus Adrenalin ,200 µl/l Krebs solution (26). Krebs plus Propranolol (6.66 g) in 1 litter Krebs solution (27). Krebs Krebs plus Verapamil 200 µl/l Krebs solution (23).

Turbidity caused by interference of protein with NO, deproteinization of plasma samples should be performed (23, 24). Plasma samples were diluted fourfold with distilled water and then 1/20th volume of zinc sulfate (300 g/l) should be added. This solution should be centrifuged at 10000 g for 5 min (24). nitrite concentration is typically measured by colorimetric Griess assay in biologic samples Because of its simplicity (24). In this method, nitrite is first treated with sulfanilamide (SA), so in acidic media a transient diazonium salt will form. This intermediate is then reacts with N-naphthylethylenediamine dehydrochloride (NED), to form a stable azo compound. The intense purple color of the product allows nitrite assay with high sensitivity and can be used to measure nitrite concentration as low as ~0.5 mM level. The absorbance of this adduct at 540 nm is linearly proportional to the nitrite concentration in the sample. This approach is adopted in most commercial kits .

Histology and staining: All samples should be stored 24 hours in formalin 10% immediately after exiting out of solutions of 30 to 90. One 3 mm ring of vessel was stored in formalin 10% immediately after harvesting as control group. Then paraffin embedded samples were sectioned at 5 µ by a microtome (Leica/ USA). H&E staining was performed. Histologically endothelial viability was classified into three categories: normal endothelium (Grade1), normal endothelium more than 50% (Grade2) and normal endothelium less than 50% (Grade3).

Statistical analysis was performed using SPSS Statistics 16. Results were expressed as means ± standard error of the mean followed by One-Way ANOVA. Post hoc analysis was performed by the Tukey test for finding best solution for preserving vessels. Repeated measurement test was used to investigate best time. In all analysis, ($P<0.05$) was considered significant.

3. RESULTS

Best preserving solution: Average concentration of NO in (K+V) compare with HB (1st control) and K (2nd control) showed higher rate in all times from 30 to 90 min (16.55±1.85). Comparing the average concentration of nitric oxide between the first and second control group showed no significant difference. Also, our investigations showed that NO concentration in Krebs plus Verapamil has the highest rate in time 90 min (Figure 1 and Table 1). Average concentration of NO in (K+A) and (K+P) compare with HB (1st control) and K (2nd control) did not show a statistically significant difference in all times from 30 to 90 min. Comparing the average concentration of nitric oxide between (K+A) and (K+P) with (K+V) showed significant difference in all times from 30 to 90 min (16.55±1.85). Again (K+V) had the highest rate of NO concentration compare with (K+A) and (K+P) (Figure 2 and Table 2).

(K+V>HB=K=K+A=K+P).

Best time: Our investigations showed that the best time for storing human saphenous veins in (K+V) preserving solution is for 90 minutes because mean of this time has higher rate compare with other 30, 45 and 60 min (10.07±0.56, p=0.002) (Figure 3).

Histologic study: Based on histologic study using light microscopy to count and determinate percentage of normal endothelial cells in different groups of storing solu-

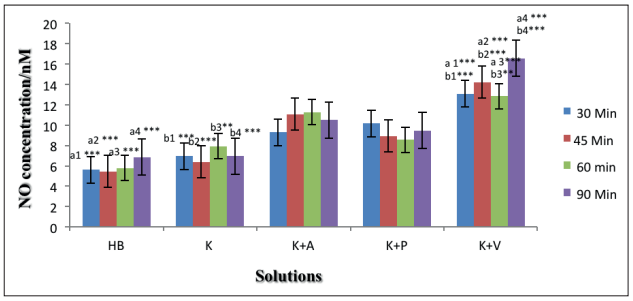


Figure 1. NO concentration in (K+V) compare with (a= HB) and (b= K) in 1: 30 min, 2: 45 min, 3: 60 min, 4: 90 min. (P= 0.01 – 0.05 *, P= 0.001-0.009 **, P= 0.0001***).

tions we obtained that more than 50 percent of endothelial cells stay normal in Krebs plus Verapamil compare with other solutions (Figure 4). Normal endothelial cells have Squamous, polygonal and stretched shape that we observed these cells in (CO) and (K+V) group. In (HB), (K), (K+A) and (K+P) groups we observed endothelial cells that were separated in some places and in some other areas were completely detached (photomicrograph 1).

K (Mean±SE)	HB (Mean±SE)	K+V (Mean±SE)	Time (min)
6.95±0.68	5.62±1.01	13.07±1.35	30
6.40±0.59	5.46±0.35	14.19±1.62	45
7.92±0.89	5.80±1.04	12.84±1.04	60
6.96±0.57	6.85±0.6	16.55±1.85	90

Table 1. Mean of NO concentration after storing saphenous vein 3 mm rings in time 30, 45, 60 and 90 min. All data mentioned based on

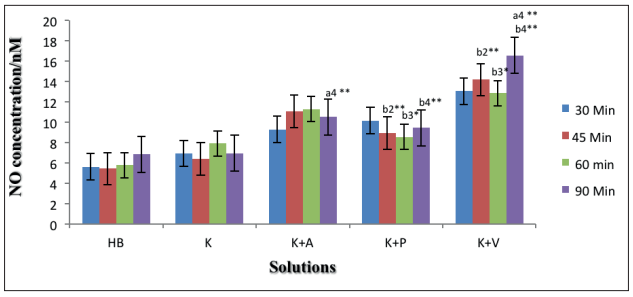


Figure 2. NO concentration in (K+V) compare with (a= K+A) and (b= K+P) in 1: 30 min, 2: 45 min, 3: 60 min, 4: 90 min. (P= 0.01 – 0.05 *, P= 0.001-0.009 **, P= 0.0001***).

(Mean±SE). K+V: Krebs plus Verapamil, HB: Heparinized blood, K: Krebs.

K+P (Mean±SE)	K+A (Mean±SE)	K+V (Mean±SE)	Time (min)
10.16±1.45	9.30±0.75	13.07±1.35	30
8.93±1.17	11.06±1.24	14.19±1.62	45
8.55±1.10	11.27±1.42	12.84±1.04	60
9.47±0.93	10.51±0.78	16.55±1.85	90

Table 2. Mean of NO concentration after storing saphenous vein 3 mm rings in time 30, 45, 60 and 90 min. All data mentioned based on (Mean±SE).. K+V: Krebs plus Verapamil, K+A: Krebs plus Adrenaline, K+P: Krebs plus propranolol.

4. DISCUSSION

Operative results significantly early re-obstruction of SVGs after CABG closely depend on preserving solutions that are used for storing these grafts. For saphenous vein grafts, protecting endothelium is vitally important. Vascular endothelium derives a number of (EDRFs) that play an important role in vaso-relaxation and in inhibition of platelet aggregation. When endothelium is impaired, the antiplatelet function of EDRFs (such as nitric oxide and prostaglandin I2) is lost and platelets attach to the areas that endothelial cells were denuded. The coag-

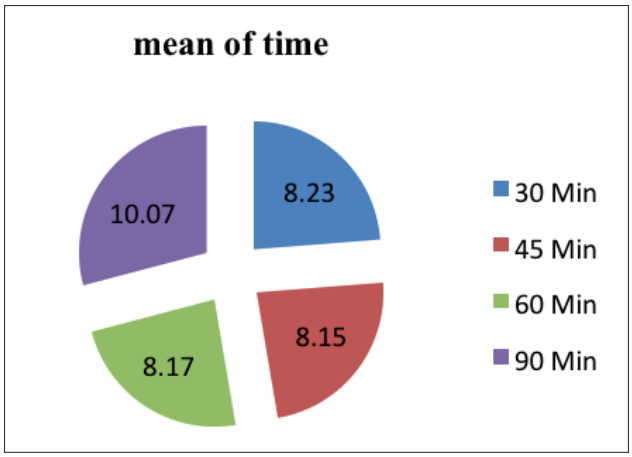


Figure 3. Comparing the average duration of human saphenous veins stored in solution

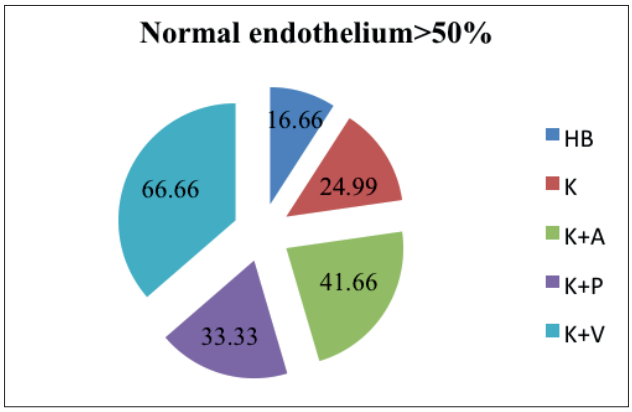
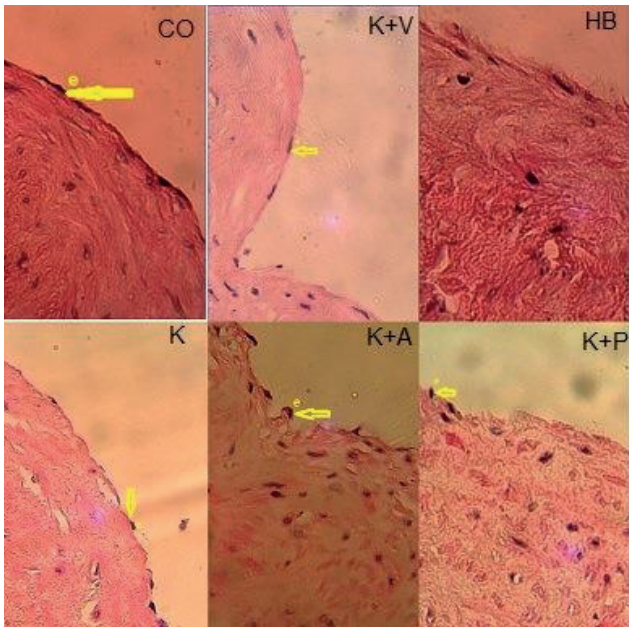


Figure 4. percentage of normal endothelial cells of saphenous vein samples



Photomicrograph 1 (X400): yellow arrows show endothelium of saphenous vein samples. (e: endothelium, CO: control, K+V: Krebs plus Verapamil, K+A: Krebs plus Adrenalin, K+P: Krebs plus Propranolol).

ulation cascade is activated by aggregating platelets and by thrombin. Thrombus then forms. This becomes the basis for later growth and development of atherosclerotic plaque and may lead to graft occlusion (24, 25). There are various types of solution used for storing conduits before anastomosis (25). Verapamil is a Ca2+ channel blocker and is highly prescribed as an anti-anginal, antiarrhythmic and antihypertensive drug (25). In our study, 4 different solutions were used for preserving 3 mm rings of saphenous veins compare with autologous heparinized whole blood as normal storage solution in operation rooms. We investigated that endothelial cells are better preserved in non-blood storage solutions. When saphenous veins were stored in oxygenated Krebs plus Verapamil solution at room temperature, we observed highest mean of nitric oxide concentration. So, the surgeon should be alert to this in the intraoperative and perioperative management of patients undergoing bypass surgery. Guo-Wei He showed that using verapamil + nitroglycerin solution for preparing radial artery can maximally preserves endothelial function in contrast to papaverine. verapamil + nitroglycerin solution may be effectively and safely used to prepare the radial artery for coronary artery bypass grafting(25,26). P. DI. NAPOLI and college evidenced that verapamil treatment may reduce endothelial dysfunction. Verapamil is also effective in protecting the integrity of cellular and membrane structures as evidenced by a reduction of CK release in heart attack (26). These two findings were agreed with our investigations and this may be because of protecting effect of verapamil on the integrity of endothelial cells. Grafts are commonly stored for 45 minutes or more. This can disrupt the integrity of the vascular endothelium in saphenous vein grafts (27). Our study has demonstrated that a storage duration of 30, 45, 60 and 90 minutes in the 4 tested storage solutions has significantly affect the viability of endothelium of vessels, as indicated by (NO) concentration compared with control rings in heparinized blood solution. However, storing rings of vessels for 90 min in (K+V) showed the highest concentration of (NO). It reveals that this time is the best time for preserving saphenous veins.

5. CONCLUSION

Protective effect of (K+V) solution on saphenous vein endothelial cells is more than (HB) solution which is the most common preserving solution that is used in cardiovascular operation rooms, and (K). So, we recommend to use(Krebs plus Verapamil) solution instead of Heparinized blood in order to protect the integrity of cellular and membrane structures of saphenous vein more efficiently. Besides, the most appropriate time for using SVG in CABG is 90 minutes after harvesting.

- Author Contribution: Mitra Shokri: contribution to acquisition, analysis and interpretation of data; revising the article; preparing the laboratory analysis of histologic samples. Reza Moradpour: contribution to acquisition, analysis and interpretation of data; revising the article. Majid Malekzade: drafting the article; preparing and diagnosing the histologic samples; analysis and interpreting the histologic data. Nourollah Rezaei: drafting the article; statistical anylysis. Shervin Ziabakhsh Tabary: doing the surgery and obtaining the veins from patients to start the research; substantial contribution to conception and design; substantial contribution to analysis and interpretation of data; critically revising the article for important intellectual content; substantial contribution to acquisition of data; final approval of the version to be published.
- Clinical trial registry number - RCT138809102799N1 .
- Conflict of interest - None.

REFERENCES

1. Liu J, Liu FH, Zhao YN, Yan DE, Gao XY, Wen LN, et al. Glucose excursion in senior patients with type 2 diabetes mellitus and coronary artery disease. Zhonghua Yi Xue Za Zhi. 2017; 97(20): 1562-7..
2. Alserius T, Hammar N, Nordqvist T, & Ivert T. Risk of death or acute myocardial infarction 10 years after coronary artery bypass surgery in relation to type of diabetes. American Heart Journal. 2016; 152: 599-605.
3. Ciloglu U, Aldag M, Albeyoglu S, Aykut V, Karakaya C, Gonul B. Central retinal vein occlusion after coronary artery bypass surgery: A case report. Acta Med Acad. 2017; 46(1): 59-62.
4. Eisenberg MS, Hallstrom AP. The problem of sudden cardiac death in the Community of New York. Praeger. 2014; 6: 5-65.
5. Chamberlain AM, Alonso A, Gersh BJ, Manemann SM, Killian JM, Weston SA, et al. Multimorbidity and the risk of hospitalization and death in atrial fibrillation: A population-based study. Am Heart J. 2017; 185: 74-84.
6. Kabil E, Banjanović B, Mujanović E. Surgical coronary revascularization on-pump versus off-pump in patients with stenosis of the main tree of the left coronary artery and carotid stenosis. Acta Med Acad. 2017; 46(1): 1-6.
7. Shirakawa M, Ishii Y, Kawase Y, Sakamoto S, Miyagi Y, Morota T, Nitta T. Coronary Artery Revascularization with V-composite Saphenous Veins Graft in a Bad Ascending Aorta;Report of a Case. Kyobu Geka. 2016; 69(13): 1110-13.
8. Mavroudis CA, Kotecha T, Chehab O, Hudson J, Rakhit RD. Superior long term outcome associated with native vessel versus graft vessel PCI following secondary PCI in patients with prior CABG. Int J Cardiol. 2017; 228: 563-9.
9. Goldman S, Zadina K, Moritz T, Ovitt T, Sethi G, Copeland JG, et al. Long-term patency of saphenous vein and left internal mammary artery grafts after coronary artery bypass surgery: results from a Department of Veterans Affairs Cooperative Study. J Am Coll Cardiol. 2014; 44: 2149-56.
10. Harky A, Balmforth D, Shipolini A, Uppal R. Is endoscopic long saphenous vein harvesting equivalent to open harvesting technique in terms of graft patency?. Interact Cardiovasc Thorac Surg. 2017. doi: 10.1093/icvts/ivx049. [Epub ahead of print]
11. Goldman S, Sethi GK, Holman W, Thai H, McFalls E, Ward HB, et al. Radial artery grafts vs saphenous vein grafts in coronary artery bypass surgery. J Am Med Assoc. 2011; 305: 167.
12. Suciu M. The role of nitric oxide (NO) and statins in endothelial dysfunction and atherosclerosis. Farmacia. 2009; 57(2): 1-10.
13. Iordanidou M, Paraskakis E, Tavridou A, Chatzimichael A, Manolopoulos VG. Endothelial nitric oxide synthase gene polymorphisms are associated with sensitization to seasonal aeroallergens in asthmatic children. World J Pediatr. 2017; 13(1): 34-40.

14. Moussa M, Banakh O. TiN x O y coatings facilitate the initial adhesion of osteoblasts to create a suitable environment for their proliferation and the recruitment of endothelial cells. Biomedical Materials, 2017; 12(12): 70-9.
15. ApostolI G, Solmon A, Smallwood MJ. Role of inorganic nitrate and nitrite in driving nitric oxide/cGMP-mediated inhibition of platelet aggregation in vitro and in vivo . Imperial Beatstream,2014; 12(1): 156-9.
16. Tsao PS, Buitrago R, Chan JR, Cooke JP. Nitric Oxide Inhibits Hetero-adhesion of Cancer Cells to Endothelial Cells: Restraining Circulating Tumor Cells from Initiating Metastatic Cascade. Scientific Reports. 2014; 4(3): 65-80.
17. Alrawi SJ, Balaya F, Raju R, Cunningham JN Jr, Acinapura AJ. A comparative study of endothelial cell injury during open and endoscopic saphenectomy: An electron microscopic evaluation. Heart Surg Forum. 2013; 4(2): 120-7.
18. Tan J, Yang L, Liu C, Yan Z. MicroRNA-26a targets MAPK6 to inhibit smooth muscle cell proliferation and vein graft neointimal hyperplasia. Sci Rep.. 2017; 7: 46602.
19. Hinokiyama K, Valen G, Tokuno S, Vedin JB, Vaage J. Vein graft harvesting induces inflammation and impairs vessel reactivity. Ann Thorac Surg. 2006; 82: 1458-64.
20. Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature. 2014; 768: 568-9.
21. Mitra AK1, Gangahar DM, Agrawal DK. Cellular, molecular and immunological mechanisms in the pathophysiology of vein graft intimal hyperplasia. Immunol Cell Biol. 2006; 84: 115-24.
22. Andrews AM1, Jaron D, Buerk DG, Kirby PL, Barbee KA, et al. Direct, real-time measurement of shear stress-induced nitric oxide produced from endothelial cells in vitro. Article info. 2010: Available online.
23. Scherzer DJ, Chime NO, Tofil NM, Hamilton MF, Singh K, Stanley RM, et al. Survey of pediatric trainee knowledge: dose, concentration, and route of epinephrine. Ann Allergy Asthma Immunol. 2017; 118(4): 516-8.
24. Moyakine AV, Spillekom-van Koulil S, van der Vleuten CJ. Propranolol treatment of infantile hemangioma is not associated with psychological problems at 7 years of age. J Am Acad Dermatol. 2017. doi: 10.1016/j.jaad.2017.01.025.[Epub ahead of print]
25. Gray CL K-KD, Solmon MB, Norvelle A, et al. Immediate post-defeat infusions of the noradrenergic receptor antagonist propranolol impair the consolidation of conditioned defeat in male syrian hamsters. Physiol Behav. 2015; 31(15): 30107-4.
26. Aysegul A, Yucel, Sebnem Gulen, Sibel Dincer, Ahmet E, Gulay I, Yetkin. Comparison of two different applications of the Griess method for nitric oxide measurement. Journal of Experimental and Integrative Medicine. 2012; 2(2): 167-71.
27. Guoa X, Dumasa M, Bonnie L. Robinsona SF, Alia MG, Paulea, Gua Q, et al. Acetyl L-carnitine targets adenosine triphosphate synthase in protecting zebrafish embryos from toxicities induced by verapamil and ketamine: An in vivo assessment. J Appl Toxicol. 2017; 37(2): 192-200.