JPP JOURNAL OF Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY
JPP Journal of Pharmacy And Pharmacology
Research Paper

Pharmacokinetics and tissue distribution profile of icariin propylene glycol-liposome intraperitoneal injection in mice

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Keywords
distribution; icariin; pharmacokinetics; propylene glycol-liposomes

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Received May 9, 2011
Accepted September 1, 2011

doi: 10.1111/j.2042-7158.2011.01388.x

Abstract

Objectives The pharmacokinetics and tissue distribution of icariin propylene glycol-liposome suspension (ICA-PG-liposomes) have been investigated.

Methods ICA-PG-liposomes or ICA-PG-solution were prepared and intraperitoneally injected to mice. Morphology and size distribution of ICA-PG-liposomes were observed by transmission electron microscopy (TEM) and laser particle sizer. Plasma and tissues were collected at different times after intraperitoneal injection and icariin concentrations were determined by HPLC.

Key findings From TEM, ICA-PG-liposomes showed spherical vesicles with a mean particle size of 182.4 nm. The encapsulation efficiency of ICA-PG-liposomes reached 92.6%. Pharmacokinetics of ICA-PG-liposomes displayed the three open compartments model. ICA-PG-liposomes enhanced icariin absorption from the abdominal cavity, prolonged mean retention time ($MRT_{(0\text{-}t)}$), increased area under curve ($AUC_{(0\text{-}t)}$) and maximum concentration in plasma. Compared with ICA-PG-solution, ICA-PG-liposomes resulted in larger amounts of icariin being distributed into spleen (60.38% total icariin), liver (16.68%), lung (6.21%), kidney (4.64%), heart (1.43%) and brain (1.83%). $AUC_{(0\text{-}t)}$ values in most tissues (except lung) of mice administered ICA-PG-liposomes were higher than those administered ICA-PG-solution, while Clearance in most tissues (except brain and lung) decreased. The $MRT_{(0\text{-}t)}$ values of ICA-PG-liposomes in all tissues and half lives of most tissues (except brain) were prolonged. From Targeted efficiency and relative uptake data, the spleen was the target tissue of the ICA-PG-liposomes.

Conclusions ICA-PG-liposomes changed the pharmacokinetic behaviour and enhanced icariin distribution in tissues. With nanometer size, high encapsulation efficiency and improved pharmacokinetics, ICA-PG-liposomes might be developed as promising carriers for icariin injection.

Introduction

Icariin is a major component abstracted from the Chinese herb Epimedium. From reported studies, icariin has extensive pharmacological activities, including improvement of erectile dysfunction, attenuation of lipopolysaccharide-induced inflammatory responses in lung, depression of neuron death from stroke, enhancement of neuronal cell survival after H_2O_2 or oxygen–glucose deprivation *in vitro*, and Aβ42-induced neurotoxicity.[1–8]

However, previous research has revealed that icariin has poor absorption and low bioavailability after intragastric administration. After being injected to rats, icariin is rapidly eliminated. For example, the bioavailability of icariin was 12% and the biological half-life was 51.7 ± 8.87 min after 100 mg/kg icariin was intragastrically administered to rats.[9] The short biological half-life and low bioavailability may be associated with the rapid metabolism in the intestine. An in-situ single-pass perfused rat intestinal model showed that icariin was rapidly metabolized into icariside I and icariside II in the intestine, which resulted in low bioavailability.[10–12] Although icariin intravenously injected increased half-life bioavailability, biological half-life of 10 mg/kg icariin single injection was 0.56 ± 0.20 h and mean retention time ($MRT_{0\text{-}\infty}$) was 0.134 ± 0.040 h in rats.[13] In another study, the half-life of icariin via intravenous injection was 170.4 ± 75.05 min.[9] These disadvantageous pharmacokinetics hinder the clinical application of icariin in most of diseases.

As an effective drug delivery system, liposomes have been developed for encapsulation of such agents as peptides, proteins and chemotherapeutics.[14–18] Loaded liposomes can be applied with intravenous injection, transdermal delivery systems and oral administration. With liposomes as the carriers, loaded drugs prolong their retention time in blood,

depress metabolism and change distribution characteristics.[19] Recently, a novel type of liposomal vesicle, propylene glycol (PG)-liposomes, was developed for the delivery of drugs through the skin. According to a report, PG-liposomes are superior to traditional liposomes and ethosomes in transdermal drug delivery.[20] As a kind of flavonoid, icariin does not dissolve in water and acid, but does dissolve in ethanol, propylene glycol and dimethyl sulfoxide (DMSO). Therefore PG-liposomes may be a candidate delivery system for icariin injection.

On the basis of a phospholipid-based microparticle preparation, new PG-liposomes were prepared in our laboratory.[21,22] With propylene glycol and trehalose (lipid membrane stabilizer) used in the formulation, propylene glycol liposomes (PG-liposomes) displayed both high flexibility as ethosomes and high stability as lyophilized liposomes. To explore the capability of PG-liposomes as an icariin delivery system, icariin-loaded PG-liposomes (ICA-PG-liposomes) were prepared. Using ICA-PG-solution as a control, ICA-PG-liposome characteristics in pharmacokinetics and tissue distribution were investigated in this study.

Materials and Methods

Materials

Hydrogenated phosphatidylcholine was purchased from Doosan Corporation Biotech BU (Kyunggi Do, Korea). Cholesterol was from Beijing Chemical Reagent Corporation (Beijing, China). Tween-80 was from Hubei Biological Technology Company (Hubei Province, China). Icariin (purity ≥ 98%) was from Tianfang Company Limited (Ningbo, China). Propylene glycol (PG) was from Sigma-Aldrich (Steinheim, Germany). Acetonitrile and MeOH (chromatographic grade) were from Tianjin Shield Fine Chemical Company (Tianjin, China).

Preparation of ICA-PG-liposomes and ICA-PG-solution

Preparation of ICA-PG-liposomes

Hydrogenated phosphatidylcholine 10 mg, cholesterol 5 mg, Tween-80 1 mg and icariin 6 mg were added into 3 ml propylene glycol, and the mixture heated to 50 ± 1°C. Three millilitres 5% trehalose solution was added slowly into 3 ml propylene glycol solution with constant stirring at 750 rev/min using a magnetic stirring apparatus (RET basic C, IKA, Guangzhou, China). ICA-PG-liposomes were formed in solution after 30 min constant stirring and the final icariin concentration of the ICA-PG-liposome solution (suspension) was 1 mg/ml.

Preparation of ICA-PG-solution

Tween-80 1 mg and icariin 6 mg were added into 6 ml propylene glycol. The final concentration of icariin in PG-solution was 1 mg/ml.

Characterization of ICA-PG-liposomes

Morphology

The morphology of ICA-PG-liposomes was observed by transmission electron microscopy (TEM, H-7500, Hitachi, Japan). A sample of ICA-PG-liposomes was diluted with phosphate buffered saline (PBS; pH 7.2), and a drop of the diluted sample was placed on the surface of a copper grid. The diluted sample was stained with 1% phosphotungstic acid and air dried. The morphology of the ICA-PG-liposomes was examined under TEM.

Size distribution

Size distribution of ICA-PG-liposomes was examined using a laser particle sizer (LS800, OMEC Co. Ltd, Zhuhai, China). The mean size was reported from at least three samples.

Liposomes encapsulation

ICA-PG-liposome suspension (2 ml) was dialysed against alcohol using a dialysis bag (molecular weight cut-off =3.5 kDa; Spectra/Por) for 2 h. Determined by HPLC, the icariin concentration outside and inside of the dialysis bag presented the loaded icariin in liposomes and free icariin, respectively. The encapsulation efficiency were calculated as follows.

$$\text{Encapsulation efficiency} = \text{loaded icariin in liposomes} / (\text{free icariin} + \text{loaded icariin in liposomes}) \quad (1)$$

Animal experiment

The animal study was performed with the approval of the ethical committee of Wenzhou Medical College and all the experiments were performed according to the guide for the Care and Use of Laboratory Animals. One hundred Swiss hanschka (ICR) male mice were divided into two groups receiving either icariin PG-liposomes or icariin PG-solution ($n = 5$ each time point). After mice received 20 mg/kg ICA-PG-liposomes or ICA-PG-solution intraperitoneally, mice were killed with 300 mg/kg chloral hydrate at 0, 0.083, 0.167, 0.333, 0.667, 1.333, 2.0, 4.0, 8.0 and 24 h. The tissues and plasma collected at 0 min served as blank samples. Blood samples were collected in heparinized tubes. Spleen, kidney, liver, lung, heart and brain were collected from all mice. Plasma was transfused into tubes after being centrifuged at 3000 rev/min for 5 min. After weighing, tissue samples were homogenated with MeOH in the ratio 1 : 9 (w/v) by ultrasound in a 4°C water bath and centrifuged at 15 000 rev/min for 10 min at 4°C. Supernatant was transfused into blank Eppendorf tubes and stored at −40°C until determination.

HPLC assay

The icariin concentrations in plasma and tissues were determined by an HPLC assay as described by Cheng *et al*.[13] with modifications. In brief, Agilent 1010 HPLC was used with the column (ZORBAX Eclipse XDB, C_{18}, 4.6 × 250 mm, 5 μm, Agilent USA). A mixture of acetonitrile : distilled water (27 : 73) was used as the mobile phase with a flow rate of 1 mg/ml. Icariin was detected at UV 270 nm using a diode array detector. The correlation coefficient of the standard curve was 0.9993 and the relative standard deviation ranged from 2.23% to 4.33%.

Pharmacokinetic analysis

Icariin concentrations in plasma and tissues were analysed with the Drug and Statistics 1.0 program (DAS 1.0, Anhui, China). Pharmacokinetics were investigated by evaluating maximum concentration (C_{max}), area under curve ($AUC_{(0\text{-}t)}$), $MRT_{(0\text{-}t)}$ and biological half-life, total body clearance (CL_{Total}) and tissue clearance (CL_{liver}, CL_{spleen}, CL_{kidney}, CL_{brain}, CL_{heart} and CL_{lung}). Targeted efficiency (TE), relative uptake (R_e), and relative value of maximum concentration (C_e), and percent of tissue $AUC_{(0\text{-}t)}$ to total $AUC_{(0\text{-}t)}$ of each formulation were calculated using the following equations:

$$TE = \frac{AUC_{(0-t)T}}{AUC_{(0-t)Non-T}} \quad (2)$$

Where $AUC_{(0\text{-}t)T}$ is the $AUC_{(0\text{-}t)}$ of icariin in the target tissue and $AUC_{(0\text{-}t)Non\text{-}T}$ is the sum of all $AUC_{(0\text{-}t)}$ of icariin in nontarget tissues.

$$R_e = \frac{AUC_{(0-t)PG-L}}{AUC_{(0-t)PG-S}} \quad (3)$$

Where $AUC_{(0\text{-}t)PG\text{-}L}$ is the $AUC_{(0\text{-}t)}$ of ICA-PG-liposomes in one organ and $AUC_{(0\text{-}t)PG\text{-}S}$ is the $AUC_{(0\text{-}t)}$ of ICA-PG-solution in corresponding organ.

$$C_e = \frac{C_{\max PG-L}}{C_{\max PG-S}} \quad (4)$$

Where $C_{maxPG\text{-}L}$ is the maximum icariin concentration of ICA-PG-liposomes in one organ and $C_{maxPG\text{-}S}$ is the maximum icariin concentration of ICA-PG-solution in one organ.

Statistical analysis

One-way analysis of variance and Student's *t*-test or Kruskal–Wallis test were adopted for statistical comparison using the SAS 8.01 (1999–2000, SAS Institute Inc., Cary, NC, USA). The data difference was considered to be statistically significant when the *P*-value was less than 0.05.

Results and Discussion

Characterization of ICA-PG-liposomes

Under TEM, most of the ICA-PG-liposomes showed spherical vesicles and no aggregation or fusion was observed (Figure 1a). Size distribution was obtained from the diameter distribution profile using a laser particle sizer. As shown in Figure 1b, mean diameter of ICA-PG-liposomes was 182.4 ± 89.3 nm, with 65% ICA-PG-liposomes ranging from 116.1 to 302.7 nm. Similar results were obtained by Jain[23] and Bendas and Tadros.[24] In their reports, the mean diameter of lamivudine ethosomes and salbutamol sulfate ethosomes were 152 ± 12 nm (phospholipid : cholesterol : ethanol = 2 : 0.15 : 45 (% w/w)) and 267.8 ± 26.3 nm (phosphatidylcholine : cholesterol : dicetylpho-

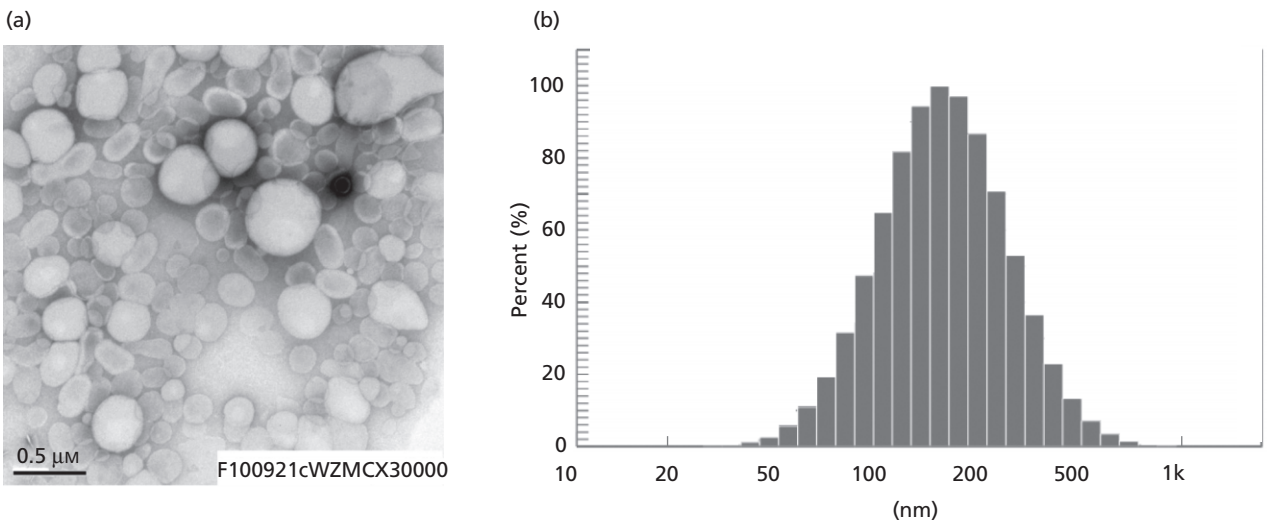

Figure 1 Transmission electron microscopy (a) and diameter distribution (b) of icariin-loaded propylene glycol-liposomes.

sphate = 60 : 20 : 5 mg, ethanol concentration was 45%), respectively.[23,24] Both studies showed the mean diameters of classic liposomes were 388 ± 14 and 411.2 ± 22.5 nm, respectively.[23,24] Compared with reported ethosomes the ICA-PG-liposomes had a similar diameter.[23,24]

From the encapsulation efficiency assay, the encapsulation efficiency of ICA-PG-liposomes reached 92.6%. Therefore, most of the icariin was encapsulated in PG-liposomes. With small vehicles, narrower size distribution and high encapsulation efficiency, ICA-PG-liposomes have the potential as a drug delivery system. Even after 30-days storage at room temperature (between 22 and 26°C), there was little change in morphology and encapsulation efficiency of ICA-PG-liposomes.

Pharmacokinetics of ICA-PG-liposomes in plasma

As shown in Figure 2, ICA-PG-liposomes changed the pharmacokinetic behaviour of icariin. After ICA-PG-liposomes were administered intraperitoneally, icariin was absorbed from the abdominal cavity and icariin concentration arrived at maximum concentration (C_{max}) in plasma at 0.083 h, which was followed by a sharp decrease at 0.167 and 0.25 h. Then icariin concentration increased gradually. After 0.50 h, icariin concentration began to decline again. At 24.0 h, icariin concentration decreased below the level of detectability (0.5 μg/ml) of the HPLC assay. The mean concentration–time curve of ICA-PG-liposomes indicated that there was redistribution of ICA-PG-liposomes. Under compartment model analysis, ICA-PG-solution (i.p.) fitted a two-compartment open model (Akaike's information criterion (AIC) = 24.934 ± 8.586, r^2 = 0.953 ± 0.045). However, ICA-PG-liposomes (i.p.) fitted the three-compartment model (AIC = 19.978 ± 11.475, r^2 = 0.977 ± 0.013), rather than the two-compartment model (AIC = 26.65 ± 13.131, r^2 = 0.915 ± 0.097).

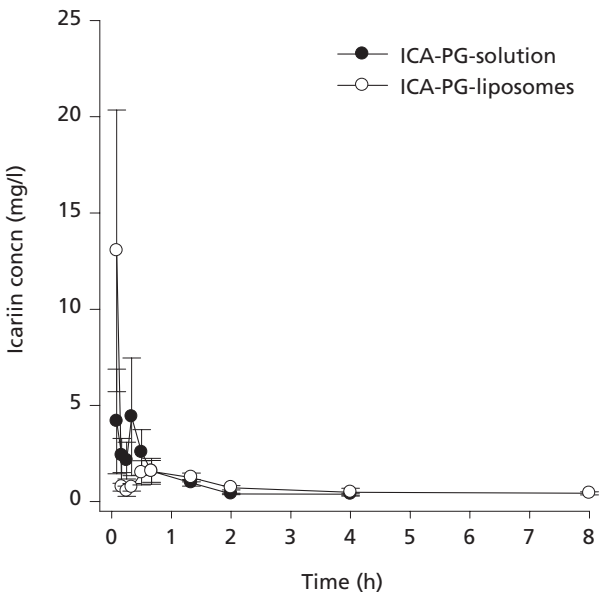

Figure 2 Mean icariin concentration–time curve of icariin-loaded propylene glycol-liposomes and icariin propylene glycol-solution *in vivo*.

In addition, as shown in Figure 3, $AUC_{(0\text{-}t)}$ and C_{max} values of the ICA-PG-liposomes in plasma were 2.37- and 2.35-times those of the ICA-PG-solution, respectively (both $P < 0.05$). These data indicated that PG-liposomes could change pharmacokinetics and distribution of icariin *in vivo*.

The results of the ICA-PG-solution in this experiment were similar to the results from Ye *et al*.[9] In that report, icariin solution presented with a two-compartment open model after the intravenous administration of 10 or 15 mg/kg icariin solution to rats. The half-lives of 10 and 15 mg/kg icariin solution were 170.4 ± 75.05 and 167.9 ± 41.67 min, respectively. In this study, however, PG-liposomes changed the pharmacokinetic behaviour of icariin compared with the reported research. The pharmacokinetics of ICA-PG-liposomes might have been a result of composition and diameter of the liposomes. Gabizon and Papahadjopoulos[25] showed that diameter and composition of a liposome formulation impacted deeply on the distribution in tissue. Most of the liposomes abided by the three-compartment model, in which blood circulation was considered as the first compartment, liver and spleen as the secondary compartment, skin and others tissue as the third compartment. According to Barza *et al*.[26] cholesterol, a main ingredient in liposomal composition, could change the pharmacokinetic behaviour in rabbit eyes. With cholesterol in the composition, ICA-PG-liposomes showed redistribution in blood as classic liposomes. Recently, studies revealed that liposomes with diameters similar to the ICA-PG-liposomes distributed into the spleen and liver mainly, and therefore resulted in the release of the drugs.[20,27,28] These factors contributed to explain the pharmacokinetic behaviour of icariin. It was indicated that ICA-PG-liposomes *in vivo* could be distributed into liver and spleen, and icariin released into blood after distribution.

Tissue distribution characteristics of ICA-PG-liposomes

After ICA-PG-liposomes or ICA-PG-solution (i.p.), icariin was detected in all tissues and the tissue pharmacokinetics of both formulations were evaluated. C_{max} of icariin in spleen was the highest among tested tissues of both ICA-PG-liposomes and ICA-PG-solution, followed by liver, kidney, lung, heart and brain. Compared with C_{max} of ICA-PG-solution, C_{max} of ICA-PG-liposomes in lung was low ($P < 0.05$). However, C_{max} of ICA-PG-liposomes in other tissues showed slight differences with C_{max} of ICA-PG-solution

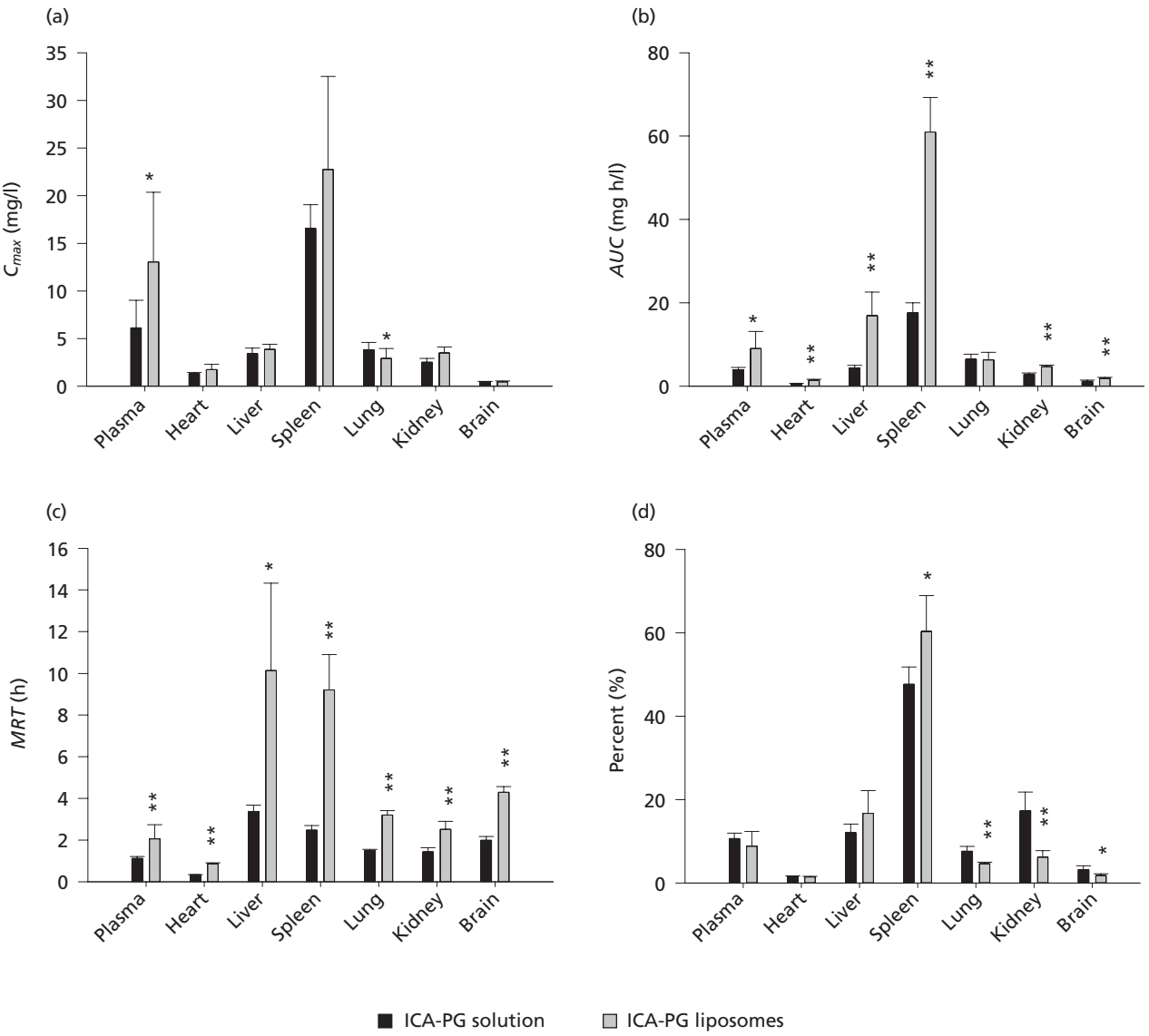

Figure 3 Pharmacokinetic parameters and percent distribution of icariin propylene glycol-solution and icariin-loaded propylene glycol-liposomes in various tissues. Percent: % of tissue *AUC* to total *AUC*. (a) C_{max} of icariin in tissues; (b) *AUC* of icariin of icariin propylene glycol (ICA-PG)-liposomes and ICA-PG-solution; (c) mean retention time (MRT) of ICA-PG-liposomes and ICA-PG-solution; (d) % of ICA-PG-liposomes and ICA-PG-solution. Compared ICA-PG-liposomes with ICA-PG-solution. $*P < 0.05$ and $**P < 0.01$.

($P > 0.05$) (Figure 3a). Compared with ICA-PG-solution, ICA-PG-liposomes had relatively higher icariin concentration at most points in tissues (Figure 4a, b, c, d) except brain and lung (Figure 4e, f).

$AUC_{(0\text{-}t)}$ of ICA-PG-liposomes in tissues were evaluated. According to Figure 3b, $AUC_{(0\text{-}t)}$ of ICA-PG-liposomes in heart, liver, kidney, spleen and brain were all significantly higher than those of ICA-PG-solution ($P < 0.01$). Especially in spleen, $AUC_{(0\text{-}24h)}$ of ICA-PG-liposomes reached approximately threefold that of ICA-PG-solution. As shown in Figure 3d, 60.383% of the total amount of ICA-PG-liposomes and 47.642% of ICA-PG-solution were distributed into the spleen. Although ICA-PG-liposomes increased icariin distribution into liver, there was no difference between formulations ($P > 0.05$). Compared with ICA-PG-solution, the percent of tissue $AUC_{(0\text{-}t)}$ to total $AUC_{(0\text{-}t)}$ of ICA-PG-liposomes in spleen increased significantly($P < 0.05$). However, the percent of ICA-PG-liposomes in kidney, lung and brain significantly decreased ($P < 0.05$). As shown in Figure 3d, the percent in the heart and liver displayed slight differences between ICA-PG-liposomes and ICA-PG-solution ($P > 0.05$).

From these results, ICA-PG-liposomes enhanced icariin distribution into spleen and liver mainly, while there was

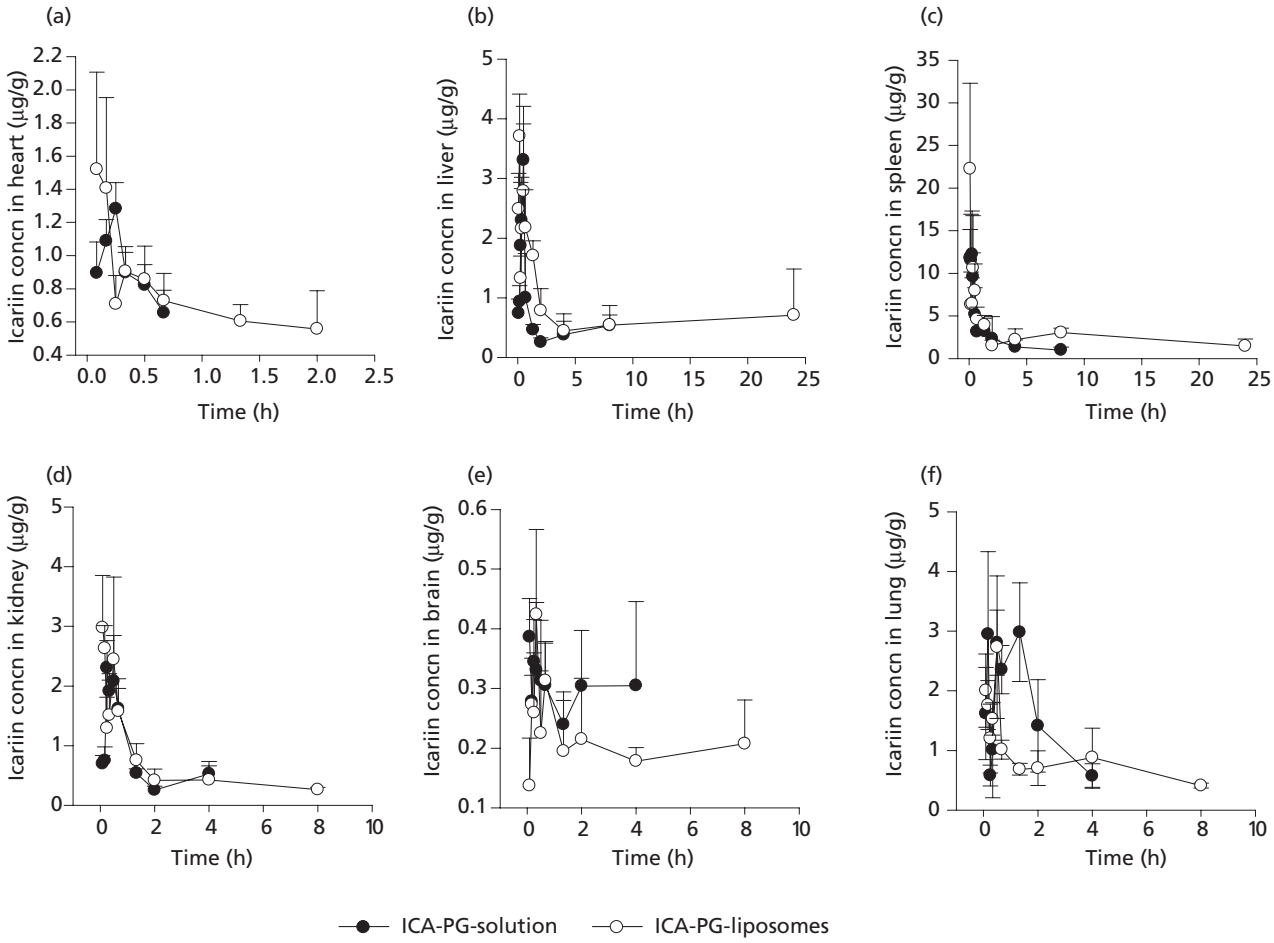

Figure 4 Mean concentration–time curves of icariin propylene glycol-liposomes and icariin propylene glycol-solution in various tissues. Icariin concentration in (a) heart, (b) liver, (c) spleen, (d) kidney, (e) brain, and (f) lung.

decreased icariin distribution in other tissues. These results could have been due to the diameter and composition of the liposomes.[20,27] With diameters of 182.4 ± 89.3 nm, ICA-PG-liposomes might be recognized as an exogenous particle by the spleen and induce higher icariin concentration in spleen. With a similar particle size to ICA-PG-liposomes, galactosylceramide liposomes increased 5-fluorouracil distribution into spleen and liver, while decreasing its distribution in heart, lung and kidney.[27] The composition of ICA-PG-liposomes also impacted on icariin distribution. In another study, a small change in the composition of the formulation, especially of cholesterol in the liposomes, could result in a dramatic impact on distribution.[25,26] Though the composition of ICA-PG-liposomes was similar to classic liposomes, the role of PG (used as solvent in ICA-PG-liposomes) in pharmacokinetics was unknown.

From the tissue distribution characteristics, ICA-PG-liposomes concentrated in the spleen. This may have caused side effects to the spleen. From reported research, the phagocytosis of liposome particles by rat splenic immature monocytes displayed immunosuppression, but the system immune function was not affected.[29] For ICA-PG-liposomes, icariin enhanced the immune function.[30,31] Therefore, further study is necessary to investigate the toxicity of ICA-PG-liposomes on spleen.

Elimination of ICA-PG-liposomes in plasma

PG-liposomes increased the $AUC_{(0\text{-}8h)}$ and C_{max} values of icariin significantly due to enhanced icariin concentration in plasma and prolongation of $MRT_{(0\text{-}8h)}$. Compared with ICA-PG-solution, $MRT_{(0\text{-}8h)}$ of ICA-PG-liposomes in plasma was prolonged significantly ($P < 0.05$). As shown in Figure 5a, the biological half-life of ICA-PG-liposomes (2.672 ± 1.538 h) was much longer than that of ICA-PG-solution (0.741 ± 0.422 h) in plasma ($P < 0.05$). These results were consistent with previous research *in vitro* and *in vivo*.[32,33] Trammer *et al*.[32] showed that liposomes displayed a

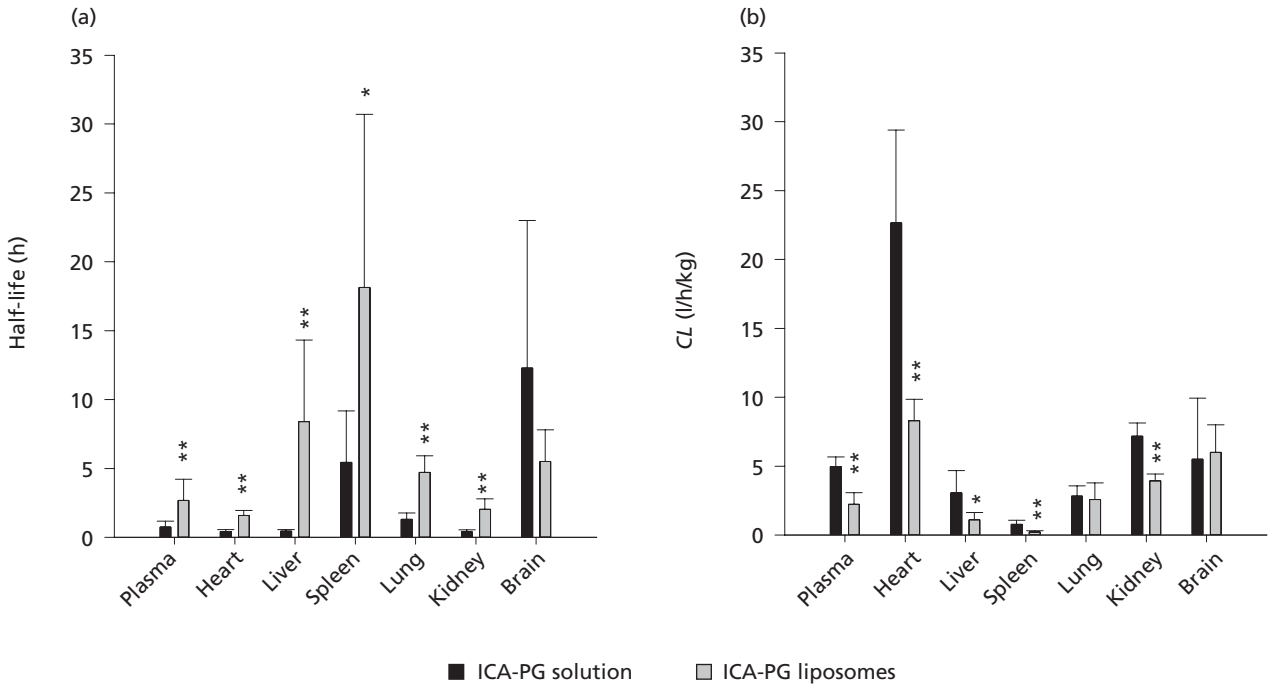

Figure 5 Clearance and biological half-life of icariin propylene glycol-liposomes and icariin propylene glycol-solution. Clearance, CL. *t*-test for the analysis of mean at $P = 0.05$ level, $*P < 0.05$ and $**P < 0.01$, comparing icariin propylene glycol (ICA-PG)-liposomes with icariin ICA-PG-solution.

prolonged release of ciclosporin and favourable longer tissue retention than PG-solution *in vitro*. Zhuang *et al*.[33] reported that liposomes prolonged the *MRT* and increased biological half-life of mitoxantrone by 1-time in plasma of rats, compared with the mitoxantrone solution.

Moreover, CL_{Total} of ICA-PG-liposomes was lower than that of ICA-PG-solution ($P < 0.05$, Figure 5b), which was consistent with the prolongation of $MRT_{(0\text{-}8h)}$ (Figure 3c). From these results, it indicated that PG-liposomes prolonged icariin retention time, and decreased elimination and excretion of icariin in tissues.

$MRT_{(0\text{-}t)}$, biological half-life and delayed elimination of ICA-PG-liposomes in tissues

ICA-PG-liposomes increased $AUC_{(0\text{-}t)}$ but not C_{max} of icariin in tissues. Since $AUC_{(0\text{-}t)}$ was related to concentration and retention time in tissues, $MRT_{(0\text{-}t)}$ and biological half-life of icariin in tissues were evaluated in this experiment. As shown in Figure 4, icariin was distributed into different tissues after the administration (i.p.) of ICA-PG-liposomes or ICA-PG-solution. From the profiles, icariin concentration could be detected in spleen and liver in 24 h, kidney, liver and brain in 8 h, and heart in 2 h. From these results, retention time of icariin in ICA-PG-liposomes was prolonged. The results of Figure 4 were consistent with the $MRT_{(0\text{-}t)}$ results of Figure 3c. Compared with ICA-PG-solution, $MRT_{(0\text{-}t)}$ of ICA-PG-liposomes was prolonged significantly in all tissues ($P < 0.05$ for liver and $P < 0.01$ for other tissues). Especially the values for $MRT_{(0\text{-}t)}$ of ICA-PG-liposomes in spleen and liver were approximately three- and four-times those of the ICA-PG-solution, respectively. As shown in Figure 5b, clearance of icariin in tissues was decreased significantly in heart, liver, spleen and kidney ($P < 0.05$). In addition, biological half-lives of ICA-PG-liposomes in heart, liver, spleen, lung and kidney were prolonged significantly ($P < 0.05$, Figure 5a). However, there was little difference in biological half-life of brain between ICA-PG-solution and ICA-PG-liposomes ($P > 0.05$, Figure 5a).

In previous studies, liposomes could prolong the biological half-life of loaded drug.[25,26] When cholesterol was used in the liposomal composition, the half-life of liposomes was prolonged compared with free-cholesterol liposomes. Large liposomes (400 nm) induced a much longer half-life than small liposomes (60 nm).[26] For ICA-PG-liposomes, both composition of cholesterol and vehicle diameter may have played important roles in the prolongation of the half-life. ICA-PG-liposomes prolonged retention time in tissues and increased the half-lives of icariin in tissue.

Targeted distribution of ICA-PG-liposomes

Pharmacokinetic data indicated that the spleen might be the main distribution tissue for ICA-PG-liposomes. Therefore,

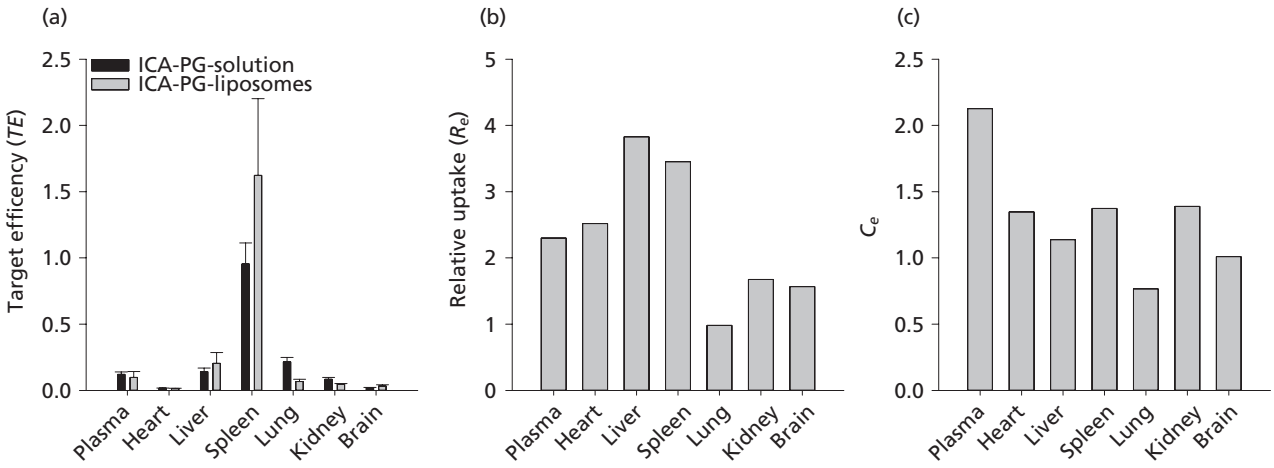

Figure 6 Targeted characteristics of icariin propylene glycol-liposomes. (a) Target efficiency (*TE*) of icariin propylene glycol (ICA-PG)-liposomes and ICA-PG-solution. (b) Relative uptake (R_e). (c) Relative value of maximum concentration (C_e).

related parameters such as R_e, *TE* and C_e, were calculated to evaluate whether the spleen was the target tissue of ICA-PG-liposomes. Target efficiency of ICA-PG-liposomes in tissues (except lung) were all beyond 1.0 that was viewed as standard value of target distribution (Figure 6b). In other words, ICA-PG-liposomes targeted distribution in tissues except lung. From C_e data, ICA-PG-liposomes tended to distribute in tissues (except lung), compared with ICA-PG-solution (Figure 6c). These results indicated that ICA-PG-liposomes increased distribution in most tissues (except lung). It was consistent with the distribution results of ICA-PG-liposomes. *TE* value of ICA-PG-liposomes in spleen was beyond 1.0, while *TE* value of ICA-PG-liposomes in other tissues were below 0.2. From these results, PG-liposomes increased uptake of icariin by tissues, especially by spleen. Although the *TE* of ICA-PG-solution in spleen was also higher than that in other tissues, the value (0.955 ± 0.158) was below 1.0. Therefore, spleen was viewed as a non-selective target for ICA-PG-solution (Figure 6a). Previous studies have shown that most kinds of liposome formulations distributed in liver and spleen, and that tissue distribution varied with composition of liposomes.[25] As mentioned above, ICA-PG-liposomes delayed the release of loaded drugs and were distributed mainly into liver and spleen. Meanwhile, ICA-PG-liposomes might be phagocytized by spleen and liver. All of these factors might contribute to the targeted distribution of ICA-PG-liposomes in spleen and liver.

Conclusions

ICA-PG-liposomes improved the pharmacokinetics, increased icariin concentration in plasma and tissues, and prolonged the MRT and biological half-life. Furthermore, ICA-PG-liposomes changed the icariin tissue distribution behaviour, resulting in the spleen and liver becoming the main distribution tissues. Though the mechanism of pharmacokinetics of ICA-PG-liposomes was not clear, composition and vehicle diameter of ICA-PG-liposomes might play important roles in icariin distribution and be phagocytized by spleen and live. Considering the nanometer size of the liposomes, high encapsulation efficiency and improved pharmacokinetics, ICA-PG-liposomes may be developed as promising carriers for icariin injection.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This research was supported by the National Natural Science Funds (30870755, 81071277, 81071164), Natural Science Foundation of Zhejiang Province (Y2110587, Y2080915), Natural Science Foundation of Beijing (7112100), Medicine and Health Grant from Wenzhou Bureau of Science and Technology (S20100049, H20100017, Y20100029), Zhejiang Province funds for Talented Youth in Medical Technology (2010ZQ007) and Zhejiang Province funds for Department of Education Research (Y201016664).

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