

## Technique Article

# Technique of Complete Heart Isolation with Continuous Cardiac Perfusion During Cardiopulmonary Bypass: New Opportunities for Gene Therapy

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Presented at the 53rd International Conference of Extracorporeal Technology, Tampa, Florida, April 15, 2015.

**Abstract:** Cardiopulmonary bypass (CPB) featuring complete heart isolation and continuous cardiac perfusion is a very promising approach for solving the problem of efficient gene delivery. In the technique presented here, separate pumps are used for the systemic and cardiac circuits. This system permits continuous isolated arrested heart perfusion through optimizing a number of delivery parameters including temperature, flow rate, driving pressure, ionic composition, and exposure time to the cardiac vessels. During complete cardiac isolation, the blood vector concentration trended from  $11.51 \pm 1.73$  log genome copies (GCs)/cm<sup>3</sup> to  $9.84 \pm 1.65$  log GC/cm<sup>3</sup> ( $p > .05$ ). Despite restructuring a very high concentration to the heart, GCs were detectable in the systemic circuit. These values over time were near negligible by comparison but detectable  $1.66 \pm .26$  during 20 minutes of recirculation and did not change ( $p > .05$ ). After the completion of the recirculation interval and subsequent washing procedure, the initial systemic blood vector GC concentration slightly increased to

$2.08 \pm .38$  log GCs/cm<sup>3</sup> ( $p > .05$ ). During the recirculation period, we supported flow via the cardiac circuit around 300 mL/min. In this technique of heart isolation with continuous cardiac perfusion, >99% of the vector remains in coronary circulation during recirculation period. The animal's non recirculation blood, or that in the system, was routinely tested during and after recirculation to contain much less than 1% of the original dose obtained via logging concentration of therapeutic over time. All of the sheep in this group recovered from anesthesia and received critical postoperative care, including all organ function, in the first 24–36 hours. Twenty-one sheep (84%) survived to euthanasia at 12 weeks. Average CPB time was  $107 \pm 19.0$  minutes and cross-clamp time was  $49 \pm 7.9$  minutes. This technology readily provides multiple pass recirculation of genes through the heart with minimal side effects of collateral expression of other organs. **Keywords:** heart isolation, gene therapy, heart perfusion, cardiopulmonary bypass. *J Extra Corpor Technol. 2018;50:193–8*

The use of cardiopulmonary bypass (CPB) technology allows performed procedures in a motionless and bloodless area within the heart chambers. Recently, the application of CPB is not limited only to the implementation of procedures that may benefit of bypass techniques where the role is simply maintaining the circulation of blood and the oxygen content

of the patient's body. Cardiac gene therapy, a relatively new translational therapeutics area, needs to maximize cardiac transfer of product into the myocardium while limiting deleterious side effects of collateral organ exposure at higher doses (1). CPB-mediated delivery technology attempts to address this delivery problem through incorporating an extracorporeal circuit to identify and modulate intrinsic and extrinsic cellular transport barriers. Gene therapies uniquely tailored and efficacious in addressing the root cause pathogenesis of many cardiac and non-cardiac diseases are rapidly expanding in applications (2).

Recently, gene therapy has been rapidly accumulating late-breaking efficacy data in numerous ongoing clinical

Received for publication November 1, 2017; accepted April 28, 2018.  
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The senior author has stated that the authors have reported no material, financial, or other relationship with any healthcare-related business or other entity whose products or services are discussed in this paper.

trials, thus expanding medical knowledge. However, gene transfer outcomes vary widely, and it has been shown that the success of any given indication will largely depend on these crucial factors: 1) selecting the proper gene construct that addresses a proper molecular target related to disease, 2) the efficiency of the delivery resulting from the combined effects of the vehicle transfer and the applied route of administration, and 3) the avoidance of a deleterious immune response and/or injury to the cardiac system (3). Delivery has now been identified as the key rate-limiting variable in both optimizing efficacy within safety limits. Therefore, it is logically reasonable to conclude that effective gene transduction is a requirement to achieve efficacy and that no perfect gene construct will result in a positive outcome without understanding its delivery parameters. Delivery applications to the myocardium have seen a dramatic increase in research over the past two decades. Experiments involving heart perfusion *ex vivo* first illustrated the basic principles of cardiac gene delivery. These were that 1) coronary flow rate and pressure correlate with performance. 2) The residence contact time of vector with the coronary circulation is the most important variable for maximizing efficiency. It has been demonstrated that a single pass of the virus solution through the heart typically transduces only 0.8% myocytes. On the contrary, 40% or higher is readily achieved when the virus-containing perfusate was recirculated for 60 minutes. 3) The composition and temperature of the perfusate impact local diffusion regulatory mechanisms at the capillary level. 4) Incorporation of endothelial permeabilizing agents such as histamine and bradykinin improved transfection efficiency by increasing the flow through the coronary arteriole system (4).

Leveraging these core concepts, at least three groups of investigators suggested that CPB may facilitate cardiac selective gene transfer to the heart and demonstrated that the arrested heart provides an optimal setting for transgene expression (5–7). Despite efforts to restrict delivery to the myocardium however, using CPB did not prevent the dissemination of gene or vector construct to collateral organs. Thus, a truly “closed loop” recirculation methodology was not achievable with CPB alone. This problem of using CPB as a standalone method for cardiac gene delivery needed to be addressed for using higher doses of gene therapy products. The first attempt in designing a completely closed system was achieved by Bridges et al. (8), who strategically added the second pump just for coronary multiple recirculation during CPB and succinctly referred to as molecular cardiac surgery with recirculation delivery (MCARD). In this study, we aim to quantitatively assess gene delivery using the technique of complete heart isolation and continuous cardiac perfusion during CPB and review the main perfusion technical features of it.

## METHODS

### Animals

All animals received humane care in compliance with the National Institutes of Health and the local Institutional Animal Care and Use Committee. Dorsett male sheep ( $n = 25$ ) weighing  $49.4 \pm 3.3$  kg were used.

### Vector Design and Production

Recombinant recombinant adeno-associated virus vectors, encoding green fluorescent protein under the control of the human cytomegalovirus promoter immediate early enhancer/promoter with a splice donor/acceptor sequence and polyadenylation signal from the human globin gene are used.

### Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

For measuring genome biodistribution, we used RT-qPCR. RT PCR was performed using the MyiQ RT-PCR Detection System (Bio-Rad Laboratories, Hercules, CA) and analyzed using MyiQ software package (Bio-Rad Laboratories). qPCR analysis was performed in optical 96-well plates using iQ SYBRGreen Supermix (Bio-Rad Laboratories).

### Statistical Methods

Comparisons between the blood vector genome copy (GC) cardiac and systemic concentrations at specific time points were performed using two-sided paired Student's *t*-tests. One-way analysis of variance was used to compare blood QPCR in the systemic and cardiac circulation at the different time points.

### Surgical Protocol

**Surgical procedures and gene delivery:** Once intubated and sedated, the animal is placed supine, prepared, and draped. Unfractionated heparin is administered (130 U/kg) and usually re-dosed to achieve and maintain an activated clotting time (ACT) of greater than 450 seconds. HemoChron 801 ACT machine (International Technidyne Corporation, Edison, NJ) with celite activator was used. Samples for blood gas analysis were drawn every 20 minutes. If ACT dropped to below 450 seconds, an additional dose of heparin was administered and ACT was checked again in 5 minutes. We used initial low dose because during our protocol development, we noted a wide variation in responses to heparin dosing and complications associated with prolonged bleeding. Besides, we added 5,000 IU heparin in CPB prime solution.

A median sternotomy is created, and a 2D-transepical echocardiogram is performed before and after weaning from CPB. The right carotid artery is then cannulated for systemic perfusion using a 14-F cannula (Medtronic, Inc., Minneapolis, MN). The aortic root vent, superior vena cava

(SVC) cannula, and retrograde cardioplegia catheter are placed. The inferior vena cava (IVC) is cannulated and full CPB is initiated. The SVC was cannulated using a 26-F right angle cannula and IVC using a 26-F straight cannula (Edwards, Irvine, CA). The target flow was 2.5 L/min-m<sup>2</sup>. The target mean arterial pressure was 60–70 mm Hg. Arterial base analysis was performed to assess the electrolytes balance, acid–base status, venous saturation, lactate, glucose, and hematocrit. Any abnormalities were corrected. The desired ranges of these parameters were as follows: pH 7.35–7.45, PaO<sub>2</sub> 20–30 kPa, PaCO<sub>2</sub> 4.6–5.5 kPa, SvO<sub>2</sub> >65%, BE arterial –2 to +2, K<sup>+</sup> 3.5–5.3 mmol/L, glucose 3.3–6.0 mmol/L, hematocrit >25%, Na<sup>+</sup> 135–144 mmol/L, Cl<sup>–</sup> 95–105 mmol/L, Mg<sup>+</sup> .7–1.0 mmol/L, and lactate .9–1.7 mmol/L. The CPB circuit was primed with 1,000 mL Normosol-R, 5,000 units heparin, 12.5 g mannitol, and 20 mg Lasix. After insertion of the arterial cannula, arterial blood was pulled retrograde through the CPB circuit to replace the priming solution in the arterial line, venous reservoir, and filter oxygenator. At the start of CPB, blood was taken back through the venous tubing to clear Normosol-R from the venous lines. To minimize hemodilution, intravenous fluid administration was minimized before bypass and cease after the initiation of CPB.

Oxygenator Sorin Inspire (Sorin Group USA, Inc., Arvada, CO) was used. In the general CPB circuit, the tubing diameter was 3/8 × 3/32 inches and in cardiac circuit 1/4 × 1/16 inches. The systemic temperature was mild to moderate hypothermia (32–34°C).

The azygous and hemiazygous veins are ligated; the IVC and SVC are snared. Vent cannulas are placed into the left ventricular cavity through the apex and into the right ventricle through the outflow tract. The left ventricle, right ventricle, and aortic root vents are connected to the venous limb of the cardiac venous return circuit. The arterial limb of the cardiac circuit is connected to the coronary sinus catheter. The cardiac circuit is primed with Normosol-R (total priming volume approximately 300 mL). The aorta and pulmonary artery are cross-clamped. Cold (4°C) del Nido cardioplegia (300 mL) is delivered antegrade. The heart is isolated by tightening the SVC and IVC snares and by cross-clamping the pulmonary artery. With the heart fully decompressed, cardiac circuit flow is initiated briefly until the coronary sinus pressure equals 40 mm Hg. During the recirculation period, we supported flow via the cardiac circuit around 300 mL/min. The virus solution is then injected into the retrograde catheter and recirculated for 20 minutes, with coronary sinus pressure of 40 mm Hg, and flow adjusted to maintain a constant coronary sinus pressure. The coronary circuit is then flushed antegrade with 1,000 mL of Hespan to wash out residual vector. Flow is restored. Re-warming is initiated. After approximately 5 minutes of systemic venting, the cross-clamps on the aorta

and pulmonary artery are removed. The aortic cannula is removed. The right ventricle (RV) cannula is removed as well. Once the heart is contracting well, the left ventricle (LV) cannula is removed. Bypass is discontinued after 30 minutes of reperfusion. Once hemodynamic stability is achieved and the heparin is reversed, the animal is weaned from CPB and closed in standard fashion. All animals are recovered from anesthesia and received critical postoperative care.

A schematic drawing of the cardiac isolation procedure is shown in Figure 1.

## RESULTS

The MCARD procedure results in complete cardiac isolation, demonstrating separation of the cardiac and systemic circulation compartments. Viral vector gene therapeutic concentration over time is delivery obtained from these experiments illustrate the proof of concept. Figure 2 demonstrates that with complete cardiac isolation, the blood vector GC concentration trended from  $11.51 \pm 1.73 \log \text{GCs/cm}^3$  to  $9.84 \pm 1.65 \log \text{GCs/cm}^3$  ( $p > .05$ ). Despite restructuring a very high concentration to the heart, GCs were detectable in the systemic circuit. These values over time were near negligible by comparison but detectable  $1.66 \pm .26$  during 20 minutes of recirculation and did not change ( $p > .05$ ). After the completion of the recirculation interval and subsequent washing procedure, the initial systemic blood vector GC concentration slightly increased to  $2.08 \pm .38 \log \text{GC/cm}^3$  ( $p > .05$ ). This observation results from the ejection of un-transduced GC from the coronary vessel system once the heart was back to normal rhythm.

Thus, in this technique of heart isolation with continuous cardiac perfusion, >99% of the vector remains in coronary circulation during recirculation period. The blood circulation routinely tested during and after recirculation to contain much less than 1% of the original dose obtained via logging concentration of therapeutic over time.

All of the sheep in this group recovered from anesthesia and received critical postoperative care, including all organ function, in the first 24–36 hours (Table 1). Twenty-one sheep (84%) survived to euthanasia at 12 weeks. Average CPB time was  $107 \pm 19.0$  minutes and cross-clamp time was  $49 \pm 7.9$  minutes. All animals had a good global ejection fraction before CPB, and echocardiographic parameters did not significantly change in postoperative period of 22 animals (92%). Moreover, we evaluated each animal for aortic and mitral valve function and did not find regurgitation in any animal. In two animals, we did have to use epinephrine at a dose of .03 µg/kg per min 2–4 hours after weaning from CPB because of low cardiac output syndrome and decreased ejection fraction. Both animals survived until euthanasia and ejection fraction returned to preoperative values after 24 hours.

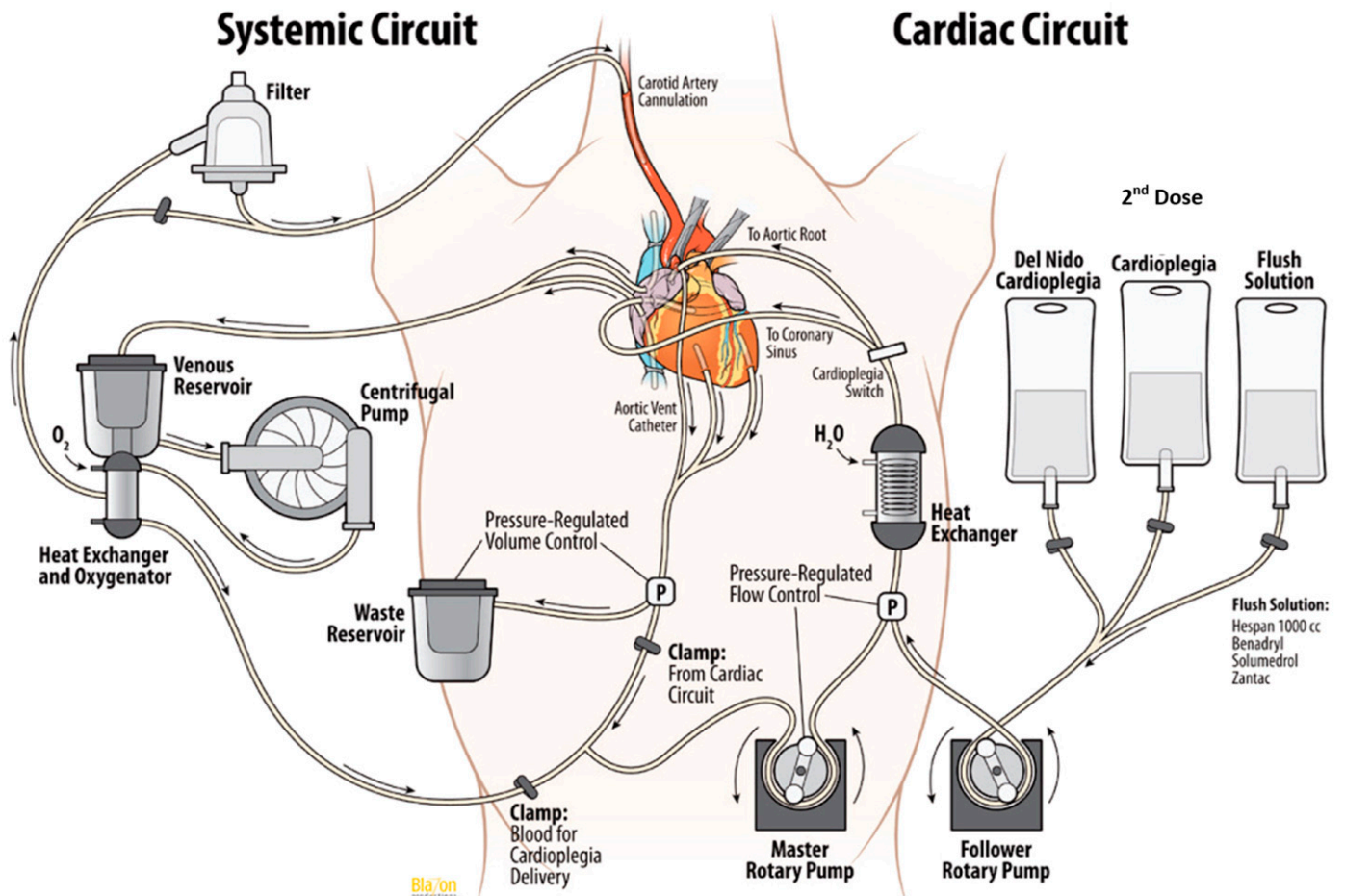


Figure 1. A schematic drawing of the cardiac isolation procedure during CPB.

### DISCUSSION

The present data demonstrate the reproducibility of using CPB and cardiac surgical procedures to achieve optimal gene transfer conditions to the heart with a complete surgical isolation of the heart in situ. This methodology results in efficient myocyte gene delivery while limiting collateral organ exposure at the same time. The perfusion circuit includes various components to maximize

safety during recirculation of a macromolecular complex solution through the cardiac circuit. We used relatively low priming volume of the cardiac circuit because the aim was to maximize the gene construct concentration to enhance myocardial gene transfer. The circuit allows for the efficient decompression of the left and right ventricles during recirculation. Compared with gene delivery during regular CPB (5), the creation of a second independent cardiac circuit can provide continuous recirculation in the context of cardiac surgery. Thus, this is a fundamentally new idea in that systemic physiology is maintained but another add-on system is used for delivery purpose only.

The system is designed to isolate the coronary circulation from the general circulation safely and efficiently with standard cardiac surgical techniques that are modified slightly for delivery concepts. However, unlike the typical bypass technique, the arrested heart after providing cardioplegia will receive a coronary supplied volume of vector which can be encoded with genes, proteins, and/or various combinations. This new technology is intended for administration and uptake of any gene therapy agent

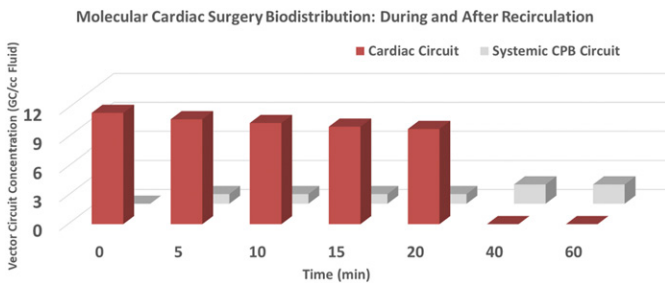


Figure 2. Molecular cardiac surgery biodistribution during and after recirculation.

**Table 1.** Postoperative care protocol: first 24–36 hours.

Monitoring	Medication	Fluids and Feed
Vital signs every 4 hours	Nasal O <sub>2</sub> 5 L/min, until stable	Hetastarch IV
SPO <sub>2</sub> monitoring until stable	Standard antibiotics	Normosol-R + dextrose + KCl
CVP every 4 hours	Furosemide if CVP > 10 cm H <sub>2</sub> O	Water when alert, feed >6 hours postoperation
EKG every 4–8 hours	K, Ca, and Mg depending on ABG/VBG	Add 5% dextrose to fluids if still NPO in AM
ABG or VBG every 2–3 hours	HCO <sub>3</sub> for metabolic acidosis	
Chest tube drainage every 1 hour	Inotropes, low cardiac output	
Urination	Amiodorone, arrhythmias	
Liver enzymes	Normosol-R 50–80 mL/hr if CVP < 4 cm H <sub>2</sub> O	
Kidney function (creatinine, urea)		
CBC chemistry panel blood		

exclusive to the myocardium. This methodology addresses the problem of obtaining intimate access to the coronary system within safety limits.

This technique essentially addresses delivery needs such as genome particles adapted for the coronary perfusion system of vessels.

The coronary perfusion system comprises arteries, arterioles, capillaries, venules, and veins. Functional arterial and venous return is necessary to maintain proper function as well as transport of key solutes and metabolites in the normal state. On the arterial side, the left coronary artery supplies the lateral and anterior walls of the left ventricle, the anterior two-thirds of the interventricular septum, and small part of the posterior wall. The right coronary artery supplies the right ventricle, the posterior wall of the left ventricle, and posterior third of the septum. We assert that venous return from the heart is equally important as arterial circulation regarding myocardial metabolic supply. Coronary venous flow starts from the cardiac capillaries and returns to the cardiac chambers through venules, which in turn are collected into the cardiac veins. Most of the blood from left ventricle and cardiac veins drains into the right atrium through the coronary sinus. Thebesian veins in the heart drain about 10% of coronary blood directly into the cardiac chambers and account for a true shunt. Therefore, during heart isolation, it is necessary to take into account this volume as well. Essentially, a steady-state delivery status is achieved with this MCARD concept by balancing flow phenomena in either direction such that macromolecules are safely transported through the dense network of capillaries accessing the entire heart.

### Flow and Pressure

During the recirculation period, we supported flow via the cardiac circuit around 300 mL/min. We were guided by the following considerations. If the flow through the cardiac circuit were, e.g., 20% of the flow rate of normal coronary blood flow in the sheep, then only 20% of the vector infused into the coronary system would recirculate on the second pass through the system. And only 4% of the vector would recirculate on the third pass and 0.8% would recirculate on the fourth pass. Thus, each virus would pass through the

coronary circulation an average of <1.3 times (9). Thus, in this technique, >99% of the vector remains in coronary circulation during recirculation period. Moreover, in previous studies, we found that this rate reduces the risk for excessive edema in the cardiac tissues following bypass procedures (10).

### Retrograde Coronary Infusion

Retrograde coronary recirculation through the coronary sinus as compared with antegrade provides a more uniform distribution of agents (11). Our coronary sinus pressure was a bit higher (>40 mm Hg) than accepted during retrograde cardioplegia in patients. We used this number because from our experience, small molecular weight substances as viral vectors can transverse the capillary endothelium when the capillary filtration pressure is high to overcome the resistance of precapillary sphincters. After the procedure, we did echocardiography, and histology after euthanasia. No myocardial edema or other complications were observed using this pressure. We believe that using higher coronary sinus pressure results in higher profiles of gene delivery. A main limitation of coronary sinus recirculation, however, is the relatively poor perfusion of the right ventricle because the pressure gradient is substantially lower. It is known that small molecular weight substances, including viral vectors, can transverse the venous capillary endothelium by different mechanisms compared with arterial endothelium because the dimensions and pressure gradients achieved are much more favorable as than those of the arterial side (12,13). Many authors consider that coronary venous infusion allows for prolonged adhesion time of the vector to the cardiac endothelium (13). Coronary sinus circulation changes the hydrostatic and osmotic pressure and increases capillary filtration coefficient, thereby augmenting viral movement through capillary barrier (14). In addition, another advantage of this procedure is its ability to overcome the resistance of precapillary sphincters located only on the arterial side of the capillary beds. Thus, less blood is shunted through into the cardiac chambers. This effect directly results in both increase in endothelial permeability and viral diffusion across the interstitial capillaries and venules. As a consequence, this promotes the transfer of macromolecular particles into the interstitial compartment of the left heart (14).

## CONCLUSIONS

CPB featuring complete heart isolation and continuous cardiac perfusion can open a new application in the field of cardiac surgery and cardiology regarding delivering novel therapies to the heart that cannot reach the clinic with regular routes of transfer. With recent advances in minimally invasive cardiac surgical solutions, there could be a unique opportunity to adapt various perfusion methodologies to maximize cardiac gene transfer.

## ACKNOWLEDGMENTS

This study was supported by NIH grant 7R01 HL083078-10. We would like to acknowledge the NHLBI Gene Therapy Resource Program. We also thank the veterinarians and veterinary technicians for their excellent care of the animals in the University of Pennsylvania and Sanger Heart and Vascular Institute.

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