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Effect of the excipient concentration on the pharmacokinetics of PM181104, a novel antimicrobial thiazolyl cyclic peptide antibiotic, following intravenous administration to mice

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ABSTRACT

Thiazolyl cyclic peptide antibiotics are known for their poor aqueous solubility and unfavorable pharmacokinetics (PK) and hence pose challenging tasks in developing these antibiotics as clinical candidates. In the current paper, we report a possible way to address these challenges with exemplification of our antibiotic PM181104. The approach was to prepare formulations with known excipients, Polysorbate 80 (Tween 80, T-80) and PEG 400 through their varied stiochiometric combination in appropriate ratio to achieve acceptable osmolarity, pH and particle size of the formulation. Two different sets of formulations were prepared with two distinct average particle diameters ranging from 32.8 to 465.4 nm. First, semi-transparent solutions with a particle size of >100 nm were achieved by keeping concentration of PEG 400 constant at 8% (w/v) and decreasing the amounts of T-80. Second, clear colorless solutions with a particle size of <100 nm were achieved by keeping concentration of T-80 constant at 8% (w/v) and decreasing the amounts of PEG 400. In PK studies, intravenous administration of formulation with particle size <100 nm to mice resulted in a two-fold increase in area under the plasma concentration-time curve (AUC_{last}) and concentration at time zero (C_0), there by facilitating the selection of suitable formulation for further efficacy studies.

1. Introduction

The increasing number of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) is a cause of great concern in antibiotic therapy and predominantly, emerging mechanisms of new resistance, making the next generation of antibiotics largely ineffective. Hence, there exists a need for the development of novel class of antibiotics with novel modes of action to overcome existing resistance mechanisms and to effectively combat these serious pathogens. Lately, thiazolyl cyclic-peptide antibiotics have been emerging as an alternative class of antibiotics. They are known for their potent *in vitro* antibacterial activity against a wide spectrum of pathogens with their unique mode of action. However, in spite of their potent *in vitro* antibacterial activity, till date these compounds have not been developed for use in humans due to low aqueous solubility and unfavorable pharmacokinetics [1–4].

We recently have reported a novel thiazolyl cyclic-peptide antibiotic, PM181104 (Fig. 1) from marine microbial source [5,6]. The compound exhibits potent *in vitro* antibacterial activity against a broad range of Gram-positive bacteria. The minimum inhibitory concentration (MIC) values evaluated for the compound were in nano-molar range. In *in vivo* studies of PM181104 in a BALB/c murine septicemia model, the compound displayed 100% effective dose (ED_{100}) value of 2.5 mg kg^{-1} of body weight against MRSA and 10.0 mg kg^{-1} against VRE, and in tissue or organ-specific infection models showed reduction in bacterial titer comparable to standard antibiotics [5,7]. In the current studies, suitable intravenous (i.v) formulation development approaches have been explored. We consider that the i.v. route of administration facilitates complete bioavailability and rapid action to treat the systemic infections associated with the Gram-positive pathogens. True to the behavior of naturally occurring thiazolyl peptide antibiotics, PM181104 too exhibited poor aqueous solubility. To overcome such difficulty, we made an effort to develop an i.v. formulation using a non-ionic surfactant with co-solvent combination approach [8]. The advantage of this approach is that the combination of surfactant and polymer may provide better protection against solvent-mediated transformation than the surfactant or polymer

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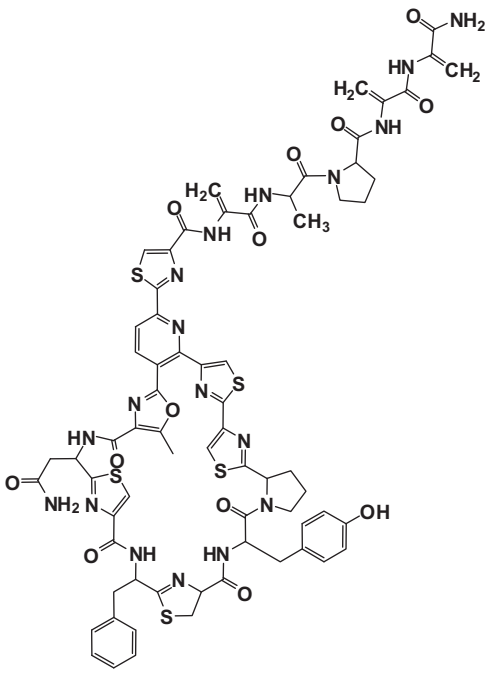

Fig. 1. Chemical structure of PM181104.

alone [9]. However, in order to avoid the possible side effects such as anaphylactic response and vascular irritability which may be caused by surfactants, it is far safe to reduce the types and stoichiometric concentration of the stabilizers used [10,11]. Indeed, there is a set-in guideline by the FDA for choosing an inactive ingredient [36]. Therefore, the major focus of our studies was to formulate a dosage form that exhibits *in vivo* efficacy with a scope to minimize the excipient composition to an acceptable extent. Initial attempts to achieve maximum drug exposure levels, also inherently delivered a proportionately higher concentration of excipients. Hence, there was a scope to bring down the excipient levels in the defined dosage delivery. Towards this effort, we embarked upon studies involving the stoichiometric alteration of two of the excipients namely, T-80 and PEG 400 which fall under generally recognized as safe (GRAS) category, and comparing their associated pharmacokinetics outcomes. We also evaluated the effect of stiochiometric variations in these excipients on the osmolarity, pH as well as particle size of the drug and its implications on *in vivo* situation. These efforts resulted in the identification of an *in vivo* efficacious formulation suitable for parenteral administration.

The current paper describes the detailed studies of the development of an *in vivo* efficacious formulation using T-80 and PEG 400 and effect of stiochiometric variation of these excipients on osmolarity, pH, particle size of the drug and the associated effect on pharmacokinetics outcomes.

2. Materials and methods

2.1. Materials

PM181104 was isolated and characterized in-house [5]. T-80 and PEG 400 were purchased from Croda Inc., USA. Sterile Water for Injection was supplied by Nirma Ltd., India. Methanol and acetonitrile solvents were of HPLC grade and were procured from Merck.

2.2. HPLC analysis

The reverse phase HPLC method was chosen for the quantitative determination of the PM181104 in the formulations. Standard and formulation samples were diluted with acetonitrile: methanol (1:1; v/v) to obtain a final concentration of 0.1 mg mL^{-1} and then injected a 10 μL injection volume directly to HPLC system. Agilent 1200 HPLC system (Agilent, USA) with a Kromasil 100 C_{18} analytical column (150 × 4.6 mm^2, particle size 3.5 μm) was used for the studies. The mobile phase was acetonitrile–water mixture (50:50, v/v). The flow rate was 1.0 mL min^{-1} and the detection wavelength was set to 309 nm. Percentage assay calculated with respective to the chromatograms of standard and sample area.

2.3. Preparation of PM181104 formulation

PM181104 nanoparticles were prepared by anti-solvent precipitation technique, using water for injection (WFI) as the anti-solvent [12]. By using this method nanoparticles can be manufactured in the absence of mechanical forces which can have influence on peptide stability [13]. For this, the specified amount of T-80 was thoroughly mixed with the specified amount of PEG 400 under vortex followed by sonication, to form the excipient mixture. The prepared excipient mixture was used to dissolve the required amount of PM181104 using sonication carried out with intermittent cooling (to maintain the temperature below 40 °C) until a turbid free solution clear of any undissolved particulate matter was obtained. The resultant clear, colorless and viscous drug excipient mixture was then injected slowly and continuously through drop wise addition using a buret to the anti-solvent under rapid mixing (1000 rpm, magnetic stirrer). Precipitation of the solid drug particles were occurred immediately upon contact with the anti-solvent. The resulting formulation suspension was sterilized by filtering through 0.2 μm filter assembly connected to vacuum. A total of eight formulations were made, and divided into two sets based on their excipient composition. The first set consisted of formulations, made with a reduced concentration of T-80. The second set consisted of reduced concentration of PEG 400. The optimization of the excipient composition in the first set of formulations (F1 – F5) was carried out using ternary compositions containing water for injection (WFI), PEG 400 8% (w/v) and a decreasing amounts of T-80 (8–0.05%) while maintaining the final concentration of PM181104 at 0.25 mg mL^{-1}. In the second set, another three formulations (F6–F8) were prepared using ternary compositions containing WFI, T-80 8% (w/v) and a decreasing amounts of PEG 400 (6–0.5%) while retaining the final concentration of the PM181104 at 0.5 mg mL^{-1}. The concentration of the drug in the described formulation was reconfirmed using HPLC analysis.

2.4. Determination of the particle size and polydispersity index

Particle size and polydispersity index (PDI) of the prepared nanoparticles of PM181104 were measured immediately after formulation filtration process by dynamic laser light scattering using particle size analyzer (Delsa Nano C, Nano-Zetasizer, Beckman Coulter, Miami, FL, USA) at back scattering measurement angle of 165°. Samples were not diluted and measured at 25 °C. The measurement was done in triplicate and size d_{90} was reported.

2.5. Osmolarity, zetapotential and pH measurements

The osmolarity, zetapotential and pH of the prepared formulations of PM181104 were measured using an osmometer (Osmomat 030-3P, Gonotec, Germany), a Delsa Nano HC, Zeta Potential Particle Analyzer (Beckman Coulter Inc., USA) and pH meter (pH