

In vitro and in vivo evaluation of novel NGR-modified liposomes containing brucine

Shu Li
Xi-Peng Wang

Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai, People's Republic of China

Abstract: In this study, a novel NGR (Asn-Gly-Arg) peptide-modified liposomal brucine was prepared by using spray-drying method. The surface morphology of the liposomes, encapsulation efficiency and particle size were investigated. The data showed that the addition of NGR did not produce any significant influence on brucine liposomes in terms of particle size or zeta potential. In addition, after 3 months of storage, no dramatic change such as visible aggregation, drug content changes or precipitation in the appearance of NGR-brucine liposomes occurred. The in vitro release results indicated that the release of brucine from NGR liposomes was similar to that of liposomes, demonstrating that the NGR modification did not affect brucine release. The in vitro drug-release kinetic model of NGR-brucine liposomes fitted well with the Weibull's equation. In vivo, NGR-brucine liposomes could significantly extend the bioavailability of brucine; however, there was no significant difference observed in the pharmacokinetic parameters between liposomes and NGR liposomes after intravenous administration. Antitumor activity results showed that NGR-modified liposomes exhibited less toxicity and much higher efficacy in HepG2-bearing mice compared with non-modified liposomes. The enhanced antitumor activity might have occurred because brucine was specifically recognized by NGR receptor on the surface of tumor cells, which enhanced the intracellular uptake of drugs.

Keywords: brucine, liposome, NGR, HepG2, in vivo, in vitro

Introduction

Brucine (CAS No 57-24-9) is an alkaloid and exists mainly in the seeds of *Strychnos nux-vomica* L. (Loganiaceae),¹ which is widely found in many southern Asian countries. Brucine itself is known as an anti-inflammatory and analgesic drug for relieving arthritic and traumatic pain.²⁻⁴ Its main pharmacodynamic actions include relief of pain, reduction of swelling and promotion of circulation.⁵ Strychnin and brucine are the two main active ingredients of the semen strychni. In addition, some research have indicated that strychnin can effectively inhibit the proliferation of several types of cancer cells, including glioma, breast cancer, colorectal cancer and others,⁶⁻⁸ with an obvious inhibitory effect on liver cancer cells. Studies involving in vitro culture of hepatoma carcinoma cells have shown that strychnin could inhibit the proliferation of HepG2 and SMMC-7721 cells.⁹⁻¹² Unfortunately, the potential use of brucine is severely limited due to high incidence of side effects. Because it is strongly fat-soluble and easily distributed in the central nervous system (CNS) in the brain and other organs, it exerts severe CNS toxicity.^{13,14} There is a narrow margin of safety between a therapeutic and a toxic dose. Thus, the key to reduce the toxicity and increase the effect of brucine is to increase the concentration of strychnin in its effect target and reduce its distribution in brain tissues to lower CNS toxicity.

Correspondence: Xi-Peng Wang
2699 West Gaoke Road, Shanghai
201204, People's Republic of China
Tel/fax +86 21 2026 1000
Email xpwangdr@163.com

Colloidal drug delivery systems, such as liposomes, represent a mature technology with considerable potential for the entrapment of both lipophilic and lipophobic drugs.¹⁵ Encapsulation or entrapment of drugs in liposomes results in distinct changes in the pharmacokinetic and pharmacodynamic properties of free drugs, and in some cases, causes an apparent decrease in toxicity and/or an increase in therapeutic efficacy.¹⁶

In recent years, the use of ligand–receptor-based system for targeted drug delivery has become a hot research topic. Use of tumors itself and receptors on newborn vascular endothelial cells as target, together with intravenous administration of targeted liposomes to promote active targeting, can effectively increase efficacy. NGR is a polypeptide which contains asparagine-glycine-arginine (Asn-Gly-Arg) sequence.¹⁷ Tumor cells and tumor newborn vascular endothelial cells exhibit high expression of aminopeptidase N (APN; CD13). NGR can integrate with high specificity, inhibit the generation of tumor newborn blood vessels and thus inhibit the growth and transfer of tumors. APN is a membrane-bound, zinc-dependent metalloproteinase that plays a key role in tumor invasion and angiogenesis.¹⁸

In this study, a novel NGR peptide-modified liposomal brucine was prepared by using spray-drying method. The surface morphology of the liposomes, encapsulation efficiency and particle size were investigated. The formulations were characterized by *in vitro* release study. The optimal formulation providing sustained drug release was selected for *in vivo* study.

Materials and methods

Chemicals and reagents

Brucine was purchased from Yuanjian Biopharma Ltd., Co. (Shanghai, People's Republic of China). The chemical structure of brucine is shown in Figure 1. Internal standard (IS) strychnine was purchased from the Shanghai Institute of Biological Products (Shanghai, People's Republic of China). Soybean phosphatidylcholine (SPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide(polyethylene glycol)-2000] (DSPE-PEG2000) and cholesterol (CHOL) were obtained from Sinopharm Chemical Reagent (Shanghai, People's Republic of China). HepG2 was purchased from Genomeditech Biopharma Ltd., Co. (Shanghai, People's Republic of China). The NGR peptide was synthesized by Ningbi Kangbei Biochemical Co., Ltd. (Zhejiang, People's Republic of China). NGR-PEG-DSPE was synthesized according to previously reported method.^{19,20} All other reagents were obtained

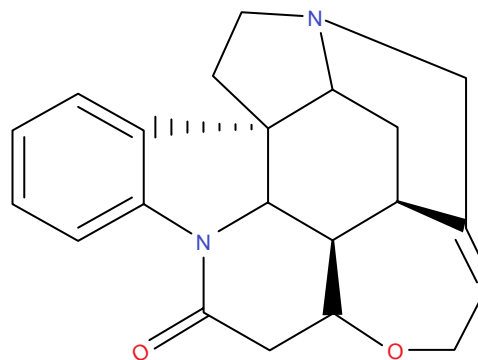


Figure 1 The chemical structure of brucine.

from Sinopharm Chemical Reagent. Methanol and acetonitrile (chromatographic grade) were obtained from EMD Millipore, Billerica, MA, USA. Water for high-performance liquid chromatography (HPLC) was double-distilled, and all other reagents were of analytical grade.

Preparation of liposomes

The NGR-modified liposomes containing brucine (NGR-brucine) were prepared by thin-film hydration method, as described previously.⁸ Briefly, a mixture of brucine, SPC, CHOL, PEG-DSPE and NGR-PEG-DSPE (the molar ratio of NGR-PEG-DSPE:PEG-DSPE:CHOL:SPC was 5:5:30:60; the weight ratio of lipid:brucine was 19:1; the modification degree of NGR in NGR-brucine was about 0.5% [molar ratio %]) was dissolved in chloroform. Then, the solvent was evaporated using an RE52 rotary evaporator (Shanghai Yarong Biochemistry Instrument Company, Shanghai, People's Republic of China) in a round-bottomed flask at 40°C for about 40 min to obtain a solid film. This film was then flushed with nitrogen gas for 30 min and stored overnight in a desiccator to remove any traces of chloroform. After that, the thin film was hydrated in a 5% glucose solution by sonication in a water bath for 10 min to produce a suspension of liposomes. Then, the liposomes were freeze-dried for 72 hours. The dry powder was rehydrated and sonicated for 3 min prior to application. For the preparation of liposomes containing brucine, a similar procedure was carried out except that the NGR-PEG-DSPE was replaced by PEG-DSPE.

Characterization

Particle size and zeta potential of the liposomes were measured by the dynamic light scattering technique using a zeta potential/particle sizer (Beckman Coulter, Brea, CA, USA). All measurements were performed in triplicate, and the

values are represented as mean \pm SD (n=3). The morphologies of liposomes were visualized by transmission electronic microscopy (TEM) (JEM-1200EX; JEOL, Tokyo, Japan). The samples were added to the surface of copper grids, and stained with phosphotungstic acid (1%, w/v). The accelerating voltage was set at 120 kV. The encapsulation efficiency was estimated from the following formula:

Encapsulation efficiency =

$$\frac{\text{Actual amount of drug loaded in liposomes}}{\text{Theoretical amount of drug loaded in liposomes}} \times 100\%$$

HPLC analysis

The concentration of brucine in the prepared liposomes was determined by HPLC. Separation was carried out at 35°C using a reverse-phase C18 column (5 μ m, 4.6 \times 250 mm). The mobile phase consisted of acetonitrile and buffer (10 mm sodium heptane sulfonate and 20 mm potassium dihydrogen phosphate, pH adjusted to 2.8 with 10% phosphonic acid). The ratio of acetonitrile/buffer (v/v) was adjusted to 24:76. The detection wavelength was 264 nm, and a flow rate of 1.0 mL/min was employed. A sample volume of 20 μ L was injected.

Storage stability studies

The NGR-brucine liposomes and non-targeted brucine liposomes were studied for stability at 4°C. These formulations were tested at regular time intervals to identify any change in particle size, zeta potential and drug content.

In vitro release

The in vitro release of NGR-brucine liposomes, brucine liposomes and free brucine was analyzed according to the published method.⁴ The liposome suspension (drug content: 2 mg) and free drug were placed in a dialysis bag with a molecular weight cut-off of 10,000 Da. The dialysis bag was suspended in 100 mL PBS (pH 7.4) which was incubated at 37°C under constant rotation at 500 rpm. At scheduled time intervals, aliquot samples were withdrawn and assayed for brucine content by HPLC as described above. The volume of dissolution medium was maintained at 100 mL throughout the experiment.

In vivo pharmacokinetic studies

Thirty Sprague Dawley rats were divided into three groups (10 rats per group). All experiments were performed in strict accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the China National Institutes of Health

(Shanghai, People's Republic of China), and legal approval was obtained from Tongji University School of Medicine. All procedures performed in studies involving animals were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Three groups were given a single dose of 2.5 mg/kg brucine solution (dissolved in PBS), brucine liposomes and NGR-brucine liposomes, respectively. Blood samples were collected at 5, 10, 30, 60, 90, 120, 180, 240, 360, 480 and 720 min after the administration, and the plasma was separated by centrifugation. The brucine concentration in the plasma was quantitatively analyzed using the HPLC method.

Briefly, plasma samples (100 μ L) were mixed with strychnine (50 μ g/mL) IS dissolved in methanol (20 μ L). To this mixture, 10 μ L of aqueous ammonia was added, and the samples were basified. Then, 3 mL of n-hexane–dichloromethane–isopropanol (65:30:5, v/v/v) was added and vortexed for 2 min. After centrifugation for 5 min at 12,000 rpm, the supernatant was collected, and the organic solvent was eliminated under nitrogen gas stream at 50°C. Then, the mixture was resuspended with the mobile phase (100 μ L). An aliquot of the supernatant (20 μ L) was injected into the HPLC system after centrifugation.

Histology studies

The histopathological changes induced by brucine liposomes and NGR-brucine liposomes after pharmacokinetic studies were evaluated. Animals were anesthetized, and their livers, spleens and kidneys were dissected and washed with cold saline. The organs were pressed between filter pads, weighed and then fixed in 10% neutral formalin using standard techniques and stained with hematoxylin and eosin for histopathological examination. All tissue samples were examined and graded under light microscopy with 500 \times magnification.

In vivo antitumor activity

The HepG2 model was established as described before.²¹ On the 8th day, the kunming mice were randomly assigned to four groups (12 animals per group): group 1 was administered a 5% glucose injection, group 2 was administered free brucine, group 3 was administered brucine liposomes and group 4 was administered NGR-brucine liposomes. The brucine formulations were all injected via the tail vein on days 8, 10, 12 and 14, at a dose of 15 mg/kg. The total dose of brucine administered in all treatment groups was 60 mg/kg. A digital caliper was used to measure the tumor

diameters, and tumor volumes (mm^3) were calculated using the following formula: tumor volume = length \times width² \times 0.5. Throughout the study, mice were weighed regularly in order to monitor the potential toxicities.

Statistical analysis

All data are presented as mean \pm SD. One-way analysis of variance was used to determine significance among groups. Statistical significance was established at $P < 0.05$.

Results

Characterization of NGR-brucine liposomes

Table 1 shows that the addition of NGR did not produce any significant influence on brucine liposomes in terms of particle size or zeta potential. The average particle size of brucine liposomes and NGR-brucine liposomes was 85.3 ± 3.2 and 92.6 ± 4.1 nm, respectively. The zeta potential of brucine liposomes and NGR-brucine liposomes was -16.2 ± 3.5 and -16.5 ± 3.3 mV, respectively. The encapsulation efficiency of brucine in liposomes and NGR-modified liposomes was $87.4\% \pm 3.1\%$ and $89.6\% \pm 2.7\%$, respectively. The high encapsulation efficiency in the formulation might be related to the strong hydrophobicity of brucine. Table 1 also gives the stability data of the particle size of NGR-brucine liposomes stored at 4°C . After 3 months of storage, no dramatic change such as visible aggregation, drug content changes or precipitation in the appearance of NGR-brucine liposomes occurred. TEM images (Figure 2) showed that the liposomes dispersed well with a uniform shape.

In vitro release

The in vitro release of brucine from the free drug, liposomes and NGR liposomes was studied in PBS (Figure 3). Over time, brucine in liposomes was released much more slowly than free drug. Table 2 shows that the in vitro drug-release kinetic model of NGR-brucine liposomes fitted well with the Weibull's equation: $\ln(1/(1 - Q)) = -2.154 \ln t + 1.12$ ($r = 0.9829$).

Pharmacokinetics

The pharmacokinetic parameters studied in rats given 2.5 mg/kg of brucine as free drug, encapsulated in liposomes and encapsulated in NGR liposomes (at brucine equivalent dose) are listed in Table 3. Figure 4 shows the mean plasma brucine concentration-versus-time curves, corresponding to the intravenous administration of free drug, liposomes and NGR liposomes, respectively. As shown in Figure 4, after a single injection of brucine injection, the plasma drug concentration quickly reached the maximum ($1,029.7 \pm 119.1$ ng/mL) in 5 min, and then it decreased rapidly and remained at around 15% of the C_{\max} value 2 h later, which implied a rapid in vivo elimination of brucine in rats. In the case of intravenous administration, the in vivo profile of liposomes was smoother than brucine-injected group. The $t_{1/2}$ and area under the curve_{0- ∞} of liposomes and NGR liposomes were 2.28- and 2.45- and 2.65- and 3.13-fold higher compared with free drug. Thus, it was reasonable to conclude that the liposomes could significantly extend the bioavailability of brucine in vivo; however, there was no significant difference in the pharmacokinetic parameters

Table 1 The particle size and zeta potential of NGR-brucine liposomes before and after storage at 4°C ($n=3$)

Preparations	Particle size (nm)	Zeta potential (mV)	Encapsulation efficiency (%)	Polydispersity index
NGR-brucine liposomes				
Day 0	92.6 ± 4.1	-16.5 ± 3.3	89.6 ± 2.7	< 0.39
Brucine liposomes				
Day 0	85.3 ± 3.2	-16.2 ± 3.5	87.4 ± 3.1	< 0.38
NGR-brucine liposomes				
Day 30	93.2 ± 3.3	-14.6 ± 2.7	88.4 ± 3.1	< 0.41
Brucine liposomes				
Day 30	86.7 ± 1.9	-15.7 ± 2.8	86.7 ± 2.9	< 0.42
NGR-brucine liposomes				
Day 60	94.1 ± 3.5	-15.1 ± 3.1	87.7 ± 3.9	< 0.44
Brucine liposomes				
Day 60	87.6 ± 3.4	-15.9 ± 3.4	86.4 ± 1.6	< 0.43
NGR-brucine liposomes				
Day 90	94.3 ± 3.9	-14.7 ± 5.2	87.2 ± 4.3	< 0.46
Brucine liposomes				
Day 90	88.5 ± 2.8	-15.3 ± 1.9	85.9 ± 4.2	< 0.45

Abbreviation: NGR, Asn-Gly-Arg.

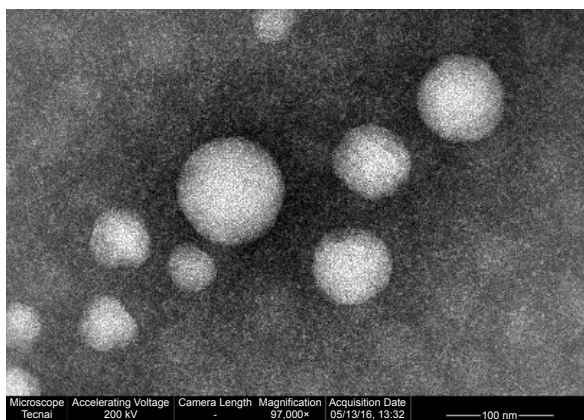


Figure 2 Transmission electron microscopy image of NGR-modified brucine liposomes (magnification 97,000 \times).

Abbreviation: NGR, Asn-Gly-Arg.

observed between liposomes and NGR liposomes after intravenous administration.

In vivo antitumor activity

As shown in Figure 5, both brucine liposomes and NGR-brucine liposomes significantly inhibited the growth of the HepG2 tumors in mice. However, NGR-modified liposomes could more effectively inhibit tumor growth than non-modified liposomes, starting from day 13. The tumor volumes of NGR-modified group were smaller than those of non-modified group. The tumor inhibition rate of NGR-modified liposomes was higher than that of non-modified liposomes ranging from 74.9% \pm 5.1% to 64.5% \pm 6.4%. Changes in the body weights of tumor-bearing mice are presented in Figure 6. The average body weights of mice injected with 5% glucose injection significantly increased after tumor cell implantation, while the weight of mice treated

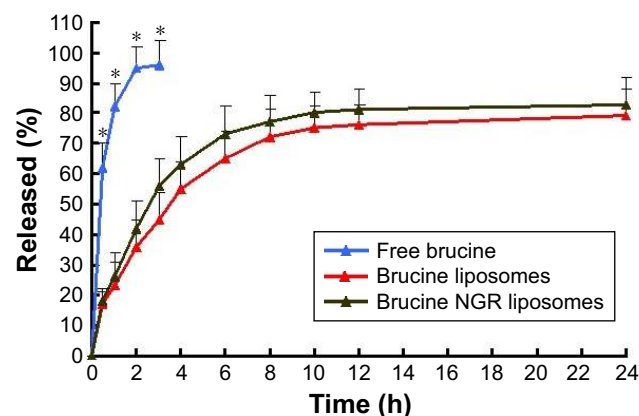


Figure 3 The release profile of free brucine, brucine liposomes and NGR-modified brucine liposomes (n=6).

Note: * $P < 0.05$, free brucine vs brucine liposomes or NGR-modified brucine liposomes.

Abbreviation: NGR, Asn-Gly-Arg.

Table 2 Dissolution kinetic parameters of brucine from NGR-modified liposomes (n=3)

Model	Formulations	
	Equation	Correlation coefficient (r)
Zero-order equation	$Q = 6.12t - 0.89$	0.9431
First-order equation	$\ln(1 - Q) = 5.27t - 1.01$	0.9152
Higuchi	$Q = 4.312t_{1/2}^{-2.212}$	0.9672
Weibull's equation	$\ln(1/(1 - Q)) = -2.154 \ln t + 1.12$	0.9829

Abbreviation: NGR, Asn-Gly-Arg.

with NGR-modified liposomes did not change significantly and the non-modified liposomes group showed a moderate increase in weight during the experiment.

Histological studies

The histopathological examination of the liver, spleen and kidney was carried out to identify any damage done to the tissues. Microphotographs of the liver, spleen and kidney were taken following their incubation with brucine formulations (Figure 7). No sign of damage such as the appearance of epithelial necrosis and sloughing of epithelial cells was detected.

Discussion

In vitro release

Both liposomes and NGR-modified liposomes showed an initial fast release of brucine within the first 4 h followed by a relatively sustained release. The burst release may be attributed to rapid diffusion of brucine from the surface of liposomes. The subsequent sustained release was due to the slow diffusion of brucine from the core of hydrophobic carrier. The in vitro release results indicated that the release of brucine from NGR liposomes was similar to that of liposomes, demonstrating that the NGR modification did not affect brucine release. After adding targeting materials, the speed of release of drug from the liposomes did not reduce

Table 3 Pharmacokinetic parameters of brucine after intravenous administration of free drug, liposomes and NGR liposomes to rats (n=6)

Parameter	Intravenous administration		
	Free drug	Liposomes	NGR-modified liposomes
$t_{1/2}$ (min)	36.2 \pm 6.5	82.6 \pm 8.5*	88.9 \pm 7.9*
AUC ₀₋₄ (μ g \cdot min/mL)	56.1 \pm 8.1	119.6 \pm 10.1*	135.3 \pm 26.5*
AUC ₀₋₂₄ (μ g \cdot min/mL)	62.5 \pm 13.8	165.9 \pm 16.4*	195.7 \pm 28.6*
MRT (min)	32.7 \pm 4.6	72.4 \pm 5.7*	85.4 \pm 8.2*
CL (L/kg/min)	0.14 \pm 0.04	0.06 \pm 0.01*	0.02 \pm 0.01*

Note: * $P < 0.05$, vs free drug.

Abbreviations: CL, clearance; NGR, Asn-Gly-Arg; MRT, mean residence time; AUC, area under the curve; $t_{1/2}$, biological half-life.

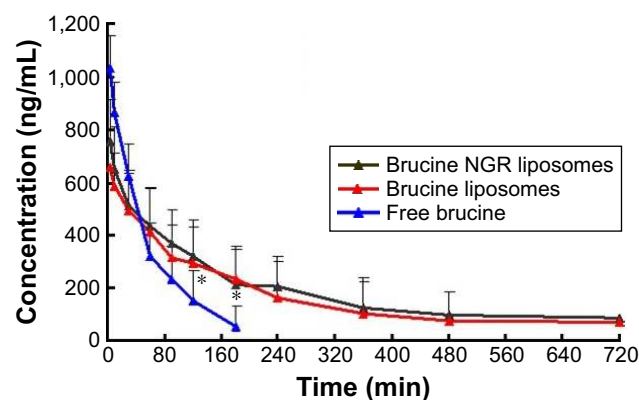


Figure 4 Concentration–time curve of brucine in different formulations: free brucine, brucine liposomes and NGR-modified brucine liposomes (n=6).

Note: * $P < 0.05$, free brucine vs brucine liposomes or NGR-modified brucine liposomes.

Abbreviation: NGR, Asn-Gly-Arg.

obviously, with a possible reason being that the quantity of added targeting material did not obviously increase the steric hindrance of the liposomes.

Pharmacokinetics

Lipid carrier systems are ideal for drug delivery because they can alter the pharmacokinetics of the associated therapeutics. Compared with the liposomes group, in the solution group, the release of brucine was instantaneous due to its moderate oil–water partition coefficient *in vivo*; after intravenous administration into blood, brucine could rapidly enter tissues through the biofilm. As the phospholipid material of liposomes group was added with DSPE-PEG known for its long recycling time, its structure could produce steric hindrance and liposomes were not easily swallowed by macrophages.

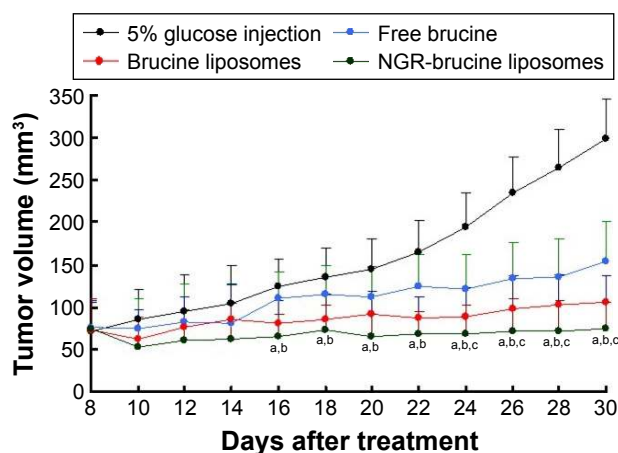


Figure 5 HepG2 xenograft tumor growth inhibition by brucine in different formulations.

Notes: Data = mean \pm SD (n=12). * $P < 0.05$, NGR-modified brucine liposomes vs 5% glucose injection; ^b $P < 0.05$, NGR-modified brucine liposomes vs free brucine; ^c $P < 0.05$, NGR-modified brucine liposomes vs brucine liposomes.

Abbreviation: NGR, Asn-Gly-Arg.

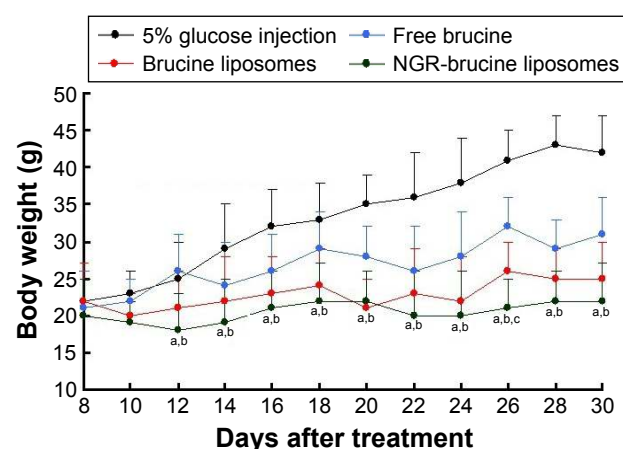


Figure 6 Animal body weights.

Notes: The body weights of treated animals were continuously monitored to investigate systemic cytotoxicity of brucine in different formulations. Data = mean \pm SD (n=12). ^a $P < 0.05$, NGR-modified brucine liposomes vs 5% glucose injection; ^b $P < 0.05$, NGR-modified brucine liposomes vs free brucine; ^c $P < 0.05$, NGR-modified brucine liposomes vs brucine liposomes.

Abbreviation: NGR, Asn-Gly-Arg.

Thus, the drug could stay for a relatively long time in blood circulation with no leakage from liposomes into tissues, and hence, the initial concentration of drug in the liposomes group was higher than the solution group. The targeting effect of NGR peptide-modified liposomes was determined by evaluating the binding capacity of target head (NGR polypeptide) and receptor (CD13, high expression in tumor cells and tumor newborn vascular endothelial cells) as well as the stability of drug during target-searching process. If the drug leaked during blood circulation from liposomes, it could easily enter the tissues because of its lipophilic nature. Thus, the main target of this research was to evaluate the stability of target materials added to liposomes. According to the pharmacokinetic results, the area under the curve of liposomes was obviously higher than that of solution group, and the mean residence time of 0.5% NGR-modified liposomes was obviously longer than unmodified liposomes, which showed good stability during transfer from targeted liposomes to target area.

In vivo antitumor activity

Overall, the antitumor activity results showed that the NGR-modified liposomes exhibited less toxicity and much higher efficacy in HepG2-bearing mice compared with non-modified liposomes.²² The enhanced antitumor activity might have occurred because brucine was specifically recognized by NGR receptor on the surface of tumor cells, which enhanced the intracellular uptake of drugs. NGR-modified liposomes exhibited high efficiency and low toxicity in the present study, which is expected to be considered in the development of other drug delivery systems. Thus, NGR-modified

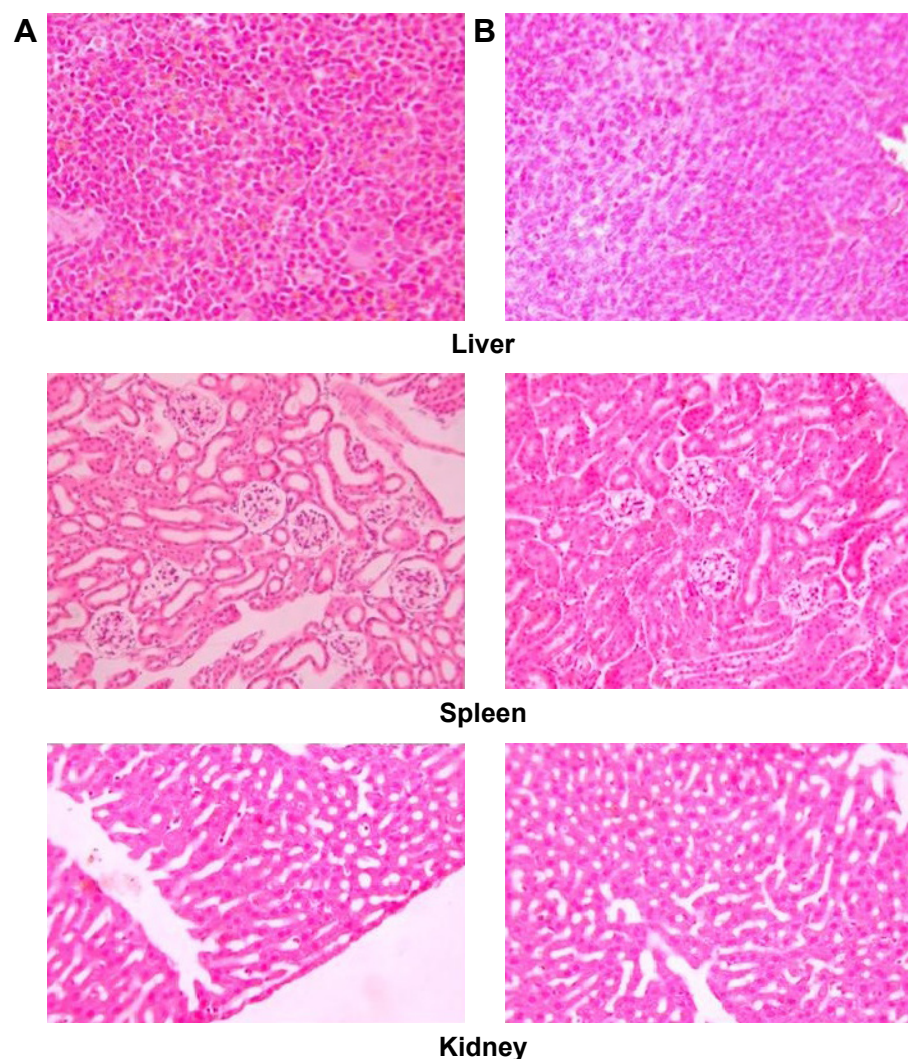


Figure 7 Histopathological studies of the liver, spleen and kidney: (A) brucine liposomes and (B) NGR-modified brucine liposomes (magnification $\times 5,000$).
Abbreviation: NGR, Asn-Gly-Arg.

liposomes establish a platform to convert a highly toxic active substance to an ideal candidate drug.

Acknowledgments

This work was supported by the Program for Outstanding Young Teachers in Tongji University (1400807). We also wish to express our thanks to Doctor Heng Lu (Department of Pharmacy, School of Pharmacy, Fudan University, Shanghai, China) for his help in the in vivo studies.

Disclosure

The authors report no conflicts of interest in this work.

References

- Chinese Pharmacopoeia Committee. *Pharmacopoeia of the People's Republic of China Volume I*. Beijing: Chemical Industry Press; 2005.
- Chen J, Hu W, Qu Y, et al. Evaluation of the pharmacodynamics and pharmacokinetics of brucine following transdermal administration. *Fitoterapia*. 2013;86:193–201.
- Chen J, Wang X, Qu YG, et al. Analgesic and anti-inflammatory activity and pharmacokinetics of alkaloids from seeds of *Strychnos nux-vomica* after transdermal administration: effect of changes in alkaloid composition. *J Ethnopharmacol*. 2012;139(1):181–188.
- Chen J, Lin A, Chen Z, et al. Ammonium sulfate gradient loading of brucine into liposomes: effect of phospholipid composition on entrapment efficiency and physicochemical properties in vitro. *Drug Dev Ind Pharm*. 2010;36(3):245–253.
- Li YL, Liu Q, Gong Q, et al. Brucine suppresses ethanol intake and preference in alcohol-preferring Fawn-Hooded rats. *Acta Pharmacol Sin*. 2014;35(7):853–861.
- Ruijun W, Wenbin M, Yumin W, et al. Inhibition of glioblastoma cell growth in vitro and in vivo by brucine, a component of Chinese medicine. *Oncol Res*. 2014;22(5–6):275–281.
- Serasanambati M, Chilakapati SR, Manikonda PK, Kanala JR, Chilakapati DR. Anticancer effects of brucine and gemcitabine combination in MCF-7 human breast cancer cells. *Nat Prod Res*. 2015;29(5):484–490.
- Luo W, Wang X, Zheng L, et al. Brucine suppresses colon cancer cells growth via mediating KDR signalling pathway. *J Cell Mol Med*. 2013;17(10):1316–1324.
- Qin JM, Yin PH, Li Q, et al. Anti-tumor effects of brucine immunonanoparticles on hepatocellular carcinoma. *Int J Nanomedicine*. 2012;7:369–379.

10. Saraswati S, Alhaider AA, Agrawal SS. Anticarcinogenic effect of brucine in diethylnitrosamine initiated and phenobarbital-promoted hepatocarcinogenesis in rats. *Chem Biol Interact.* 2013;206(2): 214–221.
11. Deng XK, Yin W, Li WD, et al. The anti-tumor effects of alkaloids from the seeds of *Strychnos nux-vomica* on HepG2 cells and its possible mechanism. *J Ethnopharmacol.* 2006;106(2):179–186.
12. Yin W, Deng XK, Yin FZ, Zhang XC, Cai BC. The cytotoxicity induced by brucine from the seed of *Strychnos nux-vomica* proceeds via apoptosis and is mediated by cyclooxygenase 2 and caspase 3 in SMMC 7221 cells. *Food Chem Toxicol.* 2007;45(9):1700–1708.
13. Liu F, Wang X, Han X, Tan X, Kang W. Cytotoxicity and DNA interaction of brucine and strychnine-two alkaloids of semen strychni. *Int J Biol Macromol.* 2015;77:92–98.
14. Teske J, Weller JP, Albrecht UV, Fieguth A. Fatal intoxication due to brucine. *J Anal Toxicol.* 2011;35(4):248–253.
15. Daeihamed M, Dadashzadeh S, Haeri A, Akhlaghi MF. Potential of liposomes for enhancement of oral drug absorption. *Curr Drug Deliv.* 2017;14(2):289–303.
16. Kloesch B, Gober L, Loebisch S, Vcelar B, Helson L, Steiner G. In vitro study of a liposomal curcumin formulation (Lipocurc™): toxicity and biological activity in synovial fibroblasts and macrophages. *In Vivo.* 2016;30(4):413–419.
17. Ma J, Zhang D, Ying X, et al. A novel NGR-conjugated peptide targets DNA damage responses for radiosensitization. *Curr Cancer Drug Targets.* 2015;15(6):533–541.
18. Arap W, Pasqualini R, Ruoslahti E. Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. *Science.* 1998; 279(5349):377–380.
19. Zhao BJ, Ke XY, Huang Y, et al. The antiangiogenic efficacy of NGR-modified PEG-DSPE micelles containing paclitaxel (NGR-M-PTX) for the treatment of glioma in rats. *J Drug Target.* 2011;19(5): 382–390.
20. Luo LM, Huang Y, Zhao BX, et al. Anti-tumor and anti-angiogenic effect of metronomic cyclic NGR-modified liposomes containing paclitaxel. *Biomaterials.* 2013;34(4):1102–1114.
21. Youssef MM, Tolba MF, Badawy NN, et al. Novel combination of sorafenib and biochanin-A synergistically enhances the anti-proliferative and pro-apoptotic effects on hepatocellular carcinoma cells. *Sci Rep.* 2016;6:30717.
22. Yin W, Wang TS, Yin FZ, Cai BC. Analgesic and anti-inflammatory properties of brucine and brucine N-oxide extracted from seeds of *Strychnos nux-vomica*. *J Ethnopharmacol.* 2003;88(2–3):205–214.

International Journal of Nanomedicine

Publish your work in this journal

The International Journal of Nanomedicine is an international, peer-reviewed journal focusing on the application of nanotechnology in diagnostics, therapeutics, and drug delivery systems throughout the biomedical field. This journal is indexed on PubMed Central, MedLine, CAS, SciSearch®, Current Contents®/Clinical Medicine,

Submit your manuscript here: <http://www.dovepress.com/international-journal-of-nanomedicine-journal>

Dovepress

Journal Citation Reports/Science Edition, EMBase, Scopus and the Elsevier Bibliographic databases. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.