International Journal of Advanced Biotechnology and Research (IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-8, Issue-4, 2017, pp1936-1941
http://www.bipublication.com

Research Article

Use of colloid nanoparticles for myocardial delivery in ultrasound-targeted micro bubble destruction

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Source(s) of support in the form of grants, equipment, drugs, or all of the above: None.

ABSTRACT

Introduction: Ultra sound (US) targeted microbubble destruction (UTMD) is a encouraging method for delivering genetic material to the heart. The aim of this study wasto test whether colloid nanoparticles can be delivered to the rat myocardium using UTMD and to determine whether tissue damage and contractile dysfunction occurs in hearts exposed to UTMD in vivo conditions. **Material and Methods:**Hearts from anaesthetized rats were exposed to perfluorocarbon-enhanced sonicated dextrose albumin (PESDA) (at two different microbubble concentrations) and US at peak pressures of 0.6, 1.2, or 1.8 MPa for 1, 3, or 9 min. During US, pairs of 30 and 100 nm fluorescent nanospheres were infused intravenously.Rats exposed to PESDA alone or US alone showed no functional abnormalities, no capillary ruptures, and no nanosphere delivery.**Results:** The data are expressed with the mean values and mean+1 standard error means (SEM). The differences in nano particulate delivery, premature ventricular contraction (PVCs), and vascular rupture between groups was assessed using a two way analysis of variance (ANOVA), examining the effect of two fixed factors. **Conclusion:**UTMD allows for colloid nanoparticles to be delivered to the rat myocardium through micro vessel rupture sites. The efficiency of ultra sound medicines supported local delivery depends on the different things which are applied peak pressure, the time duration of ultra sound exposure, and contrastconcentration.

Key words: Ultrasound, Diseases, UTMD

INTRODUCTION

In addition the well-established application of contrast echocardiography for left ventricular opacification and endocardial border augmentation in patients with suboptimal images both at rest condition and during stress are the useful tool for the assessment of myocardial perfusion (Mulvagh et al., 2000). There are two possible strategies used for delivering drugs and genes with microbubbles are emerging. The first consists of ultrasound-mediated micro bubble destruction, which is based on the cavitation of microbubbles induced by ultrasound application, and the second is the direct delivery of substances bound to microbubbles which target delivery to sites of endothelial dysfunction in the absence of ultrasound. Different drugs and genes can be incorporated onto the surface of ultrasound contrast agents. It has already been demonstrated

that per fluorocarbon filled albumin microbubbles avidly bind proteins and synthetic oligonucleotides (Miller, 2000). In a similar way, microbubbles can directly take up genetic material, such as plasmids and adenovirus (Miller, 2000), and phospholipid-coated microbubbles may have a high affinity for chemotherapeutic drugs(Fritz et al., 1997). The mechanisms by which ultrasound facilitates the delivery of drugs and genes result from a complex interplay among the therapeutic agent the microbubble characteristics, target tissue, the endothelium, and the nature of ultrasound energy. The existence of microbubbles in the insonified field decreases the peak negative pressure required to enhance the drug delivery with ultrasound. This occurs because the microbubbles act as nuclei for cavitation and decreasing the threshold of ultrasound which is energy necessary to cause this phenomenon.Another important therapeutic property of microbubblesis their increased adherence to damaged vascular endothelium.The microbubbles which are coated with albumindo not adhere to normally functioning endothelium, but their adherence to activated endothelial cells or to extra-cellular matrix of the disrupted vascular wall does occur (Villanueva et al., 1997).Because of this characteristic the delivery of genes or drugs bound to albumin-coated microbubbles could be selectively concentrated at the site of vascular injury in the presence (Porter et al., 2001) or absence of ultrasound application (Porter et al., 2003).In 1996, the first published report of targeted DNA delivery was reported using surface ultrasound and intravenously delivered microbubbles carrying antisense oligonucleotides (Porter et al., 1996).

The use of ultrasound (US)-targeted microbubble destruction (UTMD) as a tool to deliver drugs or genetic material to the heart holds considerable promise (Dijkmans et al., 2004: Price et al., 1998).Today ultrasound remains a highly desirable methodology for diagnostic imaging primarily due to its portability, and offers advantages over other imaging modalities because of its real-time, non-ionizing, high frame-rate imaging. Furthermore, ultrasound remains a low-cost modality that continues to decrease in system price with advances in manufacturing technology (Gessner, 2010). While advantageous in many respects, ultrasound currently lags behind other modalities with regard tothe ability to use contrast agents for interrogation of the interstitial space and the physiologic processes occurring there. In PET/SPECT/MRI, diagnosing complex extravascular diseases can be approached with radionuclides or paramagnetic contrast agents (Kircher et al., 2012).

Several groups have been developing drug loaded UCAs that can release drug when triggered by ultrasound at the desired target. Doxorubicin (Dox) has been loaded onto the surface of phospholipid microbubbles through electrostatic interactions (Tinkov et al., 2010) and into stabilized micelles (Husseini et al., 2007).

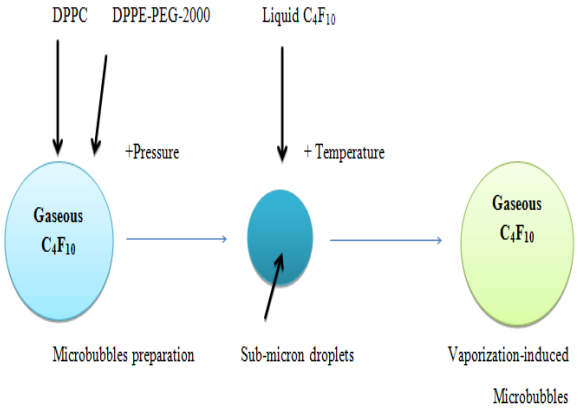

Fig 01: Diagram for making phase-change nanoparticles of submicron size distribution using pressurization and slow cooling to condense the gas inside the microbubbles.

Objectives of the study

The aim of this study wasto test whether colloid nanoparticles can be delivered to the rat myocardium using UTMD and to determine whether tissue damage and contractile dysfunction occurs in hearts exposed to UTMD in vivo conditions

MATERIAL AND METHODS

This study was ethically approved by the institution.

Preparation of Rats

Male albino rats weighted 300–400g average was selected for this study. They anaesthetized with sodium pentobarbital (60 mg per kg of the body weight). Femoral veins and the left femoral artery of rats were cannulated, to allow fluorescent nanosphere and microbubbles infusion along with the monitoring of arterial pressure.

Experimental Design

Electrodes were attached onto each leg of the rats to allow Echocardiography (ECG) triggered ultra sound emission and it record a six marginal lead ECG. Echocardiography was performed in intermittent mode (1 Hz) with a Sonos 5500 system equipped with a broadband S3 transducer that has a transmit frequency of 1.3 MHz, a bandwidth of about 25% focused at 1.3 MHz, and a greatest peak pressure of this transducer is 1.8 MPa. The transducer was functioned at a depth of 4 cm. Each frame contained 110 lines delivered over a period of 50 ms and forms a 908 sector. Each line was fired as a single eruption of ultra soundwith four cycles over 3ms. The tip of the transducer was positioned on the wall of the chest to obtain a view of shortaxis, at the level of the papillary muscles. This position was sustainedthroughout the experiments. Mechanical index (MI) was used for the calculation of peak acoustic pressure which is appeared on the system.

Preparation of microbubbles

Perfluorocarbon improved sonicated dextrose albumin (PESDA) which consist of deca-fluorobutane filled albumin microbubble whose mean diameter is 4.2±0.5 mm and a mean concentration is 0.8×10^{9}mL21 was used in this study (Porter et al., 1995). A slow bolus of 200mL of PESDA was suffusedintravenously for 30 seconds for every 3 min.

Preparation of Fluorescent Nano spheres

The efficacy of particulate delivery and the relationship between particulate delivery and ventricular function were assessed by continuously infusing a solution containing a mixture of 30 nm green-fluorescent and 100 nm blue or red fluorescent nanospheresinto one of the femoral vein catheters. Infusion rate was set at 60uL min^{-1}.

Experimental Analysis

The protocol was designed to assess the consequences of UTMD in the heartin the separate experiments. There are three phases which are as follows:

1. Instant
2. Sub-acute (24 h)
3. Long-term (7 days)

The effect of ultra sound peak pressure and the time duration of ultra sound exposure on micro vascular integrity and delivery of nano particles were examined in all groups of rats. The heart of rats were randomly exposed to the different peak pressures which are 0.6 MPa for 1 min, 1.2MPa for 3 min and 1.8 MPa for 9 min.

Two control groups were used for the study of this experiment, which are as:

1. Rats exposed to ultra sound (US) in the absence of PSEDA
2. Rats exposed to PSEDA in the absence of ultra sound (US)

Statistical analysis

The data are expressed with the mean values and mean+1 standard error means (SEM). The differences in nano particulate delivery, premature ventricular contraction (PVCs), and vascular rupture between groups was assessed using a two way analysis of variance (ANOVA), examining the effect of two fixed factors.

RESULTS

Effects of the duration of US exposure on delivery of nano particles

According to Figure 3 theeffect of the duration of US exposure on nanosphere delivery to the anterior wall of the three hearts exposed to a peak pressure of 1.8 MPa for 1.0min, 3.0min , and 9.0 min, respectively. The extent of nano particlesdelivery gradually increased with the duration of US exposure (to 0.37+0.09, 1.28+0.33, and 4.8+0.8% in hearts). These results explain on the basis of ANOVA.

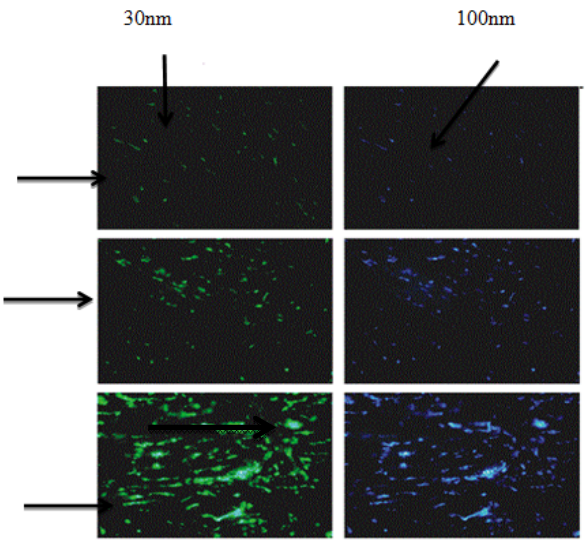

Figure 2: Fluorescent micrographs illustrating the deposition of the 30 nm green- (left) and 100 nm blue- (right) fluorescent nanospheres in hearts exposed to a peak pressure of 1.8 MPa for 1, 3, or 9 min

Effect of micro-bubble concentration on delivery of nano particles

Two groups of rats were selected for this study who received PSEDA in the diluted form or PSEDA un-diluted form. The delivery of nano particles in those hearts was low which received diluted PSEDA as compared to un-diluted PSEDA. In addition, two of the five hearts exposed to diluted PESDA which exhibited no nano sphere particles whereas the remaining three hearts showed mild to diffident nanosphere deposits that were confined to the sub-epicardial layer of the anterior wall.

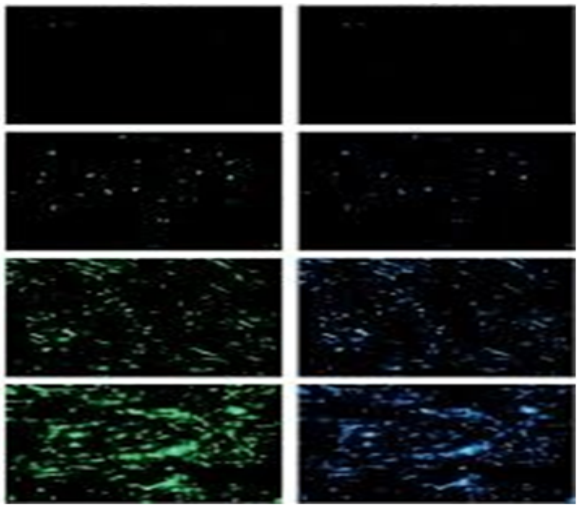

Figure 3: Fluorescent micrographs illustrating the deposition of the 30 nm green- (left) and 100 nm blue (right) fluorescent nanospheres in hearts exposed for 9 min to PESDA alone, in the absence of US or to both PESDA and US at a peak pressure of 0.6, 1.2, or 1.8 MPa.

DISCUSSION

The main aim of this study was to investigate the role of ultra sound medicines and role of micro bubbles in the delivery of colloid nano particles. Our results specify that effective myocardial delivery of these nanoparticles can be really achieved using this approach provided that the hearts are being visible for a persistent period of time to both high ultra sound peak pressure and a high concentration of microbubbles. However, thedata also indicate that UTMD induces significant bio-effects, which include transient tissue damage and micro vascular ruptures.

Skyba et al were among the first to observe microvessel ruptures in a solid organ visible in vivo to contrast microbubbles and ultra sound. Afterwards, we made very similar observations in the ex vivo setting of an isolated perfused rat heart preparation (Skyba et al., 1998).The present study confirms and these previous results by demonstrating that, in vivo as well, the simultaneous exposure of rat hearts to US and contrastmicrobubbles causes microvessel ruptures and erythrocyte extravasation.

Recently, ultrasound-targeted micro bubble devastation has been shown to be a technique that may achieve organ specific and non-invasive gene delivery with non-viral vectors. The presence of microbubbles can lower the ultrasound power can facilitate delivery of drugs or genes to a variety of tissues such as muscles, (Lu et al., 2003) lungs, (Xenariou et al., 2007) vasculature, (Akowuah et al., 2005) and tumors. Ultrasound targeted microbubble destruction has also been shown to produce cardiomyocyte injuryand to transiently impair left ventricular function (Vancraeynest et al., 2006).

Although this technology was very effective but this work had also some restrictions. The parameters for this technique, including the ultrasound acquaintance parameters, ultrasound frequency, method of ultrasound, and amount of

plasmid DNA, should be optimized for clinical use in the future. Because the advantage of this was a much less aggressive approach to cardiac gene therapy, we did not compare this technique with other invasive methods of gene delivery as in another study,such as more efficient direct left ventricular injection.

CONCLUSION

UTMD allows for colloid nanoparticles to be delivered to the rat myocardium through micro vessel rupture sites. The efficiency of ultra sound medicines supported local delivery depends on the different things which are applied peak pressure, the time duration of ultra sound exposure, and contrastconcentration.

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