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Myocardial drug distribution generated from local epicardial application: Potential impact of cardiac capillary perfusion in a swine model using epinephrine

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ABSTRACT

Prior studies in small mammals have shown that local epicardial application of inotropic compounds drives myocardial contractility without systemic side effects. Myocardial capillary blood flow, however, may be more significant in larger species than in small animals. We hypothesized that bulk perfusion in capillary beds of the large mammalian heart not only enhances drug distribution after local release, but also clears more drug from the tissue target than in small animals. Epicardial (EC) drug releasing systems were used to apply epinephrine to the anterior surface of the left heart of swine in either point-sourced or distributed configurations. Following local application or intravenous (IV) infusion at the same dose rates, hemodynamic responses, epinephrine levels in the coronary sinus and systemic circulation, and drug deposition across the ventricular wall, around the circumference and down the axis, were measured. EC delivery *via* point-source release generated transmural epinephrine gradients directly beneath the site of application extending into the middle third of the myocardial thickness. Gradients in drug deposition were also observed down the length of the heart and around the circumference toward the lateral wall, but not the interventricular septum. These gradients extended further than might be predicted from simple diffusion. The circumferential distribution following local epinephrine delivery from a distributed source to the entire anterior wall drove drug toward the inferior wall, further than with point-source release, but again, not to the septum. This augmented drug distribution away from the release source, down the axis of the left ventricle, and selectively toward the left heart follows the direction of capillary perfusion away from the anterior descending and circumflex arteries, suggesting a role for the coronary circulation in determining local drug deposition and clearance. The dominant role of the coronary vasculature is further suggested by the elevated drug levels in the coronary sinus effluent. Indeed, plasma levels, hemodynamic responses, and myocardial deposition remote from the point of release were similar following local EC or IV delivery. Therefore, the coronary vasculature shapes the pharmacokinetics of local myocardial delivery of small catecholamine drugs in large animal models. Optimal design of epicardial drug delivery systems must consider the underlying bulk capillary perfusion currents within the tissue to deliver drug to tissue targets and may favor therapeutic molecules with better potential retention in myocardial tissue.

1. Introduction

Local controlled drug delivery provides pharmacologic therapy with elevated target tissue levels and minimal peripheral side effects [1–6]. Prior studies have demonstrated pharmacologic response following epicardial (EC) application of antiarrhythmic drugs [7–14], proarrhythmic agents [15,16], vasodilators [17–21], and antiproliferative chemotherapeutics [22]. Other studies have demonstrated favorable pharmacokinetics, with elevated myocardial drug levels and minimal peripheral concentration, when drug is given to the pericardial sac [23–28]. We have shown in small animals that local EC application of inotropic

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compounds leads to elevated myocardial drug and intracellular second messenger concentrations in target ventricular tissue, enhanced contractile response with lower systemic levels, and less atrial and peripheral vascular responses than IV infusion [29,30]. EC epinephrine delivery in rats required only 1/3rd of the IV dose rate to provide an equal contractile effect without raising plasma drug levels or deleterious systemic side effects such as tachycardia and peripheral vasodilation [30].

While these phenomena from small animals are promising and intriguing, they may not necessarily translate to larger species. Indeed, the mechanisms of drug transport within and clearance from the myocardium may be more complex in larger species. Local delivery of soluble drug relies on diffusion through interstitial spaces and thus distribution may be impacted by physical dimensions [4,31–34]. Furthermore, intramyocardial capillaries may clear drug from the heart [34]. Given these concerns, we sought to extend our findings from small rodents to larger animal models, where the thickness of the heart wall and vascular networks are similar to human cardiac anatomy, and we have the means to sample venous effluent from the heart to quantify the myocardial capillary contribution to systemic loading. We used an anesthetized, adolescent swine model to study the distribution of the drug in the myocardial tissue, its route of clearance from the myocardium, and subsequent systemic levels when delivered EC or by intravenous (IV) infusion. We hypothesized that the surface area of release might impact myocardial distribution and biological response. Therefore, epinephrine was delivered to the anterior wall of the heart of swine using either a point-source release, in the form of a small 1.5 cm^2 diameter polymeric disk, or a dispersed-source release, in the form of an adherent hydrogel covering the entire anterior wall of the left ventricle. Cardiovascular function was continuously monitored, epinephrine levels in cardiac venous effluent and arterial blood were measured, and after sacrifice and tissue harvest, drug distribution in the left ventricle was quantified.

2. Methods

2.1. Fabrication and characterization of epicardial point-source alginate drug delivery platform

An alginate polymeric local drug releasing system, designed to precisely administer animal-weight based amounts of epinephrine to the epicardium, was fabricated from calcium-cross-linked alginate hydrogels [29,30]. Briefly, 0.65 ml of 2% alginate (#71238; Sigma-Aldrich) slurry in double distilled water (ddH_20) was pipetted onto the upper side of the permeable membrane of a transwell support (#3472, 24 mm, polyester, 3 μm pore size; Corning). Immersion of the transwell support in 1.5 ml of 3% $CaCl_2$ in ddH_20 using a leveled culture plate (#353047, 15.75 mm; Corning) for 30 min at room temperature cross-linked the alginate to form a flexible solid concave disk (diameter 24 mm, minimal thickness [center] 2.0 mm, maximal thickness [perimeter] 3.0 mm, lower surface area 452 mm^2, volume 700 mm^3). Free Ca^{2+} in the alginate was removed by placing the disks in ddH_2O for 30 min.

A series of *in vitro* experiments quantified the epinephrine released over time as a function of the applied concentration. The alginate disk was placed in a new transwell support and immersed in a leveled 24 well culture plate filled with 1.5 ml of normal saline representing the released drug receiving chamber. The culture plate was placed on an orbital shaker at 20 RPM (#3520; Labline). Epinephrine (10 μl of 1, 2, 5, 10 or 20 mg/ml in ddH_2O, # 0409-4921-34; Hospira) was added every 10 min to the upper free concave surface to smooth the release at the lower surface to a steady rate. These experiments were repeated in triplicate. At regular intervals, a 60-μl sample from the receiving chamber was removed to evaluate the amount of released drug and 60 μl of normal saline was added immediately to the wells to restore the receiving chamber volume. The concentration of epinephrine in each sample was determined by spectrophotometric methods (Victor^3 Multilabel Counter, PerkinElmer) [35]. Metaperiodate (6 μl of 2% $NaIO_4$ in ddH_2O, #S1878, Sigma-Aldrich) and ethanol (9 μl, 100%) were added to the samples and the absorbance at 490 nm was measured to calculate the amount of released epinephrine at each time point using a standard curve. For each concentration of applied drug solution to the alginate disk, the release rate was determined using a linear least-squares correlation. Each release rate was then linearly correlated to the applied concentration. This relationship, specific to these disks at the fixed volume and interval of applied drug solution, allows the release rate to be prescribed solely through adjustments in applied concentration [29,30]. This novel method of controlling epicardial drug release allows for precise animal weight-based dosing without any chemical modifications to the polymer platform.

2.2. Fabrication and characterization of epicardial dispersed-source poloxamer based drug delivery platform

As an alternative to the point-source alginate disk delivery platform, a poloxamer hydrogel was used to distribute the source of drug release over the entire anterior wall of the LV. Epinephrine (1 mg/ml, # 0409-4921-34; Hospira) was mixed into a 30 w/v% solution of Poloxamer 407 (#16758; Sigma) in ddH_20 at 5 °C. The resulting solution (10, 50, 100, or 200 μg/ml) was poured into a precooled beaker and mixed overnight at 5 °C with a magnetic stir plate. Poloxamer 407 solutions remain free-flowing liquids at temperatures below 15 °C; above this temperature, viscosity increases forming a hydrogel that allows for controlled drug release.

In vitro release was characterized using precooled (5 °C) 12-well culture plates (#3513; Corning) coated with 760 μl of epinephrine–poloxamer solution (10, 50, 100, or 200 μg/ml) with an average thickness of 2 mm. The poloxamer was allowed to gel at 37 °C for 5 min before 300 μl of normal saline was added to each well and the plates placed on an orbital shaker set at 80 RPM. Samples (60 μl) were drawn only once from each well at either 5, 10, 15 and 30 minute time point and were pipetted into a 96-well plate (#3799; Corning) for quantification by spectrophotometric methods as above. Thus, 12 poloxamer hydrogels were needed at each starting epinephrine concentration for the release experiment to be repeated in triplicate.

2.3. Surgical procedures

All studies were approved by the Institutional Animal Care and Use Committee at Steward St Elizabeth's Medical Center, Boston, Massachusetts. Twelve adolescent Yorkshire swine (36–41 kg) were fasted for 12 h preceding the experiment, but had access to water.

The animals were sedated with an intramuscular (IM) injection of telazol 250 mg, ketamine 125 mg, and xylazine 125 mg and restrained supine on heating pads. The airway was secured with a 6.0 mm cuffed endotracheal tube and ventilated (10 breaths per minute, initial tidal volume 500 ml, Excel 110 anesthesia machine with 7000 series ventilator, Ohmeda) to maintain end-tidal carbon dioxide between 35 and 40 mm Hg (Poet-IQ gas monitor; Criticare Systems). Anesthesia was maintained with isoflurane 1–2% during catheterization. A femoral artery catheter (#CS-04300, Arrow) was placed by the Seldinger technique for continuous blood pressure monitoring and plasma sampling. A Pressure–Volume conductance catheter (Ventri-cath 507, Millar instruments) was passed retrograde into the left ventricle under fluoroscopic guidance from the contralateral femoral artery. A pulmonary artery catheter (#746HF8, Edwards Life Sciences) was placed from the right external jugular vein to measure core temperature and continuous thermodilution cardiac output (CO). Other acquired hemodynamic parameters were mean arterial pressure (MAP), central venous pressure (CVP), heart rate, and an index of contractility (max dP/dt). Systemic vascular resistance (SVR) was calculated as (SVR = (MAP − CVP) / CO). Following catheterization, the isoflurane based anesthetic was transitioned to total intravenous anesthesia with midazolam (0.25 mg/kg/h), fentanyl