Efficient Viral Gene Transfer to Rat Hearts In Vivo

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Method Article

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Abstract

Cardiac gene transfer represents a unique strategy to alter the expression of specific genes in the heart that may result in alterations in contractile function. This approach allows us to investigate the role of these pathways on cardiac disease pathogenesis and progression. In rodents, cardiac gene transfer has been achieved predominantly by direct injection into the myocardium or perfusion of an isolated coronary segment. Either approach results in focal overexpression of the transgene and is therefore unlikely to effectively modulate global cardiac function. Here, we provide a gene transfer method that results in global transduction of the heart. We used this technique to demonstrate in vivo that beneficial effects of SUMO1 in the setting of heart failure are dependent on SERCA2a.

Procedure

Preparation of the Rat: 1. Obtain rats of various strains \(Fisher 344, Sprague-Dawley, Brown-Norway, or Wistar). The rats should be free of antibody titers against a number of routinely tested rat viruses. In addition, the rats should be free of all endo- and ectoparasites and mycoplasma species. 2. Maintain the rats in a barrier room at 72 ± 2°F with the relative humidity at 50 ± 10%, and feed them a commercial laboratory diet and water ad libitum. 3. Set the room ventilation between 12 and 15 air changes per hour of 100% pre-filtered outside air. 4. Control the light cycle period at 12 hrs of light and 12 hrs of dark with no twilight transition. 5. Have the institutional Animal Care and Use Committee approve the study. Anesthesia: 6. Anesthetize the rats with intraperitoneal pentobarbital \(40–60 mg/kg). 7. Place a rectal temperature probe in the animal, and use a heating lamp to maintain body temperature while the animal lies dorsally. 8. Intubate the animals via the larynx by using a 16-gauge soft catheter \(Angiocath, Becton-Dickinson, Sandy). 9. Place a small light source on the neck over the larynx, and lift the tongue. This allows the larynx to be clearly visualized, permitting the angiocath to be introduced accurately. 10. Ventilate the rats with tidal volumes of 2 mL at 50 cycles/min with an FiO2 of 0.21. Viral Preparation: 11. Store adenoviruses stocks at –70°C, usually in glycerol-containing solutions. Since glycerol can be toxic to the lungs, we dilute our adenoviral solutions with phosphate-buffered saline \(PBS) 1:1. 12. Thaw the solution and maintain at 37°C. 13. Depending on the mode of gene delivery, deliver 100 to 200 µL of viral solution. For in vivo gene transfer, we try to achieve a multiplicity of infection of 100 pfu/ cell. The rat heart has approximately 107 cardiomyocytes. Therefore the viral solution should have a concentration of at least 1010 pfu/mL. Calculation: Cross-clamping of the Aorta and Pulmonary Artery: 14. In an open-chested rat \(either by mid-sternotomy or through an incision from the left third intercostal space), open the pericardium, and place a 7-0 suture at the apex of the left ventricle. 15. Identify the aorta and pulmonary artery. With a forceps, pass a 2-0 suture underneath the pulmonary artery and the ascending aorta and then introduce both ends of the tube into a 16 gauge tube then tie both ends of the suture for cross clamping. \!\! NOTE that two fingers should fit between the 16-gauge tube and the tied ends of the suture. 16. Advance a 24-gauge catheter \(depending on the size of the animal) containing 200 µL of adenovirus from the apex of the left ventricle to the aortic root. \! CAUTION It is important either to feel or to visualize the catheter as it is advanced from the ventricular apex all the way to the aortic root. 17. The

aorta and pulmonary arteries are clamped distal to the site of the catheter and the solution injected. \!\! NOTE Aortic clamping alone can be performed, however expression with aortic clamping alone is low. 18. The clamp can be maintained for 10 up to 60 sec \(preferably 30-45 sec) while the heart is pumping against a closed system \(isovolumically). This allows the solution that contains the adenovirus to circulate down the coronary arteries and perfuse the heart without direct manipulation of the coronaries. \!\! NOTE Methylene blue can be injected to evaluate the perfusion and staining of the whole heart can be easily assessed. 19. After 10–60 sec, the clamp on the aorta and pulmonary artery is released. \!\! NOTE During the period of cross-clamping, the right and left ventricles become visibly pale as clear viral solution perfuses the myocardium through the coronary arteries. During the procedure, the heart rate decreases from $_{300\ \text{to}}$ 50 bpm but recovers to baseline within 30 sec of clamp release. Ventricular pressure returns to baseline within 60 sec. 20. After removal of air and blood, the chest is closed, and animals are extubated and transferred back to their cages. \!\! NOTE Pretreatment of the animals with either adenosine or serotonin by injecting a solution containing 1–10 μM into the inferior vena cava can also be used. This induces increased permeability of the capillaries, resulting in enhanced attachment of the viruses to myocardial cells. \!\! NOTE In a further refinement of this method, clamping of the aorta, pulmonary artery, and superior and inferior vena cava has been performed by tying the superior vena cava, pulmonary artery, and aorta with one 5-0 silk suture compressed with a small hollow tube. A similar compression is achieved for the inferior vena cava.

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