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Intravenous human serum albumin (HSA)-bound artemether nanoparticles for treatment of severe malaria

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ABSTRACT

Severe malaria is potentially life-threatening and requires the parenteral administration of the artemisinin-based formulation for short duration of action. Intravenous artemether formulation has shown potentially better therapeutic efficacy but its poor aqueous dissolution constitutes a challenge. This work produced human serum albumin (HSA)-bound artemether nanoparticles by emulsification and desolvation approach using nanoparticle albumin bound (nab) technology for intravenous (IV) administration. The prepared nanoparticles had a size below 50 nm. It was observed that higher HSA concentration; lower drug concentration and longer ultrasonication time (up to 60 s) favoured the production of smaller particles. Comparatively, the emulsification method allowed for higher drug entrapment per the same concentration of HSA and required less organic solvent, less water and less energy and is therefore the preferred method. HSA-bound artemether nanoparticles displayed significantly enhanced dissolution properties over raw artemether. In-vitro haemolysis studies demonstrated a significant decrease in haemolysis caused by artemether from 101% to 7% in the emulsification product and to 4% by the desolvation product.

1. Introduction

Malaria has been described as the most life threatening disease [1] with 50% of the world's population at risk of being infected with malaria. In 2015, there were estimated 214 million malaria cases and 438,000 deaths distributed in 95 countries mainly in Africa (90%), South-East Asia (7%) and the Mediterranean regions (3%), respectively [2]. Implying on the average, one person died per minute of malaria. Malaria is caused by Plasmodium parasites and transmitted through mosquito bites. Four main Plasmodium species, i.e. *P. falciparum, P. vivax, P. malariae, P. ovale* are responsible for malaria. The former two species are the most common with P. falciparum being the most deadly. Malaria is classified based on its severity into uncomplicated and severe malaria. Malaria is regarded as severe malaria if it leads to organ impairment or more than 5% of the erythrocytes are infected, or haemoglobin concentration is less than 5 g/dl (haematocrit is less than 15%) [2]. Severe malaria presents as cerebral malaria (characterized by vascular blockade of infected red blood cell in the brain interfering with blood flow) [3–6] exhibiting symptoms like severe anaemia, acute respiratory distress syndrome, and hemoglobinuria. Other symptoms include abnormalities in blood coagulation, low blood pressure due to cardiovascular, acute kidney failure, hypoglycemia and its associated metabolic acidosis [7]. Due to the high death rate and early onset of death associated with severe malaria, the primary therapeutic objective is to prevent death. Therefore, parenteral administration of intravenous (IV) artesunate, intramuscular (IM) oil-based artemether and IM quinine as first, second and third line treatments, respectively is recommended by World Health Organization (WHO) for at least the first 24 h or until the patient can tolerate oral dosage forms [6]. The primary pharmacokinetic goal is to achieve fast onset of action with a high concentration of an effective antimalarial at the shortest possible time to clear parasite load effectively thereby preventing possible recurrence or resistance. Resistance to antimalarials is a global menace and has been reported among all previously used antimalarial drugs including chloroquine, pyrimethamine, and sulphonamides [8]. Resistance leads to high death rates, high costs of treatment and loss of productivity to an economy as a whole. Resistance can be reduced by the use of combination therapy, more effective formulations and the introduction of a new medicine [9]. Faced with declining antimicrobial agents discovery pipeline with no currently introduced antimalarial drug on the market, new delivery strategies for existing antimalarial agents are urgently needed to prevent the development of resistance to currently effective drugs and increase therapeutic efficiency [9,10].

Artemisinin found in *Artemisia annua* L., has antimalarial properties but

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its low solubility in both oil and water reduces its therapeutic value. This necessitated the development of more potent semi- synthetic derivatives such as artemether, which is oil soluble and therefore of higher therapeutic value. Artemether, (3*R,5aS,6R,8aS,9R,10S,12R,12aR)*-Decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-j]-1,2-benzodioxepin ($C_{16}H_{26}O_5$) is a methyl ether ester of sesquiterpene endoperoxide lactone [11,12]. Its mechanism of action involves heme-mediated breakage of the endoperoxide linkage in the chemical structure to produce free radicals which alkylate specific malaria parasite proteins leading to the death of the parasites [13,14]. The dominance of nonpolar groups in artemether makes it practically insoluble in water. Hence, it is available on the market as oral dosage forms and oil-based IM injection. Besides the haemolytic adverse effect associated with artemether [15], the key problems associated with oil-based artemether IM injections is its erratic and non-consistent absorption profile [16]. Despite these problems, IM artemether showed comparable efficacy with IV artesunate in the treatment of severe malaria in a randomized controlled clinical trial [17] and also in a preclinical mouse model [18]. Hence, IV artemether, which eliminates the need for absorption, has the potential to give higher efficacy than IV artesunate. IV artemether offers great advantages regarding: (1) higher efficiency than artemether administered through oral, IM and other routes [19]; (2) higher plasma concentrations than IV artesunate [20]; (3) longer plasma half-life than IV artesunate [20]; (4) higher bioavailability than IM artemether [20]; (5) more consistent pharmacokinetic profile; (6) less pain on administration than the oil-based product since patients with severe malaria will already have an existing IV line which would make administration of IV artemether easier [21]; and (7) no hypoglycemic association unlike quinine which might augment hypoglycemia in severe malaria patients [7]. These unparalleled advantages make an IV artemether formulation indispensable in the stagnating fight against malaria. The only setback in tapping into the strengths of IV artemether for clinical use is its poor water solubility.

Nanoparticle albumin bound (nab) technology describes the encapsulation of hydrophobic drugs in albumin through an emulsion-evaporation cross-link method [22]. This technology exploits properties of albumin to produce safer solvent free and targeted delivery of hydrophobic drugs such as paclitaxel and artemether. Nab technology was successfully developed by Abraxis Bioscience LLC. to make Abraxane (nab-paclitaxel) which was approved by FDA in 2005 for the treatment of breast cancer. Human serum albumin (HSA) is non-immunogenic, non-toxic and biodegradable with solubilizing and cryoprotectant properties [23]. In addition, HSA is required for growth and survival of the malaria parasite in the infected erythrocytes. Therefore malaria parasites import HSA into infected erythrocytes. This provides a means for targeting artemether to malaria parasites within infected erythrocytes using the parasites own endogenous transport mechanism [24,25]. Furthermore, in the treatment of malaria, albumin has been found to be the only protein adjunct therapy that reduced death in cerebral malaria [26]. These benefits make HSA and thus nab technology great choices for an antimalarial IV formulation.

To the best of our knowledge, there has been no report about the exploration of forming artemether nanoparticles with nab technology for the treatment of severe malaria. This study reports the preparation of lyophilized HSA-bound artemether nanoparticles designed for IV administration. This preparation method has the potential to: (1) increase dissolution of artemether; (2) reduce haemolytic effect of artemether on erythrocytes; and (3) facilitated preferential uptake of artemether by malaria parasite infected erythrocytes due to the affinity of the parasite for albumin. The nanoparticles were produced via emulsification (use of water immiscible solvent) and desolvation (use of water miscible solvent) approaches of nab technology. HSA is the only excipient used in this work. Dynamic light scattering was used to characterize the particle size. Morphology of the nanoparticles was observed using scanning electron microscopy (SEM). The solid state and thermal properties of the nanoparticles were studied by powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC). The chemical structure of artemether with HSA in the formulation was analysed using Fourier transform infrared spectroscopy (FTIR). In-vitro release study was studied by 'sample and separate' method [27]. High performance liquid chromatography (HPLC) was used to analyse the amount of artemether released. Lastly, In-vitro haemolysis test was done to study the toxicity of nanoparticles produced on erythrocytes.

2. Materials and methods

2.1. Materials

Artemether was purchased from Biotain Pharma Co. Ltd, Xiamen, China, HSA was purchased from Golden West Biologicals Inc., California, USA. Dichloromethane (DCM) was purchased from Sigma-Aldrich, Singapore. Acetone was provided by Aik Moh Paints & Chemicals Pte Ltd, Singapore. Acetonitrile (HPLC grade) was supplied by Fisher Scientific. Milli-Q water was used throughout the work.

2.2. Preparation of lyophilized HSA-bound artemether nanoparticles

HSA-bound artemether nanoparticles were either produced by emulsification or desolvation approaches of nab technology [23]. In brief, 5, 10, 15, 20, 25 or 30 mg artemether was dissolved in 1 ml DCM or acetone. 125 mg HSA was dissolved in 5 ml Milli-Q water. In the emulsification approach, the artemether solution in DCM was added to the HSA solution and stirred with the aid of magnetic stirrer to produce an oil-in-water (o/w) coarse emulsion (with large and broad particle size distribution). In the desolvation approach, the artemether solution in acetone was mixed with HSA solution forming a coarse suspension. The prepared coarse emulsion or coarse suspension was ultrasonicated at 40% amplitude to produce a nanoemulsion or nanosuspension with a narrower particle size distribution. DCM or acetone was then removed from the nanoemulsion or nanosuspension by rotary evaporation under reduced pressure at 36 °C for 30 min. The resulting nanosuspension was frozen and lyophilized for 48 h to produce HSA-bound artemether nanopowders. The products obtained by emulsification and desolvation approach were designated as Art Np_Emulsification and Art Np_Desolvation, respectively. Fig. 1 shows a graphical illustration of the preparation process.

2.3. Particle size and zeta potential measurement

Dynamic light scattering (DLS) was used to study the particle size of the nanoparticles produced. DLS was done using Zetasizer Nano Z system (Malvern, UK). The samples were diluted to the equivalent of 0.2 mg/ml of artemether with Milli-Q water. Scattering angle and temperature were set as 173° and 25 °C, respectively. Measurements were performed in triplicates. Mean particle size (Z-average, diameter) and standard deviation were calculated.

Zeta potential measurement was performed by electrophoretic light scattering technology using Zetasizer Nano Z system (Malvern, UK). Reconstituted nanoparticles were diluted to the equivalent of 0.2 mg/ml artemether as was done for particle size analysis. Measurements were done in triplicates and the mean particle size (Z-average, diameter) and standard deviation were calculated.

2.4. Physical stability

The physical stability of nanoparticles after reconstitution was studied by reconstituting the nanoparticles to the original volume before lyophilization and observing them for possible particle size enlargement. Milli-Q water or normal saline solution was used as the reconstituting medium. The reconstituted samples were stored at 4 °C throughout the study. Samples were taken at regular intervals for particle size and zeta potential analysis with Zetasizer Nano Z system (Malvern, UK).