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In vitro and in vivo evaluation of PEG-conjugated ketal-based chitosan micelles as the pH-sensitive carriers

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Abstract

A novel ketal-based PEGylated chitosan conjugate, mPEG-Chitosan-Ketal (PCK), as a pH-sensitive drug carrier for cancer therapy, was found to have lower toxicity and to form self-assembled micelles for curcumin (Cur) delivery in our previous study. In this study, the PCK micelles encapsulated curcumin (Cur-PCK) with higher EE was prepared with the enhanced method with the smaller diameters about 50 nm. In vitro cellular uptake test indicated that PCK micelles could be good intracellular carriers for the anti-tumor drugs. Curcumin was incorporated into PCK micelles not only as a therapeutic drug but also a fluorescence marker. Ex vivo imaging showed the fluorescence signals of the PCK micelles in the liver and spleen was much higher from 5 h to 12 h indicated that PCK micelles improved the blood circulation time and caused the selective accumulation in the liver and spleen. Pharmacokinetic studies indicated that PCK micelles had a long circulating time. In tissue distribution, the Cur-PCK micelles were decreased in the following order: spleen > liver > heart > lung. In vivo anti-tumor efficacy assay, Cur-PCK micelles showed the most effective tumor inhibiting ability. These findings have shed some light on the PCK polymer for potential pH-sensitive carriers of curcumin.

Keywords: pH-sensitive; ketal based chitosan (PCK); curcumin; pharmacokinetics; biodistribution; anti-tumor efficacy

Introduction

Micelles can accommodate the drug in blood and to enhance its stability and bioavailability by self-assembly and perform potential carriers for the delivery small molecular drugs, proteins, and genes [1-5]. As one of the most interesting micelles, the pH-sensitive micelles could release the loading at the targeted site according to the pH stimuli [6-8]. For example, due to the slightly acidic pH of endosomes or lysosomes of tumor, the pH-sensitive micelles could release anticancer drugs in tumor and decrease the side cytotoxicity and improve the efficacy [8].

In the last few years, several strategies have been proposed to develop the pH-sensitive micelles which could be described two types. The one type was copolymer based on the protonation of amino and/or carboxyl groups. The other type was fabricated with pH-sensitive bonds such as hydrozone and acetal which could be detached under the lower pH condition. As the one type, for example, Filippov [9] reported a pH-sensitive nanoparticle fabricated by polyelectrolyte. Hsiue [10] presented a pH-sensitive micelles fabricated by poly (2-ethyl-2-oxazoline)-poly(l-lactide) copolymers. Leroux [11] showed a pH-responsive polymeric micelles fabricated by Nisopropylacrylamide (NIPAM). Lee and Kwon prepared a pH-responsive mPEG-poly(β -amino ester) block copolymer micelles [12]. As the other type, for example, Kataoka [13,14] presented poly(ethylene glycol)-poly(amino acid)s copolymers using hydrozone bonds to pH-sensitive micelles. Chen showed pH-sensitive mPEG-Hz-cholesterol copolymers fabricated with hydrozone bonds used to pH-sensitive micelles, liposomes and gels [15-18]. Frechet presented PEO-dendrimer micelles with a pH-sensitive acetal bonds [19].

Nevertheless, compared with the acidic products of most of polymers such as PLA, PLGA, polyanhydride, polyesters, ketal polymers, as new and novel potential polymers for pH-sensitive micelles, can avoid the inflammatory problems at the acidic environment of lysosomes and tumors [20-25]. Some new pH-sensitive polyketals with good pH-sensitive properties were presented, such as poly (cyclohexane-1,4-diyl acetone dimethylene ketal)(PCADK)[26], poly(1,4- Phenyleneacetone dimethylene ketal) (PPADK)[27], PK3 [28,29] that can be fabricated to the pH-sensitive micelles.

In our previous studies [30], a novel pH-sensitive mPEG-Chitosan-Ketal (PCK), was reported with interesting pH-degradable ketal linkage that could degrade under locally acidic physiological conditions. The polymeric PCK carrying pH-sensitive ketal group as hydrophobic moieties and PEG group as hydrophilic moieties was synthesized. The structure of the PCK was characterized by FTIR and ^{1}H-NMR. The resulting PCK could form self-assembled nanoparticle encapsulated curcumin with the diameter about 100 nm. The PCK nanoparticle could release encapsulated curcumin with 89% at pH 5.0. The preliminary results of cytotoxicity assay and anti-tumor efficacy might provide potential for pH-sensitive targeted curcumin delivery.

In this study, as shown in Fig.1, our aim was to develop the in vivo behavior of ketal-based PEGylated pH-sensitive chitosan (PCK) micelles. In vitro cellular uptake of PCK micelles, curcumin was chosen as the self-fluorescence indicator with MCF-7 tumor cells. Then, a series of evaluation in vivo pharmacokinetics, biodistribution and anti-tumor efficacy of PCK micelles were also evaluated.

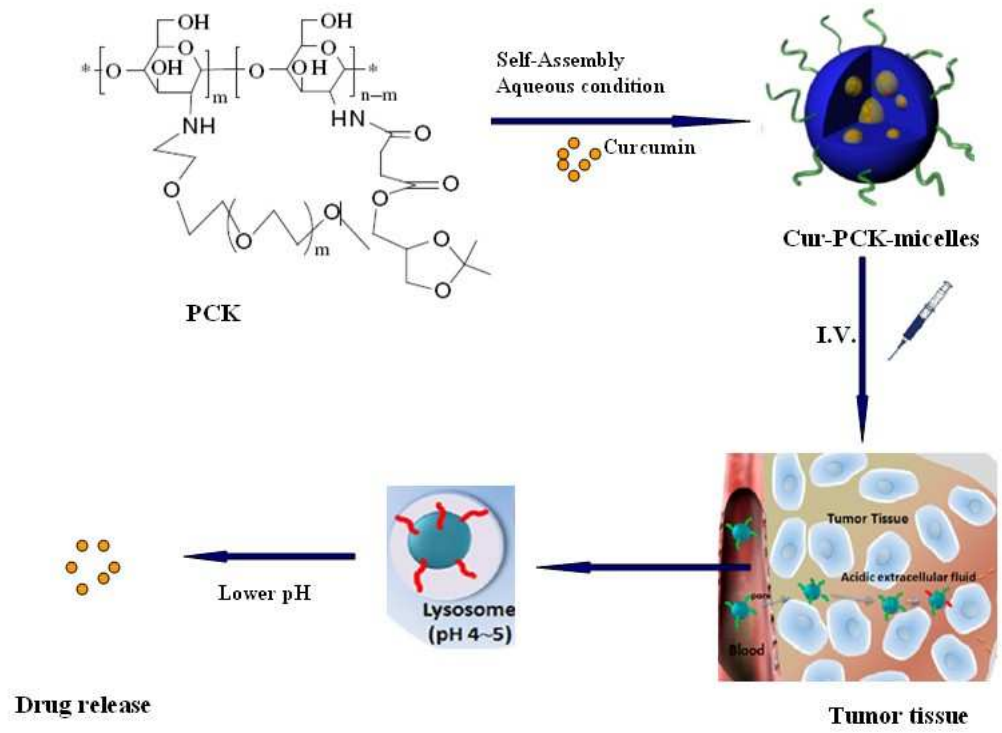

Fig.1. Schematic illustration of the formation, accumulation at tumor site and drug delivery of self-assembled Cur-loaded PCK micelles

Results and discussion

Preparation and characterization of PCK micelles

The PCK conjugate was synthesized by chemical conjugation of PEG and IPG to choitosan backbone as previously reported. Its characterization methods were also report in our previous study [30]. Briefly, chitosan of PCK was confirmed by 1HNMR with the peak at 4.70ppm(H-1), 3.0ppm(H-4), 3.9～3.6ppm(H-2，H-3，H-5，H-6). The peak at 8.00 ppm of amide bond was confirmed by the IPG conjugated to PCK. The peaks at 2.75 ppm and 2.90ppm of imino group were confirmed by the mPEG conjugated to PCK. The CMC of PCK was 0.024 mg/ml used by pyrene emission spectra (shown in Fig.2.).

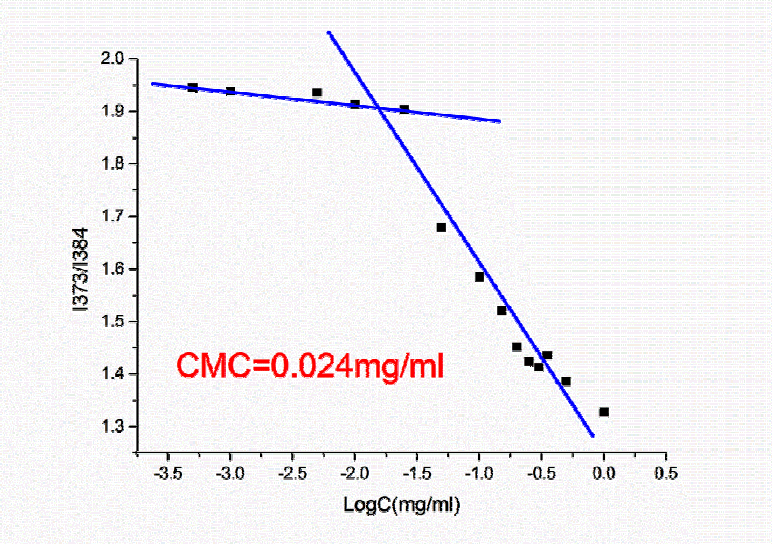

Fig.2. The intensity ratio (I_{373}/I_{384})of the pyrene emission spectra versus the log concentrations of PCK

As our previous study shown, the pH-sensitive property of PCK conjugate could be controlled by pH condition. The PCK micelles could release encapsulated curcumin with accelerated rate under lower pH condition. The encapsulated curcumin PCK micelles were stable and have less toxicity compared to curcumin suspension [30]. As shown in Fig.3, the PCK micelles encapsulated curcumin (Cur-PCK) with higher EE was prepared with the enhanced method with the samller diameters about 50 nm than the previous 100 nm [30].

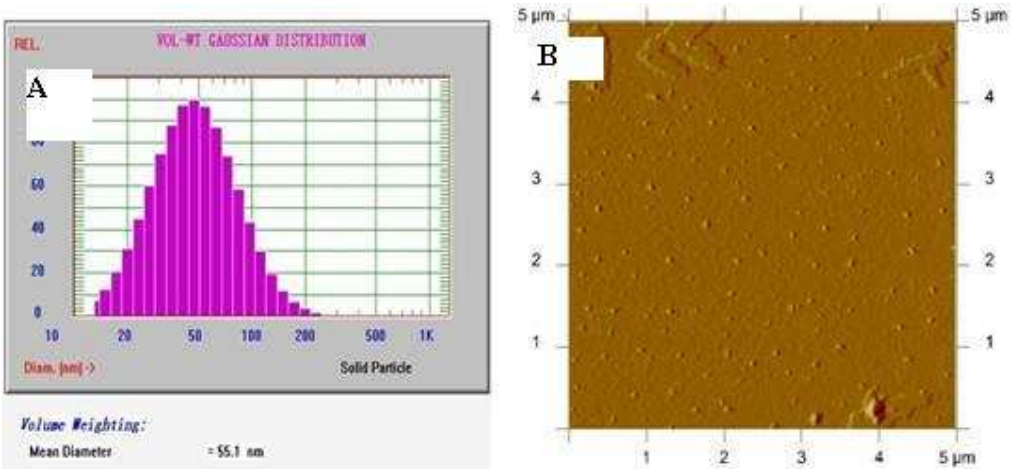

Fig.3. Size distribution (A) and AFM image (B) of Cur-PCK micelles

In vitro cellular uptake of PCK micelles

During our research, the studies of in vitro cellular uptake and intracellular behavior of PCK micelles have also taken other markers, such as coumarin-6, doxorubicine. But there is no innovation. When the Cur-PCK micelles were used into the cellular uptake test, the good pictures of cellular uptake were obtained. There is little report about Cur labeled nanoparticle. As shown in Fig.4, the fluorescent signals treated with Cur-PCK micelles were detected in the MCF-7 cancer cells from 0.5-6 h. However, the fluorescent signals treated with the control were not detected due to the Cur dissolved in the ethanol. The fluorescent intensity was higher at 4 h increasingly by the cellular uptake. Then, the cells presented the atrophy or apoptosis decreasingly at 6 h due to the encapsulated Cur therapeutic effect. This finding demonstrated that PCK micelles could be good intracellular carriers for the anti-tumor drugs.

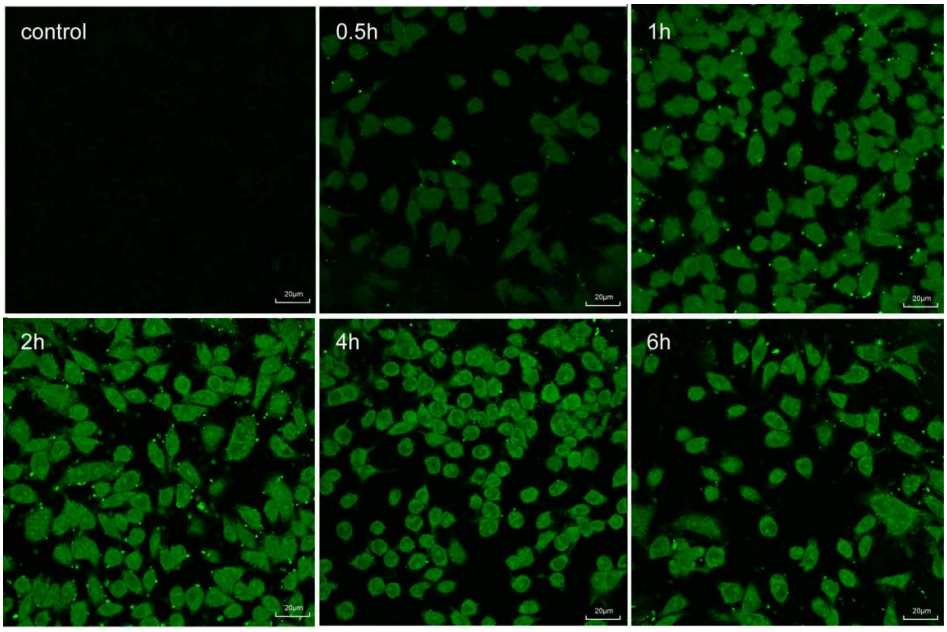

Fig.4. Fluorescent imaging of cellular uptake of Cur-PM at different times after incubation with MCF-7 cell

Ex vivo imaging of PCK micelles

In this study, the preliminarily biodistribution and tissue targeting efficiency of PCK micelles were presented with the noninvasive near infrared optical imaging technique. Fig.5. showed the real-time images of DiR labeled PCK micelles and the free DiR in the mice at 4 h. Due to the blood circulation of micelles, the abundant fluorescence signals of PCK micelles were founded in liver about 4 h. However, a little fluorescence signals of free DiR were founded in liver about 4 h. This could indicate that the pH-sensitive PCK micelles will have a longer blood circulation after 4 h detection.

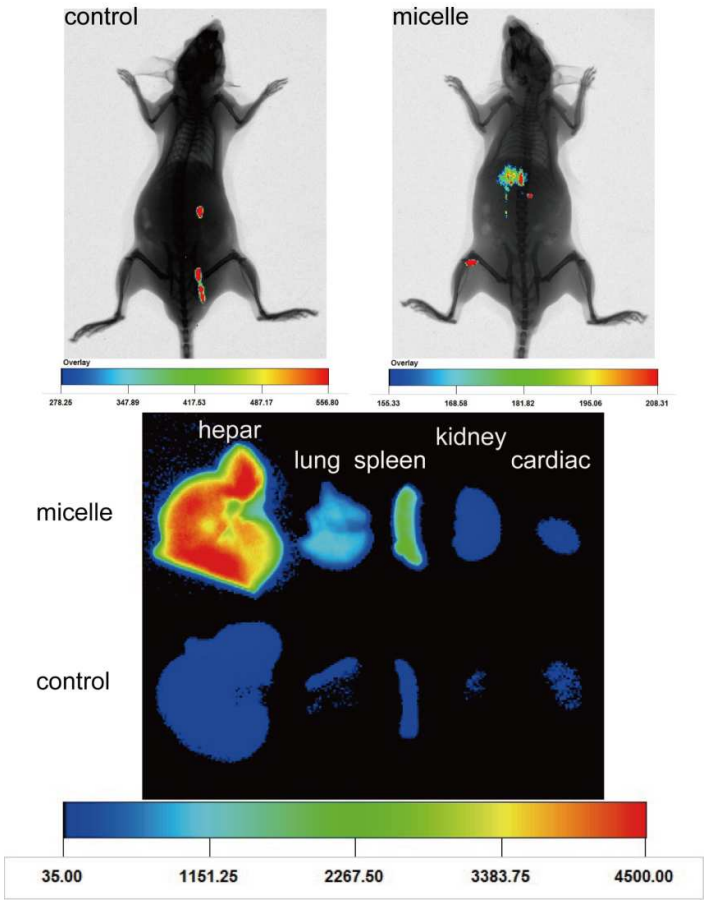

Fig.5. In vivo non-invasive NIRF images of free DiR (control) and DiR labeled PCK micelles in mice at 4 h

Then, the further studies in different time were carried out. Fig.6. showed the fluorescence signals of free DiR reduced more quickly. Interestingly, the fluorescence intensity of the PCK micelles in the liver and spleen was much higher from 5 h to 12 h [30, 31]. These results indicated that PCK micelles improved the blood circulation time and caused the selective accumulation in the liver and spleen.

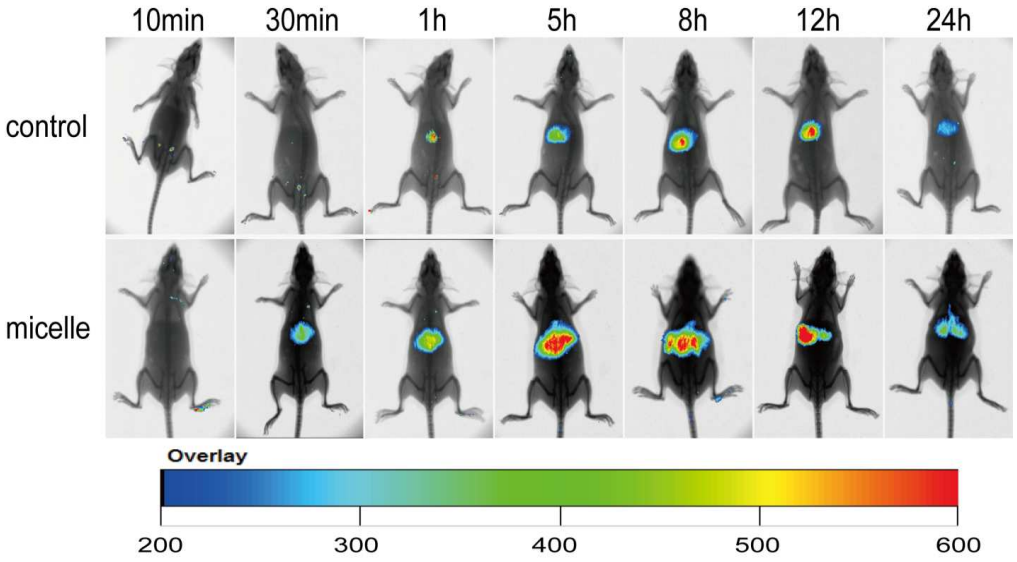

Fig.6. Time-dependent whole body images after intravenous injection

Pharmacokinetic Studies of Cur-PCK micelles

To assess the pharmacokinetic behavior of Cur-PCK micelles, each formulation was administrated at a dose of 10 mg/kg. The pharmacokinetic parameters for Cur in plasma were tested by the compartmental method under HPLC analysis. The plasma concentration profiles of Cur after intravenous injection are shown in Fig.7.

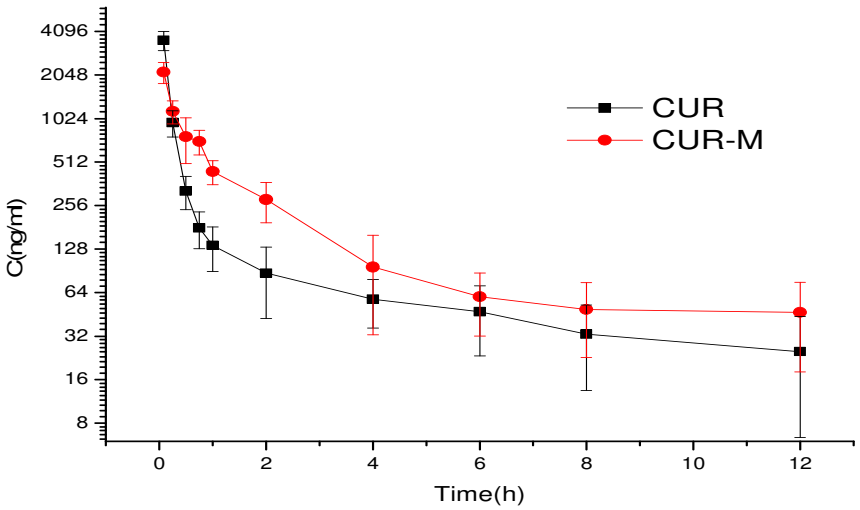

Fig.7. Time courses of Cur levels in rat plasma after i.v. administration of Cur-PCK and Cur suspension. Each point represents the mean ±SD (n =5)

Table 1 Pharmacokinetic parameters of Cur in the two formulations after i.v. administration

Parameter	Mnits	CUR	CUR-PCK
AUC	h*μg/ml	1.25±0.26	2.53±0.44*
K01	1/h	0.123±0.033	1.009±0.032
K10	1/h	6.32±2.09	1.07±0.39
K12	1/h	4.18±1.52	2.02±0.81
K21	1/h	2.059±0.658	0.586±0.137
α	1/h	10.41±1.81	3.77±1.30
β	1/h	1.315±0.417	0.164±0.040*
$t_{1/2\alpha}$	h	0.068±0.013	0.208±0.092*
$t_{1/2\beta}$	h	0.559±0.152	3.722±1.921*
A	μg/ml	7.39±1.15	2.20±0.53
B	μg/ml	0.584±0.191	0.324±0.046
CL_F	L/h	4.12±0.83	2.03±0.36*
Tmax	h	0.026±0.007	0.039±0.011
Cmax	μg/ml	5.05±1.70	2.34±0.57
Vc_F	L	0.793±0.209	1.997±0.469*

Data represent mean value ±SD, n =5.
*p<0.05, compared with Cur

The pharmacokinetic parameters are shown in Table 1. Both Cur-PCK micelles and Cur control presented the similar distribution trends at the same dose of 10 mg/kg. However, the plasma AUC of Cur-PCK was 2.0-fold higher than that of the control; and the Vc, t1/2α, and t1/2β of Cur in Cur-PCK were increased by 2.5-, 3.06-, and 6.66-fold, respectively. Free Cur in DMSO was quickly removed from the circulating system after intravenous injection with a rapid distribution phase (t1/2 α (h) =0.068±0.013) and a rapid terminal elimination phase (t1/2 β (h)= 0.559±0.152 h), and was under the HPLC detection limit after 6 h. The Cur-PCK micelles have a slow distribution phase (t1/2 α (h) =0.208±0.092) and a slow terminal elimination phase (t1/2 β (h)= 3.722±1.921 h). But the Cur-PCK micelles could be detected after 12 h and 24 h. However, Cur-PCK micelles signifycantly changed the Cur pharmacokinetic parameters in comparison with Cur suspension. Pharmacokinetic studies indicated that PCK micelles had a good and long circulating time and will obtain the good therapeutic effect [15, 30, 31].

Tissue Distribution Studies of Cur-PCK micelles

The tissue distribution profiles of Cur-PCK and Cur after i.v. administration were studied in mice. The concentration of Cur in each tissue was tested by HPLC assay. As shown in Fig.8, 9, Cur was widely and rapidly distributed into most tissues, and the highest concentration of Cur-PCK micelles was found in lung, followed by liver and spleen at 15 min after administration. However, after 6 h, the Cur concentration was decreased in the following order: spleen > liver > heart > lung (Fig.9.). Therefore, it is interesting that the pH-sensitive Cur-PCK micelles might have lower accumulation in the liver and spleen than the Cur suspention and have the potential lung-targeted effect [31].

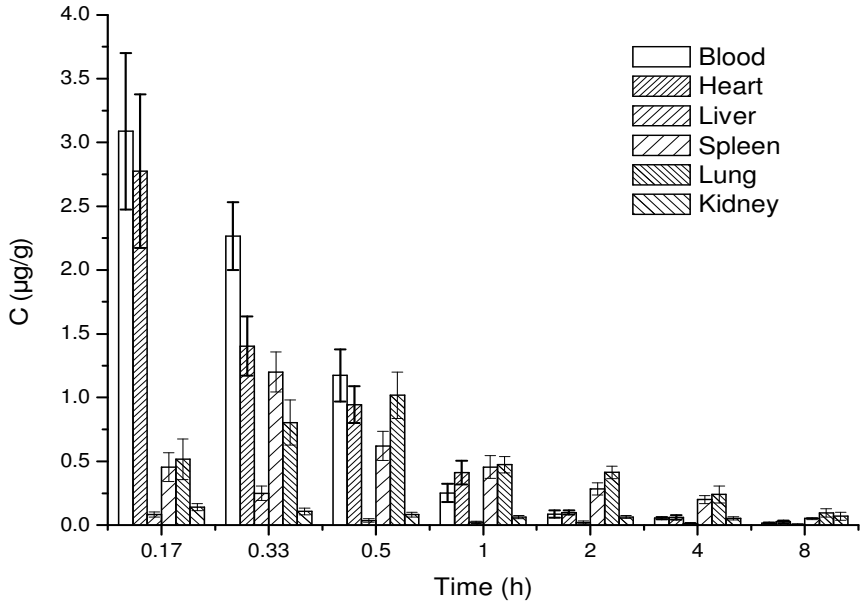

Fig.8. Cur suspension (DMSO) concentrations in rats at a dose of 10 mg/kg at different sample times. Each column represents the mean± SD (n =6).

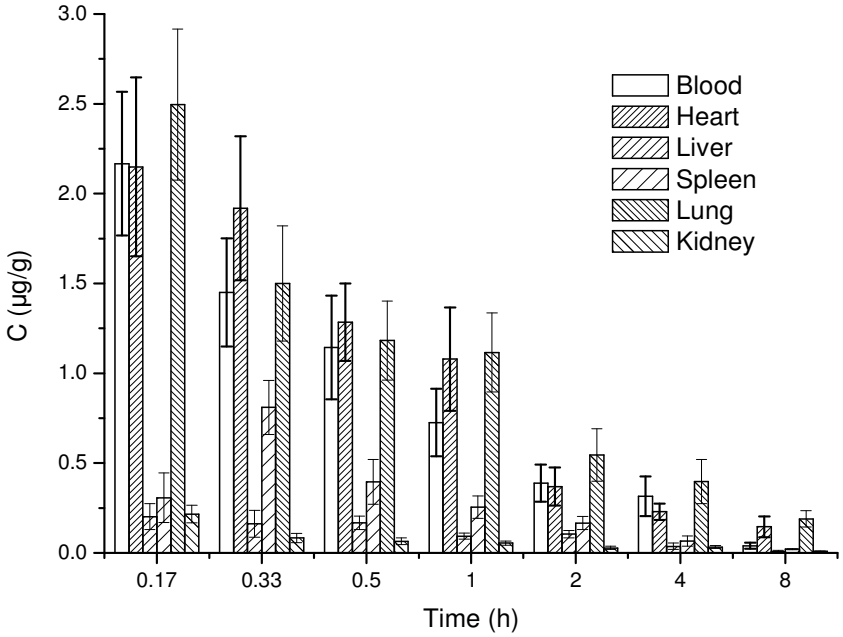

Fig.9. Cur-PCK micelles concentrations in rats at a dose of 10 mg/kg at different sample times. Each column represents the mean± SD (n =6).

In vivo anti-tumor efficacy assay

In the previously study, the primary of anti-tumor efficacy assay was assayed. As shown in Fig.10, the pathological image is the tissue sections in different mice group[15]. The saline group showed typical pathological characteristics of tumor with closely arranged tumor cells. However, tumor tissue in the Cur control presented spotty necrosis and intercellular blank. Moreover, Cur-PCK micelles showed the most effective tumor inhibiting ability[31]. The findings indicated the Cur-PCK nanoparticle is a potential carrier for Cur anti-tumor delivery.

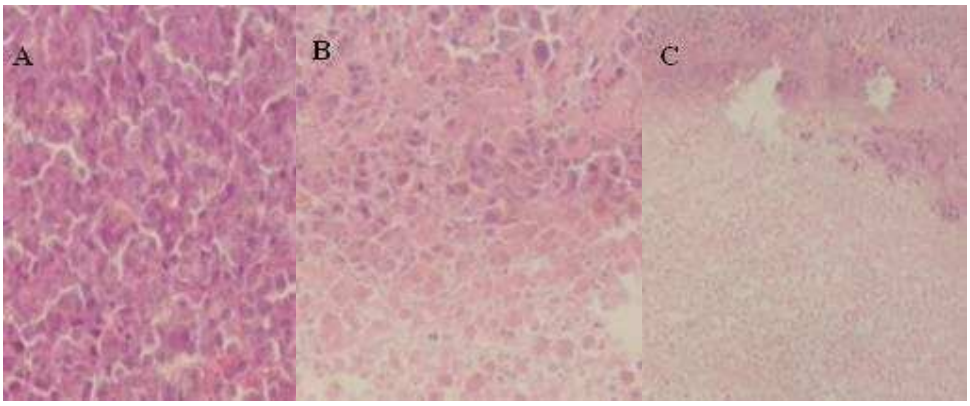

Fig.10. Pathology section of tumor with different Cur formulation. Normal saline as a control (A), Cur control (B), Cur-PCK(C)

Conclusion

In this study, we have prepared a novel curcumin-loaded pH-sensitive ketal based chitosan polymer, mPEG-Chitosan-Ketal (PCK). The mechanism of pH-sensitive ketal based chitosan polymer drug delivery system could be explained that the PCK micelles can deliver drugs to the acidic environments of tumors, inflammatory tissues[30].

The PCK micelles encapsulated curcumin (Cur-PCK) with higher EE was prepared with the enhanced method with the smaller diameters about 50 nm than the previous 150 nm. In vitro cellular uptake test indicated that PCK micelles could be intracellular carriers for the anti-tumor drugs. As a novelty, curcumin (Cur), was incorporated into PCK micelles not only as a therapeutic drug but also a fluorescence marker. Ex vivo imaging showed the fluorescence signals of the PCK micelles in the liver and spleen was much higher from 5 h to 12 h indicated that PCK micelles improved the blood circulation time and caused the selective accumulation in the liver and spleen. Pharmacokinetic studies indicated that PCK micelles had a longer circulating time and will obtain the good therapeutic effect.

In tissue distribution, the Cur-PCK micelles were decreased in the following order: spleen > liver > heart > lung. It is interesting that the pH-sensitive Cur-PCK micelles might have lower accumulation in the liver and spleen and have the potential lung-targeted effect. In vivo anti-tumor efficacy assay, Cur-PCK micelles showed the most effective tumor inhibiting ability. Overall, the findings have shed some light on the PEG conjugated ketal-based chitosan Cur micelles (Cur-PCK) will be a potential pH-sensitive carrier for nano-drug delivery

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Disclosure

The authors report no conflicts of interest in this work.

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