ORIGINAL ARTICLE

Preparation of Neogambogic Acid Nanoliposomes and its Pharmacokinetics in Rats

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ABSTRACT

Objective: To prepare neogambogic acid nanoliposomes (GNA-NLC) and study its pharmacokinetics (PK) in rats.
Study Design: An experimental study.
Place and Duration of Study: Mudanjiang Medical University, Mudanjiang, China, from January 2016 to October 2017.
Methodology: GNA-NLC was prepared by emulsion evaporation-low temperature solidification. The entrapment efficiency, average particle size, and zeta potential were investigated. Male Wistar rats were injected with 1 mg/mL gambogic acid and GNA-NLC into the caudal vein respectively, and the plasma concentration was determined by UPLC-MS/MS. The pharmacokinetic parameters of the two agents were compared.
Results: GNA-NLC prepared in this study were mostly spherical spheroids with an average particle size of 146.35 ±1.72 nm, polydispersity coefficient of 0.26 ±0.02, zeta potential of -28.24 ±0.13 MV, entrapment efficiency of 84.63%, and drug loading capacity of 4.23%. DSC showed that neogambogic acid nanoparticles had formed and neogambogic acid was amorphous in the matrix. The pharmacokinetics results in rats showed that GNA-NLC plasma concentration was significantly higher than that of common preparation of gambogic acid, with a half-life period of 10.14 ±0.03 hours, 4.57 times that of gambogic acid. $AUC_{0 \sim 24h}$ of gambogic acid in GNA-NLC lipidosome was 58.36 ±0.23 μg/h/mL, 4.83 times that of gambogic acid.
Conclusion: GNA-NLC can be prepared successfully by emulsion evaporation-low temperature solidification. The method is simple and easy to control. The GNA-NLC has a long cycle, and high blood concentration, sustained release compared with the raw material gambogic acid.

Key Words: *Neogambogic acid nanostructured lipid carrier, Emulsion evaporation-low temperature solidification, Rat, Pharmacokinetic behaviour.*

INTRODUCTION

Gamboge is a colloidal resin secreted by gamboge tree, *garcinia maingayii*. As a traditional Chinese medicine, gamboge is mainly used for attacking toxic, detumescence, removal of dental caries, closing sores, hemostasis, insecticide, etc. It is mainly used to treat ulcer, pyogenic infections, anabrosis, eczema, tumors, stubborn dermatitis, traumatic injury and hemorrhage.[1] Neogambogic acid (GNA) monomer has been extracted from gamboges. Studies have confirmed that GNA is a major component for anti-tumor effects of gamboges, which can inhibit liver cancer HepG2 cells, lung cancer A549 cells, breast cancer cells and other tumor cells in varying degrees.[2] It is not sensitive to normal cells like HUVEC cell lines, human umbilical vein endothelial cells, and does not affect the spleen, kidney and other organ functions. However, GNA is a strong fat-soluble ingredient that is insoluble in water and freely soluble in organic solvents such as methanol, ethanol and acetone. GNA pharmacokinetic study found that, GNA can be rapidly eliminated from rats after intravenous administration with very short half-life period.[3] These features lead to restricted GNA development and clinical application.

With the development of nanotechnology, the research of nanometer drug-loading system is also becoming more and more mature. Especially, in the field of anticancer, nanometer drug-loading system plays an indispensable and unique role. In particular, lipid nanoparticles (such as solid lipid nanoparticles, nanostructured lipid carriers, *etc.*) are widely studied.[4] Nanostructured lipid carrier (NLC) is a colloidal drug loading system with special nanostructures prepared by mixing solid lipids and liquid lipids that are spatially incompatible with each other at a certain temperature. Belonging to the field of aqueous dispersed system, it supports administration in a variety of ways to solve difficult drug delivery problems of many poorly water soluble medicines.[5]

At the same time, as the second generation of lipid nanostructured carrier system, NLC inherits various advantages of traditional nano-carriers (nanoemulsion,

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Received: January 27, 2018; Accepted: July 30, 2018

liposome, *etc*.) and solid lipid nanoparticles,[6] and has the additional advantages of high loading capacity, good stability, and sustained release on target.[7]

The purpose of this study was to prepare GNA-NLC and study its pharmacokinetics in rats for references of GNA-NLC in clinical practice.

METHODOLOGY

This study was carried out in Mudanjiang Medical University, China, from January 2016 to October 2017. The research was conducted after approval from the Committee on Animal Research and Ethics. The experiment prepared GNA-NLC by emulsion evaporation-low temperature solidification. The prescription dose of GMS, SA, MCT (the total mass of the three lipids is 200 mg, mass fraction of MCT accounts for 20% of total lipid), lecithin (5.0 mg/mL mass concentration) and GNA was dissolved in 5mL anhydrous ethanol, heated in water bath to 68 ~ 70°C to form an oil phase. Polysorbate-80, F68 at a ratio of 1: 1 was dissolved in 20 mL of water, heated in water bath to the same temperature as the oil phase. The oil phase was slowly and uniformly injected into a stirred (1000 r / minute) aqueous phase with a 1 mL syringe, and stirring was continued for 3 hours. After the organic solvent was completely volatilised, the emulsion was quickly poured into 40 mL of ice water (ice bath) while stirring, and stirring was continued for 2 hours to obtain GNA-NLC.

In this experiment, GNA-NLC entrapment efficiency was determined with reference to micro-column centrifugation in literature.[8] GNA-NLC 100 μL were loaded it on the top of the micro-column. After elution for six times with 0.3 mL distilled water added each time, continuously elution was done for 17 times. Filtrate containing GNA-NLC was collected in 1~6 tubes. Methanol was added to dissolution set to 25 mL. The mass (M1) of encapsulated GNA in the nanoparticles was analysed and calculated by HPLC. Another GNA-NLC 100 μL without column chromatography was added with methanol for dissolution and set to 25 mL. By sample analysis, the total mass of GNA in the dispersed system (M0), entrapment efficiency and drug loading capacity were calculated.

$$\text{Entrapment efficiency} = M_1 / M_0 \quad (1)$$

$$\text{Drug loading capacity} = W_{administration} \times \text{entrapment efficiency} / (W_{administration} \times \text{entrapment efficiency} + W_{fat}) \quad (2)$$

In formula (2), $W_{administration}$ is the total drug mass in the system; and W_{fat} is matrix mass. In this study, three batches of GNA-NLC were prepared by the same process, and their entrapment efficiency and drug loading capacity were measured.

Appropriate amount of GNA-NLC suspension was diluted by 30 times. Particle size and zeta potential were measured at room temperature using a zetasizer nano-laser particle analyser.

Five to six grams samples were placed in an aluminum pot subjected to scanning at a rate of 10°C/min within a certain temperature range. With blank aluminum pot as a control, the pyrolysis curve of each sample was filled for DSC analysis using Origin 7.5 software.

The GNA-NLC was dissolved in ethanol to prepare a solution with a mass concentration of 100 mg/mL, and then diluted to 1 mg/kg with PEG 400. Twelve male Wistar rats were randomly divided into two groups. One group was treated by tail vein injection of 1 mg/kg gambogic acid solution and the other group was by injection of 1mg/kg GNA-NLC solution. Then 0.2 mL blood was collected from rat orbit at 5, 10, 20, 30 minutes and at 1, 2, 4, 6, 8, 10, 12 and 24 hours after administration, added with heparin sodium anticoagulation and centrifuged at 2 827 × g for 10 minutes. Plasma was separated and reserved. Fifty μL of plasma and 10 μL of 300 ng / mL tripterine standard solution were mixed using vortex mixer for two minutes. One mL of ethyl acetate was added, followed by mixing using a vortex mixer for one minute, and centrifuging at 6,000 r / minute for 10 minutes. The upper organic phase was taken, dried under nitrogen at 37°C, redissolved in 100 μL of 50% methanol and centrifuged at 6,000 r/minute for 10 minutes. Eighty μL samples were taken for detection. Plasma gambogic acid was determined by UPLC-MS/MS method. The feasibility of UPLC-MS/MS method was validated with good specificity and no endogenous interference. The lowest limit of quantification was 5 ng/mL. The standard curve was plotted with integral value of peak area of gambogic acid/integral value of peak area of tripterine as Y-axis (Y), and with mass concentration of gambogic acid as X-axis (X). Linear relationship of gambogic acid was fine at 5-1 000 ng/mL. The regression equation was $Y = 0.00352X + 0.02$, $R^2 = 0.9970$. Pharmacokinetic parameters were calculated by Winnonlin 5.2 pharmacokinetic analysis software.

Data was entered and analysed by using SPSS 20.0. Measurement data were given as mean ±standard deviation (SD). Independent samples t-test was used to review relationship of the plasma concentration and main pharmacokinetic parameters. The p-value of less than 0.05 was considered significant.

RESULTS

The results showed that the three batches of entrapment efficiencies were 84.12%, 84.46% and 85.31%, respectively; the average entrapment efficiency was 84.63 % for three batches. The drug loading capacity of three batches was 4.19%, 4.23% and 4.28%, respectively; the average drug loading capacity was 4.23%, showing that the process repeatability is fine.

The particle size of GNA-NLC obtained in this experiment was 146.35 ±1.72 nm for three batches and the polydispersity coefficient (PI) was 0.26 ±0.02 for three

batches, indicating small particle size and even distribution of GNA-NLC obtained in this experiment.

The zeta potential of GNA-NLC measured by Zetasizer was -28.24 ±0.13 mV for three batches. Analysing from electrostatic repulsion, the resulting nanodispersion system has good physical stability.

The melting point of the GNA raw material was 108.1°C, the melting peaks of GMS and SA were 61.4 and 57.3°C, respectively; in the GNA-NLC curve, GNA melting peaks disappeared, and GMS and SA melting peaks were not seen, but a new melting peak was shown at 42.9°C. Thus, GNA-NLC constituted a new phase, GNA no longer existed in a crystalline state, possibly distributed in the matrix in amorphous state. At the same time, the crystal lattice orderness of GMS and SA lipids decreased.

The drug-time curve of gambogic acid solution and GNA-NLC treated by tail vein injection is shown in Figure 1. The main pharmacokinetic parameters were calculated by non-compartmental model, with results shown in Table I. The results showed that the plasma concentration of GNA-NLC was significantly higher than that of the common preparation of gambogic acid ($p<0.001$), with a half-life period of 10.14 ±0.03 hours, which was 4.57 times that of gambogic acid. $AUC_{0\sim24\ h}$ of gambogic acid in GNA-NLC liposomes was 58.36 ±0.23 μg/h/mL, which was 4.83 times that of gambogic acid.

Table I: Main pharmacokinetic parameters of gambogic acid and GNA-NLC in rats (n = 6 mice).

Parameters	Gambogic acid	GNA-NLC	t-value	p-value
Half-life period (h)	2.22 ±0.06	10.14 ±0.03	-265.98	<0.001
V (mL)	67.53 ±0.40	40.31 ±0.23	143.92	<0.001
CL (mL·h¹)	20.43 ±0.28	3.75 ±0.25	108.81	<0.001
$AUC_{0\text{-}24\ h}$ (μg·h/mL)	12.06 ±0.34	58.36 ±0.23	-276.70	<0.001

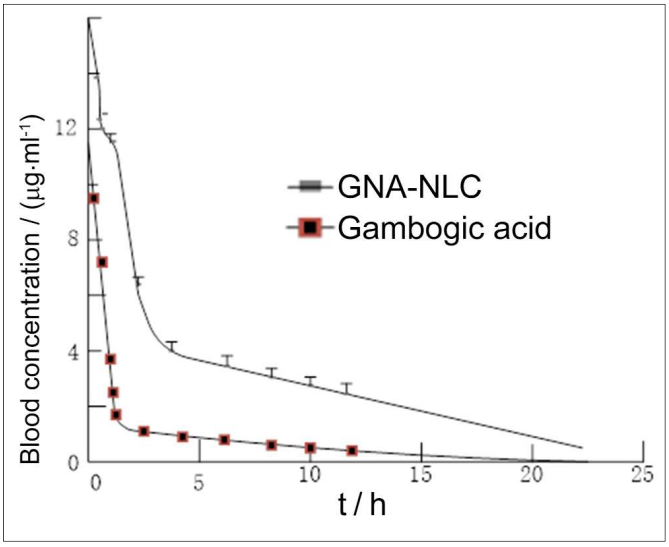

Figure 1: Drug-time curve of gambogic acid solution and GNA-NLC treated by tail vein injection.

DISCUSSION

NLC is the second generation of lipid nano-drug loading system.[9] Due to the liquid lipid loading, its entrapment efficiency and drug loading capacity are greatly improved compared with solid lipid nanoparticles.[10] GNA-NLC was successfully prepared in this experiment, with entrapment efficiency significantly improved compared to neogambogic acid solid lipid nanoparticles.

Lecithin mainly aids emulsification in the prescription. The experiment found that, entrapment efficiency and drug loading capacity increased with the increase of lecithin mass; at the same time, the particle size of the prepared nanoparticles also increased significantly. The main reason may be that lecithin as a co-emulsifier also plays the role of matrix.[11] With increase of lecithin content, the matrix increased as the loaded drugs increased, so the entrapment efficiency and drug loading capacity also increased.[12] On the other hand, when the matrix concentration was increased, the viscosity of the dispersed system increased and the surface tension increased correspondingly, so the diameter of the nanoparticles formed under the same emulsification conditions was large.[13]

NLC belongs to the thermodynamically unstable homogeneous dispersed system, which is prone to particle size increases, flocculation, precipitation, delamination and other instabilities because of collision of molecules with each other.[14] Zeta potential is one of NLC property characterisations, also an indicator of long-term stability.[15] Analysing from electrostatic repulsion theory, the colloidal system is in a stable state when the absolute value of zeta potential is greater than the critical value 30.[16] However, many hydrophilic emulsifiers in NLC exert a steric stabilising effect due to their special structure, which can stabilize the colloidal system with lower potential.[17]

Micro-column centrifugation is mainly used for the separation and purification of biological macromolecules.[18] This micro-column centrifugation is mainly used for the separation and purification of biological macromolecules. Micro-column centrifugation is mainly used for the separation and purification of biological macromolecules. Method utilises the dual action of gel molecular sieve and centrifugal force to achieve rapid separation, which is currently mainly used for the purification of liposomes.[19,20] The experimental results show that this method is simple, fast, reproducible, and can be used for the purification of NLC nanoparticles. During the experiment, it was found that more foam was produced at a stirring speed of 1200 r/minute, which may affect the full contact of surfactant and oil phase, thus affecting the emulsification effect. Hence, 1000 r/minute stirring speed was adopted in this experiment.

In this experiment using F68, polysorbate-80 and lecithin composite emulsifier, it was found that NLC prepared

using composite emulsifier had better stability and entrapment efficiency, which was consistent with the literature reports.[21]

The pharmacokinetics results in rats showed that the GNA-NLC plasma concentration was significantly higher than that of the common preparation of gambogic acid, with a half-life period of 10.14 ±0.03 hours, which was 4.57 times that of gambogic acid. $AUC_{0\sim24\ h}$ of gambogic acid in GNA-NLC liposomes was 58.36 ±0.23 μg/h/mL, 4.83 times that of gambogic acid. It indicated that GNA-NLC can effectively prolong the drug's retention time in blood and maintain a high plasma concentration, suggesting that GNA-NLC can increase the anti-tumor activity of gambogic acid.

CONCLUSION

GNA-NLC can be prepared successfully by emulsification evaporation-low temperature solidification. The process is simple and easy to control. The GNA-NLC has a long cycle, and high blood concentration, sustained release compared with the raw material gambogic acid, with a good clinical application prospect.

Acknowledgements: This study was supported by the Scientific Research Project of Heilongjiang Health Planning Committee (No.2016-370); Scientific Research Projects for Basic Scientific Research in Heilongjiang Provincial Universities (No.zs201517); Natural Science Foundation of Heilongjiang Province (No.H2016090); University Nursing Program for Young Scholars with Creative Talents in Heilongjiang Province (No.UNPYSCT-2017172).

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