Local Interstitial Delivery of Estradiol by Micellar Drug Release for Cardioprotection in vitro

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ABSTRACT SUMMARY
An amphiphilic hydrophilic-hydrophobic block copolymers of methoxypoly (ethylene glycol)-co-poly (valerolactone-co-lactic) (mPEG-PVLA) were synthesized by the ring-opening polymerization of methoxy poly (ethylene glycol), α-Valerolactone, and D.L-lactide. This material showed a low critical micelle concentration and small particle size. The purpose of this study is to characterize a novel 17β-estradiol (E2) drug delivery carrier for anti-cardiomyocyte apoptosis. The release rate of E2 entrapped in mPEG-PVLA micelle was examined in vitro. Release profiles of the E2 showed that there was diffusional release. E2 micelle inhibited H9c2 cardiac myoblast apoptosis caused by ischemia/reperfusion injury. The non-cytotoxicity, biodegradability and biocompatibility of mPEG-PVLA appear to be a promising estradiol delivery carrier.
Keywords: micelle, in vitro drug release, anti-apoptosis

INTRODUCTION
Although early reperfusion of the ischemic myocardium is important for preservation of tissue viability, it’s apparent that reperfusion may in itself be harmful to the surrounding tissue [1]. Thus, a paradoxical situation develops where reoxygenation, which is essential for survival of the tissue, may in fact be harmful. Damage due to the restoration of blood flow is termed “reperfusion injury.”

Until menopause, age-dependent increases in cardiovascular disease are less pronounced in women than in men. The corresponding decrease in estrogen levels and increase in cardiovascular disease associated with menopause has generated interest in the actions of estradiol (E2) on the cardiovascular system. Administration of estrogen is reported to be protective in animal models of myocardial ischemia and reperfusion, atherosclerosis, and arrhythmia [2]. However, adverse effects such as depression, breast tenderness, headaches, and nausea are associated with both oral and transdermal E2 formulations [3].

Biocompatible polymers have been used to deliver 17β-estradiol. Poly (lactide-co-glycolide) (PLGA) micro-spheres have been used to load and release E2 [4]. However, the large sizes of microspheres are not ideal for avoiding the body’s defense mechanisms, i.e., the reticuloendothelial system (RES).

Block copolymers made from polycaprolactone and poly lactide have been used to create microspheres [5]. Block copolymers have also been used in the preparation of micellar drug delivery systems. Amphiphilic self-assembled systems are attractive drug delivery vehicles, mostly due to their size, stability, versatility, and biocompatibility. In this study, methoxy poly(ethylene glycol)-co-poly (valerolactone-co-lactic) (mPEG-PVLA) copolymer micelles have been explored as a drug delivery carrier.

EXPERIMENTAL METHODS
Biodegradable micelle of mPEG-PVLA were synthesized by the ring-opening polymerization of monomers and mPEG in the of presence stannous 2-ethylhexanoate. A typical synthetic procedure was shown in figure 1. The chemical structures were identified by 1H NMR. Physic-chemical characterization was evaluated, including the critical micelle concentration (CMC), particle size of micelle. The morphology of micelles was determined by transmission electron microscopy (TEM) and scanning electron microscope (SEM).

The loading efficiency of E2 into the micelles was determined by high-performance liquid chromatography (HPLC). The solution of the micelles with the incorporated E2 used for the release experiment were placed into dialysis chambers (MWCO: 1000 g/mol), PBS was then added to each dialysis chamber at 37 °C. At various time points, a sample was measured by HPLC.

RESULTS AND DISCUSSION
The chemical structures were identified by 1H NMR (Figure 2). The CMC and particle size were measured by pyrene and Dynamic Light scattering methods. The CMC is 3.64×10^3 mg/ml and average particle size of micelles was about 200 nm. The loading efficiency and drug content was shown in table 1. The morphology of micelles was determined by TEM and SEM and the results are shown in Figure 3. There was change of size in E2 drug loading.

![Figure 1 A typical synthetic procedure of mPEG-PVLA](image)

<table>
<thead>
<tr>
<th>weight ratio of drug/polymer (w/w)</th>
<th>0.5</th>
<th>0.2</th>
<th>0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loading efficiency (%)</td>
<td>91</td>
<td>68</td>
<td>15</td>
</tr>
<tr>
<td>Drug content % (w/w)</td>
<td>45</td>
<td>14</td>
<td>1.5</td>
</tr>
<tr>
<td>Table 1 The loading efficiency and drug content</td>
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The E2 release profiles were measured by HPLC. E2 from mPEG-PVLA micelles with different E2/polymer weight ratio is released at different rates (Figure 4). The linearity of the plot is indicative of a diffusional release. The bioactivity of E2 micelles were measured by MTT (Figure 5) and LHD analysis (Figure 6), both of results indicative E2 micelles protected H9c2 cell under hypoxia/reperfusion injury model. The
anti-apoptosis capability of E2 micelles were measured by JC-1 staining. In healthy cells, JC-1 forms J-aggregates which display strong fluorescent intensity. In apoptotic or unhealthy cells, JC-1 exists as monomers which show strong fluorescence intensity (Figure 7). The ratio of fluorescent intensity of monomers to fluorescent intensity of J-aggregates can be used as an indicator of cell apoptosis (Figure 8). E2 micelles inhibited H9c2 cardiac myoblast apoptosis caused by hypoxia/reperfusion injury mold.

Figure 2 Chemical structures identify by ¹NMR

Figure 3 (a) TEM of micelles. (b) TEM of E2-micelles. (c) SEM of micelles. (d) SEM of E2-micelles.

Figure 4 E2 release profiles

Figure 5 Results of MTT assay. H9c2 cell were treated with 1 ppm of E2, 10 ppm of polymers. The weight ratio of E2 micelle was 0.1

Figure 6 Results of LDH assay. H9c2 cell were treated with 1 ppm of E2, 10 ppm of polymers. The weight ratio of E2 micelle was 0.1

Figure 7 JC-1 staining results. In healthy cells, JC-1 forms J-aggregates which display strong fluorescent intensity. In apoptotic cells, JC-1 exists as monomers which show strong fluorescence intensity. H9c2 were treated with 1 ppm of E2, 10 ppm of polymers. The weight ratio of E2 micelle was 0.1

Figure 8 Results of JC-1 staining assay. H9c2 cell were treated with 1 ppm of E2, 10 ppm of polymers. The weight ratio of E2 micelle was 0.1

CONCLUSION
The biological activity of E2 in vitro was retained after the preparation of the micelles. The incorporation and release of 17β-estradiol from mPEG-PVLA micelles were investigated as a potential drug delivery system.

REFERENCES