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·述 评· ·Editorial·

Cardiac Protection during Open Heart Surgery: A View from Coronary Endothelial Function

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Abstract: Cardioplegic (and organ preservation) solutions were initially designed to protect the myocardium (cardiac myocytes) during cardiac surgery (and heart transplantation). Due to the differences between the cardiac myocytes and vascular (endothelial and smooth muscle) cells in structure and function, the solutions may have adverse effect on coronary vascular cells. However, such effect is often complicated by many other factors such as ischemia-reperfusion injury, temperature, and perfusion pressure or duration. In evaluation of the effect of a solution on the coronary endothelial function, a number of points should be taken into consideration. First, the overall effect on endothelium should be identified. Second, the effect of the solution on the individual endothelium-derived relaxing factors (nitric oxide, prostacylin, and endothelium-derived hyperpolarizing factor) must be distinguished. Third, the effect of each major component of the solution should be investigated. Fourth, the effect of a variety of new additives in the solution may be studied. In the last decades, we have focused our research on the endothelial function during open heart surgery and have for the first time found that high potassium concentration impairs the EDHF-mediated function. This review attempts to discuss the above issues based on available literature in order to provide information for further development of cardioplegic or organ preservation solutions.

Key words: cardioplegia; endothelium; endothelium-derived hyperpolarizing factor; cardiac surgery; coronary artery

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从冠脉内皮细胞功能看心脏直视手术中的心脏保护

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摘 要:心脏停跳液(器官保存液)最初是为了保护在心脏外科手术(心脏移植手术)中的心肌(心肌细胞)

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而设计的。由于心肌细胞和血管细胞（内皮细胞和平滑肌细胞）在结构和功能上的差异，此类液体对冠脉的血管细胞有不利的作用。同时，这种不利的作用往往由于许多其他的因素而变得复杂化，例如缺血再灌注、温度、灌注压或者灌注持续时间。在评估一种停跳液对冠脉内皮功能的影响时，我们需要考虑很多方面。第一，需要确定停跳液对内皮的整体影响。第二，必须区分停跳液对每种血管内皮细胞舒张因子（一氧化氮，前列环素，内皮源性超极化因子）的作用。第三，需要研究停跳液里各个主要成分的作用。最后，需要研究各种新型添加剂的作用。在过去十余年中，我们的研究集中在心脏外科手术中内皮细胞的功能上并首次发现了高钾对内皮细胞超极化因子的损害作用。本综述拟在现有文献基础上讨论以上的问题，希望能够为心脏停跳液或器官保存液更进一步的发展提供信息。

关键词：心脏停跳液；内皮；内皮源性超极化因子；心脏外科；冠状动脉

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1 Introduction

During open heart surgery using cardiopulmonary bypass, the heart is usually arrested for precise intracardiac repair or coronary grafting. Ischemia-reperfusion injury is the major problem in open heart surgery; cardioplegia was initially designed to protect the myocardium from this injury. In the case of heart transplantation, cardioplegia or organ preservation solutions are used to preserve the donor heart or other organs. It is now well known that coronary circulation plays a key role in myocardial perfusion. Injury to the coronary circulation may change the coronary resistance and therefore affect the coronary flow. The reduction of coronary flow may damage the myocardium perfusion that, in addition to the ischemia-reperfusion injury to the myocardium, may further damage the myocardial function. Due to the differences between the cardiac myocytes and vascular (endothelial and smooth muscle) cells in structure and function, cardioplegia may have adverse effect on coronary circulation. In addition, for organ transplantation, cardioplegic or organ preservation solutions are used to preserve the heart or other organs. The endothelium-smooth muscle interaction may also be changed during the preservation. Endothelium is critical in regulating vascular tone and the endothelium-dependent relaxation is mediated by three different endothelium-derived relaxing factors (EDRFs) - nitric oxide (NO), prostacyclin (PGI_2), and endothelium-derived hyperpolarizing factor (EDHF)[1]. Although some studies suggested the preservative effect of crystalloid cardioplegic or organ preservation solutions on the endothelial function, numerous studies provided evidence for functional or histological endothelial damage after exposure to these solutions.

2 The Possible Mechanisms Under-lying the Damage of Cardioplegia and Organ Preser vation on Endothelial Function

The "so-called" effect of solutions on coronary endothelium is often mixed with the effects due to other factors combined with cardioplegia procedure as follows: (1) Direct action of the solutions due to their intrinsic characteristics (the components of the solution, such as hyperkalemia). This has been discussed in our recent review(Fig.1)[2]; (2)Adjuncts to the cardioplegic procedure such as hypothermia or the infusion pressure or duration; (3) The effect of ischemia-reperfusion injury. The ischemia-reperfusion injury may involve both myocytes and coronary endothelium-smooth muscle. Therefore, the protection of the heart should also involve these two aspects. The injury to the coronary circulation may involve both NO and EDHF mechanisms. The NO mechanism is susceptible to ischemia-reperfusion whereas the EDHF mechanism may be altered by the hyperkalemic cardioplegia. To further protect the heart, supplemental therapy for NO and optimizing the components of cardioplegia to restore the EDHF-mechanism may be important. (4)Other factors involved in isolated working heart models or *in vivo* models when these models are used to study endothelial function.

Taken together, the "true" effect of the solutions on

the endothelium should be carefully distinguished from other factors in order to identify the possible damaging effect due to the intrinsic characteristics of the solutions.

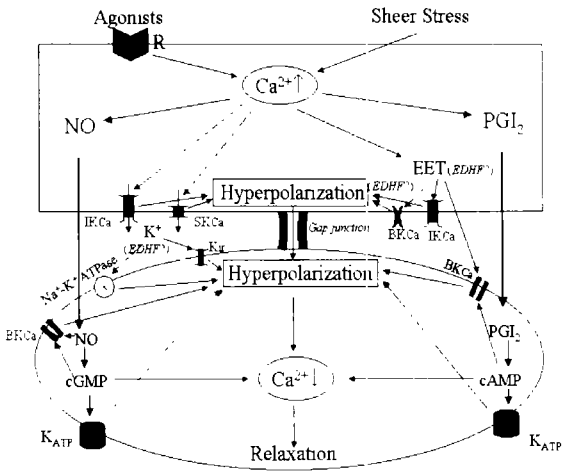

Fig.1 Schematic diagram describing the pathways of endothelium-dependent vasorelaxation

Under sheer stress or the stimulation of agonists, $[Ca^{2+}]i$ rise in endothelial cells that leads to the release of prostacyclin (PGI_2), nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF). These three endothelium-derived relaxing factors decrease the $[Ca^{2+}]i$ in smooth muscle cells through different mechanisms and ultimately relax the smooth muscle. BKCa: large conductance calcium-activated potassium channel; IKCa: Intermediate conductance calcium-activated potassium channel; SKCa: small conductance calcium-activated potassium channel; Kir: Inward rectifier potassium channel; KATP: ATP-sensitive potassium channel; cAMP: cyclic 3′, 5′-adenosine monophosphate; cGMP: cyclic 3′, 5′-guanosine monophosphate; EET: epoxyeicosatrienoic acid. (Reproduced from Reference 92 with permission)

3 The Influence of Cardioplegic and Organ Preservation Solutions on Individual $EDRF_s$

3.1 The influence of the solutions on PGI_2

The increase of release of PGI_2 during myocardial ischemia with / without cardioplegic arrest was demonstrated. Gene expression of COX-1 and PGI_2 synthase were not altered after cardioplegia but COX-1 protein level was significantly reduced accompanied with increased expression of COX-2 [3]. However, the combined effect of ischemia-reperfusion was not excluded. It is also unknown how long this increase would maintain under hypoxic condition.

3.2 The influence of the solutions on NO

Studies have suggested impaired NO-related endothelial function during cardiopulmonary surgery. We have demonstrated when the effect of ischemia-reperfusion is excluded, the NO-related, endothelium-dependent vasorelaxation after exposure to oxygenated crystalloid hyperkalemic cardioplegia to acetylcholine or substance P is well preserved in either porcine epicardial coronary arteries[4] or neonatal rabbit aorta[5]. Although in these studies, the indomethacin-resistant relaxation is actually mediated by both NO and EDHF, the unchanged endothelium-dependent response and the susceptibility of EDHF to cardioplegic solution[6-8] provide convincible evidence for the minimal impact of hyperkalemic cardioplegic solution on the NO-related function after exposure for a certain period (1 or 2 hours).

3.3 The influence of cardioplegic or organ preservation solutions on EDHF

We have conducted a series of experiments to investigate the effect of cardioplegic solution and organ preservation solutions on EDHF -mediated function. With exclusion of the effect of ischemia/reperfusion and elimination of the effect of PGI_2 and NO, we have demonstrated that hyperkalemia [6,7], St Thomas' Hospital cardioplegia (ST) [8] and UW solution [9] impair EDHF-related function either in porcine or human coronary arteries[6,7]. The mechanism is due to the opposite effect of EDHF and hyperkalemia. Hyperkalemia depolarizes whereas EDHF hyperpolarizes the smooth muscle membrane. The persistent depolarizing effect of hyperkalemia even after wash-out of cardioplegic solution restricts the hyperpolarizing effect of EDHF [7,9,10]. Use of potassium channel openers as hyperpolarizing cardioplegia may overcome this shortage of hyperkalemic cardioplegia [11].

4 The Influence of Different Components in Cardioplegic and Organ Preservation Solutions on Endothelial Function

4.1 Effect of K^+ on endothelial function

K^+ is the key component in cardioplegic / organ

preservation solutions. The concentration of K^+ varies in different solutions. In UW solution, it is as high as 125 mmol/L whereas only 20 mmol/L in ST and 10 mmol/L in Histidine-Tryptophan-Ketoglutarate (HTK) solution, respectively. The importance of K^+ concentration with regard to coronary endothelial impairment was revealed. K^+ at 30 mmol/L but not at 20 mmol/L abolished the endothelial-dependent, 5-hydroxytryptamine-induced vasodilatation[12].

In contrast, studies from others [13] and us have demonstrated that hyperkalemia *per se* does not significantly alter the endothelium-dependent relaxation as a whole to acetylcholine or substance P in porcine coronary arteries (to K^+ 50 mmol/L) [4] and neonatal rabbit aorta (to K^+ 100 mmol/L) [5]. These contradictory results stimulate further investigations as to the effect of hyperkalemia on individual relaxing factors derived from endothelial cells. Up to date, there is little evidence showing that the reduction of the production of NO is due to hyperkalemia. Rather, it is most likely due to the combined ischemia-reperfusion injury. When the capability of the endothelium to release NO is preserved or there is no presence of specific NO inhibitors, the endothelium is tolerant to hyperkalemia as far as the endothelium-dependent relaxation is concerned as shown above [4, 5, 13]. Most recently, with measurement of NO by a NO-specific electrode, we for the first time provided direct evidence that NO release is not affected by 1 hour exposure to 20 mmol/L K^+ [14].

On the other hand, in contrast to NO, susceptibility of EDHF to high concentration of K^+ has been demonstrated in accumulating studies. When the effect of PGI_2 and NO is inhibited by indomethecin and N^G-nitro-L-arginine ($_L$-NNA), the endothelium-dependent relaxation/hyperpolarization (mediated by EDHF) to a number of EDRF stimuli is impaired by incubation with K^+ ranging from 20 to 125 mmol/L in porcine and human coronary arteries [6,7,10]. Realizing that $_L$-NNA cannot abolish the production of NO, we further added oxyhemoglobin, a scavenger of NO, to abolish the effect of residual NO and demon-strated again the detrimental effect of hyperkalemia on the EDHF-mediated relaxation and hyperpolarization in porcine coronary micro-arteries[15].

The mechanism of the reduced EDHF-mediated relaxation in hyperkalemic solutions is two-fold. First, hyperkalemia depolarizes the coronary smooth muscle membrane and the prolonged depolarization increases the difficulty for subsequent hyperpolarization. Second, EDHF hyperpolarizes the vascular smooth muscle cell through opening K^+ channels, the function of which may be blocked by K^+ that is a natural K^+ channel blocker [7,10].

4.2 Effect of Mg^{2+} on endothelial function

The introduction of Mg^{2+} into cardioplegia helps to achieve immediate heart arrest during cardiac surgery and the enrichment of Mg^{2+} may counteract the unfavorable effect of hypocalcemia on sarcolemmal membrane by preventing calcium influx and thus obtain better membrane stabilization. In addition to the protective effect on myocardium, Mg^{2+} has been proven to be a potent vasodilator through both endothelium-dependent and independent mechanisms. In addition, a recent study from our laboratory demonstrated that in porcine coronary arteries, Mg^{2+} preserves the EDHF -mediated relaxation and hyperpolarization and restores the EDHF function impaired by hyperkalemia [15].

4.3 Effect of procaine on endothelial function

Similar to Mg^{2+}, the local anaesthetic procaine is added to cardioplegia to induce asystole and obtain membrane stabilization. We further showed that procaine does not affect EDHF function in the coronary circulation [16] despite of the fact that it depolarizes the membrane of vascular smooth muscle cells by reducing K^+ conductance [17].

5 The Effect of Different Additive to Cardioplegic or Organ Preser-vation Solutions on Endothelial Function

The additives that are aimed to protect endothelial function may be categorized as follows: ① NO substrates or donors. ② PGI_2 analogues. ③EDHF analogues: epoxyeicosatrienoic $acid_{11,12}$ (EET^{12}_{11})[18,19]. ④ K^+ channel openers (KCOs): Aprikalim [20,21],

KRN4884 [22]. ⑤ Scavengers of oxygen-derived free radicals. ⑥ Sodium-hydrogen ion exchange (NHE) inhibitors [23]. ⑦ Other substances: Adenosine, phosphodiesterase Ⅲ-inhibitor (E-1020), 17beta-estradiol, Ca^{2+} antagonists, metabolic substrates (i.e. glutamate, aspartate, fumarate etc.).

6 Conclusion

In summary, due to the differences between the cardiac myocytes and vascular (endothelial and smooth muscle) cells in structure and function, the cardioplegic or organ preservation solutions primarily designed to protect the myocardium may have detrimental effect on coronary vascular endothelial cells, as demonstrated in the last decades. One must be aware of that under either experimental or clinical settings, reported effect of the solutions on coronary endothelium is often mixed with many other factors such as ischemia-reperfusion injury, temperature, and perfusion pressure and duration, not only due to the components of the solutions per se. In evaluation of a clinically used solution and in development of a new solution, these factors should be carefully distinguished from the effect of the solution. The key component of the cardioplegic and heart preservation solutions that causes cardiac arrest-high concentrations of potassium ion (hyperkalemia) is the major component that has been studied regarding the endothelial function. The primary contributor of the endothelium-dependent relaxation, NO-pathway, is mainly impaired due to ischemia-reperfusion injury. The resistance of NO-pathway to the moderately increased potassium concentrations used for cardioplegia (~20 mmol/L) explains the excellent clinical results by using either crystalloid or blood cardioplegia. On the other hand, the second endothelium-dependent relaxation pathway-the EDHF pathway that is usually a "back-up" of the NO pathway, is significantly altered (damaged) by hyperkalemia even at the moderately high concentration of potassium. This is because hyperkalemia inhibits potassium channels in the endothelium and smooth muscle that are related to either the release of EDHF from the endothelium or the target of the action of EDHF. Magnesium has a protective effect on this pathway because it hyperpolarizes the coronary smooth muscle membrane and therefore has "synergetic" effect with EDHF. When combined with ischemia-reperfusion and other factors that significantly impair the NO-pathway, the effect of hyperkalemia on the EDHF pathway becomes important issue in the protection of coronary endothelium. Variety of new additives aimed to protect these two major endothelium-dependent pathways may further improve the protection of the coronary endothelium from other factors such as ischemia-reperfusion injury. All these issues should be taken into account in the development of new cardioplegic and heart preservation solutions in the future in order to provide "perfect" cardiac protection.

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