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High concentrations of drug in target tissues following local controlled release are utilized for both drug distribution and biologic effect: An example with epicardial inotropic drug delivery

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ABSTRACT

Local drug delivery preferentially loads target tissues with a concentration gradient from the surface or point of release that tapers down to more distant sites. Drug that diffuses down this gradient must be in unbound form, but such drug can only elicit a biologic effect through receptor interactions. Drug excess loads tissues, increasing gradients and driving penetration, but with limited added biological response. We examined the hypothesis that local application reduces dramatically systemic circulating drug levels but leads to significantly higher tissue drug concentration than might be needed with systemic infusion in a rat model of local epicardial inotropic therapy. Epinephrine was infused systemically or released locally to the anterior wall of the heart using a novel polymeric platform that provides steady, sustained release over a range of precise doses. Epinephrine tissue concentration, upregulation of cAMP, and global left ventricular response were measured at equivalent doses and at doses equally effective in raising indices of contractility. The contractile stimulation by epinephrine was linked to drug tissue levels and commensurate cAMP upregulation for IV systemic infusion, but not with local epicardial delivery. Though cAMP was a powerful predictor of contractility with local application, tissue epinephrine levels were high and variable — only a small fraction of the deposited epinephrine was utilized in second messenger signaling and biologic effect. The remainder of deposited drug was likely used in diffusive transport and distribution. Systemic side effects were far more profound with IV infusion which, though it increased contractility, also induced tachycardia and loss of systemic vascular resistance, which were not seen with local application. Local epicardial inotropic delivery illustrates then a paradigm of how target tissues differentially handle and utilize drug compared to systemic infusion.

1. Introduction

Local controlled drug delivery provides pharmacologic therapy with elevated target tissue levels and minimal peripheral side effects [1–3]. There is, however, little direct relationship between applied dose and biologic response [4–7] as seen and expected from systemic infusion. While intravascular injection brings drug into a tissue through dense uniform capillary networks [8,9] providing uniform distribution [10], local application only applies drug to the target tissue surface. Locally applied drug must enter the tissue and diffuse through the target area down concentration gradients (Fig. 1) [4,10–13]. The very high supratherapeutic drug concentration near the point of release may lead to saturation of proximal binding sites or exceed their capacity for binding. The remaining unbound drug in a tissue participates in diffusive spread [14]. Supratherapeutic concentrations of drug near the release device ensure that a large fraction of the drug is unbound, and provides a driving gradient for trans-target distribution. This unbound fraction is not biologically active until drug diffuses to a region where receptors are unsaturated and available. Progression of effect, through longer periods of treatment or higher doses, therefore, depends on the geographic extent of therapeutic concentrations to the target tissue and the extent of saturated binding. While increasing administered systemic dose increases target organ concentrations and biologic effect as long as receptors are available (Fig. 1B), tissue capillaries remove locally released drug from a tissue [11,13] rather than deliver it. Thus, capillary clearance and enzymatic degradation limit the extent of tissue under pharmacologic control even with substantial increments of local drug release and, unlike systemic delivery, extra steps are required for drug to reach receptors suggesting a non-linear nature of the dose-response [4–6].

We examined the hypothesis that local application dramatically reduces systemic circulating drug levels, but leads to significantly higher

Abbreviations: EC, Epicardial; IV, Intravenous; Max dP/dt, Maximum rate of change of pressure; SVR, Systemic vascular resistance; HR, Heart rate; LVAW, Left ventricular anterior wall; LVIW, Left ventricular inferior wall.

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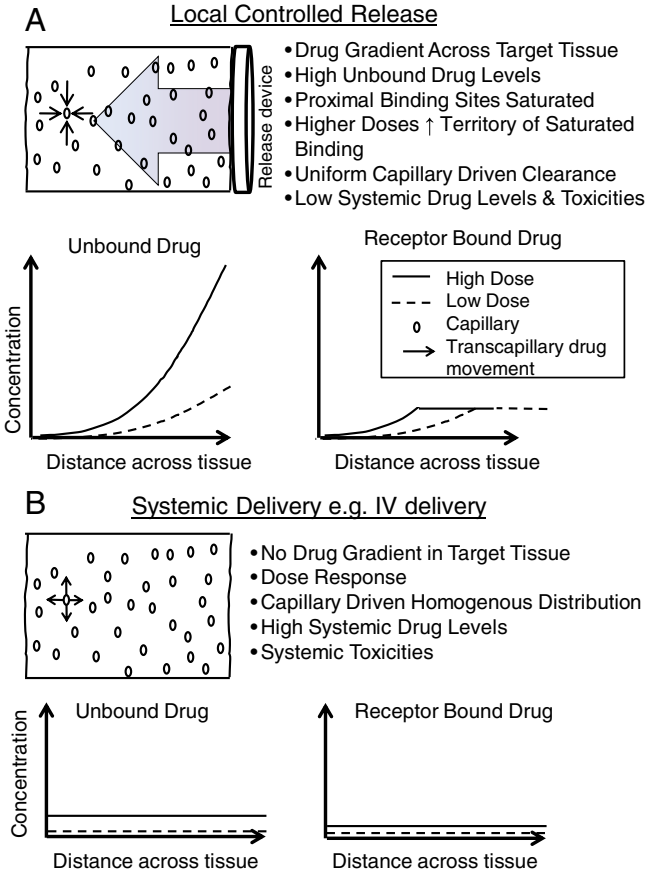

Fig. 1. A paradigm of drug distribution in local and systemic delivery. Panel A: Local drug delivery leads to a gradient in drug distribution across the target tissue. The unbound fraction may exceed by many fold the bound active drug fraction. Close to the release matrix where unbound concentrations are high, receptors are saturated and the bound phase uniform. Increased release rate may increase the extent of saturated binding which might increase biologic effect. Panel B: Systemic drug delivery provides homogenous target tissue drug distribution from capillary networks with low levels of unbound drug that are comparable to bound drug fractions. Higher doses lead to higher concentrations of drug in tissue and increased effect, provided receptors are not saturated.

tissue drug concentration than might be needed with systemic infusion when dosed to similar therapeutic endpoints. A biologic system including a drug with a cell surface receptor mediated function, detectable intracellular intermediates, and a functional organ-wide response was needed to test this hypothesis and provide underlying mechanisms. For these reasons, epinephrine, a drug with well described systemic pharmacokinetics [15], was administered to a focal region of the epicardial surface of the anterior wall of the rat heart in a preparation that measures indices of global left ventricular (LV) contractility in real time, and tissue levels of drug and cyclic adenosine monophosphate (cAMP) at the time of organ harvest. This intracellular intermediate was measured as a marker of the intracellular biological effect of epinephrine, as cAMP is upregulated by adrenergic receptor activation and increases calcium release into the cytosol of the myocyte leading to more forceful contractions [16,17]. These experiments required a novel, finely tunable polymeric platform that allows precise, steady animal weight based controlled release. Epicardial administration of epinephrine at a single weight based dose rate was compared to IV systemic infusion at an equivalent dose rate at which preliminary data suggested would provoke an equivalent global LV contractile response.

2. Methods

2.1. Fabrication and characterization of the epicardial drug delivery platform

A novel polymeric local epicardial (EC) drug releasing system designed to permit precise animal-weight based epinephrine release to the anterior wall of the beating rat heart was fabricated from calcium-cross-linked alginate hydrogels [18]. Briefly, 45 μl 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, 6.5 mm, polyester, 3 micrometers pore size; Corning). Immersion of the transwell support in 1 ml of 3% $CaCl_2$ in ddH_20 using a leveled 24 well culture plate (#353047, 15.75 mm; Corning) for 25 min at room temperature cross-linked the alginate to form a solid but flexible disk. Free Ca^{2+} in alginate disks was removed by placing the disks in ddH_2O for 60 min. The end product had the shape of a concave disk with diameter of 6.5 mm, with minimal thickness of 0.52 mm at the center and maximal thickness of 1.2 mm along the perimeter, lower surface area of 32 mm^2 and the volume of the concave of 30 mm^3. These devices were formed without embedded drug. When epinephrine is applied in solution to the upper free concave surface at regular intervals they serve to smooth the release at their lower surface to a linear rate and disperse drug over an area approximately the size of the disk.

A series of *in vitro* experiments quantified the epinephrine released over time as a function of the applied concentration to the upper surface of the concave disk (Fig. 2A). The formed alginate disk was placed in a new transwell support and immersed in a leveled 24 well culture plate filled with 320 microliters (μl) of ddH_2O representing the released drug receiving chamber. The culture plate was placed on an orbital shaker at 80 RPM (#3520; Labline). Epinephrine (Hospira) solution (10 μl of 0.05, 0.1, 0.25, 0.5, 0.75 or 1 μg/μl in ddH_2O) was added every 10 min onto the upper surface of the alginate disks. These experiments were repeated in triplicate. At regular intervals, a 60 μl sample from the receiving chamber was removed to evaluate the amount of the released drug and 60 μl of ddH_2O was added immediately to the wells to restore receiving chamber volume. The concentration of epinephrine in each sample was determined by spectrophotometric methods [19]. 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 (Fig. 2A). 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 (Fig. 2B). 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 [18]. This novel method of controlling epicardial drug release allows for precise animal-weight based dosing without any chemical modifications of the platform.

2.2. Surgical procedures

All studies were approved by the Institutional Animal Care and Use Committee at Steward St Elizabeth's Medical Center, Boston, Massachusetts, USA. Twenty four adult male Sprague–Dawley rats (375–475 grams) underwent identical surgical procedures but different routes of epinephrine administration. Four groups of animals ($N = 6$) were studied: 0, 0.1 and 0.3 μg/kg/min IV and 0.1 μg/kg/min epicardial (EC). All animals had an identical alginate disk applied to the anterior wall of the heart. Hemodynamic measurements were made prior to treatment and at steady-state. The hearts were harvested during steady state treatments and transmural tissue cores (6 mm, Miltex biopsy punch, VWR, #21909-144) from the inferior wall and the anterior wall