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Polymeric nanoparticles as a platform for permeability enhancement of class III drug amikacin

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ABSTRACT

Amikacin (A), a water soluble aminoglycoside antibiotic is commercially available for intravenous administration only. Present investigation is aimed at the development of poly-lactic-co-glycolic acid (PLGA) nanoparticles (A-NPs).[1] for oral permeability enhancement of amikacin. The pharmaceutical attributes of the A-NPs revealed particle size, 260.3 ± 2.05 nm, zeta potential, −12.9 ± 1.12 mV and drug content, 40.10 ± 1.87 μg/mg with spherical shape and smooth surface. *In vitro* antibacterial studies showed that the A-NPs were active against *P. aeruginosa*, *K. pneumoniae* and *E. coli*. The permeation study across rat ileum showed 2.6-fold improvement in P_{app} for A-NPs than A-S[2] This increase in permeability is due to the uptake of nanoparticles by Peyer's patches of intestinal epithelium and endocytic uptake via enterocytes. Flow cytometric analysis demonstrated 2.2-fold higher uptake of Rh B-NPs[3] than Rh B-S[4] and elucidated the dominance of enterocytes mediated endocytosis of nanoparticles. Furthermore, stability data collected as per ICH guidelines for three months under accelerated conditions had shown that the A-NPs were stable. The purported drug delivery system hence, seems a promising tool to replace successfully the current intravenous therapy and is used to support relevant patient compliance thereby, adding value to the "patient care at home".

1. Introduction

Amikacin is an aminoglycoside antibiotic [1] with an outstanding feature of its resistance to bacterial aminoglycoside inactivating enzymes, leading to widest spectrum of anti-microbial activity including many organisms that are resistant to other aminoglycosides. It is bactericidal in nature and acts through "binding irreversibly" to the specific 30S-ribosomal subunit proteins thereby, inhibiting protein synthesis that results in cell death [2].

Owing to short half-life (114 ± 16.7 min), amikacin is needed to be administered frequently at higher doses leading to progressive adverse effects [3]. Therefore, researchers investigated intravenous novel formulations of various antibiotics to combat their adverse effects and to increase the therapeutic efficacy. Varshosaz et al. [4] reported reduced accumulation of amikacin in kidney using amikacin loaded solid lipid NPs in comparison to free drug via intravenous administration thus, reducing the probability of nephrotoxicity. Similarly, Xiang et al. [5] also demonstrated 17.8 folds improved AUC of dexamethasone using solid lipid NPs. The above studies thus, indicate that the delivery system based novel nano preparations provide sustained release and protect drugs from metabolism induced degradation thereby, enhancing the drug's residence time and consequently bioavailability, which in turn reduce the dose and dosing frequency leading to decreased dose related toxicity and side effects. These characteristics ultimately make novel formulation an ideal candidate to deliver toxic drugs [6].

Clinically available formulations of amikacin are meant for intravenous and intramuscular administration, as it belongs to class III drugs of biopharmaceutics classification system. Oral administration of any drug for the treatment of chronic diseases that require long and continuous treatment has always remained fascinating owing to its several advantages. These include better quality of life, non-invasive painless therapy, flexibility in fabrication of dosage form as well as

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[1] Amikacin encapsulated poly-lactic-co-glycolic acid nanoparticles.
[2] Amikacin solution.
[3] Rhodamine B encapsulated poly-lactic-co-glycolic acid nanoparticles.
[4] Rhodamine B solution.

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frequency of dosing and low healthcare cost etc. In addition, it could also preclude the requirement of hospitalization, medical and nursing care. Consequently, the oral administration of drugs contributes towards better patient compliance. With high solubility in water and low permeability however, leads to poor bioavailability of this class of drugs in general and amikacin in particular. Therefore, oral delivery of amikacin is highly challenging and needs novel therapeutic interventions.

The findings in previous studies indicate that the nanoparticulate systems could be better substitutes for the effective and safe delivery of therapeutic molecules that are pharmaceutically challenging. Therefore, it is expected that PLGA NPs of amikacin would be able to improve the permeability of amikacin across biological barriers and its biodisposition. At the same time, sustained release profile of drug would maintain the therapeutic concentration for extended period at lower dose that would result into reduced systemic toxic effects thus improved safety and efficacy. Present investigation hence, intends a comparative assessment of amikacin conventional formulation versus novel nano formulation through oral route.

2. Materials and methods

2.1. Materials

Amikacin sulphate, Dulbecco's Modified Eagle Medium (DMEM), Dulbecco's Phosphate Buffer Saline (PBS), Fetal Bovine Serum (FBS, 10%), Penicillin and Streptomycin (0.1% Penstrap), Trypsin-EDTA (0.25%) and Polyvinyl alcohol (MW-25,000) were procured from Sigma-Aldrich Co., USA. Poly (D, L-lactide-co-glycolide), lactide:glycolide (50:50, Resomer 50 3H, MW-7000-17,000, PLGA) was procured from Sigma-Aldrich Co., Germany. The rest of the chemicals used were of analytical grade.

Human colon adenocarcinoma cell line (HT-29, NCCS, Pune) was gifted by Professor S. A. Hussain, Department of Biosciences, Jamia Millia Islamia, New Delhi, India.

2.2. Encapsulation of amikacin in PLGA NPs

Amikacin encapsulated in PLGA nanoparticles (A-NPs) was prepared employing the method of Tariq et al. [7]. The method was slightly modified wherein 0.5% w/v polyvinyl alcohol (PVA) and 10 mg of amikacin were added in 200 µl of Milli Q water (Internal Aqueous Phase, IAP). Organic Phase (OP) was prepared by dissolving 100 mg of PLGA in 4 ml dichloromethane. Thereafter, IAP was emulsified in OP solution using sonication on an ice bath for 60 s at 25 W, 40% duty cycles and 30% amplitude (Sonopuls, Germany). The primary emulsion (w_1/o) thus, formed was added dropwise in 16 ml external aqueous phase (1% PVA, w/v) along with homogenisation (Silent Crusher M, Heidolph, Germany) at 10,000 rpm for 30 s over an ice bath. The secondary emulsion ($w_1/o/w_2$) formed was then, sonicated at 25 W, 40% duty cycles and 30% amplitude for 2 min over an ice bath. The solvent was evaporated at room temperature (25 ± 2 °C) from the dispersion obtained under mild stirring at 400 rpm. Finally, the nanoemulsion obtained was centrifuged at 15,000 rpm for 15 min at 4 °C in Sorvall Centrifuge RC6+ (Thermo Scientific, USA). It was followed by the washing of pellet thrice with ice cold Milli Q water and subsequent lyophilization in freeze dryer (Lab Conco., USA) for 24 h.

The above procedure was optimised statistically using Box-Behnken design (DOE, Stat Ease Design Expert software). Placebo NPs without amikacin were also prepared by the above method.

2.2.1. Preparation of fluorescent labelled PLGA NPs

Rhodamine B (Rh B) was used as a fluorescent probe for the preparation of fluorescent NPs (Rh B-NPs). 2 mg of Rh B was dissolved in water and used in place of amikacin using the procedure as described above for the preparation of A-NPs.

2.3. Nanoparticle characterisation

2.3.1. Entrapment efficiency and drug loading

To determine the entrapment efficiency and drug loading in PLGA NPs, 10 mg amikacin were dissolved in 200 µl dimethyl sulfoxide and the volume was made to 10 ml with Milli Q water. The resulting suspension was subjected to centrifugation at 10,000 rpm for 10 min. 100 µl aliquot from the above sample was added to 100 µl of 0.0625% ortho-phthalaldehyde (OPA) reagent. Fluorescence was measured immediately (within 10 min) at λex/λem of 340/450 nm by spectrofluorimeter [8]. The experiment was performed in triplicate. The following formula was used to calculate drug loading and entrapment efficiency [9]:

$$\text{Drug loading}(\%w/w) = \frac{\text{Amount ofdrug quantified in nanoparticles}}{\text{The total amount of polymer (PLGA) added}} \times 100$$

$$\text{Entrapment efficiency}(\%w/w) = \frac{\text{Amount ofdrug quantified in nanoparticles}}{\text{The total amount of drug}} \times 100$$

2.3.2. Particle size and zeta potential

The particle size, polydispersity index (PDI) and zeta potential of A-NPs were determined using the dynamic light scattering (DLS) method with a computerized analytical system (Malvern Zetasizer, UK) and deduced by 'DTS nano' software. The sample was prepared by dispersing 1 mg of A-NPs in 2 ml Milli Q water for the determination of size, PDI and zeta potential.

2.3.3. Size and surface morphology analysis

The surface morphology and size of A-NPs were analysed using Scanning Electron Microscope (SEM) and Transmittance Electron Microscope (TEM), respectively. The evaluations of size using TEM (TOPCON, Tokyo, Japan) and surface morphology using SEM (EVO LS 10, Carl Zeiss, Germany) were carried out as per the method described earlier [7].

2.3.4. X-ray diffraction studies

The X-ray diffraction measurements of amikacin, placebo NPs and A-NPs were evaluated using nickel filtered CuKα radiation at 1.54 Å with X-ray diffractometer. The intensity data obtained was analysed using in-built PCAPD diffraction software for the correct detection of peak position and its corresponding intensity data. The measurements were taken continuously from 10° to 50° angles at intervals of 10° of the samples placed on a glass slide.

2.3.5. Drug-excipient compatibility studies

Drug-excipient compatibility was analysed by Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared spectroscopy (FTIR).

For DSC, accurately weighed amikacin (1 mg), placebo NPs and A-NPs (equivalent to 1 mg amikacin) were loaded in a DSC pan with a DSC loading puncher and sealed. The samples were thereafter, scanned within a heating range of 40–300 °C at the rate of 10 °C per min under nitrogen atmosphere employing a differential scanning calorimeter (Perkin Elmer Pyris 6 DSC, U.S.A). The DSC curves were then compared.

For FTIR analysis, adequate amount of amikacin, placebo NPs and A-NPs were placed into the ATR unit of the spectrophotometer. These were scanned between 4000–400 cm^{-1} and graphs were compared.

2.4. In vitro release studies

The *in vitro* release study of amikacin from A-NPs and free drug solution (A-S) was evaluated using dialysis bag method. 2 mg of