COMMUNICATION

Pharmacokinetics and Nephrotoxicity of Amphotericin B-Incorporated Poly(Ethylene Glycol)-*Block*-Poly(*N*-Hexyl Stearate l-aspartamide) Micelles

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ABSTRACT: The purpose of this investigation was to study the pharmacokinetics and nephrotoxicity of amphotericin B (AmB), incorporated in poly(ethylene glycol)-*block*-poly(*N*-hexyl stearate l-aspartamide) (PEG-*b*-PHSA) micelles (AmB/PEG-*b*-PHSA). After AmB/PEG-*b*-PHSA or AmB for injection, United States Pharmacopeia (USP), was dosed intravenously in rats (0.8 mg/kg), serum was collected over 72 h, and organs collected at 72 h for AmB analysis. To test for the nephrotoxicity caused by AmB, renal markers of damage were assessed 24 h after a single injection of AmB/PEG-*b*-PHSA or AmB for injection, USP, focusing on detection of urinary enzymes. PEG-*b*-PHSA micelles caused a significantly lower area under serum concentration curve and higher clearance relative to AmB for injection, USP. PEG-*b*-PHSA micelles lowered the distribution of AmB in liver and lung tissues, but did not significantly lower the level of AmB in the kidneys relative to AmB for injection, USP. However, urine levels of *N*-acetyl-β-glucosaminidase and γ-glutamyltransferase were significantly lower for AmB/PEG-*b*-PHSA relative to AmB for injection, USP. In summary, PEG-*b*-PHSA micelles reduced the nephrotoxicity of AmB, the dose-limiting toxicity of this important antifungal agent. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:2064–2070, 2011

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INTRODUCTION

Amphotericin B (AmB) is a powerful intravenous antifungal therapeutic with proven efficacy against many pathogenic fungi. However, a major limitation of its clinical application remains the high incidence of renal impairment caused by AmB for injection United States Pharmacopeia (USP) (AmB-D).[1] Strides have been made toward reducing drug toxicity by lipid-based formulations, which shift the distribution of AmB away from the kidneys.[1] Unfortunately, these formulations have lower antifungal efficacy than AmB-D, mandating dose escalation, and mortality rates for systemic mycoses remain high.[2] Aiming for an increase in the therapeutic index of AmB, that is, reduced renal toxicity and retained efficacy relative to AmB-D, we have prepared poly(ethylene glycol)-*block*-poly(*N*-hexyl-stearate-l-aspartamide) (PEG-*b*-PHSA) micelles that effectively increase the water solubility of AmB and show similar antifungal efficacy as AmB-D in a mouse model of invasive candidiasis.[3] Herein, we report on the differences in the pharmacokinetics and biodistribution of AmB formulated in PEG-*b*-PHSA micelles and as AmB-D, which is a colloidal dispersion of AmB with sodium deoxycholate and a buffering agent. Previous findings indicated that AmB-loaded PEG-*b*-PHSA micelles have excellent *in vitro* stability in the presence of serum proteins.[4] On the basis of these findings, it was hypothesized that PEG-*b*-PHSA micelles loaded with AmB will increase circulation time of AmB in rats, preventing dose dumping upon injection and resulting in reduced nephrotoxicity for AmB.

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MATERIALS AND METHODS

Materials

AmB was a gift from X-GEN Pharmaceuticals Inc. (Big Flats, New York). AmB-D was purchased from X-GEN Pharmaceuticals Inc. (Northport, New York). Piroxicam, glacial acetic acid, high-performance liquid chromatography (HPLC)-grade acetonitrile, methanol, water, and chloroform were purchased from Sigma–Aldrich Inc. (St. Louis, Missouri). Oxygen USP was purchased from A-L Compressed Gases Inc. (Spokane, Washington). Silastic® laboratory tubing was purchased from Dow Corning Corp. (Midland, Michigan). Intramedic® polyethylene tubing was purchased from Becton Dickinson Primary Care Diagnostica, Becton Dickinson and Company (Sparks, Maryland). Monoject® 23 gauge ($0.6 \times 25\,mm^2$) polypropylene hub hypodermic needles were purchased from Sherwood Medical (St. Louis, Missouri). Undyed braided-coated polyglycolic acid synthetic absorbable surgical suture was purchased from Surgical Specialties Corporation (Reading, Pennsylvania). PEG-*b*-PHSA was synthesized as described previously.[4] The molecular weight of the PEG block was 12,000 g/mol, and the core forming block (PHSA) had 11 repeating units, nine substituted with stearic acid.

Preparation of AmB-Incorporated PEG-*b*-PHSA Micelles

AmB-incorporated PEG-*b*-PHSA micelles were prepared using a simple solvent evaporation method described previously.[5–7] Briefly, PEG-*b*-PHSA was dissolved in chloroform (20 mL) in a 50 mL round bottom flask at a 6 mg/mL concentration, and AmB was dissolved in methanol at 0.3 mg/mL concentration (11 mL) and added at a 2:1 molar ratio (PEG-*b*-PHSA/AmB). The organic solvents were removed at 65°C under reduced pressure. Flasks were purged with dry nitrogen gas for 5 min to eliminate any residual solvent. Sterile dextrose 5% was added, and the sample flask was rotated for 10 min at 65°C. Samples were then sonicated for 30 s and filtered through a sterile 0.22 μm nylon syringe filter. The dimensions of AmB-incorporated PEG-*b*-PHSA micelles was determined by dynamic light scattering using a ZETASIZER Nano-ZS (Malvern Instruments Inc., Worcestershire, UK) equipped with He–Ne laser (4 mW, 633 nm) light source and 173° angle scattered light collection configuration. PEG-*b*-PHSA micelles were diluted in double-distilled H_2O, and the samples were equilibrated for 2 min at 25°C before measurements. The final PEG-*b*-PHSA concentration was approximately 0.5 mg/mL. Correlation function was curve-fitted by the cumulants analysis method to calculate mean size and polydispersity index (PDI), which is a measure of relative variance of the *Z*-average size assuming a Gaussian distribution. All measurements were performed in triplicate, and volume-weighted particle sizes are presented as the average diameter and PDI.

The content of AmB in PEG-*b*-PHSA micelles was quantified by reverse-phase HPLC. The HPLC system used for quantifying was a Shimadzu prominence HPLC system (Shimadzu Corporation, Kyoto, Japan), consisting of a LC-20AT pump, SIL-20AC HT autosampler, CTO-20AC column oven, and a SPD-M20A diode array detector. A similar method as previously described by Echevarria *et al*.[8] was used. Micelle sample (50 μL) and 50 μL of piroxicam at a concentration of 100 μg/mL in methanol (internal standard) were dried by nitrogen purge and reconstituted with 200 μL of mobile phase consisting of acetonitrile, 10% acetic acid, and water (41:43:16), which was filtered, degassed, and sonicated prior to use. AmB content was quantified by injecting 50 μL of sample into two Phenomenex® Luna® C18(2) ($250 \times 4.6\,mm^2$, 5 μm pore size; Phenomenex®, Torrance, California) in sequence. Columns were kept at room temperature. AmB and piroxicam were detected at 406 and 356 nm, respectively, and eluted at 10.2 and 15.8 min, respectively.

Surgical Procedures

Male Sprague–Dawley rats (250–320 g) were obtained from Simonsen Labs (Gilroy, California) and given food (Teklad F6 rodent diet; Teklad, Harland Laboratories, Madison, Wisconsin) and water *ad libitum* in the vivarium for at least 1 week before use. Rats were housed in temperature-controlled rooms with a 12 h light/dark cycle. The day before the pharmacokinetic experiment, jugular veins of the rats were catheterized with sterile silastic cannula (Dow Corning Corp.) under anesthesia [IsoFlo®, (isoflurane, USP) Abbott Laboratories, North Chicago, Illinois) coupled with an oxygen regulator, periodically monitoring pedal reflex and respiration rate to ensure that a surgical plane of anesthesia was maintained. Jugular vein catheterization involved exposure of the vessel prior to cannula insertion. After cannulation, the Intramedic PE-50 polyethylene tubing (Becton, Dickinson and Company, Franklin Lakes, New Jersey) connected to the cannula was exteriorized through the dorsal skin. The cannula was flushed with 0.9% sodium chloride irrigation USP (saline; Baxter Healthcare, Deerfield, Illinois). The animals were transferred to individual metabolic cages with access to food and water *ad libitum* for recovery. Ethics approval for animal experimentation was obtained from the Institutional Animal Care and Use Committee at Washington State University in accordance with "Principles of Laboratory Animal Care" [National