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Berberine-loaded Nano-Liposomes Generated with Ethanol-Injection and Thin-film Hydration Methods

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1. Introduction

The poor solubility in water is very often a problem for active pharmaceutical substances of plant origin. The formulation of such drugs as liposomal preparations enables to improve the bioavailability of these drugs. Berberine (BBR) is a quaternary isoquinoline alkaloid derived from many native plant species (*Coptis* spp., *Berberis* spp., *Hydrastis canadensis* etc.). BBR has been traditionally used for the treatment of different disorders including hyper-cholesterolemia and cardiovascular diseases [1,2]. BBR has a strong antimicrobial activity enabling the use of it as an anti-diarrheal, anti-protozoal, fungal, candida, yeast, and parasitic intestinal active ingredient [3]. In addition, BBR has shown an anti-inflammatory, anti-diabetic, lipid peroxidation, and neuroprotective activity [3,4]. Unfortunaterly, BBR is poorly soluble in water and has a low bioavailability (<10%) due to the induced activity of multidrug efflux transporter P-glycoprotein (P-gp) in the intestine itself [2]. Such limitations associated with a poor oral bioavailability of BBR could be overcome by nanoformulating BBR to liposomes.

Pharmaceutical liposomes can be fabricated by ethanol-injection and thin-film hydration methods. The lamellarity, size, shape and ultra-structure of liposomes can be determined by using different advanced techniques, such as cryogenic electron microscopy (Cryo-EM), dynamic light scattering (DLS), size-exclusion chromatography (SEC), and atomic force microscopy (AFM) [5]. Confocal laser scanning microscopy (CLSM) has been also used for such imaging [6].

The aim of our study is to investigate ethanol-injection and film hydration methods for generating BBR-loaded liposomes and to study the structure, size, size distribution and entrapment efficiency of the liposomes. The liposomes are ultimately intended for the oral treatment of hypercholesterolemia.

2. Materials and methods

BBR (Sichuan Weikeqi Biological Technology Co., Ltd., China) was used as an active ingredient, and hydrogenated soy phosphatidyl choline (HSPC), distearoyl phosphatidylglycerol (DSPG), alpha-tocopherol (a-TP), and sodium deoxycholate (SDC) were used as liposome forming agents. The BBR-loaded liposomes were prepared by ethanol-injection and thin film-hydration methods. Liposomes were characterized (including polydispersity index, PDI) by Zetasizer Nano ZSP (Malvern Instrument Limited, UK), cryogenic electron microscopy (Cryo-EM) and coherent anti-stokes Raman scattering (CARS) microscopy (Leica TCS SP8 CARS, Germany). The entrapment efficiency (EE) was assessed with an UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan).

3. Results

The size, size distribution and EE of the BBR-loaded liposomes studied are summarized in Table 1. The morphology and internal structure of BBR-loaded liposomes fabricated by an ethanol-injection method were imaged by means of Cryo-EM. The liposomes were spherical and unilamellar

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Table 1 *The size, size distribution and entrapment efficiency (EE) of liposomes fabricated by ethanol injection and thin-film hydration method (n=3, mean ±SD)*

Exp.	Ethanol-injection			Thin-film hydration		
	Mean size (d.nm)	PDI	EE (%)	Mean size (d.nm)	PDI	EE (%)
1	133.6 ± 1.1	0.209 ± 0.008	55.9±2.4	248.7 ± 3.3	0.272 ± 0.053	60.5±2.6
2	117.3 ± 1.3	0.186 ± 0.004	57.8±2.1	236.0 ± 2.0	0.248 ± 0.009	64.0±1.4
3	82.34 ± 1.3	0.113 ± 0.004	51.0±2.5	-	-	-
4	-	-	-	182.8 ± 2.6	0.297 ± 0.022	77.5±1.5
5	120.4 ± 1.1	0.243 ± 0.001	58.4±2.5	292.0 ± 3.9	0.181 ± 0.033	67.6±3.1
6	-	-	-	209.0 ± 1.1	0.317 ± 0.037	90.6±1.4
7	243.6 ± 4.1	0.195 ± 0.012	81.8±1.4	208.2 ± 3.2	0.312 ± 0.031	87.3±0.8
8	91.2 ± 2.0	0.232 ± 0.015	88.2±1.2	448.6 ± 6.6	0.276 ± 0.016	91.9±4.2
9	50.9 ± 1.2	0.259 ± 0.009	64.9±2.4	239.2 ± 2.4	0.243 ± 0.009	78.0±1.7

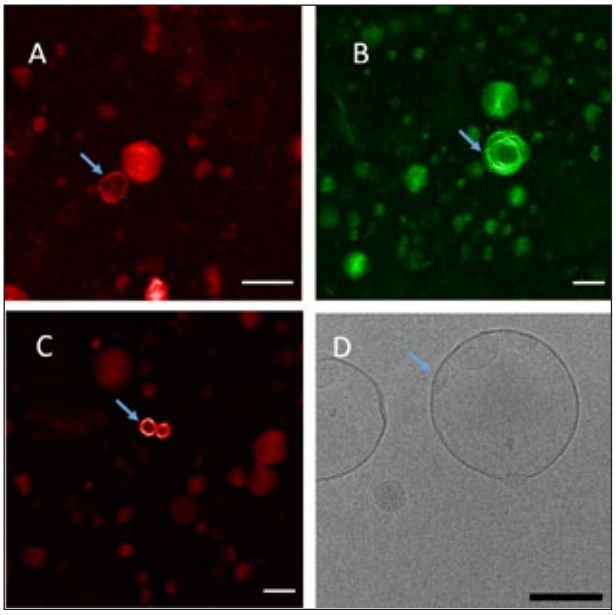

Figure 1 *CARS microscopy images (A-C) of the liposomes fabricated by a thin film hydration method (initial liposomes before extrusion). Scale bar = 10 µm. The Cryo-EM image (D) of the liposomes fabricated by an ethanol injection method. Scale bar = 100 nm*

vesicles with a single thin layer surrounding the entrapped aqueous phase (***Figure 1D***). The BBR-loaded liposomes fabricated by a thin-film hydration method exhibited different liposome forms (structures): multivesicular vesicles (MVV) (***Figure 1A***), multilamellar vesicles (MLV) (***Figure 1B***), and unilamellar vesicles (UV) (***Figure 1C***). In the background of the CARS micrographs (***Figure 1A-C***), numerous liposomes (with a spherical shape) can be distinguished, but due to the position of these liposomes in relation to a CARS laser source, their internal structure were not able to be imaged.

The BBR-loaded liposomes prepared by an ethanol-injection method presented the mean liposome size ranging from 50 nm to 244 nm, while the liposomes generated with a thin-film hydration method (before extrusion) exhibited a larger micron-scale size and variation in structure (***Figure 1A-C***). After extruding 60 times through a polycarbonate membrane (0.4 µm), however, the mean size in diameter of the present liposomes reduced significantly ranging ultimately from 182 nm to 449 nm (***Table 1***).

4. Conclusions

Ethanol-injection and thin-film hydration are feasible methods for generating BBR-loaded liposomes. The structure of the liposomes generated with an ethanol injection method is UV, and the liposomes obtained by a thin-film hydration method are MLV, MVV and UV types. The BBR-loaded liposomes with an uniform size and PDI can be fabricated. The EE of liposomes ranged from 51.0 ± 2.5% to 88.2 ± 1.2% in an ethanol-injection method and from 60.5 ± 2.6% to 91.9 ± 4.2% in a thin-film hydration method.

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References

1. *Neag, M.A., Mocan, A., Echeverria, J., Pop, M.R., Bocsan, C., Crisan, G., Buzoianu, A.D., Berberine: botanical occurrence, traditional uses, extraction methods, and relevance in cardiovascular, metabolic, hepatic, and renal disorders, Front. Pharmacol., 9: 557 (2018). doi:* 10.3389/fphar.2018.00557.
2. *Zhang X., et al., Intestinal absorption mechanisms of berberine, palmatine, jateorhizine, and coptisine: involvement of P-glycoprotein. Xenobiotica, 41(4): 290-296 (2011).*
3. *Do Tat Loi, Vietnamese medicinal plants and herbs, 8th Publication - Medical Publishing, 195 (2006).*
4. *Zhou J., Zhou S. Berberine regulates peroxisome proliferator activated receptors and positive transcription elongation factor b expression in diabetic adipocytes, Eur. J. Pharmacol. 649(1): 390-397 (2010).*
5. *Tonggu L., Wang L., Cryo-EM sample preparation method for extremely low concentration liposomes, Ultramicroscopy, 208:112849 (2020).*
6. *Ruozi B., Belletti D., Tombesi A., Tosi G., Bondioli L., Forni F., Vandelli M.A., AFM, ESEM, TEM, and CLSM in liposomal characterization: a comparative study, Int. J. Nanomedicine 6: 557–56 (2011).*