*Panax notoginseng*을 이용한 리보좀 운반체

김명훈*, 김선남[1], 김광수[2], 이수덕[3]

*캠브리지대학교 화학과, [1]나드리화장품㈜ 기술연구소, [2](주)에이티랩 기술연구소, [3](주)라누베 기술연구소
(mhk22@cam.ac.uk, myunghunkim@ymail.com*)

Liposome-Based Delivery Systems in *Panax notoginseng*

Myung Hun Kim*, Seon Nam Kim[1], Kwangsoo Kim[2], Sudok Yi[3]
*Department of Chemisty, University of Cambridge, Cambridge, CB2 1EW, UK
[1]Technology Institute, The Nadree Co. Ltd., Pyongtaik-Si, 451-863, Korea
[2]AT LAB Co. Ltd., SNU Business Incubator#5-105, Suwon-Si, 441-853 KOREA
[3]Technology Institute, LaNube Co. Ltd., Seoul 152-080, Korea
(mhk22@cam.ac.uk, myunghunkim@ymail.com*)

The herbal formulation *Panax notoginseng* (PN) is derived from the root of the traditional Korean herb *Panax notoginseng* which exhibits activities in modulating vascular tone such as promotion of blood circulation, removal of blood stasis, and alleviation of pain. Panax notoginsenoside (PNS) is the major active component of PN, including notoginsenoside R1 (R1, 2.74%), ginsenoside Rb1 (Rb1, 29.86%), ginsenoside Rg1 (Rg1, 20.46%), and ginsenoside Rd (Rd, 7.96%) [1-2]. Several studies on the physiological effects of PNS have been reported, such as anti-inflammatory action, vasodilator effect, protective effect on microcirculatory disturbance induced by lipopolysaccharides, hepatic fibrosis, and antioxidant effect by increasing the superoxidase activity in the blood [3-5]. However, PNS is poorly absorbed when administrated orally. Little R1 and Rb1 were absorbed from the digestive tract by oral administration to rats. It was also reported that the amount of Rg1 absorbed via oral administration was within 1.9%–20.0% of the dose. The low bioavailability of PNS could be explained by the following reasons: (1) the decomposition in the stomach, metabolism in the intestine, and elimination in the liver; (2) PNS are highly water-soluble substances with high molecular weight, which lead to low membrane permeability; (3) Rg1 and Rb1 can aggregate into micelles in PNS aqueous solution and be salted out in gastrointestinal tract fluid which contains electrolytes. Such an aggregation limits the permeation of PNS through the cell membrane of the gastrointestinal tract. To overcome these obstacles, PNS delivery using novel drug carriers will likely yield more promising clinical applications.

Liposomes as a novel platform technology provide an alternative to improve the drug delivery, which composed of a flexible bilayer and surrounded by an aqueous core domain. Liposome-based delivery systems plays an important role owing to easy preparation, increasing the bioavailability, and also offers drug targeting and controlled release [6]. In addition, charged liposomes could be as carriers to enhance the permeation through the skin in the transdermal drug delivery which are administered by the percutaneous route. Recently, it has been reported that liposomes had been employed in the field of *Panax notoginseng* and made encouraging successes [7]. Liposomes possess unique physical and chemical properties, which not only improve *Panax notoginseng* stability, bioavailability, and difficulty in penetration to some cells

but also enhance the pharmacodynamic action and induce the target.

The development of liposomes of *Panax notoginseng* is promising, which will be effectively used in the clinical application in the near future. This paper will concentrate on the recent research of *Panax notoginseng* liposomes, such as their experiment design method, preparation and formulation, characterization, and quality control, as well as *in vivo* and *in vitro* studies. We intend to summarize the progress in *Panax notoginseng*s liposomes with the aim to provide reference for research and development of *Panax notoginseng*.

Experimental

To prepare liposomes of *Panax notoginseng*, lots of factor should be considered such as extraction time of *Panax notoginseng*, the amount of water added into the plant, excipients to *Panax notoginseng* ratio, as well as emulsifiable time by ultrasound. Therefore, an ideal experiment design seems important. So far, orthogonal test is widely used to optimize the preparation conditions of *Panax notoginseng* liposomes. Zuozhen et al. take advantage of orthogonal design to investigate the preparation factor for the cordyceps sinensis Sacc polysaccharide liposomes [8]. The optimum preparation process received was emulsifiable time by ultrasound with 15 min, the plant extracted by water 3 times with 3 h for each time, 8 times in all the water was as much as the plant. Further study found that preparation conditions of astragalus *Panax notoginseng* liposomes optimized by orthogonal design; that is, lecithin to drug ratio was 10 : 1, lecithin to cholesterol ratio was 8 : 1, and ultrasonic time was 20min. The author concluded that the astragalus *Panax notoginseng* liposomes prepared under the optimized conditions had high encapsulation efficiency and active ingredients-loading rate, uniform shape, and particle size, as well as reproducible quality.

Results and Discussion

Determination of encapsulation efficiency

Liposome encapsulation efficiency (EE%) was determined using the ultrafiltration technique for separating the nonentrapped drug from liposomes.33 For this, 500 μL drug-loaded liposomal dispersion was placed in an ultrafiltration tube (Nanosep MF; Pall Corporation, Port Washington, NY) which was fitted with a filter membrane (molecular weight cut off: 10,000). The free drug in the underlayer solution was collected by centrifugation at 8000 rpm for 15 minutes (3-18K high-speed refrigerated centrifuge; Sigma, Germany) and the drug content (R1, Rb1, and Rg1) in the ultrafiltrate (Cfree) was determined by high-pressure liquid chromatography (HPLC) on a C18 Hypersil column (Thermo, Finnigan, UK) (250 × 4.6 mm, 5 μm) at 203 nm. Gradient elution was employed using solvent A (acetonitrile) and solvent B (water) at 25°C; the gradient program used was as follows: initial 0−15 minutes, linear change from A−B (20:80, v/v) to A−B (21.5:78.5, v/v); 15−36 minutes, linear change to A−B (40:60, v/v). The flow rate was kept at 1.0 mL · min−1 and the sample injection volume was 20 μL. Then 0.5 mL of liposomal suspension was diluted with 2.0 mL of a mixture (acetone:chloroform = 2:1, v/v) to determine the total drug (c_{total}) by HPLC. The EE% was calculated by:

$$\text{EE } \% = \frac{c_{total} - c_{free}}{c_{total}} \times 100$$

Stability

The physical stability of the products protected from light at 4°C was assessed by evaluation of the suspensions at predetermined time points.

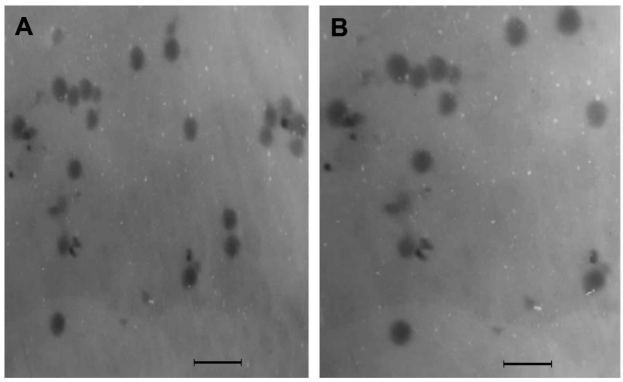

Fig. 1 Transmission electron microscopy images of (A) PNS-NP and (B) PNS-LP. Note: Bar is 500 nm.

In vitro release

The releases of R1, Rb1, and Rg1 from different PNS preparations, PNS solution, PNS-NP, PNS-LP, and PNS-HLV, were all evaluated. PNS-loaded nanoparticles were suspended in 10 mL

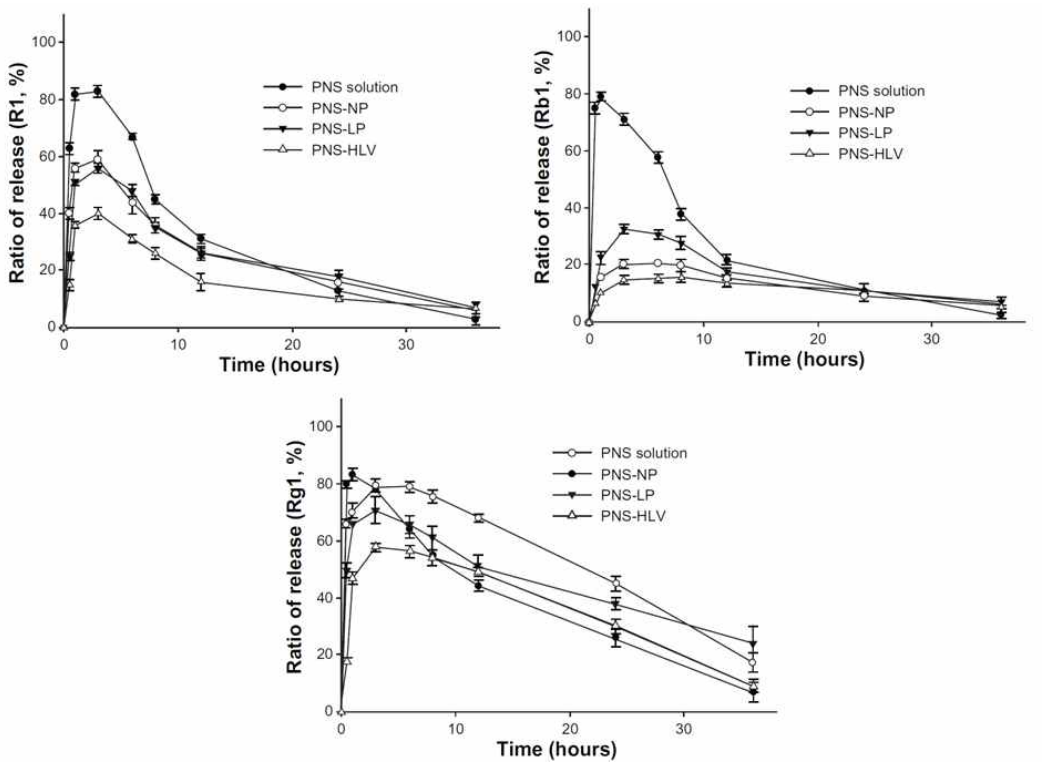

Fig. 2 Cumulative percentage release profiles of R1, Rb1, and Rg1 from PNS preparations at pH = 2 (n = 3). Abbreviations: PNS, panax notoginsenoside; PNS-HLV, panax notoginsenoside-loaded hybrid liposomal vesicles; PNS-LP, PNS-loaded liposomes; PNS-NP, PNS-loaded nanoparticles;

Table. 1 Physicochemical characterizations of PNS formulations (n = 3)

Type	Median particle size diameter (nm)	Zeta potential (mV)	EE%			
			R1	Rb1	Rg1	RSD
PNS-NP	117.1 ± 9.7	−18.7 ± 0.44	31.7 ± 1.4	72.4 ± 1.2	15.2 ± 2.0	74.0%
PNS-LP	147.0 ± 12.4	−22.5 ± 0.39	42.8 ± 1.8	65.6 ± 1.9	28.3 ± 1.1	41.3%

Abbreviations: PNS, panax notoginsenoside; PNS-LP, PNS-loaded liposomes; PNS-NP, PNS-loaded nanoparticles

EE% of all the three components of PNS was increased, especially for Rg1, increasing from 15.2% to 40.5%, dependent on the applied method. The water-in-oil-in-water double emulsion solvent evaporation method for the nanoparticle preparation was designed for the components with relative higher polarity and the thin film hydration method was applied for the components with relative lower polarity. Since the logP values of major active components of PNS, Rb1, R1, and Rg1, are -0.5618, 0.034, and 0.8, respectively, the EE% of Rb1 after first encapsulation in nanoparticles was the highest. Furthermore, the differences among the EE% of these three components of PNS, R1, Rb1, and Rg1, were shortened with the relative standard deviation (RSD) decreasing from 74.0% to 35.5%

Conclusion

Liposome-based *Panax notoginseng* delivery system has been considered as a promising carrier for *Panax notoginseng*, because it can improve *Panax notoginseng* stability and bioavailability, enhance the pharmacodynamic action, and induce the target.

Acknowledgements

This study was supported by a grant of Agriculture-Industry-Commerce Fusion R&D Project in the Korea Small & Medium Business Administration (SMBA) in Republic of Korea (Grant No. SA114199).

Reference

1. 1. Lei XL, Chiou GC, *Am J Chin Med.*, **14(3−4)**, 145−152 (1986).
2. Cicero AF, Vitale G, Savino G, Arletti R, *Phytother Res.*, **17(2)**, 174−178 (2003).
3. Chen ZH, Li J, Liu *J, Am J Chin Med.*, **36(5)**, 939−951 (2008).
4. Peng XD, Dai LL, Huang CQ, He CM, Yang B, *Biochem Biophys Res Commun.*, **388(1)**, 31−34 (2009).
5. Luo FC, Wang SD, Qi L, Song JY, Lv T, Bai J., *J Ethnopharmacol.*, **133(2)**, 448−453 (2011)..
6. R. Rajera, K. Nagpal, S. K. Singh, D. N. Mishra, *Biological and Pharmaceutical Bulletin*, **34(7)**, 945−953 (2011).
7. L. Ni, H. Caixia, Z. Cheng, *Chinese Journal of Modern Applied Pharmacy*, **26(8)**, 620−623 (2009).
8. F. Zuozhen, G. Dongxiu, Z. Xiaoting, *China Pharmaceuticals*, **14(8)**, 50−51 (2005).