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Etomidate-loaded micelles for short-acting general anesthesia: Preparation, characterizations, and *in vivo* studies

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ABSTRACT

Etomidate is one of the most commonly used intravenous (*i.v.*) general anesthetics with rapid onset, minimal impact on breathing and excellent hemodynamic stability. But its application is usually coupled with several disadvantages including inadequate water-solubility for injection and adrenal toxicity in particular. Selective delivery of etomidate to brain via some water-soluble nanocarriers provides a potential way to settle these problems. In this study, etomidate-loaded poloxamer micelles (Eto-SOPM) were prepared using a thin-film hydration method. Soybean oil (SO) was used to enhance the encapsulation of etomidate, which resulted in acceptable drug concentration (2 mg/mL) for injection. The drug release of Eto-SOPM was not significantly hindered by encapsulation or the addition of soybean oil, and was faster than that of the commercially available etomidate fat emulsion ($P < 0.001$). The *in vivo* study revealed that the drug potency of etomidate was not significantly affected by encapsulation, and that Eto-SOPM had notably shortened action period as compared with etomidate fat emulsion ($P < 0.001$). As rapid onset and short action period are two essential requirements of general anesthetics, these results suggested that Eto-SOPM represents a promising etomidate preparation for short-acting general anesthesia and warrants more study in the future.

1. Introduction

Etomidate, an imidazole derivative, is one of the most commonly used *i.v.* general anesthetics for surgical operations. The anesthetic effect of etomidate is realized through its subunit-dependent interaction with γ-aminobutyric acid type A ($GABA_A$) receptors in the brain [1]. It is highly favored for its rapid onset, minimal impact on breathing and excellent hemodynamic stability, which make it a promising anesthetic agent for patients who are critically ill or have cardiovascular diseases [2].

However, etomidate has some disadvantages that have restricted its clinical applications. The poor water-solubility of etomidate makes it unsuitable for direct *i.v.* injections. In addition, etomidate can intensively bind to 11β-hydroxylase, significantly hindering the synthesis of adrenocortical steroids that play important roles in the regulation of immune function and homeostasis, etc [3,4]. The adverse effects of etomidate exhibited in its clinical application have raised great concerns. Even a single bolus injection of etomidate for anesthetic induction has become controversial, since it can suppress adrenocortical function for more than one day and may increase mortality in the critically ill [3]. Therefore, prolonged continuous infusion of etomidate to maintain anesthesia has long been precluded to prevent severe adverse effects and to improve survival. Compared with other general anesthetics, it is more used for anesthetic induction, through the administration of a single bolus dose.

So far, some etomidate preparations such as Etomidate-Lipuro® (B-Braun, Germany), Forry™ (Nhwa, China), Amidate® (Abbott Laboratories, USA), and Hypnomidate® (Janssen-Cilag, UK), etc., have been developed for acceptable drug concentrations (typically 2 mg/mL) by either forming oil-in-water (o/w) fat emulsions or using propylene glycol as a cosolvent. However, propylene glycol was reported to cause obvious adverse effects, such as pain on injection and lactic acidosis [5]. Additionally, in our previous investigation, some etomidate fat emulsions were found to have sustained drug release and prolonged drug effects which go against the essential requirements of central nervous system (CNS) drugs, namely rapid onset and short action period for safety concerns. More importantly, very few of the existing formulations have taken into account the adverse effects brought about by the drug itself. The inhibition of adrenocortical steroids synthesis remains a large obstacle to expanding the clinical application of

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etomidate. Therefore, it is a need to develop novel formulations to minimize the adverse effects of etomidate and avoid the defects of the existing formulations.

Lately, formulations based on hydrophilic nanocarriers such as liposomes and polymeric micelles have been actively pursued to improve the water-solubility of hydrophobic drugs, achieve selective drug biodistributions, and control drug release, thus to enhance the efficacy and reduce the side effects of drugs [6,7]. Some targeted nanocarriers were also used to prepare fluorescent nano-probes for *in vivo* imaging [8]. Therefore, encapsulation of etomidate using proper nanocarriers could be a promising way to solve the problems of this drug. So far however, very few of the nanocarriers can well satisfy the requirements of general anesthetics, since hydrophilic formulations often meet with problems in penetrating blood-brain barrier (BBB) due to their hydrophilicity and unsuitable sizes [9]. In addition, the interactions between the loading materials and drugs, including hydrophobic interactions, electrostatic interactions, hydrogen bonding, etc., can hinder the escape of payload from the nanocarriers, thus slowing down the release of drugs. Generally, an ideal nanocarrier for etomidate should not only solubilize the drug, but also have good biocompatibility, be able to facilitate etomidate across the BBB, and can release the drug at a proper rate.

During our previous investigations, it was found that micelles formed by poloxamer (Pluronic®) could be good candidates for the delivery of etomidate. Poloxamer-based nanocarriers have long been used for the solubilization and targeted delivery of various anticancer drugs [10,11]. In recent years, the trials using different kinds of poloxamers for the delivery of CNS drugs have produced wonderful results [12–16]. It was reported that, poloxamers are able to inhibit the drug efflux transporters such as P-glycoprotein (P-gp) and multidrug-resistant proteins (MRPs) in the brain [15,17]. Therefore, the encapsulation of etomidate using poloxamer micelles can be a good way to enhance drug delivery to brain, which may consequently alleviate the side effects of this drug.

In the present study, we prepared etomidate-loaded poloxamer micelle, Eto-SOPM, using a thin-film hydration method. Eto-SOPM was characterized as regard to its size distribution, zeta potential, drug entrapment efficiency, and drug release profile. Finally, the *in vivo* anesthetic effects were tested in rat.

2. Materials and methods

2.1. Materials and animals

Poloxamer 338 (Pluronic® F108, average Mn 14600 Da), etomidate (crystals, purity > 98%), and SO were purchased from Aladdin Industrial Co., Ltd. (Shanghai, China). Etomidate fat emulsion was purchased from Nhwa Pharmaceutical Co., Ltd. (Jiangsu, China). All other chemicals were of analytical grades. The Sprague-Dawley (SD) rats were from the Laboratory Animal Center, Southwest Medical University (Luzhou City, China).

2.2. Preparation of Eto-SOPM

Eto-SOPM was prepared using thin-film hydration method, as shown in Fig. 1. 10 mg etomidate, 120 mg poloxamer, and 0.5 mg SO were well codissolved in 2 mL chloroform. The organic solvent was then removed using a rotary evaporator (YRE-2000, Yuhua Instrument, China) to form a thin film of mixture. The film was further dried under high vacuum for 1 h to remove residual solvent. Eto-SOPM was formed by adding 5 mL phosphate-buffered solution (PBS, pH 7.3) to hydrate the film followed by ultrasonic homogenization for 10 min using a probe sonicator (Scientz-IID, Ningbo Scientz Biotechnology, China) with a power of 90 W. Drug-loaded micelles without SO (Eto-PM) were similarly prepared without the addition of SO. Blank poloxamer micelles (SOPM) were similarly prepared without the addition of drug.

2.3. Characterizations of Eto-SOPM

The particle size distribution and zeta potential of Eto-SOPM were measured using Dynamic Light Scattering (DLS) and electrophoretic light scattering (ELS), respectively. Sample solutions were diluted to reach a suitable concentration, followed by the measurement on a Malvern Zetasizer (Nano ZS, Malvern Instruments, UK). Each sample was measured for three times under the same conditions. For stability study, Eto-SOPM samples were stored at room temperature for 3 months, and the size distribution and zeta potential of Eto-SOPM were measured.

The morphology of Eto-SOPM was observed using a transmission electron microscope (TEM) (H-600, Hitachi, Japan). Sample solution was properly diluted and was placed on an ultra-thin carbon film-coated copper grid, and was negative-stained using phosphotungstic acid. After dried at room temperature, the copper grid was put onto the sample holder and fixed, followed by the visualization of Eto-SOPM.

The percentage of drug loading capacity (DLC%) and drug entrapment efficiency (EE%) of Eto-SOPM was determined using a filtration method. 4 mL sample solution was filtered using a ultra centrifugal filter unit (MWCO 3000 Da, Amicon®, Merck KGaA, Germany). The filtration was stopped when 0.2 mL filtrate was obtained. The filtrate was properly diluted, followed by photometric determination of free etomidate concentration at 243 nm wavelength using a UV-vis spectrophotometer (A390, Shanghai Onlab Instruments, China). The DLC% and EE% of Eto-SOPM were calculated as follows: DLC% = (weight of loaded etomidate ÷ weight of Eto-SOPM) × 100%; EE% = (weight of loaded etomidate ÷ weight of input etomidate) × 100%.

2.4. In vitro drug release profile of Eto-SOPM

The *in vitro* drug release profiles were investigated through dynamic dialysis method as previously reported [18]. The dialysis was performed under sink conditions at 37 ± 0.5 °C. Briefly, sample solution of Eto-SOPM, Eto-PM, etomidate fat emulsion, or free etomidate dissolved in PBS (pH 7.3) was transferred into a dialysis membrane (MWCO 2500 Da), and was dialyzed against 400 mL PBS. Each sample solution contained 10 mg etomidate, with initial drug concentration of 2 mg/mL for Eto-SOPM, Eto-PM, and etomidate fat emulsion, respectively, and 0.4 mg/mL for PBS-dissolved etomidate. At predetermined intervals, the accumulative amount of released etomidate was determined using a UV-vis spectrophotometer and the calibration curve of etomidate obtained at 243 nm wavelength.

2.5. In vivo anesthetic effect of Eto-SOPM

The protocol of the study was approved by the Ethics Committee at the First Affiliated Hospital of Chongqing Medical University (approval number: 2017-068). The European Community guidelines as accepted principles for the use of experimental animals, were adhered to. The median effective doses (ED_{50}) for etomidate-induced loss of righting reflex (RR) were estimated using an 'up and down' method for small samples, with an effective sampling size (N) of at least 6 [19,20]. Adult male SD rats (200–300 g) were injected with an initial dose of Eto-SOPM, commercially available etomidate fat emulsion or free etomidate dissolved in PBS (all doses were calculated based on etomidate), and were placed in their supine position. The loss of RR is defined as failing to right themselves onto all four paws. If the rat did not have loss of RR, the next rat was given an increased dose (by a 0.1 log interval). If the rat showed loss of RR, the next rat was given a decreased dose (by a 0.1 log interval). Each rat was used for only one injection. The test was terminated when the 'nominal' (evaluable) sample size reached six (n = 6). In this study, the actually used etomidate doses for the test were 0.50, 0.63, 0.79, and 1.00 mg/kg. The tested dose values and the consequence of events (success or failure to induce loss of RR) were then used for the estimation of ED_{50}, according to the method