Research & Reviews: Pharmacy and Pharmaceutical Sciences

e-ISSN: 2320-1215
p-ISSN: 2322-0112
www.rroij.com

Novel Curcumin Intravenous Injection System: Preparation, Characterization, Improved Bioavailability and Safety *In Vitro* and *In Vivo*

Liandong Hu[1,2*], Na Zhao[1,2], Jianli Zhang[1,2], Yanjing Shi[1,2], Qiaofeng Hu[1,2], Yan Jiao[1,2], Xiaoyun Xiong[3] and Jianying Su[3]

[1]School of Pharmaceutical Sciences, Hebei University, China

[2]Key Laboratory of Pharmaceutical Quality Control of Hebei Province, Hebei University, China

[3]Xi'an Lijun Pharmaceutical limited liability company, China

[*]**Correspondence to author:** Liandong Hu, School of Pharmaceutical Sciences and Key Laboratory of Pharmaceutical Quality Control of Hebei Province, Hebei University. No.180, WuSi Road, Baoding 071002, China, Tel: +86-22-60203347; E-mail: hbupharm@126.com (L. Hu).

Rec date: Oct 12, 2016; **Acc date:** Nov 02, 2016; **Pub date:** Nov 07, 2016

Research Article

ABSTRACT

Curcumin (CUR), a naturally bioactive component in the *Curcuma longa* have a wide range of biological and pharmacological actions. However, the poor water solubility with poor bioavailability limits its application. The aim of this study was to develop a novel CUR intravenous injection to improve CUR absorption and enhance drug therapy effect. The physical characteristics of CUR intravenous injection such as particle size, zeta potential and drug content were all investigated in detail. This formulation did not cause hemolysis and intravenous irritation. Pharmacokinetics results showed the Cmax and area under the curve (AUC) of CUR after intravenous administration was than those of oral administration. This study demonstrated the CUR intravenous injection may be a promising delivery system of CUR to improve bioavailability and clinical therapeutic effect in the future.

Keywords: Curcumin, Intravenous injection, Physical characteristics, *In vitro* release, Safety, Pharmacokinetics

INTRODUCTION

CUR is a naturally bioactive ingredient of *Curcuma longa* rhizome [1]. CUR has not only been used as food coloring agents [2], but also as a traditional drug, exhibiting a variety of biological functions such as antitumor, anti-inflammatory and antioxidant activities [3,4] which has been used as a herb drug in China and India [5] for a long time, no significant toxicity was found in human even at several grams orally daily for months [6].

Although CUR has good biological activities and pharmacological actions [7,8] , the water-insolubility limits its clinical application. Various oral formulations have been developed to overcome CUR low solubility and poor bioavailability such as solid dispersions [9], complexations [10], nano emulsions [11], prodrug [5], nanoparticles [12] and so on. Our laboratory has also prepared CUR micro emulsion to promote the oral absorption of CUR [13]. In these studies, the oral bioavailability of CUR was improved about 3-20 times compared with the pure CUR powder. The absolute oral bioavailability of CUR powder was only 0.9% in rats [14,15], So the 3-20 times oral improvement cannot produce satisfactory clinical therapeutic effect, the CUR absorption problem has not been fundamentally solved.

In this study, a novel CUR intravenous injection (CUR injection) was successfully prepared. The physicochemical characteristics such as particle size and morphological observation were carried out. The hemolytic test and irritation assessment were further performed. Pharmacokinetic properties of CUR injection were carried out and compared with those of CUR suspension.

e-ISSN: 2320-1215
p-ISSN: 2322-0112

EXPERIMENT

Materials and methods

CUR was supplied by Sino pharm Chemical Reagent Co., Ltd. (Shanghai, China) and Kolliphor RH40 was purchased from BASF Co., Ltd. (Germany). Dehydrated alcohol (Tianjin, China) was purchased from Kemel chemical reagents Ltd. All other chemicals were of analytical grade, and used without any further purification. Distilled water was used throughout the study.

Chromatographic conditions: The concentration of CUR was quantified by a Shimazu HPLC system (Kyoto, Japan). The HPLC system consisted of LC-20A pumps and a SPD-20A UV/VIS detector with a C18 analytical column (5 μm, 250 Ч 4.6 mm). The mobile phase was composed of acetonitrile and H_2O (containing 5.0% glacial acetic acid) at the ratio of 60:40 (v:v). The detected wavelength was 428 nm with a flow rate of 1.0 mL/min.

Preparation of CUR injection

In brief, CUR was firstly dissolved into Kolliphor RH40 at 40°C, and then dehydrated alcohol was added and stirred continually for 20 min to form a clear solution. The final ratio was CUR: Kolliphor RH40: dehydrated alcohol =1:40:12 (w/w). Normal saline was added to the above solution before used. The formulation can be diluted infinitely and no precipitation was observed.

Particle size and zeta potential

The droplet size and zeta potential of CUR injection were measured by NICOMP particle sizing system (CW380, Santa Barbara, CA). The analysis data of droplet size were evaluated using intens-wt gaussian distribution. All the samples were diluted with distilled water to get a suitable concentration for examination.

Transmission electron microscopy (TEM)

TEM (JEM-100SX, JEOL, Japan) was used to observe the morphology of CUR injection. The diluted CUR injection was spread onto a copper grid. The grid was then dried at room temperature before examination.

Drug content of CUR injection

Drug content is an important characteristic to evaluate the property of formulations. A 0.1 mL samples of the CUR injection were transferred to a 100-mL volumetric flask, and then distilled water was added to the solution to dilute to 100 mL. All the solution was filtered through a 0.45 μm filter membrane to remove drug without encapsulating. The concentration of CUR remaining in solution was measured by HPLC mentioned above.

Hemolytic test

Rabbit blood was used to assess the hemolytic effect of the CUR injection. Blood was obtained from the ear vein of rabbits, and fibrin was removed by stirring with glass rod for several minutes. Then, appropriate amount of normal saline was added to the fibrin-free rabbit blood. The supernatant was removed after centrifuged at 3500 rpm for 10 minutes. Red erythrocyte cells were washed (after centrifuged) several times with normal saline solution. Finally, a suitable amount of normal saline solution was added to the red blood cells to gain 2% erythrocyte dispersion. Varied concentrations of CUR injections (0.1, 0.2, 0.3, 0.4, and 0.5 mL) were added to the tubes along with 2.5 mL volumes of the erythrocyte dispersion. Then normal saline was added to the tubes to obtain a final volume of 5 mL. Normal saline and distilled water was used as negative and positive controls, respectively. The tubes were incubated at 37°C and observed for 3 hours. Then all the samples in the tubes were centrifuged at 4000 rpm for 10 min. The percentage of hemolysis was measured by UV-vis analysis of the supernatant at a wavelength of 545 nm. Hemolysis percent were calculated per the following equation:

Hemolysis percent (%)=(ABS sample-ABSNS)/(ABS water-ABSNS)*100%

Intramuscular irritation

New Zealand rabbits (2.5 kg to 3.0 kg) were randomly assigned to two groups: CUR injection group and negative control (0.9% normal saline) group. For CUR injection group, 0.5 mL diluted solutions were injected intramuscularly via left side of gluteus; for the negative control group, the same amount of normal saline was injected to the rabbits, once a day for three days. After the last administration, the muscle tissues were collected, fixed in 10% neutral buffer-formalin solution for histopathological examination.

e-ISSN: 2320-1215
p-ISSN: 2322-0112

Intravenous irritation

Intravenous irritation assessment was performed on New Zealand rabbits (2.5 kg to 3.0 kg) Rabbits were randomly divided to two groups: CUR injection group and negative control (0.9%, normal saline) group. CUR injection was diluted with normal saline to a final concentration of 6.25 mg/mL, then 0.5 mL diluted solution was injected intravenously via the left ear-border vein, for negative control group, the same amount of normal saline was injected. The injection site was carefully examined. The rabbits were sacrificed 24 h after the last administration, and the ears were cut and fixed in 10% neutral buffer-formalin solution for histological examination.

Pharmacokinetic evaluation

Healthy male SD rats (250 g ± 20 g) were used for the study. All the following animal experiments met the requirements of the national act of the people's republic of China with the use of experimental animals. The animals were fasted overnight prior to the experiment but had free access to water. They were randomized to be separated into two groups. For the control group, pure CUR was suspended in sodium carboxymethyl cellulose aqueous solution (1%, w/v) and given orally (1000 mg/kg). For the experiment group, CUR injection was intravenously injected (50 mg/kg) at the site of femoral vein. Blood samples were collected in tubes containing heparin at pre-determined intervals, separated by centrifugation and frozen at -20°C until analysis.

RESULTS AND DISCUSSION

Physical characteristics of CUR injection

The particle size and morphology of prepared CUR injection were shown in **Figure 1.** It was obvious that the particles exhibited spherical shape and non-adherent to each other. The result of particle agreed with TEM examination. The average particle size was 15.0 nm. The results showed that the CUR injection was prepared successfully and the size distribution was uniform.

Figure 1. The transmission electron micrograph of curcumin injection.

Hemolytic test

The hemolytic test was performed to evaluate the safety of CUR injection for intravenous administration. The results of hemolytic test were shown in **Figure 2.** The positive control tube exhibited complete hemolysis, the upper solution was a transparent red; while in other five sample tubes, the upper solution was transparent, and erythrocytes were precipitated at the bottom just as the normal saline group. The hemolysis percent were calculated in **Table 1.** All the hemolysis percent were below 5%. These results demonstrated that the CUR injection did not cause hemolysis.

e-ISSN: 2320-1215
p-ISSN: 2322-0112

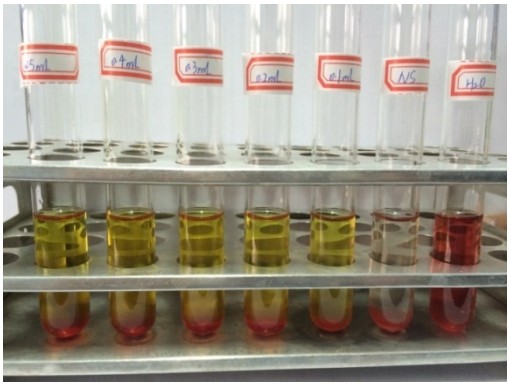

Figure 2. The results of hemolytic test.

Table 1. The results of hemolytic experiment.

The number of test-tube	1	2	3	4	5	6	7
2% erythrocyte dispersion (mL	2.5	2.5	2.5	2.5	2.5	2.5	2.5
normal saline (mL) distilled water (mL)	2.0	2.1	2.2	2.3	02.4	2.5	2.5
CUR injection (mL)	0.1	0.2	0.3	0.4	0.5	0	0
UV absorption (Abs)	0.048	0.062	0.062	0.045	0.046	0.035	0.720
Hemolysis percent (%)	1.90	3.94	3.94	1.46	1.60	-	-

Intramuscular Irritation assessment

The histopathological examinations of muscle irritation were shown in **Figure 3.** After intramuscular injection of normal saline, no obvious visible damage at the injection site or surrounding tissues were observed, which was same as that in CUR injection group. The skeletal muscle fibers were smooth both in normal saline group and CUR injection group.

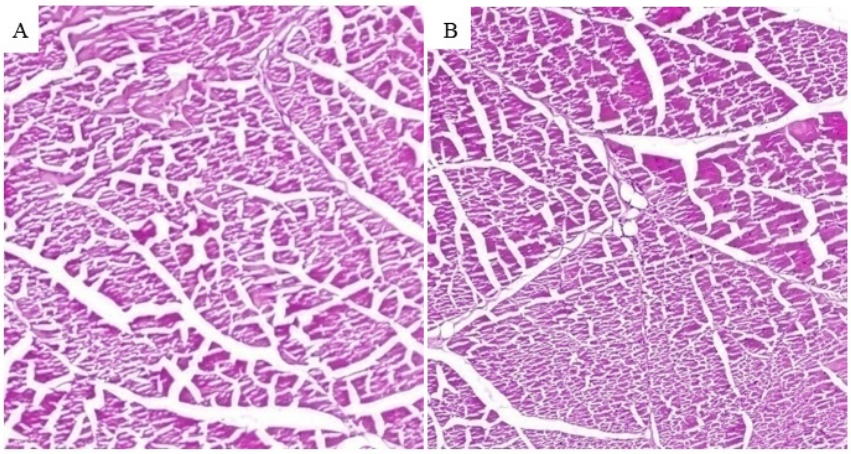

Figure 3. The histopathology examination of rabbit muscle curcumin intravenous injection group (A), normal saline (B).

Intravenous Irritation assessment

Figure 3 showed the appearance and histopathology examination of rabbit ear vein after intravenous injection of CUR injection. In CUR injection group **(Figure 4C),** no venous irritation was observed, including vascular engorgement, edema and indurations. The vessel wall was undamaged. In normal saline group **(Figure 4D),** no dropsy, congestion and blood clot were observed at the injection site. No inflammatory phenomena were found in both groups.

e-ISSN: 2320-1215
p-ISSN: 2322-0112

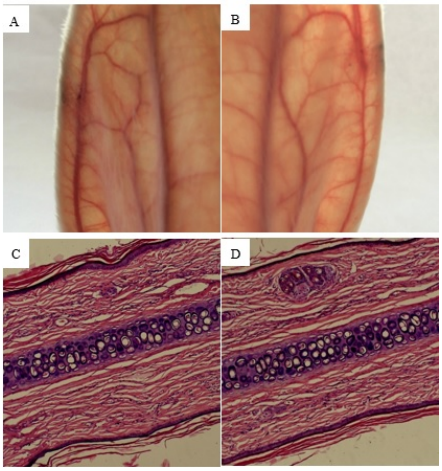

Figure 4. The appearance examination of rabbit ear vein, curcumin intravenous injection group (A), normal saline (B) and histopathology examination of rabbit ear vein, curcumin intravenous injection group (C), normal saline (D).

Pharmacokinetic evaluation

The blood concentration-time curves were shown in **Figure 5** and the pharmacokinetic parameters were also listed in **Table 2.** It was found that The C_{max} and AUC of CUR injection given intravenously were significantly higher than those of CUR suspension and CUR injection given orally. The AUC0-6 h of CUR injection gave intravenously was 1593.82 μg·min/mL ± 279.98 μg·min/mL significantly greater than 208.80 μg·min/mL ± 35.40 μg·min/mL of oral CUR suspension ($p<0.05$).

Table 2. Pharmacokinetic data of different CUR formulations in rats (n=5)

Parameters	Dosage (mg/kg)	C_{max} (μg/mL)	T_{max} (min)	AUC (μg·min/mL)
CUR injection (i.v.)	50	212.24 ± 36.62	5.00 ± 0	1593.82 ± 279.98
CUR suspension (oral)	1000	0.95 ± 0.12	86.40 ± 33.00	208.80 ± 35.40

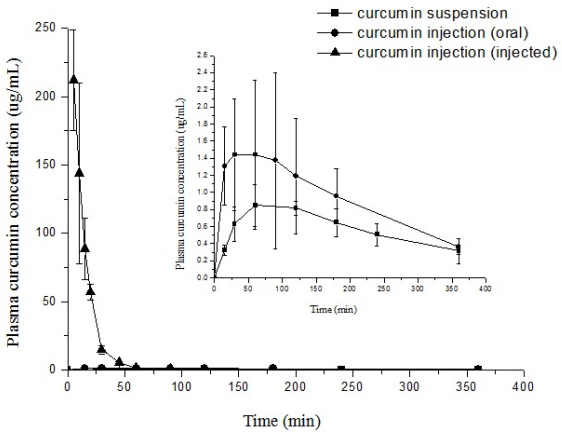

Figure 5. Plasma concentration-time profile of curcumin in rats after oral administration of curcumin suspension, curcumin injection and i.v. administration of curcumin injection (mean ± SD, n=5), the profile upright was the magnified curcumin suspension profile.

Calculated by the different administration dose **(Table 2),** the absolute bioavailability of oral pure CUR was 0.66% compared to intravenous CUR injection, and which was corresponding to 0.9% absolute bioavailability reported by Onoue [14]. The result indicated that CUR absorption was significantly improved by intravenous injection than that of oral administration in rats.

Intravenous administration is a medical method that the liquid was injected into blood directly. While oral administration is a process that drug was transported from gastro intestine to blood circle system. Compared to intravenous injection, drug via oral administration needed a longer time to reach blood and subjected to the first pass

e-ISSN: 2320-1215
p-ISSN: 2322-0112

effect. Some papers [16-22] previously reported other experiments of CUR via oral administration in **Table 3,** although the bioavailability of CUR in these studies was improved to some extent from the documents and our study, based on the absolute bioavailability results, we can conclude that oral administration is not the optimal delivery route for CUR administration and intravenous route is a potential delivery system for CUR clinical application.

Table 3. Literature data of numerous other CUR formulations developed to enhance CUR bioavailability via oral administration

Animals	Formulation	Dosage (mg/kg)	AUC (μg·min/mL)	RB
Wistar rats [16]	CUR	1000	79.2	About 4.47
	CUR–phospholipid complex	1000	354	
SD rats [17]	CUR	100	1.45	7.1
	CUR dry emulsion	100	10.26	
SD rats [18]	CUR	50	0.92	5
	CUR solid dispersion	50	4.38	
Wistar rats [19]	CUR	50	9.36	7.02
	CUR solid dispersion	50	71.5	
SD rats [20]	CURoid	24	1.12	9.6
	CUR micro emulsion	24	10.86	
SD rats [21]	CUR	100	9.48	7.49
	CUR microspheres	100	71.05	
SD rats [22]	CUR	250	14.69	About 10.62
	CUR liposome	40	25.00	
RB: Relative bioavailability of corresponding formulation to CUR				

The hemolytic and intravenous irritation test both indicated that CUR injection was safe for the potential parenteral application. Although CUR injection contains some Kolliphor RH40, we think it is safe for clinical use. As we all know, paclitaxel is a medication used to treat several types of cancer. Paclitaxel injection (Taxol) is a clear, colorless to slightly yellow viscous solution. It is supplied as a no aqueous solution intended for dilution with a suitable parenteral fluid prior to intravenous infusion. Each milliliter of Taxol solution contains 6mg paclitaxel, 527 mg of purified Cremophor ELP (polyoxyethylated castor oil) and 49.7% (v/v) dehydrated alcohol. Patients need a dose about 300 mg paclitaxel every day, and the content of corresponding Cremophor EL is 26.35 g. In our study, if a patient takes the same amount of CUR, the content of Kolliphor RH40 is 12 g, the dose is lower than Cremophor EL. Cremophor EL and RH40 in concentrations corresponding to clinical doses caused endothelial and epithelial toxicity. Endothelial cells were more sensitive to surfactant treatment than epithelial cells, and Cremophor EL was more toxic than RH40 in both cell types [23]. Above all, CUR injection may be a good clinical choice soon.

CONCLUSION

CUR injection was successfully and firstly prepared by Kolliphor RH40 and ethanol. Various characteristics of CUR injection were all investigated in detail. The formulation was stable and can be diluted infinitely. What's more, no hemolysis occurred, and no muscle irritation and intravenous irritation was observed. The pharmacokinetic experiment proved that the plasma AUC of CUR injection was significantly higher than that of pure CUR. This study demonstrated the potential clinical use of CUR via intravenous injection delivery system.

ACKNOWLEDGEMENT

This work was supported by the talent introduction program of Hebei University (No. Y2005064), the medical and engineering science research center of Hebei University (No. BM201109). Hebei provincial natural science foundation of

China-Shijiazhuang pharmaceutical group (CSPC) Foundation (No. H2013201274) and the Top Young Talents Program of Hebei Province.

REFERENCES

1. Wegiel LA, et al. Curcumin amorphous solid dispersions: the influence of intra and intermolecular bonding on physical stability. Pharm Dev Technol. 2013;19:976-1986.
2. Li B, et al. Both solubility and chemical stability of curcumin are enhanced by solid dispersion in cellulose derivative matrices. Carbohydr Polym. 2013;98:1108-1116.
3. Gao M, et al. Preparation and characterization of curcumin thermosensitive hydrogels for intratumoral injection treatment. Drug Dev Ind Pharm. 2013;40:1557-1564.
4. Wang L, et al. Encapsulation of curcumin within poly (amidoamine) dendrimers for delivery to cancer cells. J Mater Sci Mater Med. 2013;24:2137-2144.
5. Wei XL, et al. Oily nanosuspension for long-acting intramuscular delivery of curcumin didecanoate prodrug: Preparation, characterization and in vivo evaluation. Eur J Pharm Sci. 2013;49:286-293.
6. Yu H, et al. Improving the oral bioavailability of curcumin using novel organogel-based nanoemulsions. Agric Food Chem. 2012;60:5373-5379.
7. John MK, et al. Development and pharmacokinetic evaluation of a curcumin co-solvent formulation. Anticancer Res. 2013;33:4285-4291.
8. Yoysungnoen P, et al. Anti-cancer and anti-angiogenic effects of curcumin and tetrahydrocurcumin on implanted hepatocellular carcinoma in nude mice. World J Gastroenterol. 2008;14:2003-2009.
9. Kim SA, et al. Enhanced systemic exposure of saquinavir via the concomitant use of curcumin-loaded solid dispersion in rats. Euro J Pharm Sci. 2013;49:800-804.
10. Gupta NK, et al. Bioavailability enhancement of curcumin by complexation with phosphatidyl choline. J Pharm Sci. 2011;100:1987-1995.
11. Ganta S, et al. Curcumin enhances oral bioavailability and anti-tumor therapeutic efficacy of paclitaxel upon administration in nanoemulsion formulation. Pharm Nanotechnol. 2010;99:4630-4641.
12. Dandekar PP, et al. Curcumin-loaded hydrogel nanoparticles: Application in anti-malarial therapy and toxicological evaluation. Pharm Nanotechnol. 2010;99:4992-5010.
13. Hu L, et al. Preparation and enhancement of oral bioavailability of curcumin using microemulsions vehicle. Agric Food Chem. 2011;60:7137-7141.
14. Onoue S, et al. Formulation design and photochemical studies on nanocrystal solid dispersion of curcumin with improved oral bioavailability. J Pharm Sci. 2009;99:1871-1881.
15. Yang KY, et al. Oral bioavailability of curcumin in rat and the herbal analysis from Curcuma longa by LC-MS/MS. J Chromatogr B. 2007;853:183-189.
16. Maiti K, et al. Curcumin-phospholipid complex: Preparation, therapeutic evaluation and pharmacokinetic study in rats. Int J Pharm. 2007;330:155-163.
17. Jang DJ, et al. Enhanced oral bioavailability and antiasthmatic efficacy of curcumin using redispersible dry emulsion. Biomed Mater Eng. 2014;24:917-930.
18. Seo SW, et al. Preparation and pharmacokinetic evaluation of curcumin solid dispersion using Solutol ®; HS15 as a carrier. Int J Pharm. 2012;424:18-25.
19. Wan DH, et al. Fusion as an Approach to Enhance Dissolution Rate of a Poorly Water-Soluble Drug (Curcurmin). Adv Mater Res. 2011;393-395:119-122.
20. Xiao Y, et al. Preparation and oral bioavailability study of curcuminoid-loaded microemulsion. J Agric Food Chem. 2013;61:3654-3660.
21. Zhang J, et al. Development and evaluation of a novel phytosome-loaded chitosan microsphere system for curcumin delivery. Int J Pharm. 2013;448:168-174.
22. Chen H, et al. N-trimethyl chitosan chloride-coated liposomes for the oral delivery of curcumin. J Liposome Res. 2012;22:100-109.

23. Kiss L, et al. Kinetic analysis of the toxicity of pharmaceutical excipients cremophor EL and RH40 on endothelial and epithelial cells. Int J Pharm. 2013;102:1173-1181.