Oral Nanomedicines for the Treatment of Parasitic Diseases

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ABSTRACT SUMMARY:

Self-nanoemulsifying drug delivery systems (SNEDDs) prepared from GRAS excipients (Labrafil M1944CS: Labrasol: Capryol 90: BPQ 30: 59 : 10 : 1% w/w/w/w) enhance the oral bioavailability of a poorly soluble antiparasitic drug, Buparvaquone (BPQ), while maintaining in vitro efficacy against *L. infantum* promastigotes. BPQ-SNEDDs possess high loading and excellent stability to tropical temperatures while allowing for the complete dissolution of BPQ in simulated gastrointestinal media. We hypothesise that the enhanced solubilisation capacity of BPQ in the GI tract is responsible for the enhanced oral bioavailability. Adsorption of prepared BPQ-SNEDDs on solid carriers (glycol chitosan) and lyophilisation resulted in a solid nanomedicine that can be reconstituted to yield stable BPQ-SNEDDs of nanomolar in vitro activity.

INTRODUCTION:

Visceral Leishmaniasis (VL) is the second deadliest parasitic disease after malaria [1]. Since there are no effective vaccines to prevent Leishmania infections, management of VL relies on parenteral antimonials (first-line), pentamidine, paromomycin, and amphotericin B or its lipid formulations. Miltefosine is the only oral therapy but, the risk of resistance is very high which reduces its clinical usefulness [1]. Treatment of parasitic diseases are hampered by the lack of an oral technology platform able to deliver antiparasitic poorly soluble drugs in adequate amounts to the tissues of interest (liver and spleen), along with increased cost, low safety margin and thermal instability of available effective therapies. Buparvaquone (BPQ), an antiprotozoal hydroxylnaphtoquinone with known anti-Leishmaniasis activity in vitro (ED_{50}: 0.05-0.1μM), has not been translated into an effective therapy due to its low aqueous solubility (< 30 ng mL^{-1}, BCS Class II drug) [1,2]. The current project is aimed at enhancing the solubilisation capacity of BPQ in the gut and its oral bioavailability by encapsulation in a lipid based self-nanoemulsifying drug delivery systems (SNEDDs) and develop an oral thermally stable and ideally solid nanomedicine for the treatment of VL.

EXPERIMENTAL METHODS:

Pseudo-ternary phase diagrams were constructed to identify the microemulsion regions and to optimize the concentrations of oil (Oleoyl macrogol-6 glycerides, Labrafil M1944CS), surfactant (Caprylocaproyl macrogol-8 glycerides, Labrasol), and cosurfactant (Propylene glycol monocaprylate, Capryol 90) [2]. BPQ was solubilised in Labrasol and the oil and co-surfactant were added. BPQ SNEDDs were left stirring (50 rpm) over 24h in a waterbath at 37°C. BPQ loading was quantified after centrifugation of prepared SNEDDs containing 10, 20, 50 mg g^{-1} of SNEDDs (14,000 rpm for 15 minutes) using a modified RP - HPLC method [Phenomenex Onyx Monolithic C18 column (5μm, 4.6 mm x 10+100+100 mm), flow rate 1.5 mL min^{-1}, injection volume: 40 μL] [2].BPQ SNEDDs were characterised for particle size (PCS), zeta potential and morphology (TEM). The dissolution of BPQ from BPQ-SNEDDs loaded capsules (NP Caps™, pullulan, Capsugel) was studied in the flow through cell (USP, Apparatus IV) at various pH levels [USP 2013 simulated gastric fluid without pepsin (1.2), acetate buffer (4.5) and phosphate buffer (6.8), flow rate 6ml min^{-1}] [3]. Stability studies of BPQ SNEDDs were performed at 40± 2°C and 75±5% relative humidity (RH) over 3 months. BPQ - solid SNEDDs were prepared by adsorption of BPQ - SNEDDs (2mls, BPQ 1% w/w) on glycol chitosan (200kDa, 0.4g) and mixed in a mortal with lactose (3.1g) prior to the addition of 60mls of water and lyophilisation. Solid SNEDDs were characterised using PXRD and DSC. The in vitro anti-leishmanial activity against *L. infantum* promastigotes was performed for both BPQ-SNEDDs and BPQ - solid SNEDDs [4]. RP-HPLC was used to analyse acetonitrile extracted plasma samples obtained after oral administration of BPQ SNEDDs and BPQ aqueous suspension prepared by probe sonication (50% output, 200 watt for 10 minutes).

RESULTS AND DISCUSSION:

Phase diagrams illustrated that the optimal microemulsion region can be achieved using an oil to a constant surfactant: co-surfactant ratio of 1:6.8 w/w or above. BPQ SNEDDs (Labrafil M1944CS: Labrasol: Capryol 90: BPQ 30: 59 : 10 : 1% w/w/w/w). BPQ-SNEDDs and reconstituted BPQ - solid SNEDDs were quasispherical and below 400nm and (228 ± 1nm and 279 ± 52 nm, polydispersity index: 0.121 ± 0.017 and 0.365 ±0.042, zeta potential: -22 ±

4.4mV and -8.94 ± 4.2 mV respectively) (Figure 1A, B) [5]. The maximum loading of BPQ was identified to be 16.92 ± 1.59 mg g^{-1} in BPQ- SNEDDs and 5.71 ± 0.54 mg g^{-1} for BPQ - solid SNEDDs. Near complete release (89.3±1.9%) was observed in flow-through cell studies with BPQ-SNEDDs filled capsules within 30 minutes (pH1.2) (Figure 1C). Drug loading and particle size of BPQ-SNEDDs remained unaltered over 3 months (40 ± 2°C, 75±5% RH, p<0.05).

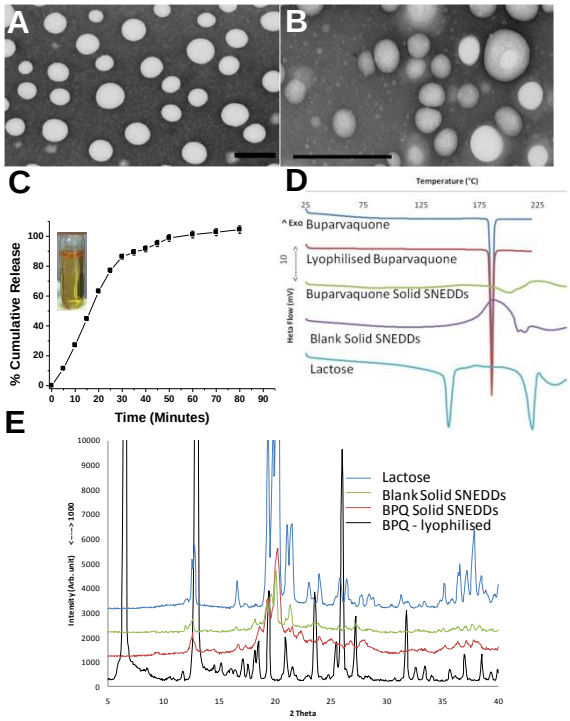

Figure 1: A,B; Transmission electron microscopy images of BPQ-SNEDDs (1:200 dilution) and reconstituted BPQ - Solid SNEDDs (1µg mL^{-1} in water – 200kDa glycol chitosan) (Bar: 500 nm), C; % Cumulative BPQ release from BPQ-SNEDDs (pH 1.2: 0-30min, pH 4.5: 30-60 min, pH 6.8: 60-80 min), D; DSC curves at 10°C min^{-1} of BPQ - solid SNEDDs and E; PXRD analysis of BPQ - solid SNEDDs

BPQ and lyophilized BPQ are crystalline materials characterized by a strong endothermic peak at 183°C corresponding to the melting point of the drug (Figure 1D). BPQ-SNEDDS exhibit an endothermic peak at the same onset temperature that the unmodified drug but also glass transition and crystallization peaks which indicate that the drug is at least partially amorphised. BPQ exhibits Bragg peaks characteristic of crystalline material even after lyophilisation (Figure 1E). BPQ – solid SNEDDs resulted in fewer Bragg peaks which were broader and of lower intensity. The absence of the characteristic peaks of BPQ (2θ°: 6.55) indicates the amorphisation of BPQ.

BPQ – SNEDDS and BPQ – solid SNEDDs showed potent in vitro efficacy in the nanomolar range against *L infantum* similar to BPQ in DMSO (IC_{50} for all <37nM) while having negligible cytotoxicity. BPQ-SNEDDs significantly enhance the bioavailability of BPQ compared to aqueous dispersions of BPQ after oral administration (55% increase in plasma AUC $_{0-24}$).*

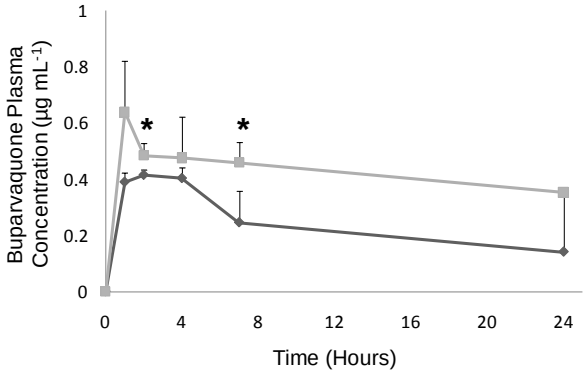

Figure 2: Buparvaquone plasma concentration after oral administration of BPQ (aqueous suspension – probe sonicated, rhombus) and BPQ-SNEDDs (1.1% w/w, square) formulations to CD-1 mice (n=3, Student t-test: *p<0.05, dose: 6 mg kg^{-1} of BPQ).

CONCLUSION:

The present study demonstrates for the first time enhancement of the oral bioavailbility of a poorly soluble antiparasitic drug, buparvaquone, in mice with SNEDDs. BPQ-SNEDDs illustrated high loading, particle size below 400nm, quasispherical morphology, excellent stability at tropical conditions and in vitro efficacy in the nanomolar range. Reconstitution of prepared BPQ - solid SNEDDs by adsorption of BPQ-SNEDDs on glycol chitosan and lyophilisation resulted in a liquid nanoemulsion with comparable in vitro efficacy. Studies on the dissolution properties and the oral bioavailbility and efficacy of BPQ- solid SNEDDs are underway. Developed SNEDDs or solid-SNEDDs prepared from GRAS excipients are cost-effective, stable oral alternatives for the delivery of poorly soluble antiparasitic drugs.

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