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Toxicity evaluation of methoxy poly(ethylene oxide)-*block*-poly(ε-caprolactone) polymeric micelles following multiple oral and intraperitoneal administration to rats

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ABSTRACT

Methoxy poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL) copolymers are amphiphilic and biodegradable copolymers designed to deliver a variety of drugs and diagnostic agents. The aim of this study was to synthesize PEO-*b*-PCL block copolymers and assess the toxic effects of drug-free PEO-*b*-PCL micelles after multiple-dose administrations via oral or intraperitoneal (ip) administration in rats. Assembly of block copolymers was achieved by co-solvent evaporation method. To investigate the toxicity profile of PEO-*b*-PCL micelles, sixty animals were divided into two major groups: The first group received PEO-*b*-PCL micelles (100 mg/kg) by oral gavage daily for seven days, while the other group received the same dose of micelles by ip injections daily for seven days. Twenty-four hours following the last dose, half of the animals from each group were sacrificed and blood and organs (lung, liver, kidneys, heart and spleen) were collected. Remaining animals were observed for further 14 days and was sacrificed at the end of the third week, and blood and organs were collected. None of the polymeric micelles administered caused any significant effects on relative organ weight, animal body weight, leucocytes count, % lymphocytes, liver and kidney toxicity markers and organs histology. Although the dose of copolymers used in this study is much higher than those used for drug delivery, it did not cause any significant toxic effects in rats. Histological examination of all the organs confirmed the nontoxic nature of the micelles.

1. Introduction

Nanomedicine is an emerging field with a great potential to improve the diagnosis and treatment of human diseases (Li et al., 2015; Weissig and Guzman-Villanueva, 2015). Numerous types of nano delivery systems are being developed for this purpose; such as polymeric nanoparticles, polymeric micelles, polymersomes, liposomes, and dendrimers (Bozzuto and Molinari, 2015; Koudelka et al., 2015; Lukowiak et al., 2015; Mahmud et al., 2007). Preclinical studies have shown the positive impact of the nanocarriers on the payloads. Problems such as aqueous solubility, limited oral absorption or bioavailability, poor pharmacokinetic profile, intolerable toxicity were solved by applying nano delivery systems (Farokhzad and Langer, 2009; Haley and Frenkel, 2008).

Methoxy poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL) copolymer (Fig. 1) is among the extensively explored block copolymers that are used for drug delivery applications (Aliabadi et al., 2005a). PEO/PCL copolymers are amphiphilic and biodegradable, and have been shown to form micelles, polymersomes, polymeric nanoparticles, and thermoresponsive and pH-sensitive gels (Aliabadi and Lavasanifar, 2006). Moreover, several drug-loaded PEO-*b*-PCL nanocarriers were investigated both *in vitro* and *in vivo* (Wei et al., 2009). The majority of the reported studies showed that PEO-*b*-PCL nanocarriers were able

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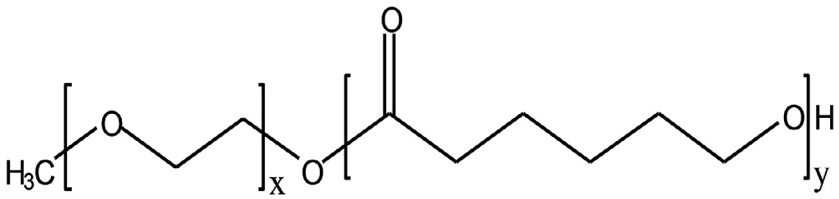

Fig. 1. Chemical structure of methoxy poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL) (x = 114; y = 30–200).

to significantly enhance the solubility of the loaded drug and favorably modify its pharmacokinetic/biodistribution profile (Aliabadi et al., 2005a; Binkhathlan et al., 2010; Xiong et al., 2008). Nonetheless, there is only a limited number of toxicity studies on drug-free PEO-*b*-PCL copolymers/nanocarriers.

The aim of the current study was to synthesize PEO-*b*-PCL block copolymers with different molecular weights and assess the probable toxic effects, following oral and intraperitoneal (ip) administration of drug-free PEO-*b*-PCL micelles after multiple-dose treatments in rats. For that purpose, PEO-*b*-PCL block copolymers with four different molecular weights of PCL were synthesized and made into micelles. The influence of all four different polymeric micelles on blood and organs (liver, kidney, heart and spleen) of the rats was assessed. Toxicological evaluations were incorporated to confirm the candidacy of these four different micelles, especially through the oral route, as safe nanocarriers.

2. Materials and methods

2.1. Chemicals and reagents

Methoxy PEO (M_n 5,000), stannous octoate (~95%), ε-caprolactone (97%), and THF (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Kits for estimation of aspartate aminotransferase, gamma glutamyl transpeptidase, creatinine, and blood urea were purchased from Giesse Diagnostics, Italy. Deionized water was prepared in-house using Millipore system. All chemicals used were of highest purity grade including sodium chloride, potassium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate.

2.2. Methods

2.2.1. Synthesis and characterization of PEO-b-PCL block copolymers

PEO-*b*-PCL block copolymers were synthesized by ring opening polymerization of ε-caprolactone using methoxy PEO (M_n 5000) as an initiator and stannous octoate as a catalyst, as previously reported (Aliabadi et al., 2005b; Binkhathlan et al., 2010). Briefly, methoxy PEO, ε-caprolactone and stannous octoate were added to a previously flamed ampoule, nitrogen purged, then sealed under vacuum. The reaction proceeded at 140 °C for 4 h. Different ε-caprolactone to methoxy PEO feed ratios were used to synthesize PEO-*b*-PCL block copolymers with varying degrees of ε-caprolactone polymerization. ^{1}H NMR spectrum of PEO-*b*-PCL in $CDCl_3$ at 500 MHz (Bruker Ultra shield 500.133 MHz spectrometer) was used to determine the number average molecular weight of the block copolymers. The degree of polymerization of ε-caprolactone was estimated by comparing the peak intensity of PEO (—O—CH_2—CH_2; δ = 3.65 ppm) to that of PCL (—O—CH_2; δ = 4.075 ppm). The number-averaged molecular weights, weight-averaged molecular weights and molecular weight distributions of the synthesized copolymers were determined by gel permeation chromatography (Viscotek TDA 305-040 Triple Detector Array, Viscotek Corp., Houston, TX, USA). Samples (100 μL from 15 mg/mL polymer stock solutions in THF) were injected into an 8.0 × 300 mm Viscotek T6000 M column (Viscotek Corp., Houston, TX, USA) with guard column. The mobile phase (THF) was delivered at a flow rate of 1 ml/min. The calibration curve was established with six polystyrene standards (molecular weight range: 1570–46,500).

2.2.2. Preparation and characterization of PEO-b-PCL micelles

Assembly of block copolymers was achieved by co-solvent evaporation where PEO-*b*-PCL (30 mg) dissolved in acetone (0.5 mL) was added in a drop-wise manner (1 drop/15 s) to stirring distilled water (3 mL). The remaining acetone was removed by evaporation at room temperature under vacuum. Mean diameter and polydispersity of self-assembled structures in aqueous media were measured by dynamic light scattering (Zetasizer Nano ZS, Malvern Instrument Ltd., UK). The concentration of block copolymers was 10 mg/mL. To adjust the tonicity for ip injections, sucrose was added to the polymeric micellar solution to achieve a final sucrose concentration of 95.76 mg/mL.

2.2.3. Animals

Pathogen-free healthy male rats of Wistar strain were used in this study. Animals were obtained from Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The rats were approximately 10 weeks old (weighing in the range of 180–200 g) at the start of the study. All animals were housed in polypropylene cages, six rats per cage, and were kept in a room maintained at 25 ± 2 °C with a 12 h light/dark cycle. Animals were given free access to standard laboratory animal feed and water ad libitum. All the procedures were approved by The Experimental Animal Care Centre Review Board (Ref. No. C.P.R.-3625) and performed according to NIH guidelines.

2.2.4. Experimental design

To investigate the toxicity profile of PEO-*b*-PCL micelles, 60 animals were divided into the following groups (six rats/group):

1. Group I and II served as control and received vehicle only by oral gavage or ip injection, respectively, daily for seven days.
2. Groups III, IV, V and VI received 100 mg/kg of PCL_{30}, PCL_{60}, PCL_{120} and PCL_{200}, respectively, by oral gavage daily for seven days.
3. Groups VII, VIII, IX and X received 100 mg/kg of PCL_{30}, PCL_{60}, PCL_{120} and PCL_{200}, respectively, ip injections daily for seven days.

Twenty-four hours following last administration, half of the animals were sacrificed and blood and organs (lung, liver, kidneys, heart and spleen) were collected. The remaining half of the animals were observed for further 14 days and were sacrificed at the end of third week, and blood and organs were collected for further investigation.

2.2.5. Blood collection

Blood was collected in heparinized vacutainer tubes from retro-orbital plexus while rats were anesthetized. A part of the blood was centrifuged (300*g* for 10 min) to obtain plasma for various organ