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Soy Lecithin Liposome as Potential Platform for Intravenous Delivery of Quercetin in Cancer Treatment

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Abstract

Naturally occurring compounds having anticancer properties are considered as attractive candidates for the treatment of cancer. Quercetin (QR; 3,3′,4′,5,7- pentahydroxylflavone), one of the most important and abundant flavonoid in the human diet, recently gained much attention for its direct proapoptotic effect on various types of tumors and little to no toxicity to normal cells, which were well-documented in numerous *in vitro* and *in vivo* studies. In spite of the common presence in many edible fruits and vegetables, QR has poor oral absorption, resulting in very low bioavailability. Besides, the poor aqueous solubility, rapid body clearance, and instability in physiological environment also hinder the intravenous administration of QR. In this study, liposome formulated from eco-friendly soy lecithin (SLP) was developed and exploited for QR encapsulation, creating an aqueous intravenous delivery platform to overcome QR's limitations. QR-SLP was prepared by thin film hydration method, followed by the investigation into the influence of hydration media (deionized water, PBS pH 5.7, and PBS pH 7.4) on QR-SLP's size, polydispersity index (PDI), zeta potential, and drug encapsulation capacity. Transmission electron microscopy (TEM), dynamic light scattering (DLS), zeta potential measurement, differential scanning calorimetry (DSC), and UV-Vis spectrophotometer method were used for the characterizations. Results showed that hydration media had a strong influence on the size, PDI, and encapsulation capacity of the formulations, in which QR-SLPs hydrated with PBS pH 7.4 possess the smallest size (93.7 ± 4.9 nm) with low polydispersity and the highest drug loading efficacy (~95.18% for loading efficiency (LE) and ~8.65% for loading capacity (LC)) whereas those hydrated with PBS pH 5.7 have the largest size (303.7 ± 3.5 nm) with high PDI value and the lowest loading efficacy (~87.33% for LE and ~7.99% for LC). Moreover, all synthesized formulations were spherical in shape and had negative surface charges from -40 to -50 mV. QR was slowly released from QR-SLPs up to 24 h, showing a controlled and long-lasting release. Taken together, all of the obtained results demonstrated that QR-SLP hydrated with PBS pH 7.4 could serve as a potential platform for intravenous QR delivery, thus improving the applicability of QR in cancer treatment.

Keywords:

Quercetin, Soy Lecithin,

Liposome,

Drug Delivery System,

Cancer Treatment.

1. Introduction

Anticancer agents derived from naturally occurring plant-based compounds has gained increasing attention for cancer treatment and prevention [1]. Among various phytochemicals found in plants, Quercetin (QR) is an important and ubiquitous flavonoid in the human diet that owns antiproliferative and direct proapoptotic effect on multiple malignant tumors such as breast, ovarian, cervical, prostate, pancreas, colon, liver, and lung. It exerts anticancer properties also in the cells of osteosarcoma, melanoma, several leukemia and lymphoma [2-5]. In *in vivo* studies, the inhibitory effect of QR on carcinogen-induced tumors has been demonstrated in several animal models and considered as Phase I clinical

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trial anticancer drug [2, 5]. Moreover, its little to no toxicity to normal cells with lack of adverse side effects have been proved in human [6, 7]. Hence, there is no doubt that QR could become a highly potential anticancer drug. Unfortunately, dietary consumption of QR cannot provide sufficient amount to compatible with the anticancer effectiveness since oral bioavailability of QR is only ~2% [6, 8] in spite of its common presence in many edible fruits and vegetables (i.e. apples, onions, capers, berries, lovage leaves, etc.) [6, 7]. Besides, the poor aqueous solubility, rapid body clearance, and instability in physiological environment also hinder the intravenous administration of QR [2, 6, 9]. In this case, higher dose together with increased administration frequency of QR are needed, leading to increased tendency of side effects and cost of treatment. Various strategies have been taken into consideration to overcome those limitations, including chemical modification of QR to improve its solubility. However, the drug potency could be lost [6, 10]. Therefore, utilization of drug delivery systems for QR delivery has been focused.

Liposomes – spherical vesicles comprising of at least one lipid bilayer enclosed aqueous core have been widely known as versatile carrier for drug delivery application by virtue of their biodegradability, high compatibility, ability to entrap both hydrophilic and hydrophobic agents, preferential accumulation at tumor site through enhanced permeation and retention (EPR) effect, and release the cargo in controlled manner. In addition, drug entrapped inside liposomes can be better protected from the degradation in physiological condition [11, 12]. Properties of liposomes are considerably altered with reference to lipid composition, synthetic method, and particle size and surface charge. Regarding the choice of bilayer components, the rigidity/fluidity of liposomes' lipid bilayer were strongly influenced as well. Among the choices, the use of soy lecithin (SL), which is a natural and unsaturated phospholipid obtained from soybean, for liposomes preparation not only yields much more permeable liposomes but also facilitates large-scale industrial production thanks to the reduced production costs compared to saturated phospholipids [11]. Several studies have been carried out on the benefit of using SL to prepare soy lecithin liposomes (SLPs) [11, 13, 14].

Previous study reported that in the presence of lipids, the absorption of QR is improved and the elimination of QR is also delayed. QR displayed a high affinity towards liposomes as well which was attributed to QR's planar configuration, thus facilitating the embedding of QR into organized structure of phospholipid bilayer of liposomal vesicles [15, 16]. As revealed in other studies, better entrapment of QR in lipid layers during film formation would be refer to the apparent lipophilicity of QR [17]. Furthermore, liposomes are considered as one of the most successful nano-platform as compared to other nanocarriers with many liposomal formulations that are underwent clinical trials and approved. Thus, encapsulation of QR into liposomes, specifically SLPs, would be of great interest.

Herein, liposome formulated from eco-friendly SL (SLP) was developed and exploited for QR encapsulation, creating an aqueous intravenous delivery platform to overcome QR's limitations, improving the applicability of QR in cancer treatment. QR-SLP was prepared by thin film hydration method. Notably, the influences of different hydration media at different pH (deionized water, PBS pH 5.7, and PBS pH 7.4) on QR-SLP's size, polydispersity index (PDI), zeta potential, and drug encapsulation capacity were investigated. Differential scanning calorimetry (DSC), transmission electron microscopy (TEM) image and release behavior of entrapped QR from optimized QR-SLP were characterized.

2. Materials and methods

2.1. Materials

Quercetin and cholesterol were purchased from Sigma-Aldrich (St Louis, MO, USA). Lecithin from soybean (SL) and Tween 80 (polyoxyethylene sorbitan monooleate) were obtained from Tokyo Chemistry Industry Co., Ltd. (Kita-ku, Tokyo, Japan). Cetyltrimethylammonium bromide (CTAB) was purchased from Merck (Darmstadt, Germany). Methanol and chloroform were supplied by Fisher Scientific (Houston, TX, USA). All chemicals and solvents were of highest analytical grade and used without further purification.

2.2. Preparation of QR-SLPs using different hydration media

QR-SLPs were prepared by thin film hydration method. Briefly, SL and cholesterol (molar ratio 9:1) together with 1% CTAB dissolved in chloroform was mixed with QR dissolved in methanol at room temperature (chloroform: methanol = 2:1 v/v), in which the weight ratio between lipid phase and QR is 10:1. The mixture was dried to thin film using a rotary evaporator (Buchi Rotavapor R-144, Essen, Germany) for 4 h at 45^{o}C and maintained under vacuum condition overnight to remove all traces of solvents. Next, the thin film was hydrated with different hydration media (deH_2O, PBS pH 5.7, or PBS pH 7.4) containing Tween 80 (0.5%, v/v) by stirring at 60^{o}C for 2 h, followed by the intermittent probe sonication of 30 min. Finally, the suspension was homogenized using ULTRA-TURRAX® Tube Drive (IKA, Germany) to give QR-SLPs.

2.3. Characterizations

Differential scanning calorimetry (DSC) of SLPs, QR, QR-SLP prepared with deH_2O as hydration medium (QR-SLP-1), QR-SLP prepared with PBS pH 5.7 as hydration medium (QR-SLP-2), and QR-SLP prepared with PBS pH 7.4

as hydration medium (QR-SLP-3) were measured using TGA/DSC 3^+ (Mettler Toledo, USA). Samples were heated in range of 30-400°C at a scanning rate of 5°C/min.

Hydrodynamic size, polydispersity index (PDI), and zeta potential of synthesized QR-SLPs were determined by dynamic light scattering (DLS) Zetasizer (SZ-100, Horiba, Japan). All samples were sonicated for 15 min and measured at the detection angle of 90° and the temperature of 25°C using helium-neon (He-Ne) at 633 nm as incident beam. Zeta potential of QR-SLPs was also analyzed using SZ-100. Experiment was performed in triplicate.

Transmission electron microscopy (TEM) (JEM-1400; 300 kV, JEOL, Tokyo, Japan) was utilized to illustrate the morphology of optimized batch. Sample was prepared by placing a drop of sample solution (1 mg/mL in deH_2O) onto a carbon-copper grid (300-mesh, Ted Pella, Inc., USA) and air-drying for 10 min.

2.4. Determination of QR loading capacity

The loading capacities of QR-SLP-1, QR-SLP-2, and QR-SLP-3 were determined by indirect method regarding the initial fed QR and loaded QR, which was calculated indirectly from unloaded QR. QR-SLPs suspension was transferred to centrifuge tubes and centrifuged at 1000 rpm for 10 min in refrigerated ultracentrifuge (HERMLE Z 32 HK, Germany). The drug loading capacity including loading efficiency (LE) and loading content (LC) of QR-SLPs were quantified from supernatant after centrifugation by UV-Vis spectrophotometer (Shimadzu UV-1800, USA) at a wavelength of 372 nm and calculated according to equations as follows:

$$\text{LE (\%)} = \frac{\text{weight of fed QR - weight of unloaded QR}}{\text{weight of fed QR}} \times 100 \quad (1)$$

$$\text{LC (\%)} = \frac{\text{weight of fed QR - weight of unloaded QR}}{\text{weight of SLPs and QR}} \times 100 \quad (2)$$

2.5. In vitro QR release study

Dialysis method was used to analyze the release behavior of QR from optimized batch of QR-SLPs. A dialysis bag (MWCO 6-8 kDa) filled with 1 mL of QR-SLPs was immersed into vial containing 20 mL of PBS buffer (0.01 M, pH 7.4) containing 2% Tween 80. Then, the vial was placed in an orbital shaker bath for the horizontal shaking at 50 rpm and maintained temperature of 37°C. At specific time intervals, release medium (1 mL) was collected and equally substituted with fresh media. The collected samples were filtered (pore size = 0.22 µm) before being measured in triplicate by UV- Vis spectrophotometer.

3. Results and discussion

Regarding two aromatic rings linked by oxygen-containing heterocyclic ring, QR has lipophilic nature and a tendency to be entrapped within liposomal bilayer [3]. After being encapsulated into QR-SLPs, determination of the efficiency and the content of QR that are successfully entrapped inside SLPs is needed; and these parameters are important characteristic that directly affect the therapeutic effect of delivery nano-platform. Three formulations QR-SLP-1, QR-SLP-2, and QR-SLP-3 prepared using three different hydration media at different pH including deionized water, PBS pH 5.7, and PBS pH 7.4, respectively, were investigated firstly for the QR loading capacity. As shown in Figure 1, the overall loading capacity of SLPs for QR are high, which is mainly attributed to the lipophilicity of QR that ease the entrapment of it into SLPs' lipid phase and minimize the loss of QR to external aqueous medium. QR-SLP-3 hydrated with PBS pH 7.4 possess the highest LE (95.18 ± 2.91%) and highest LC (8.65 ± 0.91%) whereas those hydrated with PBS pH 5.7 have lowest loading efficacy (87.33 ± 1.27% for LE and 7.99 ± 0.27% for LC). This difference might be explained by the effect of pH of hydration media on the phospholipid membrane of QR-SLPs. At acidic pH (PBS pH 5.7), polar head groups of phospholipid chains are protonated and gained positive charge, causing destabilization of liposomal lipid layer and thus the leakage of entrapped QR within lipid bilayer. This phenomenon was also observed in cation lipids at low pH [18]. In physiological pH of 7.4, QR remains largely ionized since QR is a weak acid with a pK_a (negative log of dissociation constant) of 6.74. Proton from ionized QR now can partly compensate for the negative charge-gained phospholipid chains, thus stabilizing liposomal membrane. As expect, QR-SLP-3 has the most effective QR entrapment, impressively high as compared to previously reported QR-loaded liposomal systems [17, 19].

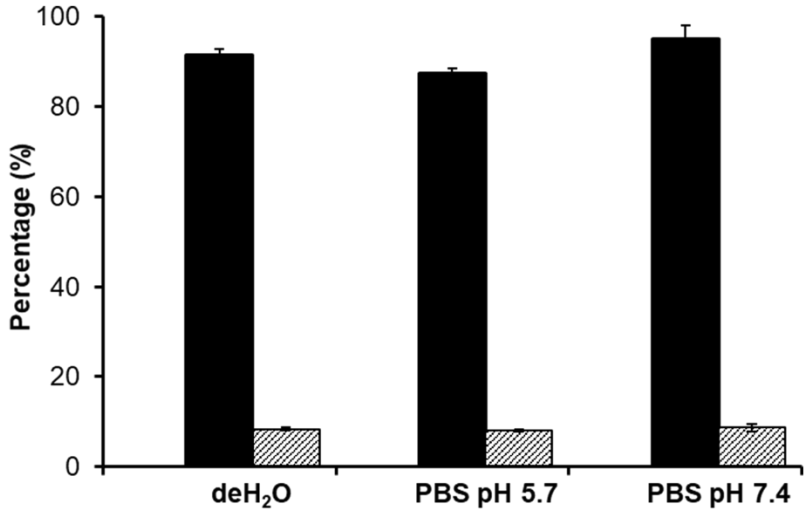

Figure 1. LE (black) and LC (diagonal) of QR-SLPs hydrated with deH_2O (QR-SLP-1), PBS pH 5.7 (QR-SLP-2), and PBS pH 7.4 (QR-SLP-3), respectively.

Others important properties that affect *in vivo* integrity and biological fate of a nano-platform are particle size and surface charge. As reported, nano-platforms with the size in range of 10-200 nm can be entrapped easily by endosome during endocytosis, thus facilitating the passive targeting and intracellular accumulation of nano-platforms within tumor cells through the EPR effect [20]. Consequently, both QR-SLP-1 (99.93 ± 3.45 nm), and QR-SLP-3 (93.70 ± 4.97 nm) have the potential to deliver QR more effectively to tumors, excepted for QR-SLP-3 with the size larger than 200 nm (Figure 2a). PDI values of them were very close to 0.4, indicating relatively homogenous nano-platforms.

The result showed that hydration media had a slight influence on the zeta potential of synthesized QR-SLPs. QR-SLP-1 hydrated with deH_2O depicted no significant change in zeta potential (~ -42 mV) in comparison with blank SLPs (-41.7 mV). Those hydrated with PBS media, especially at pH 7.4, showed drastic increased in zeta potential, which was around -50.8 mV. Negative surface charge not only averts the aggregation of nanoparticles but also prevent them from being phagocytized, improving the stability of QR-SLPs. Taken together, QR-SLP-3 with highest loading capacity as well as desirable particle size and stability might serve as optimized platform among three synthesized formulations.

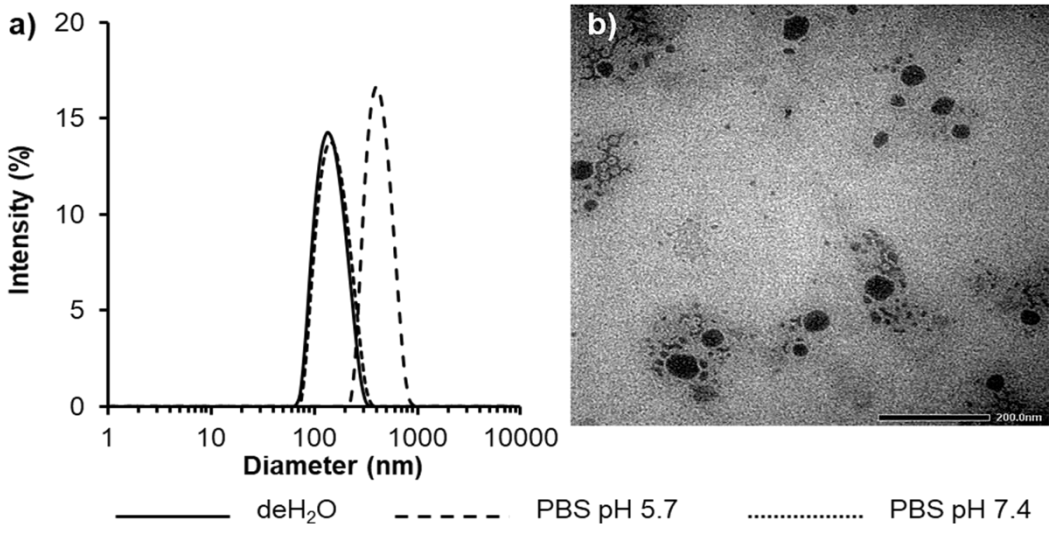

Figure 2. Particle size distribution of QR-SLP-1, QR-SLP-2, and QR-SLP-3 (a) and TEM image of QR-SLP-3 (scale bar = 200 nm) (b).

In the corresponding TEM image of QR-SLP-3 (Figure 2b), it is apparent that all particles are nearly spherical in shape and well separated. Further, DSC analysis confirmed the encapsulation of QR into SLPs (Figure 3). The complete absence of melting peak of QR at 308^{o}C implied the interaction of entrapped QR with liposomal lipid components, which is in accordance with previous results [17, 21, 22]. Moreover, an endotherm at 109^{o}C indicated the dehydration of QR as well as the evaporation of water from liposomal platform as aqueous dispersion [22].

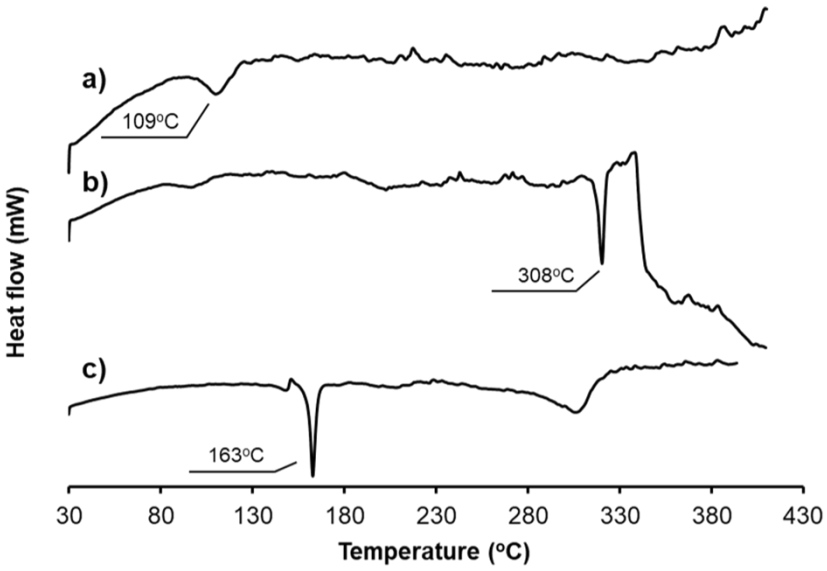

Figure 3. DSC thermograms of QR-SLP-3 (a), QR (b), and SLP (c).

Besides the ability to efficiently load the drug, efficient platform for intravenous delivery of QR should be capable of releasing them at controlled and sustained rate at the target site. As demonstrated in Figure 4, about 26.05% of QR was released in the first hour. This could be explained by the release of QR molecules that are absorbed into the outer phospholipid layer or loosely bound at lipid-water interface at the surface of SLPs. The cumulative amount of released QR increased up to 41% for the next 5 h, followed by a slower sustained release with merely 3.5% of cumulative QR detected more in the media for the last 18 h. It is clear that the entrapment of QR into SLPs platform led to sustained release manner thanks to the well-known reservoir effect of liposomes, as expected [17, 21]. This result suggest that it would be promising for utilizing SLPs to control and sustain the release rate of QR in intracellular delivery.

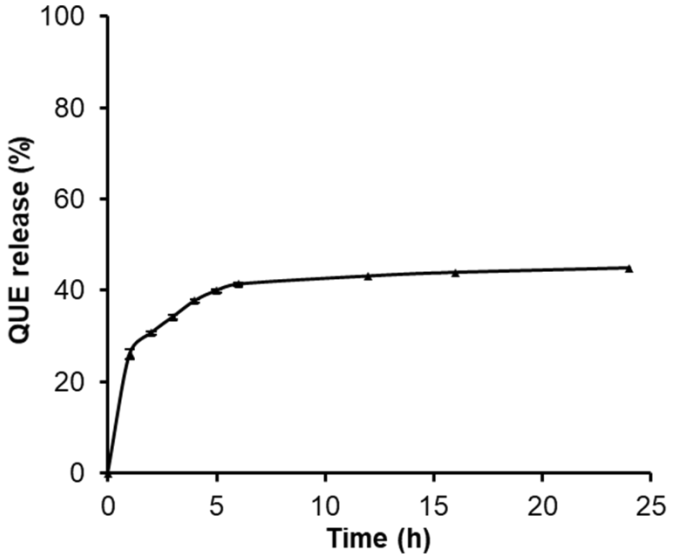

Figure 4. *In vitro* release profile of QR from QR-SLP-3.

4. Conclusion

To overcome QR's limitations, poorly soluble QR was successfully encapsulated into liposomes formulated from eco-friendly SL prepared by thin film hydration method using different hydration media at different pH values. Overall, QR-SLP-3 prepared with PBS pH 7.4 is the most promising one with the desirable size, low PdI, and good stability. More importantly, highest amount of QR (~95%) was detected in QR-SLP-3, which was impressively high as compared to previously reported QR-loaded liposomal systems. QR was released from QR-SLP-3 in a sustained manner as well. These obtained results demonstrated that QR-SLPs hydrated in PBS pH 7.4 could be a potential platform for QR delivery.

5. Conflict of Interest

The authors declare no conflict of interest.

6. Acknowledgements

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