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The effect of cardioplegia on structure and function of saphenous vein: An *in vitro* study

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Abstract
Purpose and aim: the mechanisms of cardioplegia on the endothelial and smooth muscle cells membrane are controversial, in this *in vitro* study we evaluated the effect of cardioplegia on structure and function of the saphenous vein.
Materials and Methods: In this *in vitro* study, ten segments were gently harvested and were placed in 10ml organ bath chamber containing Krebs solution. After careful washout, the venous segments were first contracted with 10^{-6} mM phenylephrine to reach %75 of maximal contraction. Then the endothelial-dependent and endothelial-independent relaxations were assessed in the presence of acetylcholine (10^{-6}mM) and sodium nitroprusside (SNP) 10^{-5}mM) respectively. The vein strips were mounted again in the organ bath chamber contained cardioplegia solution with different KCl concentrations (5, 20 and 40meq) at 25°c for 30 minutes to assess contraction and relaxation response just right the procedure that previously mentioned.
Results: Endothelium-dependent relaxation to acetylcholine showed the significant difference after incubation in 40meq of KCL cardioplegic solution (p value<0.05), but there was no difference in segments incubated in 5meq and 20meq KCL cardioplegic solution. There was no significant difference in relaxation to sodium nitroprusside between segments. Dilated group: no difference was seen after incubation in the groups. (P value>0.05).Constriction with phenylephrine also showed no significant difference in all the groups. (P value>0.05). Vasorelaxation with acetylcholine and SNP showed no significant changes in the groups. Conclusion: high concentration cardioplegic solutions can depolarize endothelium and decrease endothelial-dependent vasodilation, moreover, concentrations less than 20mMol of normothermic storage solution should be used to maintain normal endothelial function.

Keywords: cardioplegia, saphenous vein, endothelial function, depolarization, KCL

1. Introduction
The coronary artery diseases are more common and it is responsible for 33.3% of population deaths in people more than 35 years around the world [1]. The most common heart surgery is coronary artery bypass grafting (CABG) and its successful rate depends on several factors including the disease, graft, injury during operation and ischemia-reperfusion injury [2]. A typical CABG is usually performed through a midline incision over the sternum. The internal mammary artery will then be released followed by a longitudinal incision over the GSV path along the leg medial part to harvest a sufficient length of the vein. The aorta and right atrium are cannulated simultaneously and connected to cardiopulmonary bypass (CPB) machine [3]. After aortic cross-clamp, cardioplegia solution containing 20 meq/l KCl is injected via aortic root cannula, this solution flows to the cardiac muscles and causes total cardiac arrest for preparing a bloodless operation field [4]. The cardioplegic arrest is the gold standard of cardioprotection and needs a potassium-rich solution for depolarization arrest, and is evitable for the most of cardiac surgeries [4]. The cardioplegia was introduced in 1957 by Lam, by injection of KCl intraventricular that led to the cardiac arrest in the hypothermic dog [5]. More than 99% of cardioplegic and most preservation solutions contain K+ concentrations > 15 mEq/L and the myocardial cell membrane from a resting voltage of about −85 mV to −50 mV [6]. High potassium level in cardioplegia protects myocardial tissue through membrane depolarization of myocytes and cardiac asystole and stops ischemia-induced ATP depletion and finally reserve myocardial energy [6]. However, the studies indicated that depolarization is a first step of membrane damage and activates and produces oxidant agents, leads to increasing of voltage sensitive endothelial NADPH oxidases and other oxidants) [7]. Regarding mentioned studies, it appears the mechanisms of cardioplegia on endothelial are controversial, therefore, in this *in vitro* study we evaluated the effect of cardioplegia on structure and function of the saphenous vein.

2. Materials and Methods
In this *in vitro* study, ten demographically matched patients aged 40-70 years old candidate for CABG were enrolled. A written consent was taken according to Tehran heart center ethics committee to GSV remnants for *in vitro* study. Ten

segments were finely harvested by a cardiac surgeon and immediately kept in a test tube floated in ice chips containing heparinized blood. The segments were excluded if they had more than 2 branches, passing more than 30 minutes of harvestation and trauma during harvestation. The segments were carried to the laboratory and transferred into oxygenated Krebs solution equilibrated with carbogen at 37°C. Adhering connective tissues and fats were removed. Non-branched rings of 3-4mm were placed in 10ml organ bath chamber containing Krebs solution with constant oxygenation at 37°C. The isometric contraction force of each segment was measured using force transducer (MLT) and recorded (AD instrument). A preload of 2g was applied to the venous rings and kept constant for 1-hour equilibration. Bath fluid was replaced every 20minutes during this period. Each venous segment was precontracted with 100meq KCl twice to prove tissue viability. Those preparations that sufficiently responded to the KCl were used for the next experiment. Precontraction with KCl lasted 45minutes till a plateau contraction was achieved. After washout, segments were contracted with accumulative doses (10^{-9}up to 10^{-5}mM) of phenylephrine in a logarithmic order of 0.5 intervals. Contractions were repeated till maximum stabilized and reproducible contractions were obtained.After careful washout, the venous segments were first contracted with 10^{-6}mM phenylephrine to reach %75 of maximal contraction. Then the endothelial-dependent and endothelial-independent relaxations were assessed in the presence of acetylcholine (10^{-6}mM) and sodium nitroprusside (SNP) (10^{-5}mM) respectively. After an initial assessment dilated and nondilated segments were equally divided into 3 groups of 5 samples each. The vein strips were mounted again in the organ bath chamber contained cardioplegia solution with different KCl concentrations (5, 20 and 40meq) at 25°c for 30 minutes to assess contraction and relaxation response just right the procedure that previously mentioned.

3. Data analyses

Data were analyzed using SPSS version 20.Categorical data are presented as numbers (%), and continuous data as mean ± SD. We used the Chai_2 test to compare categorical variables and the Student's t test to compare continuous variables. $\alpha < 0.05$ was considered significant.

4. Results

Non-dilated group: Maximum contraction of segments with 100 meq KCL in Krebs solution before and after incubation in cardioplegic solutions, also EC50 values of contraction with phenylephrine are presented in table 1, the response to KCL was not significant in all groups, also EC50% dose-response changes was not significant (p value>0.05).Endothelium-dependent relaxation to acetylcholine showed the significant difference after incubation in 40meq of KCL cardioplegic solution (p value<0.05), but there was no difference in segments incubated in 5meq and 20meq KCL cardioplegic solution (table 2). Moreover there was no significant difference in response to sodium nitroprusside relaxation before and after incubation (table 3).

Dilated group

Maximum contraction with 100meq of KCL in dilated veins is demonstrated in table 4. No difference was seen after incubation in the groups. (P value>0.05) Constriction with phenylephrine also showed no significant difference in all the groups. (P value>0.05). Endothelial-dependent relaxations in the presence of acetylcholine (10^{-6}mM) is demonstrated in table 5 also endothelial-independent relaxations response to SNP shown in table 6 in which dilated segments showed non-significant difference regarding different cardioplegic concentrations before and after incubation.

Maximum contraction to Kcl in non-dilated group in absence of cardioplegia was1.8g±0.2 (n=15) and in dilated group was 0.6±0.1 (n=15) which was significantly reduced in dilated group (pvalue< 0.001).

Maximum contraction to Phenylephrine

Endothelium-dependent relaxation with Ach in the non-dilated group in the absence of cardioplegia was 77.8%=6.1 (n=15) and in dilated group was 43.2%=7.2 (n=15). Endothelium-independent relaxation with sodium nitroprusside was 134.1%=7.1 (n=15) in the non-dilated group and 37.7=3.2 (n=15) in dilated group in the absence of cardioplegia. Relaxation with Ach was significantly reduced in dilated group (P value<0.05), also relaxation with Snp was significantly reduced (p value<0.001).

Table 1: Maximum contraction of non-dilated segments with 100 meq KCL in Krebs solution before and after incubation in cardioplegic solutions, and EC50 values of contraction with phenylephrine

Concentration of storage solution	Vasocontriction with 100Meq of Kcl(g)		P value	N	EC50 values of contraction with phenylephrine dose reponse		LOGEC50 values of contraction with phenylephrine dose reponse	
Cardioplegia with 40 Meq of Kcl	Before incubation	After Incubation	>0.05	5	Before incubation	After incubation	Before incubation	After incubation
	2.26±1.31	2.76±1.56			2.2960e+006	13.49	6.361	4.116
Cardioplegia with 20 Meq of Kcl	Before incubation	After incubation	>0.05	5	Before incubation	After incubation	Before incubation	After incubation
	1.46±0.64	1.36±0.62			1.3500e+006	102714	6.130	5.012
Cardioplegia with 5 Meq of Kcl	Before incubation	After incubation	>0.05	5	Before incubation	After incubation	Before incubation	After incubation
	1.44±0.45	1.23±0.29			9.4350e+006	1.6080e+006	6.975	6.206

Table 2: Endothelial-dependent relaxation response to acetylcholine in non-dilated segments before and after incubation in cardioplegic solutions

Concentration of cardioplegic solution	Before incubation	After incubation	P
5meq kcl	85.3 ± 11.6	74.9 +12.6	>0.05
20meq kcl	73.4 ± 9.9	65.7 ± 12	>0.05
40meq kcl	71.8 ±9.4	42.4 ±10	<0.05

Table 3: Endothelial-independent relaxation response to SNP in non-dilated segments before and after incubation in cardioplegic solutions

Concentration of cardioplegic solution	Before incubation	After incubation	P
5meq kcl	135.8±18.4	122±25.1	>0.05
20meq kcl	137.3±12.1	115.6±7.7	>0.05
40meq kcl	138.8±11.6	121.1±16	>0.05

Table 4: Maximum contraction of dilated segments with 100 meq KCL in Krebs solution before and after incubation in cardioplegic solutions

Concentration of storage solution	Vasocontriction with 100Meq of Kcl(g)		P value
Cardioplegia with 40 Meq of Kcl	Before incubation	After Incubation	>0.05
	0.4±0.14	0.22±0.1	
Cardioplegia with 20 Meq of Kcl	Before incubation	After Incubation	>0.05
	0.75±0.25	0.17±0.05	
Cardioplegia with 5 Meq of Kcl	Before incubation	After Incubation	>0.05
	0.68±0.16	0.48±0.78	

Table 5: Endothelial-dependent relaxation response to acetylcholine in dilated segments before and after incubation in cardioplegic solutions

Concentration of storage solution	Vasorelaxation with ACH		P value
Cardioplegia with 40 Meq of Kcl	Before incubation	After incubation	>0.05
	46.7±15	31.8±14	
Cardioplegia with 20 Meq of Kcl	Before incubation	After incubation	>0.05
	48.4±13	38.1±12.5	
Cardioplegia with 5 Meq of Kcl	Before incubation	After Incubation	>0.05
	34.5±11.2	32.6±12	

Table 6: Endothelial-independent relaxation response to SNP in dilated segments before and after incubation in cardioplegic solutions

Concentration of storage solution	Vasorelaxation with Snp		P value
Cardioplegia with 40 Meq of Kcl	Before incubation	After incubation	>0.05
	36.8±12	32.6±16.5	
Cardioplegia with 20 Meq of Kcl	Before incubation	After incubation	>0.05
	43.7±11.9	34.5±17.2	
Cardioplegia with 5 Meq of Kcl	Before incubation	After Incubation	>0.05
	32.8±20.9	31.6±14	

5. Discussion

Cardioplegic solution is a high potassium storage solution that causes cardiac arrest. However, vascular reactivity can be impaired when exposed to high potassium concentration solutions [8]. In the present study, we did not find significant difference in constriction response before and after incubation in modified St. Thomas cardioplegic solution with different potassium concentrations. Moreover, endothelium-independent vasodilation was unchanged in response to sodium nitroprusside in all samples but, endothelial dependent vasodilation with acetylcholine was impaired in the samples that were incubated in 40 mMol of potassium for 15 minutes, this wasn't seen in lower concentrations of potassium. So, we concluded that up to 20 mMol of potassium in normothermic cardioplegia usually doesn't impair endothelium-dependent vasodilation, but concentrations of potassium higher than 20 mMol would probably impair endothelial reactivity. In a review, Qin *et al*. indicated that the most important component of the cardioplegic and heart preservation solutions that induce cardiac arrest is hyperkalemia. They revealed that a combination of agents plus hyperkalemia induce endothelial impairment and there is little evidence that show hyperkalemia impact on NO-related endothelial function. They concluded that in the absence of ischemia-reperfusion, the NO-pathway of the endothelium of coronary is resistant to the cardioplegic solution with potassium about 20 mEq/L. However, the endothelium is damaged in this level of potassium via "back-up" of the NO pathway, due to inhibition of K+ channels and smooth muscle [9]. High potassium storage solutions are used to preserve myocardial function, but the impact of these solutions on vascular endothelial function is controversial. The studies revealed that increase in extracellular potassium as a result of hyperpolarization can cause vasodilation in vessels [10]. Also, increase in extracellular potassium produce sustained endothelial-independent relaxation of vascular smooth muscle equivalent to acetylcholine [11]. Moreover, the acute rising of potassium in concentrations above 12 mMol can soften endothelial cells and increase NO production, therefore, can

cause vascular relaxation [12]. All these findings explain the reason of vascular relaxation when incubated in cardioplegia. On the other hand, direct depolarization of the vascular smooth muscle or underlying endothelium which is electrically related to smooth muscle induces vasoconstriction [13]. The vasoconstrictive and vasorelaxive factors balance, regulate the coronary vascular tone which in turn modify end effector" potassium channels including KCa, Kv, and KATP channels and etc., in underlying vascular smooth muscle [13, 14]. The studies indicated that incubating small resistance coronary arteries of porcine in either St. Thomas's solution or Celsior preservation solutions caused a significant potassium-induced depolarization and loss of endothelial-derived hyperpolarizing factor (EDHF)-mediated function [15, 16]. Other studies also indicated that the endothelium-derived vasoconstrictor Endothelin-1 induces vasoconstriction [17]. In line with these findings and in contrast to our results, Higashi *et al* revealed that potassium as low as 10 mM can contribute in endothelial damage including vascular depolarization, inflammation, neutrophil adherence, leaky junctions, platelet activation, pro-oxidant production, and coagulopathy [18]. It is supposed that the opening of potassium channels in endothelium by acetylcholine induces hyperpolarization of the endothelium, which flows to the smooth muscle via myoendothelial gap junction, leading to inhibition of calcium entry into smooth muscle cells and relaxing the vessels [19, 20]. Blocking the calcium-activated potassium channels can eliminate acetylcholine vasodilatory effects but not sodium nitroprusside [21]. High potassium concentration is believed to have a depolarizing effect on the endothelium membrane and by interrupting with endothelium hyperpolarization and the opening of calcium-activated potassium channels, inhibits acetylcholine vasodilatory effect [22]. In our study, we also found that manual dilation of the vein significantly reduces the vessel response to vasodilators and vasoconstrictors. This can be a predictable matter as the pressure can disrupt vein endothelium and media and the damage can cause physiologic dysfunction. Okon *et al*. reported that although isometric distention in organ bath didn't impair vein contraction or relaxation but, manual distention significantly abolished its contraction and distention [23]. Dilation trauma also reduces NO production and may occlude late patency of the grafts [24]. In summary, the current practice and previous experiences evaluating here indicated that Saphenous vein is still the most frequent conduit used in coronary artery bypass grafting moreover high potassium induces intimal damage and occludes the patency in the 16–25% of saphenous vein conduits during one year after surgery [25, 26].

This was an *in vitro* study with relatively small sample size, that limit to that limit the ability to generalize the result of our survey. Further investigations are recommended with larger series to validate the findings reported here.

Conclusion: we revealed that high concentration cardioplegic solutions can depolarize endothelium and decrease endothelial-dependent vasodilation, moreover, we indicated that concentrations less than 20mMol of normothermic storage solution should be used to maintain normal endothelial function.

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7. References

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