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Novel self-assembled nano-tubular mixed micelles of Pluronics P123, Pluronic F127 and phosphatidylcholine for oral delivery of nimodipine: *In vitro* characterization, *ex vivo* transport and *in vivo* pharmacokinetic studies

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ABSTRACT

Subarachnoid hemorrhage (SAH) is a major cause of death in patients suffering from stroke. Nimodipine (NM) is the only FDA-approved drug for treating SAH-induced vasospasm. However, NM suffers from poor oral bioavailability (5–13%) due to its low aqueous solubility, extensive first pass metabolism and short elimination half-life (1–2 h). The objective of this study was to develop NM-loaded Pluronic/phosphatidylcholine/polysorbate 80 mixed micelles (PPPMM) that can solubilize NM in aqueous media even after dilution, prolong its circulation time, improve its bioavailability and eventually help in targeting it to the brain tissue. PPPMM formulations were prepared using the thin film hydration technique, and evaluated for drug payload, solubilization efficiency (SE), micellar size, zeta potential, transmission electron microscopy (TEM) and *ex vivo* transport through rat intestine. The selected NM-loaded PPPMM, containing PC to Pluronics® molar ratio of 75:25, showed a drug payload, SE, micellar size and zeta potential of 1.06 ± 0.03 mg/mL, 99.2 ± 2.01%, 571.5 ± 11.87 nm and −31.2 ± 0.06 mv, respectively. The selected formulation had a much larger hydrophobic core volume for solubilization of NM and exhibited the highest NM transport. TEM micrographs illustrated the formation of highly flexible nano-tubular mixed micelles (NTMM). The *in vivo* pharmacokinetic study showed greater bioavailability of NM in plasma (232%) and brain (208%) of rats from NM-loaded PPPMM compared to that of the drug solution due to the efficiency of flexible NTMM to enhance absorption of NM from the intestinal mucosa. The significant increase in drug solubility, enhanced drug absorption and the long circulation time of the NTMM could be promising to improve oral and parenteral delivery of NM.

1. Introduction

Subarachnoid hemorrhage (SAH) is a serious, life-threatening type of stroke where cerebral vasospasm remains a serious complication and a major cause of death and disability in these patients. Nimodipine (NM) is a dihydropyridine calcium antagonist with therapeutic indications for cerebrovascular spasm, stroke and migraine (Gelmers, 1985; Langley and Sorkin, 1989). Recently, NM has been shown to be effective in ameliorating memory degeneration and preventing senile dementia in the old age (Pantoni et al., 2000; Zhang, 1993). NM is also used for cerebral malaria, cerebral vasospasm, acute ischemic stroke, and migraines (Ahmed et al., 2000; Cabrales et al., 2010; Togha et al., 2012; Wolf et al., 2010). NM is the only FDA-approved drug for treating SAH-induced vasospasm. The oral administration of NM has several limitations and disadvantages. NM belongs to Class II of the Biopharmaceutical Classification System (BCS) with the typical characteristics of high permeability and poor solubility (Fu et al., 2013). The substantial factors that limit its oral bioavailability (5–13%) and clinical efficacy are the very low aqueous solubility (3.86 μg/mL) and the extensive first pass metabolism in the liver (Soliman et al., 2010; Sun et al., 2008). NM was also found to have a very short half-life (1–2 h) with subsequent need for frequent dosing (every 4 h) (Langley and Sorkin, 1989).

Among several drug carriers currently under investigation for improved drug absorption and efficacy, nanocarriers hold the greatest promise (Soliman et al., 2010). Inclusion of poorly soluble drugs into polymeric mixed micelles has been found to be very attractive concept for solubilization and bioavailability enhancement (Wei et al., 2009; Zhao et al., 2012). Polymeric micelles are

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nanocarriers based on amphiphilic block copolymers of hydrophilic and hydrophobic chains that self-assemble in water above the critical micelle concentration (CMC) (Abdelbary and Tadros, 2013). Pluronic mixed micelles have a core–shell structure which enables the system to incorporate poorly soluble drugs. These systems exhibit many advantages such as targeting ability, long circulation and easy production. In addition, they can also inhibit P-gp enhancing drug absorption (Chiappetta and Sosnik, 2007). However, the low stability of these polymeric micelles upon dilution in the bloodstream or gastrointestinal fluids and the consequent drug precipitation circumvent their use in drug delivery.

Pluronics® P123/F127 mixed micelles (PMM) are considered to be kinetically stable due to the stabilization effect of long polyethylene oxide (PEO) chains of hydrophilic F127 blended with P123 in micelles which might prevent the stacking of cylindrical aggregates formed by the long polypropylene oxide (PPO) chains of P123 (Wei et al., 2009). However, these micelles will finally separate since they are thermodynamically unstable. It was assumed that more hydrophobic mixed micelles display lower CMC and concentrations remain above those values even after high dilution (Chiappetta and Sosnik, 2007). A small concentration of vegetable oil was introduced into Pluronic solutions to decrease micelle degradation upon dilution while not compromising the drug loading capacity of oil-stabilized micelles (Rapoport, 1999). The ability of mixed micelle formulation to keep the drug in solubilized form after dilution in the GI tract is a key factor in bioavailability enhancement rather than solubility of the drug in the formulation itself. Therefore, it seems necessary to develop new hydrophobic thermodynamically stabilized PMM that could resist precipitation upon dilution through incorporation of phosphatidylcholine (PC) in micelle structure.

Herein, NM-loaded Pluronic/PC/polysorbate 80 mixed micelles (PPPMM) were developed after incorporation of PC and Polysorbate 80 as examples of hydrophobic and hydrophilic molecules, respectively in an attempt to increase the thermodynamic and kinetic stabilities of these micelles. PC has the ability to enhance oral absorption and bioavailability through improving portal blood absorption and lymphatic delivery. (Marczylo et al., 2007; Mourao et al., 2005; Shanmugam et al., 2011; Sugawara et al., 2001). Moreover, it was reported that polysorbate 80 acts also as *p*-glycoprotein and/or CYP450 enzymes inhibitors decreasing the intestinal efflux and drug biotransformation (Basalious et al., 2010). It was assumed that the incorporation of PC would increase the thermodynamic stability of the micelles due to the tight hydrophobic interactions with hydrophobic PPO blocks and the consequent reduction of CMC. Increasing the hydrophobic character of these copolymers favors also the transition of morphology of micellar systems in aqueous solutions from spherical into worm-like (Khimani et al., 2012). To the best of our knowledge, no attempt has been reported to increase the solubilizing efficiency of PMM through incorporation of PC in the micellar structure.

The objective of this study was to develop NM-loaded PPPMM that can solubilize NM in aqueous media at clinically relevant concentrations even after dilution, prolong its circulation time, reduce its frequency of administration and eventually target it to the brain tissue. Investigations have been carried out to elucidate the formation of spherical and tubular micelles and studying the effect of the different morphologies on drug permeation. NM-loaded PPPMM were evaluated for drug payload, solubilization efficiency (SE), micellar size, zeta potential and *ex vivo* transport through intestinal membranes of rats. The *in vivo* pharmacokinetic behavior of the optimum PPPMM formulation in plasma and brain tissue was compared to NM solution following oral administration to rats.

2. Materials and methods

2.1. Materials

NM and amlodipine besylate (IS) were kindly donated by Marcyrl for pharmaceutical industries (Cairo, Egypt). Difunctional block copolymers of ethylene oxide/propylene oxide [Pluronic® F127 and Pluronic® P123] were purchased from Sigma chemicals company (St. Louis, USA). L-α-Phosphatidylcholine (PC) from soybean was purchased from MP Biomedicals (Santa Ana, California, USA). Spectra/Pore® dialysis membrane (12,000–14,000 molecular weight cut off) was purchased from Spectrum Laboratories Inc. (CA, USA). Disodium hydrogen phosphate was procured from Merck (Darmstadt, Germany). Ethanol and Polysorbate 80 were from El-Nasr Chemical Co. (Cairo, Egypt). All the materials were used as received without any further modifications.

2.2. Preparation of nimodipine-loaded Pluronic/PC/polysorbate 80 mixed micelles (PPPMM)

NM (10 mg), phosphatidylcholine (33, 66, and 100 mg) and Pluronic® (F127 and P123) mixture in the ratio 2:1 (100, and 200 mg) were accurately weighed and dissolved in ethanol (10 mL) in a round-bottom flask. Polysorbate 80 (20% of the phosphatidylcholine content) was dissolved in the solution. The solvent was slowly evaporated at 60 °C under reduced pressure using a rotary evaporator (Buchi R-110 Rotavapor, Flawil, Switzerland) revolving at 120 rpm for 1 h until a thin dry film was formed on the inner wall of the flask. The dried film was treated with distilled water (~9 mL) and the flask was allowed to revolve at a fixed hydration temperature of 60 °C for 30 min under normal pressure. The mixture was then sonicated for 1 min and the volume was adjusted into 10 mL at room temperature (25 °C) to obtain nanocarrier dispersions. For comparative purpose, liposomes containing 100 mg of phosphatidylcholine and PMM containing 200 mg

Table 1
Composition of nimodipine-loaded PPPMM, PMM and liposome and their characterization values.

Formula	Total Pluronics mixture (mg)	PC (mg)	PC: Pluronics molar ratio	Tween 80 (mg)	Payload (mg/mL)	SE (%)	PS (nm)	PDI	Zeta potential (mV)
PPPMM-1	100	33	75:25	6.6	1.10 ± 0.01	93.7 ± 2.31	665.2 ± 12.56	0.55 ± 0.09	−23.1 ± 0.09
PPPMM-2	100	66	85:15	13.2	0.95 ± 0.02	92.5 ± 1.48	550.1 ± 14.12	0.55 ± 0.08	−22.3 ± 0.05
PPPMM-3	100	100	90:10	20	1.05 ± 0.03	94.1 ± 1.97	458.6 ± 9.08	0.53 ± 1.02	−25.6 ± 0.04
PPPMM-4	200	33	60:40	6.6	0.97 ± 0.01	98.6 ± 1.99	549.4 ± 8.47	0.79 ± 1.10	−24.4 ± 0.05
PPPMM-5	200	66	75:25	13.2	1.06 ± 0.03	99.2 ± 2.01	571.5 ± 11.87	0.43 ± 0.06	−31.2 ± 0.06
PPPMM-6	200	100	80:20	20	1.08 ± 0.05	97.4 ± 2.09	761.1 ± 15.89	0.35 ± 0.03	−30.5 ± 0.04
Liposome	–	100	–	–	0.97 ± 0.04	81.8 ± 2.35	359.4 ± 11.24	0.24 ± 0.01	−28.4 ± 0.04
PMM	200	–	–	–	0.98 ± 0.05	87.9 ± 2.87	123.56 ± 4.56	0.21 ± 0.02	−2.3 ± 0.01

All formulations contained 10 mg NM.
PC: phosphatidylcholine, SE: solubilization efficiency, PDI: polydispersity index, PS: particle size, PMM: Pluronic p123/F127 mixed micelles.