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The Use of Lipid-Based Formulations to Increase the Oral Bioavailability of Panax Notoginseng Saponins Following a Single Oral Gavage to Rats

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Purpose: **This article was intended to improve the absorption of ginsenoside Rg1 and Rb1 of Panax notoginseng saponins (PNS).** ***Methods:*** **PNS-Phospholipid complex and a lipid-based formulation by dissolving the complex in the medium chain fatty glycerides were prepared, and their oral relative bioavailability was determined in rats and compared with an aqueous solution of PNS for each component.** ***Results:*** **The study gave evidence that the phospholipids could combine with the two active constitutes of PNS and form a PNS-phospholipid complex. The complex efficiently increased the solubility of hydrophilic ginsenoside Rg1 and Rb1 in some selected hydrophobic esters, such as fatty glycerides, and constructed the lipid-based formulations of PNS. The experimental result in rats in vivo showed that the oral relative bioavailability was enhanced remarkably by these lipid-based formulations composed of the PNS-Phospholipid complex and various esters. The absorption enhancement of the medium-chain glyceride (Labrafac cc and Capmul MCM (3:1)) was somewhat greater than that of other fatty glyceride. The area under the plasma concentration-time curve (AUC) of ginsenoside Rg1 and Rb1 of the PNS-complex in the medium-chain glyceride were 27.38 μg.mL-1.h and 600.08 μg.mL-1.h, compared with 2.52 μg.mL-1.h and 92.29 μg.mL-1.h of the PNS aqueous solution, respectively.** ***Conclusions:*** **The oral relative bioavailability of ginsenoside Rg1 and Rb1 of PNS was enhanced remarkably by the lipid-based formulations. These findings reveal a new strategy to increase oral bioavailability by lipophilicity enhancement for some highly water-soluble but poorly absorbed drugs.**

Keywords Panax notoginseng saponins; ginsenoside Rg1; ginsenoside Rb1; PNS-phospholipid complex; oil solution; oral relative bioavailability

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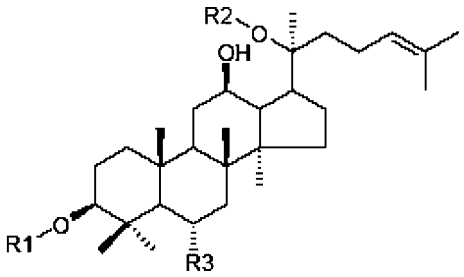
INTRODUCTION

Panax notoginseng is used as a general health remedy in many countries and also as a therapeutic agent in traditional Chinese medicine. Various pharmacologic effects of Panax notoginseng have been demonstrated in the literature (Shen, 2000; Zheng, 1994). The Panax notoginseng saponins (PNS), containing ginsenosides saponins, mainly triterpenoid dammarane derivatives, are phytochemically extracted from Panax notoginseng, and have been regarded as the principal components manifesting the pharmacologic activities. Ginsenosides can be structurally classified into two groups, namely, the protopanaxadiol ginsenosides and the protopanaxtriol ginsenosides (Figure 1).

Ginsenoside Rg1, one of the main triol saponins, possesses the properties of exciting the central nervous system, antifatigue, and hemolysis. Ginsenoside Rb1, which belongs to the diol saponins, shows effective anti-inflammatory action, an obvious vasodilator effect, and tranquilizing function to the central nervous system (Benishin, Lee, Want, & Liu, 1991; Takino, 1994).

PNS are very soluble in water but poorly absorbed when administrated orally. Odani and colleagues (1983) report that the amount of ginsenoside Rg1 absorbed via oral administration was within the range of 1.9% to 20.0% of the dose. It was also reported that little ginsenoside Rb1 was absorbed from the digestive tract by orally administering it to rats (Takino, Odani, Tanizawa, & Hayashi, 1982). The low oral bioavailability of PNS was caused by the hydrolysis in the stomach acid environment (Hasegawa, Sung, Matsumiya, & Uchiyama, 1996; Kanaoks, Akao, & Kobashi, 1994), metabolism in the intestine, and elimination in the liver. The bioavailability of ginsenoside Rg1 and Rb1 after portal venous administration was 50.56% and 59.49%, respectively (Han & Fan, 2006; Han, Sha, Wu, & Fang, 2006).

There are few reports about the pharmaceutical formulation study to enhance the oral absorption of PNS. In this paper,



Protopanaxdiol group

Compounds	R1		R2	R3
Ppd	H	H	H	460
Rb1	Glc2-Glc	Glc6-Glc	H	1108
Rc	Glc2-Glc	Glc6-Ara(f)	H	1078
Rd	Glc2-Glc	Glc	H	946
Ppt	H	H	-OH	476
Re	H	Glc	-O-Glc2-Rha	946
Rg1	H	Glc	-O-Glc	800

Glc = Glucose; Xyl = xylose; Ara(f) = α-L-arabinofuranosyl; Rha = rhamnopyranosyl.

FIGURE 1. The structure of main ginsenosides of PNS.

PNS-phospholipid complex was investigated, which was expected to increase its lipophilicity. The lipid-based formulations of PNS were obtained by dissolving the PNS-phospholipid complex in the fatty glycerides. The solubility of ginsenoside Rg1 and Rb1 was examined while the PNS-phospholipid complex was prepared and dissolved in fatty glycerides in the absence of water. Dissolution in vitro of ginsenoside Rg1 and Rb1 from the PNS-phospholipid complex and its oil solution was studied. The oral relative bioavailability of ginsenoside Rg1 and Rb1 from the complex alone and from the lipid-based formulation was determined in rats in vivo and was compared with the PNS aqueous solution.

MATERIALS AND METHODS

Materials

PNS was purchased from Kunming Phytopharmaceutical Co., Ltd. (Yunnan, PR China), which was phytochemically extracted from the roots of Panax notoginsneg. The percentage contents of Rb1 and Rg1 in PNS were 36.95% and 30.45%, respectively. Phospholipids were purchased from Tai-wei-yao-ye Ltd.; the phosphatidyl content was approximately 82% (w/w), and the ratios of C18 to C16 and saturated lipids to unsaturated lipids were 4.88 and 0.30 (w/w), respectively. Labrafil M 1944cs, Labrafac cc, and Plurol oleique CC 497 (Polyglyceryl-6 dioleate) were kindly gifted by GATTEFOSSE (France). Olive oil purchased from Nanjing Chemical Reagent Co., Ltd. is oral Pharmaceutical excipient. Capmul MCM was supplied by Karlshamns Lipid Specialties (USA). Acetonitrile (Merck, Germany) and methanol (Sandong Yuwang Industrial & Commercial Co., Ltd.) were of High Performance Liquid Chromatography (HPLC) grade. Tetrahydrofuran was purchased from Nanjing Chemical Reagent Co., Ltd., and other chemicals were of reagent grade.

Sprague–Dawley male rats weighing about 220 g to 250 g were obtained from the Jiangsu animal breeding center in Nanjing, and the studies were approved by the Animal Ethics Committee of China Pharmaceutical University.

Methods

Preparation of PNS-Phospholipids Complex

PNS of 1.0 g and phospholipids of 1.2 g were placed in a 100 mL round-bottom flask and dissolved in anhydrous tetrahydrofuran of 10 mL. The complex was kept at 55°C under magnet stirring for two hours. After tetrahydrofuran was evaporated under vacuum at 40°C, the dried residues were gathered and placed in desiccators overnight, then crushed in the mortar and sieved with an 80 mesh. The resulting PNS-phospholipid complex was transferred into a glass bottle, flushed with nitrogen, and stored at room temperature.

Solubility Determination of PNS

Solubility in Chloroform. When 20 mg of PNS powder, phospholipid complex, and physical mixture of PNS and phospholipids equivalent to 20 mg of PNS were each dissolved in 10 mL of chloroform by vortexing for one minute at room temperature, the clarity and state of the resultant solutions was observed.

Solubility of PNS in the Mix Oil of Labrafac cc and Capmul MCM (3:1). The ginsenoside Rg1 and Rb1 were determined when excessive amounts of PNS, PNS-phospholipid complex, and physical mixture of PNS and phospholipids were each added to a 2 mL mixture of Labrafac cc and Capmul MCM (3:1) in sealed glass containers at 37°C. Each experiment was performed in triplicate. The liquids were agitated for 24 hours and then centrifuged to remove the excess PNS (10 minutes, 12000 rpm). The 1 mL supernatant was mixed with 9 mL of methanol. A 20 μl aliquot was injected into HPLC and the concentration of Ginsenoside Rgl and Rb1 was measured.

Preparation of PNS Lipid-Based Formulations

PNS lipid-based formulations were obtained by wetting the PNS-phospholipid complex with plurol oleique CC 497 and then dissolving it in different oil vehicles at 60°C to produce an homogeneous and clear lipid-based formulation with a concentration of 90.9 mg PNS in 1.0 g of oil.

Dissolution Test In Vitro of Ginsenosde Rg1 and Rb1 from PNS-Phospholipid Complex and its Oil Solution of Labrafac cc/Capmul MCM (3:1)

Accurately weighed PNS-phospholipid complex and its oil solution of Labrafac cc and Capmul MCM (3:1) were

dispersed in water and retained in an incubator at 37°C with successive shaking (200 rpm) to evaluate the dissolution profiles of ginsenoside Rg1 and Rb1. The weight ratio of the aqueous phase and the formulation was 1:10. At predetermined time intervals, the aqueous phase was taken and the concentrations of ginsenoside Rg1 and Rb1 were analyzed by HPLC.

Relative Bioavailability Study of PNS Aqueous Solution, PNS-Phospholipid Complex, and Lipid-Based Formulations in Rats

PNS aqueous solution (120 mg/mL), PNS-phospholipid complex in aqueous solution (120 mg/mL), and the PNS-phospholipid complex in different oil vehicles (120 mg/g) were each administered orally at a dose of 600 mg/kg to fasted rats with free access to water. Blood samples were collected at 5 minutes, 20 minutes, 40 minutes, 60 minutes, 1.5 hours, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours, 48 hours, and 72 hours, postadministration, under light ether anaesthesia.

HPLC Assay of Ginsenoside Rg1 and Rb1

Blood samples were pretreated with solid phase extraction (SPE) cartridges (OASIS®, Waters, USA). The mean recoveries for Rg1 and Rb1 were 91.88% and 92.06%, respectively, using 0.2 mL of plasma for extraction. The lower limits of quantification were 26.7 ng and 40 ng in each 50 μl injection on the HPLC column for Rg1 and Rb1, respectively. The lower limits of concentration were 534 ng/mL and 800 ng/mL for Rg1 and Rb1, respectively. The mean recoveries for Rg1 and Rb1 were 91.88% ± 5.27% and 92.06% ± 5.93%. The intra- and inter-day assay precision for ginsenoside Rg1 and Rb1 were less than 5.0%. Linearity of the calibration curves was found over the range of 80 ng to 2000 ng for ginsenoside Rg1, and 120 ng to 2000 ng for Rb1. The equation of the calibration curve of Rg1 was, where M is drug mass and A is peak area, $M = 13.179A+20.522$ ($r = 0.9991$); the Rb1 equation was $M = 16.42A+47.591$ ($r = 0.9993$). This method had been demonstrated to be highly sensitive and accurate for the determination of Rg1 and Rb1 in rat plasma.

HPLC was carried out using a Diamonsil C18 reverse phase column (5 μm, 4.6 mm × 250 mm). An Agilent 1100 liquid chromatography system, equipped with a binary solvent delivery system, an autosampling device, and a UV detector, was used. UV absorption was measured at 203 nm.

Gradient elution of the analytes was performed using water (A) and acetonitrile-water (65:35 v/v) (B). Initial condition was A:B (62:38 v/v), linearly changed to A:B (36:64 v/v) at 32.5 minutes. The post time was six minutes. The column temperature was maintained at 35°C, and the flow rate was 1 mL/min.

Pharmacokinetic Data Analysis

The AUCs for ginsenoside Rg1 and Rb1 after administration were obtained using the linear trapezoidal rule from time zero to the last measured time point, followed by the addition of the extrapolated tail area, calculated by dividing the last measured plasma concentration by the terminal rate constant.

The results are expressed as the mean ± *SD*, and statistical analysis was performed by the ANOVA test with $p = .05$ as the minimal level of significance.

RESULTS

Formation of PNS-Phospholipid Complex

The different quantity ratios of PNS to phospholipids were tested for obtaining an optimized complex. The complex prepared with the quantity ratio of 1:1.2 was a kind of fine, flaxen powder in appearance, which completely dissolved in chloroform to form a clear solution. The content of ginsenoside Rg1 and Rb1 in the complex were 46.74 mg/g and 51.59 mg/g (w/w), respectively.

When the ratio phospholipids to PNS was lower than 1:2, the material could not be dissolved completely in chloroform, which indicated that the PNS had not been completely bound with the phospholipids. However, while the quantity ratio was greater than 1:2, the appearance of resultant materials appeared viscous and it was hard to be further formulated.

The PNS powder or the physical mixture of PNS and phospholipids in the same ratio as the complex was directly added into chloroform, after which the solution became turbid and visible precipitation of PNS was observed.

Solubility of PNS in the Mix Oil of Labrafac cc and Capmul MCM (3:1)

Table 1 shows the solubility of ginsenoside Rg1 and Rb1 when PNS powder, physical mixture of PNS and phospholipids, and PNS-phospholipid complex were each separately dispersed into the mix oil of Labrafac cc and Capmul MCM (3:1). The data show that solubility of ginsenoside Rg1 of the PNS-phospholipid complex in the mix oil was 16.93-fold that of PNS powder, and was 1.84-fold that of the physical complex. The solubility of ginsenoside Rb1 was improved as well. Ginsenoside Rb1 of PNS powder could not be detected in the mix oil, but was determined to be as high as 0.709 ± 0.0266 mg/g compared with 0.515 ± 0.032 mg/g in the physical mixture when the PNS-phospholipid complex was dissolved in the mix oil.

TABLE 1
Solubility of Ginsenoside Rg1 and Rb1 in Labrafac cc/Capmul MCM (3:1)

	Solubility (mg/g; $n = 4$)	
	Rg1	Rb1
PNS powder	0.065 ± 0.00189	ND
Physical complex	0.596 ± 0.0505	0.515 ± 0.032
Phospholipids complex	1.095 ± 0.05	0.709 ± 0.0266

Oral Relative Bioavailability In Vivo in Rats

The Absorption of Ginsenoside Rg1 and Rb1 in Different Lipid-Based Formulations of PNS-Phospholipid Complex

Table 2 shows the relative bioavailability of ginsenoside Rg1 and Rb1 in rats after oral administration of the PNS-phospholipid complex dissolved in different oils. It was found that the oil vehicles enhanced the absorption of ginsenoside Rg1 and Rb1 of the complex in different degrees. The order of absorption enhancing for Rg1 was, from first to last: Labrafac cc/Capmul MCM (3:1), olive oil, Capmul MCM, and Labrafil M 1944cs. The improved absorption of ginsenoside Rb1 was, from greatest to least: Labrafac cc/Capmul MCM(3:1), Capmul MCM, olive oil, and Labrafil M 1944cs. The relative bioavailability of ginsenoside Rg1 to the PNS aqueous solution was 1086.51%, 848.41%, 569.44%, and 281.90%, respectively. The relative bioavailability of ginsenoside Rb1 compared with the PNS aqueous solution was 650.21%, 377.6%, 195.52%, and 120.92%, respectively.

Absorption of Ginsenoside Rg1 and Rb1 in PNS-Phospholipid Complex and the Complex in the Mix Oil of Labrafac cc/ Capmul MCM (3:1)

The absorption of ginsenoside Rg1 and Rb1 is summarized in Table 3. Figures 2 and 3 respectively show the mean plasma concentration versus time profiles of ginsenoside Rg1 and Rb1 obtained with PNS aqueous solution, PNS-phospholipid complex, and the complex dissolved in Labrafac cc/Capmul MCM (3:1). It was apparent that the markedly higher plasma levels of ginsenoside Rg1 and Rb1 were obtained when administered as dissolved complex in Labrafac cc/Capmul MCM (3:1), compared with those obtained as an aqueous solution. It was found that the peak plasma levels of ginsenoside Rg1 and Rb1 administered as PNS-phospholipid complex were even lower than that given as aqueous solution. Both the PNS-phospholipid complex and the oil solution of the complex appeared to be absorbed at a slower rate and much longer residence time, so as to achieve higher extent of absorption. However, no statistically significant difference was observed between the $AUC_{0-\infty}$ values of the PNS aqueous solution and the PNS-phospholipid complex alone.

The $AUC_{0-\infty}$ of ginsenoside Rg1 after oral administration of PNS aqueous solution, PNS-phospholipid complex, and the oil solution of the complex were 2.52 μg/ml.h, 4.02 μg/ml.h, and 27.38 μg/ml.h, respectively. A statistically significant difference was observed between the $AUC_{0-\infty}$ values of aqueous solution and oil solution groups ($p < 0.05$). The relative bioavailability of ginsenoside Rg1 in oil solution and PNS-phospholipid complex at a dose of 120 mg/mL was 1086.51% ($p < .05$) and 159.52% to PNS aqueous solution.

The $AUC_{0-\infty}$ values of ginsenoside Rb1 after oral administration of PNS aqueous solution, PNS-phospholipid complex, and the oil solution of the complex were 92.29 ± 34.29 μg/ml.h, 146.32 ±

TABLE 2
Effect of Oil Vehicles on the Oral Absorption of Ginsenoside Rg1 and Rb1 in PNS-Phospholipid Complex in Rats

Media (numbers of C atoms in fatty acid chain)	$AUC_{0-\infty}(\mu g^{-1}mL\ h)$		Relative Bioavailability	
	Rg1	Rb1	Rg1	Rb1
Aqueous solution	2.52 ± 0.94	92.29 ± 34.29	100%	100%
Labrafac cc/Capmul MCM (3:1) (C_8/C_{10})	27.38 ± 10.42	600.08 ± 207.80	1086.51%[a]	650.21%[a]
Capmul MCM (C_8/C_{10})	14.35 ± 9.49	348.49 ± 203.04	569.44%	377.60%[a]
Labrafil M 1944cs (C_{18})	7.23 ± 4.33	111.60 ± 51.32	286.90%	120.92%
Olive oil (C_{18})	21.38 ± 9.06	180.45 ± 42.89	848.41%	195.52%

[a]$p < 0.05$ compared with the control. Each value represents the $M \pm SD$ of four animals.

TABLE 3
$AUC_{0-\infty}$ Values of Ginsenoside Rg1 and Rb1 Following Administration as Aqueous Solution, Phospholipids Complex, and Complex in Labrafac cc/Capmul MCM (3:1)

Dosage Form	$AUC_{0-\infty}(\mu g^{-1}mL\ h)$		Absorption-Enhancing Factor	
	Rg1	Rb1	Rg1	Rb1
Aqueous solution	2.52 ± 0.94	92.29 ± 34.29	1	1
PNS-phospholipid complex	4.02 ± 1.80	146.32 ± 43.40	1.59	1.58
Oil solution of complex	27.38 ± 10.42	600.08 ± 207.80	10.86[a]	6.5[a]

[a]$p < 0.05$ compared with the control. Each value represents the $M \pm SD$ of four animals.

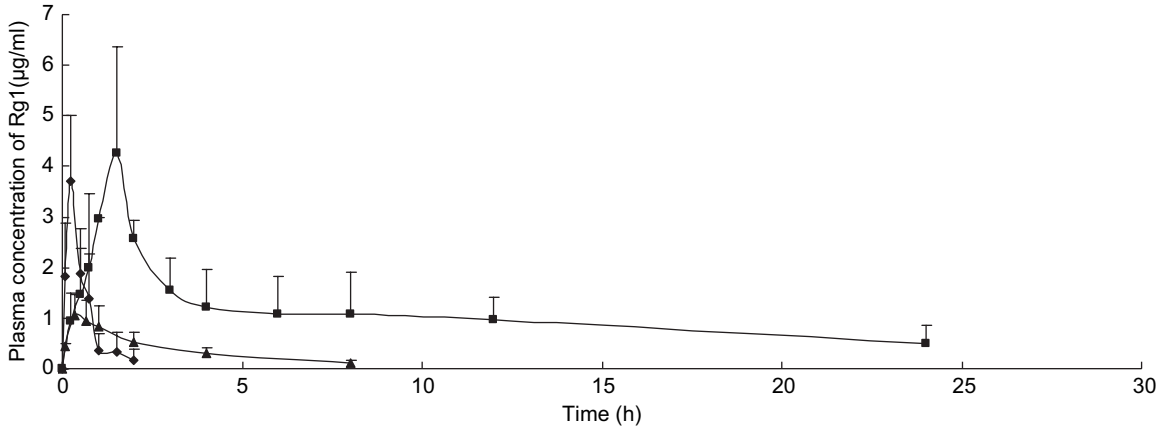


FIGURE 2. Plasma concentration time profiles of ginsenosides Rg1 after oral administration of PNS (600 mg/kg) to rats (each point represents the $M \pm SD$ of four animals): aqueous solution (◆—◆), phospholipids complex (▲—▲), phospholipids complex in Labrafac cc/Capmul MCM (3:1; ■—■).

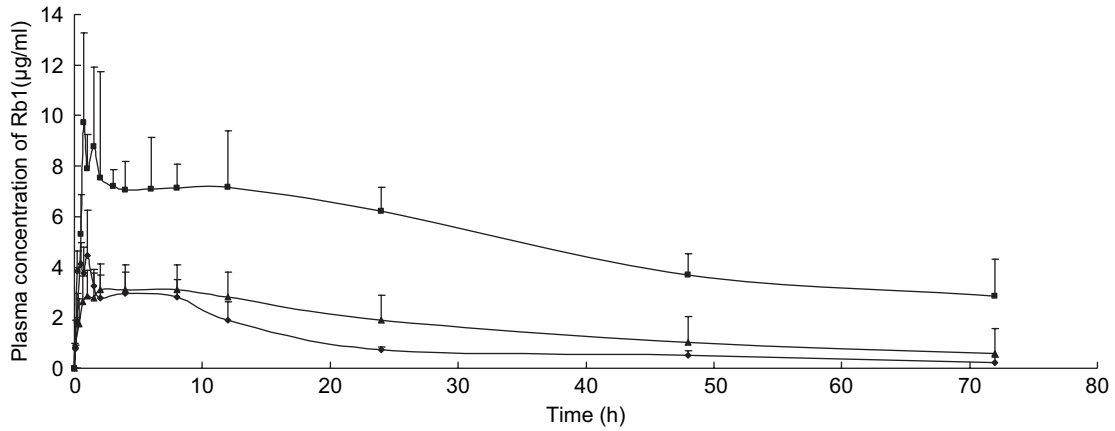


FIGURE 3. Plasma concentration time profiles of ginsenosides Rb1 after oral administration of PNS (600 mg/kg) to rats (each point represents the $M \pm SD$ of four animals): aqueous solution (◆—◆), phospholipids complex (▲—▲), phospholipids complex in Labrafac cc/Capmul MCM (3:1; ■—■).

43.40 μg/ml.h, and 600.08 ± 207.80 μg/ml.h, respectively. The relative bioavailability of ginsenoside Rb1 in the PNS-phospholipid complex and oil solution of the complex were 158.54% and 650.21% ($p < 0.05$) compared with the PNS aqueous solution.

Dissolution In Vitro of Ginsenoside Rg1 and Rb1 from PNS-Phospholipid Complex and its Oil Solution of Labrafac cc/Capmul MCM (3:1)

When the PNS-phospholipid complex was dispersed in water, PNS was immediately dissolved, and the aqueous phase was homogeneous and clear. Therefore, no release data of PNS-phospholipid complex was obtained. The resultant solution was lyophilized, and the lyophilization product could not be dissolved again in chloroform, which may be associated with the instability of PNS-phospholipid complex in water.

The release profiles of ginsenoside Rg1 and Rb1 from the complex oil solution of Labrafac cc/Capmul MCM (3:1) are presented graphically in Figure 4. It was shown that ginsenoside Rg1 and Rb1 are released from the formulation gradually at almost the same rate, and are released completely within one hour.

DISCUSSION

The Ways of Improving Oral Absorption of Highly Hydrophilic Drugs

The inefficient absorption is one of several serious problems in the delivery of highly hydrophilic drugs. Low membrane permeability is an important factor dominating the poor absorption. The permeability is proportionally related to molecular size (molecular weight) or partitioning into lipid cell membrane. PNS is a highly water-soluble substance and the molecular weights of ginsenoside Rg1 and Rb1 are larger than 500 dalton (800 dalton and 1108 dalton, respectively). There are also more than 5 H-bond donors in their structures. According to

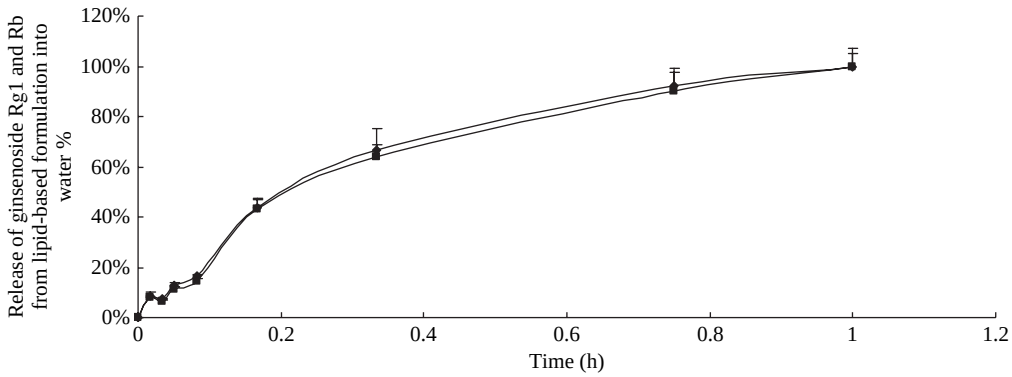


FIGURE 4. In vitro release of ginsenoside Rg1 and Rb1 in phospholipids complex with Labrafac cc/Capmul MCM (3:1) in aqueous phase (n = 3). Results were the $M \pm SD$: ginsenoside Rg1 (◆—◆), ginsenoside Rb1 (■—■).

the "rule of 5" (Lipinksi, Lombardo, Dominy, & Feeney, 1997), these characteristics are limiting factors for the absorption or permeability of the agents. To improve oral absorption and bioavailability of these compounds, many different strategies have been used.

One of the strategies used was chemical modification to increase the partitioning coefficient of these compounds. A-72517, a dipeptide renin inhibitor, was a structural relative of A-64662 (a first generation renin inhibitor). The latter was intravenously efficacious, but poor bioavailability had been shown for oral administration (Kleinert et al., 1992). With P3-site residue of A-64662 modified with sulfonamide moiety, A-72517 was the more lipophilic compound with a log P of 4.6 (in octanol-water, pH 7.4) and the oral bioavailability was been improved 10-fold (Buhlmayer et al., 1988).

Another alternative to enhance oral absorption was to coadminister these compounds with absorption enhancers. These enhancers may act as the openers of the transcellular or paracellular pathways or both (Lee, 1990). A variety of exogenous compounds, including Ca^{2+} chelators, surfactants, bile salts, fatty acids, and so on, had been identified to increase transcellular or paracellular permeability of these compounds (Swenson & Curatolo, 1992).

Nonparenteral routes of administration such as nasal and rectal (Sasaki et al., 2003), or other novel approaches such as liposomes (Muramatsu, Maitani, & Nagai, 1996) and nanoparticles (Cournarie, Auchere, & Chevenne, 2002) were developed to overcome the oral absorption restriction.

The method of utilizing a phospholipid-drug complex was used for some insoluble and poorly absorbed drugs. It was a relatively simple and safe way for pharmaceutical design and formulation, comparing with other methods described as above. For example, the oral bioavailability of silybin–phospholipid complex in rats was 4.33-fold higher than that of silybin-*N*-methylglucamine (Yanyu, Yunmei, Zhipeng, & Qineng, 2006). However, few efforts were made for those drugs, which have the properties of the high water solubility but poorly oral absorption. Our results show that the phospholipid-complex formation of ginsenoside Rg1 and Rb1 in PNS, and the two active agents of the complex, had the much higher solubility in both chloroform and oils than that of PNS powder. Ginsenosides are hydrophilic and cannot dissolve in chloroform, so the solution became turbid and visible precipitation of ginsenoside Rg1 and Rb1 was observed. However, once the complex was formed, it completely dissolved in chloroform, and the clear solution was formed. It suggested that the hydrophilic property and the partitioning coefficient of the components had been changed to some extent, which implied a possibility for the improvement of oral absorption. As shown in our results, the instability of the complex in water may prevent its practical application in pharmaceutical preparations. On the other hand, the results indicated the good solubility and better stability of the complex in oil vehicles, which made the pharmaceutical usage of the complex possible.

Effect of Different Oil Vehicles on the Oral Absorption of Ginsenoside Rg1 and Rb1 in the Phospholipids Complex in Rats

As shown in Table 4, when oil vehicles were used for the PNS-phospholipid complex, the absorption of ginsenoside Rg1 and Rb1 were improved significantly. As the medium-chain fatty glyceride, the enhanced mechanism of Labrafac cc/Capmul MCM (3:1) (C_8/C_{10}) may be different from that of the long-chain fatty glyceride, such as olive oil and labrafil M 1944 cs. The literature on lipid absorption suggests that the molecules of long-chain fatty glycerides access the intestinal lymph in preference to the portal bloods (Caliph, Charman, & Porter, 2000; Fukui et al., 1989; Nankervis, Davis, Day, & Shaw, 1996; Porter & Charman, 1997). The digestion products of long-chain triglycerides were preferentially resynthesized in the enterocyte, assembled into lipoproteins, and secreted into the mesenteric lymph, whereas medium chain triglycerides were primarily absorbed directly into the portal blood. And the digested lipids of medium chain triglycerides with bile salts formed lipophilic particles, and overcame the barrier of

aqueous diffusion layer in the gastrointestinal (GI) tract (Aungst et al., 1996; Kiyasu, Bloom, & Chaikoff, 1952; Lokikangas, Wilen, Einarsson, & Artursson, 1994; New & Kirby, 1999). In addition, it was reported that the medium-chain fatty glyceride had an enhanced effect on the intestinal cells to allow the lipid particles to pass across the cell layer (Beskid et al., 1988; Constantinides et al., 1994; Ueda, Shimojo, & Kozatani, 1983; Unowsky et al., 1988; Yeh, Berenson, & Samowitz, 1995). The results of the relative bioavailability indicate the existence of the possible absorption routes for the medium fatty glyceride, which would contribute to the improved absorption of ginsenoside Rg1 and Rb1, compared with long-chain fatty glyceride lipid vehicles.

Formulation made of Labrafac cc alone produced a turbid suspension. Proper amounts of Capmul MCM were employed in order to solubilize the PNS-phospholipid complex in Labrafac cc. It was found that the transition of formulation from turbidity to clarity occurred with increasing Capmul MCM, and the threshold point was about 3:1 (Labrafac cc/Capmul MCM).

In Vivo and In Vitro Study of PNS-Phospholipid Complex and its Oil Solution

The relative bioavailability of ginsenoside Rg1 in rats was slightly increased after oral administration of the PNS-phospholipid complex compared with the PNS aqueous solution. It was clear that PNS-phospholipid complex was unstable in water, so the effect may be due to the weak enhancement effect of phospholipids as one of the surfactants or emulsifiers. The relative bioavailability of ginsenoside Rg1 was remarkably improved after administration of the oil solution, which was 6.81-fold higher than that observed after administration of phospholipids complex.

Similar to the result of ginsenoside Rg1, the absorption of ginsenoside Rb1 was slightly enhanced as the PNS-phospholipid complex was used alone. The relative bioavailability was 158.54% after administration as a PNS-phospholipid complex and 650.21% ($p < .05$) after given as its oil solution of Labrafac cc/Capmul MCM (3:1) compared with aqueous solution.

Besides of the stability of the complex in an oil solution and the enhancing effect of the medium fatty glyceride, the prolonged absorption time due to the slow movement of the oil in the GI tract may be related to the improved relative bioavailability. The dissolution study demonstrated in vitro that ginsenoside Rg1 and Rb1 released much more rapidly in the aqueous phase when the PNS-phospholipid complex was directly dispersed in water than the solubilized complex in oil did. The oil could slow the diffusion rate of these active molecules to aqueous phase more effectively than the complex itself. The prevention of ginsenoside Rg1 and Rb1 molecules from diffusing into aqueous phase may be an important factor for the absorption enhancement. Both the enhanced lipophilicity and the prolonged residence time of these components in the GI tract were contributed to the increased absorption.

Furthermore, it's speculated that bile salt or mucous proteins in the GI tract may emulsify the oil when the oil solution iss diluted by GI liquid. As the absorption of PNS in aqueous solution was poor, the ginsenoside Rg1 and Rb1 molecules of PNS to be absorbed may be either partly wrapped by the phospholipid molecules in the oil drops, or at the interface between the oil drops and the water phases.

In addition, the lipid-based formulation may effectively protect against the degradation of GI glucoside hydrolase by preventing these molecules from diffusing to the aqueous phase.

Potential Application to Therapeutic Agents Classified as Class III Compounds

As PNS is highly water-soluble but has low permeability, it is classified as a Class III drug according to the Biopharmaceutics Classification System. For Class III drugs, bioavailability will be very much independent of the drug dissolution properties of the formulation, while permeation through the intestinal membrane will be the rate limiting process for drug absorption. The phospholipid complex of the hydrophilic drugs created a possibility in which these drugs can be soluble in selected oils. The combination of phospholipid complex of hydrophilic agents and lipophilic solution containing selected medium fatty esters may be a useful method for their potent enhancement of poor absorption in the GI tract for Class III compounds.

CONCLUSIONS

The enhanced relative bioavailability of PNS had been demonstrated to be associated with the lipid phase. The oral absorption of ginsenoside Rg1 and Rb1 was improved dramatically, compared with aqueous solution and complex alone after administration in rats. The significant increase may not only be due to the soluble complex in oils, but also due to more comprehensive factors in the GI tract and decreased enzyme degradation, and so forth.

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