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Development and Evaluation of a Novel Drug Delivery: Pluronics/SDS Mixed Micelle Loaded With Myricetin *In Vitro* and *In Vivo*

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ABSTRACT

This study is to prepare and evaluate Pluronics-modified mixed micelle (MM) to deliver polyphenolic myricetin (MYR) across the blood-brain barrier. MYR has been proven to be an effective anticancer agent against glioblastoma cells in our previous studies. However, the poor solubility of MYR limits its access to the brain. In this study, the feasibility of preparing lipid-based MM that combined sodium dodecyl sulphate (SDS) with Pluronic F68 (F68) and Labrasol was investigated. Furthermore, the nonionic surfactant coating technology for the protection of MYR against oxidation, and its attainment in oral bioavailability was examined. On account of the altered biomaterial properties of F68/SDS–modified lipid-based micelles, myricetin-loaded mixed micelles (MYR-MMs) were prepared by solvent-evaporation method to self-assembly into MMs. The average size of MYR-MMs was 96.3 nm, with negatively charged potential and spherical in shape. The drug loading of MYR-MMs was high with the increased grafting ratio, the more prolonged drug release profile, and more effective killing glioblastoma cells *in vitro*. Moreover, MYR-MMs showed a higher preference for the brain than free MYR alone, suggesting the novel MMs loaded with MYR could promote absorption and increase relative bioavailability. Taken together, the F68/SDS–modified and Labrasol-modified lipid-based micelles may provide a promising method to deliver polyphenolic compounds across the brain to treat brain tumor.

Introduction

Myricetin (MYR) is a potential chemopreventer because of its involvement in the suppression of tumor-related processes, including oxidative stress, apoptosis, proliferation, metastasis, and inhibition of mutant p53 expression.[1] Although much less researched, MYR appears to be a promising flavonoid for the treatment of cancers, such as blocking migration and invasion of lung cancer and colon cancer cells, predominantly through inhibition of MMP-2 expression and ERK phosphorylation.[2,3] MYR has also received greater attention as a proapoptotic flavonoid with specific and almost exclusive activity on glioblastoma cell lines. As reported by Chiu et al.,[4] MYR exhibits similar inhibitory effects on phorbol myristate acetate–induced U87 cells. However, the poor bioavailability and aqueous solubility of MYR limit its application for the treatment of brain tumor. Moreover, the polyphenolic compounds are easily decomposed in factors of light, high temperature, microorganism, moisture, and pH level; for example, MYR is easy to degradation and very unstable at high pH or temperature.[5] Several traditional methods, such as auxiliary solvent, the use of mixed solvents, prodrug formation by chemical modification, liposomal preparation, or use of cyclodextrin inclusion compounds, have been used to improve the solubility of MYR.[6,7] However, only the enhancement of the aqueous solubility and dissolution rate of MYR cannot prevent its rapid degradation at high pH and temperature; therefore, it should be considered for further formulation development of MYR to enhance both the solubility and prevention from its rapid degradation so as to exert its bioactivity.

In recent years, a novel drug delivery, mixed micelle (MM) has gained increasing attention as drug delivery to enhance the solubility and bioavailability of poorly soluble drugs, most of these chemotherapeutics.[8,9] Based on biodegradable polymeric micelles, the novel nanocarriers with favorable properties to treat brain tumor should have the ability to deliver across physiological barriers, such as the blood-brain barrier (BBB).[10,11] It is reported by Contal et al. that the interfacial cross-linking "when the polymeric micelles are self-assembling nanoconstructs of amphiphilic copolymers replaced by

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an oligomer or polymer. In this case, amphiphilic polymers are able to self-organize into a supramolecular structure in an aqueous solution that possessing a hydrophobic central core and a hydrophilic crown."[12] To reduce the polydispersity and improve the stability of polymeric micelles, the cross-linking could happen either in the hydrophilic part or in the core after the micelles are formed. Studies also have shown that sodium dodecyl sulphate (SDS) is the immobilization of micelles that combined with solubilization for antioxidation phenomenon.[13] Furthermore, polyphenolic fractions in SDS-micelles could reduce α-tocopheroxyl radical to effectively protect α-tocopherol (α-TOH).[14] It is important to choose the surfactants for specific device to improve the biocompatibility of polymeric micelles. These nonionic surfactants, such as Pluronic F68 (F68), F108, and F127, are commonly used in drug delivery systems for pharmaceuticals and proteins[15-18] and as viscous injectable polymeric gels for drug delivery.[19,20] Composite solutions of biopolymers and surfactants are of significant importance because their mechanical and transport properties can be precisely tuned with appropriate combinations. Effective transport within tissue and drug delivery is critical to the success of such devices.[21,22]

In this study, to improve the water solubility and therapeutic efficacy of MYR, the incorporation of Pluronic F68, SDS, and Labrasol was used as biopolymers and surfactants, respectively, for the myricetin-loaded mixed micelles (MYR-MMs), and the novel F68/SDS—modified lipid-based micelles loaded with MYR were prepared by using the solvent-evaporation method. The characterizations, such as morphology, zeta potential, particle size, grafting ratio, encapsulation efficiency, and the *in vitro* releasing profile of the MYR-loaded micelles, were investigated. The antibrain tumor activity of MYR-MMs was evaluated *in vitro*. Moreover, the distribution and the pharmacokinetic parameters of MYR-MMs *in vivo* were also evaluated.

Materials and Methods

Materials

SDS and Pluronics F68 were purchased from BASF Aktiengesellschaft (BASF, Germany). PEG-8 caprylic and/or capric glycerides (Labrasol) were purchased from Gattefossé (St-Priest, France). Soy lecithin and polysorbate 80 were purchased from Shanghai Youngsun Foods Co., Ltd. (Shanghai, China). MYR was obtained from National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, Beijing, China); Annexin V and pyridine iodide (PI) were obtained from Sigma Chemical Co. (St. Louis, MO); Roswell Park Memorial Institute 1640 medium (RPMI1640), penicillin-streptomycin, trypsin-EDTA, and fetal bovine serum were obtained from GIBCO. U251 glioblastoma cells were purchased from American Type Culture Collection (Rockville, MD). U251 cells were grown as recommended at 37°C, 5% CO_2. All other chemicals used in the study were of analytical grade.

Preparation of Self-Aggregating Myricetin-Loaded Mixed Micelles

The MYR-MMs were prepared using the method of solvent evaporation. Briefly, MYR and phospholipids of soy lecithin (ratio of 1:10, w/w) were dissolved in 10.0 mL of ethanol-acetone mixed solvent (ratio of 1:1, v/v) at 40°C, with agitation at 200 rpm for 60 min, until a clear mixture was formed. Next, Labrasol and polysorbate 80 (ratio of 4:1, w/w) were diluted with 8.0-mL deionized water and added into the mixture with agitation at 200 rpm for 60 min until the MMs formed. Finally, 12.0-mL prewarmed distilled water was added into the MMs, and the solution was evaporated in a rotator evaporator at 100 rpm at 70°C to obtain a thin film, then vacuum dried overnight at room temperature. The thin film was dissolved in 10.0-mL deionized water, and the micelles were produced by adding water-soluble SDS and Pluronics F68 to obtain the MYR-loaded F68/SDS—modified lipid-based MMs. The mixture solution was dialyzed against deionized water using dialysis membrane (molecular weight cutoff 6000-8000 Da) for 24 h to obtain MYR-MMs preparation.

The control micelles were also prepared by the same method without adding MYR at any stage of the preparation.

Characterizations

The zeta potential and particle size of MYR-MMs were determined by laser size scattering determinator (Zetasizer Nano-ZS90, Malvern, UK). The MYR-MMs were filtered through a filter membrane (0.45 μm) and were negatively stained using 0.5% uranyl acetate, and images were taken using transmission electron microscopy for morphologic characterization.

Measurement of Encapsulation Efficiency and Drug Loading

The drug loading content (DLC) and drug loading efficiency (DLE) of MYR-MMs were separated by using a Sephadex LH-20 (GE Healthcare Biosciences) column. Briefly, Sephadex G-50 is pretreated with 60% alcohol, squeezed into a 5-mL syringe. Distilled water is run through the injector 1 h 0.2 mL/min to remove air bubbles. Then, pretend to sample list, with 95% ethanol as mobile phase at the flow rate of 1.0 mL/min. The eluate was collected from 0-30 min, and the concentrations of MYR were determined by HPLC (DIONEX Utimate-3000 with a dual λ absorbance detector) at 364 nm. A flow of 1.0 mL/min was used for the eluent, which was made up of methanol and/or 0.3% phosphoric acid (55:45) from 0-15 min. The DLC and DLE were calculated by using the following equations:

$$\mathrm{DLC}(\%) = \text{amount of drug}/(\text{amount of polymer} + \text{drug}) \times 100\%$$

$$\mathrm{DLE}(\%) = \text{amount of drug in micelles}/\text{amount of the feeding drug} \times 100\%$$

Peak area (A) as ordinate, concentration (C) as abscissa of linear regression, the standard curve equation: $A = 1.96C + 14.46$, ($R = 0.9996$), limit of quantitation: 5.0~500 μg/mL, reproducibility for loading content and entrapment efficiency of MYR (RSD = 1.19%, $n = 5$).

In Vitro *Release*

The drug release was performed in simulated gastric fluid (SGF, 0.1-N HCl) conditions. For the SGF experiment, the drug release was performed under buffer solutions. A typical procedure in the present study was as follows: an aqueous dispersion of MYR-MMs (1.0 mg/mL, 2.0 mL) was transferred to a dialysis bag (molecular weight cutoff = 6000-8000 Da; Thermo Fisher Scientific Inc., Waltham, MA), and then the dialysis bag was immersed in 50 mL of SGF solutions (with pH values of 2, 4, 6, and 7). The samples (2.0 mL) were taken at predetermined time intervals for estimating the amount of drug released from 0.25 to 48 h, except for the use of pure PBS solution to replace the SGF solution. The amount of MYR in the supernatant was determined by HPLC. A series of parallel experiments was conducted. Each measurement was done in triplicate, and the data were shown as the mean value plus a standard deviation (±SD).

Anticancer Effect on Glioblastoma Cells

MTT Assay

The cell viability and the sensitivity to drugs was determined in triplicate using the MTT assay, the 96-well microplates were seeded