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Chitosan oligomers as drug carriers for renal delivery of zidovudine

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ABSTRACT

Chitosan oligomers (COS) are water soluble and can be a potential drug carrier for renal targeting delivery. Zidovudine (AZT), a FDA approved antiretroviral drug, has a very short half life and is eliminated very quickly in human plasma and kidney after administration. In this study, AZT-COS conjugates were prepared and evaluated in terms of renal targeting. The *in vitro* release of AZT from AZT-COS conjugates was confirmed in mice plasma and renal homogenate. The pharmacokinetics study indicated longer mean retention time of AZT-COS conjugates with the values of about 1.5 h than 0.59 h of AZT. The AZT-COS conjugates were found accumulated in kidney other than heart, liver, spleen, lung and brain after *i.v.* administration, in line with the evidence of the fluorescence imaging of FITC labeled COS in 12 h. In conclusion, AZT-COS conjugates have the potential to be developed into a renal-targeting drug delivery system.

1. Introduction

Several approaches are available for the improvement of the solubility of chitosan in aqueous systems: for example trimethyl chitosan (Mourya & Inamdar, 2009; Muzzarelli & Tanfani, 1985; Sieval et al., 1998) shows much higher aqueous solubility than chitosan in a broader pH range as the intermolecular hydrogen bond between the amine and the hydroxyl groups of the chitosan is weakened. This derivative considerably increases the permeation of neutral and cationic peptide analogs across Caco-2 intestinal epithelia (Thanou, Verhoef, & Junginger, 2001). Another approach for improving the solubility of chitosan is to prepare the oligomers of chitosan (COS, degree of polymerization 2–10) from chitosan either chemically or enzymatically (Aam et al., 2010; Kim & Rajapakse, 2005). Various lipases hydrolysize chitosan to water-soluble COS (Lee, Xia, & Zhang, 2008; Muzzarelli, Stanic, & Ramos, 1999; Muzzarelli, Xia, Tomasetti, & Ilari, 1995).

Chitins and chitosans are non-allergenic drug carriers (Muzzarelli, 2010) whose safety has been amply assessed (Kean & Thanou, 2010). Randomly 50% N-acetylated chitosan labeled with fluorescein isothiocynate (FITC) and administered intraperitoneally to mice accumulated in kidneys more than in blood, liver, spleen, lung and muscle (Onishi & Machida, 1999). Using water-soluble chitosan as a vehicle for renal targeting delivery, Yuan et al. (2007) synthesized randomly 50% N-acetylated low molecular weight chitosan (LMWC)-prednisolone conjugate and administrated to mice by *i.v.* injection. It was confirmed that the drug-carrier conjugate was uptaken by more than 10 folds than the controlled prednisolone in kidney. Further research indicated that this derivative of chitosan was mainly uptaken by the megalin receptors of renal proximal tubules (Yuan et al., 2009). Recent study of the N-deacetylation degree (DD) of chitosan revealed that chitosan with a high DD would be more easily uptaken by kidney than that with a low DD (Yuan et al., 2011).

Based on the results described above, COS, the water-soluble chitosan oligomers with a very high DD (≥98%), can be used as a drug carrier targeted to kidney, aiming to the tubular accumulation mediated via the megalin receptors (Christensen & Verroust, 2002). The candidate drug selected for this study is zidovudine (AZT), the first antiretroviral drug approved by FDA for the treatment of acquired immune deficiency syndrome (AIDS). AZT can reduce both the mortality and the morbidity of human immunodeficiency virus (HIV) infection, and has been widely used in clinically treatment of AIDS (Broder, 2010). Although AZT has been proved to be beneficial to the patients with human immunodeficiency virus associated nephropathy (HIVAN), one of the most common complication found in HIV infected persons (Cohen, Sun, Shapshak, & Imagawa, 1989; Lochner & Wolf, 2006), and could slow down the evolution of HIVAN to end stage renal disease (ESRD) (Ifudu et al., 1995; Michel, Dosquet, Ronco, Mougenot, & Mignon,

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1992; Takahashi, Nakamura, Kanda, & Iwamoto, 2004), the therapeutic effect was severely limited due to its rapid elimination from blood circulation and scarce accumulation in kidney after administration (de Miranda, Burnette, & Good, 1990; de Miranda et al., 1989). AZT has been reported in the form of various conjugates, such as poly(HEMA-zidovudine) conjugate (Neeraj, Chandrasekar, Sara, & Rohini, 2011), poly(ethylene glycol)-AZT conjugate (Li et al., 2010), dextrin-AZT conjugate (Wannachaiyasit, Chanvorachote, & Nimmannit, 2008) and lipid-AZT conjugate (Watal, Shukla, & Misra, 2000). Among these, polymer-AZT via a succinic spacer could sustain the release of AZT from less than 1 h to approximately 24 h, which satisfies the requirement to a controlled release of AZT to overcome the problem of its rapid elimination in blood circulation. And what's more, the combination of COS with AZT is considered to treat the infected renal cells through COS-renal targeting delivery of AZT.

2. Materials and methods

2.1. Apparatus and materials

Maximum excitation wavelength (Ex), maximum emission wavelength (Em) and fluorescence intensity of samples were determined using a RF-5301PC spectrofluorophotometer (Shimadzu, Japan). Fluorescence imagings were performed in a macro imaging system LT-9 equipped with illumatool dual light system LT-99D2 (Lightools Research, Encinitas, USA). FTIR spectra were recorded using a Thermo Nicolet NEXUS 670 FTIR mainframe (Thermo Fisher Scientific, USA). HPLC analysis was performed using Shimadzu instruments (Chiyoda-Ku, Japan) consisted of a CTO-10A column thermostat, two LC-10AT pumps and a SPD-10A UV detector. A Scienhome ODS column (5 μm, 150 × 4.6 mm, Tianjin, China) was used to separate samples.

Chitosan oligomers (base form, DD ≥98%; COS-1: polymerization degree 2–8, average molecular weight ≤1 kDa; COS-2, polymerization degree 2–10, average molecular weight ≤2 kDa; COS-3, polymerization degree 2–15, average molecular weight ≤3 kDa) were purchased from Dalian Glycobio Co., Ltd (China). Zidovudine (USP28) was supplied by Guangzhou Eastbang Pharmaceuticals Science & Technology Co., Ltd (China). Fluorescein isothiocyanate, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), N-hydroxysuccinimide (NHS) and 4-(dimethylamino)-pyridine (DMAP) were purchased from Sigma–Aldrich Co. (USA). All other chemicals used were of reagent grade. Wistar rats (male, 200–220 g) and kunming mice (male, 18–22 g) were provided by West China Experimental Animal Center of Sichuan University (China).

2.2. Fluorescece imaging of FITC-COS

Three kinds of FITC-COS were prepared according to Onishi and Machida (1999) with some modifications. Briefly, a solution of FITC (6 mg, 15.4 μmol) in 0.6 ml of dimethyl sulfoxide was added dropwise to a solution of COS (120 mg, 0.73 mmol of amino group) in 12 ml of carbonate buffer (0.05 M, pH 10) with stirring. The mixture was stirring at room temperature for 16 h, then precipitated into 100 ml of acetone. The obtained FITC-COS was washed by acetone for several times until uncoupled FITC was totally removed (monitored by TLC) then drying in vacuum at 40 °C overnight. All the procedures described above were manipulated away from light. The Ex, Em and FITC labeling ratios of FITC-COS were detected in a carbonate buffer (0.05 M, pH 10).

Kunming mice were divided into four groups randomly and injected intravenously with 3 mg/kg of FITC or about 100 mg/kg of FITC-COS (equivalent to 3 mg/kg of FITC) dissolved in physiological saline, respectively. The mice were exsanguinated and sacrificed at prearranged intervals, then the tissue samples including brain, heart, liver, spleen, lung and kidney were collected, washed with physiological saline, and the fluorescence imagings were recorded. Another mouse served as the blank control was processed simultaneously following the same procedures except that physiological saline was given instead of FITC or FITC-COS solution.

2.3. Synthesis of AZT-COS conjugates

5′-O-succinylzidovudine (AZT-suc) was synthesized according to the method described by Giammona, Cavallaro, Fontana, Pitarresi, & Carlisi (1998) with some modifications. Briefly, 5.34 g (20 mmol) of AZT, 4.00 g (39.7 mmol) of succinic anhydride and 4.00 ml (28.7 mmol) of triethylamine were dissolved in 100 ml of anhydrous tetrahydrofuran. The solution was heated at reflux in an oil bath with stirring for about 2 h until there was no existence of free AZT (monitored by TLC). The solvent was rotary evaporated, and the residue obtained was dissolved in 100 ml of water. The aqueous solution was acidified to pH 2 by dilute hydrochloric acid (1 M) and extracted by 50 ml of dichloromethane for four times. The organic solution was dried by anhydrous sodium sulfate overnight, then filtered and evaporated to gain AZT-suc. All the procedures described above were manipulated away from light.

Three kinds of AZT-COS were synthesized following the same procedures described below. A solution of AZT-suc (0.496 g, 1.42 mmol) in 1 ml of dimethylformamide was added dropwise to a solution of 0.5 g (3.04 mmol of amino group) of COS in 20 ml of water, then 0.196 g (1.7 mmol) of NHS, 0.4 g (2.09 mmol) of EDC·HCl and 0.257 g (2.10 mmol) of DMAP were added sequentially. The reaction mixture was stirring for 16 h at room temperature away from light, then precipitated into 150 ml of acetone. The obtained residue was washed by acetone for several times until the reagents and uncoupled AZT-suc were totally removed (monitored by TLC and HPLC), then dried in vacuum at 40 °C overnight. The conjugates were obtained in a yield of 97.4%, 98.9% and 96.7% based on the starting materials COS-1, COS-2 and COS-3, respectively. FTIR (KBr) was performed to confirm the coupling of AZT with COS, and the amount of AZT linked to COS was evaluated by HPLC after alkaline hydrolysis of the conjugates.

2.4. In vitro drug release of AZT-COS

The *in vitro* drug release of various AZT-COS were investigated in 35% mouse plasma (diluted with PBS, pH 7.4, v/v) and 35% mouse renal homogenate (homogenized and diluted with physiological saline, g/ml). An aqueous solution of AZT-COS (200 μl) was added to 4 ml of preheated hydrolysis medium. The mixture was maintained in a water bath at 37 ± 0.1 °C under continuously stirring, and two portions of 100 μl were sampled each time at suitable time intervals. An aqueous solution of sodium hydroxide (0.3 M, 50 μl) was added to one portion of sample to hydrolyze residual AZT-COS for 5 min with vortexing, then neutralized by 50 μl of dilute hydrochloric acid (0.3 M). After deproteinized by 400 μl of acetonitrile and centrifugated at 10,000 rpm for 10 min, the samples were analyzed by HPLC. Another portion of sample was determined following the same procedures with the replacements of NaOH and HCl by water. The AZT contents of residual AZT-COS in each time point were calculated by subtracting the AZT contents of unhydrolyzed samples from that of corresponding hydrolyzed samples. Each experiment was repeated in triplicate.

2.5. Pharmacokinetics studies

Wistar rats were divided into four groups randomly, and each group had 5 rats. The control group was administered intravenously with 37.5 mg/kg of AZT dissolved in physiological saline,

and the three test groups were intravenously given various AZT-COS (equivalent to 37.5 mg/kg of AZT) dissolved in physiological saline, respectively. The blood samples were collected at predetermined intervals, and the plasma was separated by centrifugation. Each plasma sample of the test groups was divided into two portions and followed the same procedures described in Section 2.4 to determine the plasma concentrations of released AZT and total AZT of the conjugates (both released and unreleased), whereas the plasma samples of the control group were treated as unhydrolyzed samples.

2.6. Biodistribution of AZT-COS conjugates

Kunming mice were divided into four groups randomly, and each group had 30 mice. The control group was administered intravenously with 50 mg/kg of AZT dissolved in physiological saline, and the three test groups were intravenously given various AZT-COS (equivalent to 50 mg/kg of AZT) dissolved in physiological saline, respectively. The mice were exsanguinated and sacrificed at prearranged intervals, then the tissues including heart, liver, spleen, lung, kidney and brain were collected, washed with physiological saline, weighed and homogenized with two fold of physiological saline (g/ml). The samples of three test groups were treated as hydrolyzed samples described in Section 2.4, whereas those of the control group were treated as unhydrolyzed samples.

2.7. HPLC analysis of AZT

A mixture of methanol and phosphate buffer (0.05 M, pH 2.5) in the ratio of 30:70 (v/v) was served as the mobile phase. 300 μl of each sample was concentrated in nitrogen gas flow at 40 °C away from light, redissolved in 300 μl of the mobile phase and centrifugated at 10,000 rpm for 10 min. 50 μl of each treated sample was injected into the HPLC instruments, and the effluent was detected at 265 nm.

2.8. Data analysis

The data of pharmacokinetics and biodistribution study were processed using the Drug and Statistics Software 2.0 (DAS 2.0, Shanghai, China).

3. Results and discussion

3.1. Fluorescence imaging of FITC-COS

Fluorescence imaging of the carrier in organs and tissue becomes a convenient tool. Recent investigations indicated that the DD and the molecular weight (MW) of water-soluble chitosan derivatives may play a role in manipulating renal targeting retention time. Aiming to understand the potential of COS with a high DD and low MW as a renal drug delivery carrier, three kinds of COS with a DD of more than 98% and a MW of less than 3 kDa were investigated. FITC labeled COS was synthesized and examined, the Ex and Em of various FITC-COS were identical to FITC with the value of 490 ± 1 nm and 520 ± 1 nm. The FITC labeling ratios of FITC-COS-1, FITC-COS-2 and FITC-COS-3 were 29.7, 29.4 and 30.1 μg/mg, respectively. After intravenous administration, a significant portion of FITC-COS accumulated in kidney, whereas the other tissues showed no notable difference with blank samples (Fig. 1). The renal fluorescence intensity of three kinds of FITC-COS were significantly higher than that of the FITC control within 12 h (Fig. 2), and the brightest renal fluorescence was found in the FITC-COS-2 group among three test groups. With a DD of more than 98% and a MW of less than 2 kDa, the renal fluorescence of the FITC-COS-2 group showed the maximal brightness in 5 h, indicating the maximal accumulation of the carrier in kidney. Through the urinary excretion and *in vivo* enzymatic digestion, the fluorescence was faded off after 12 h.

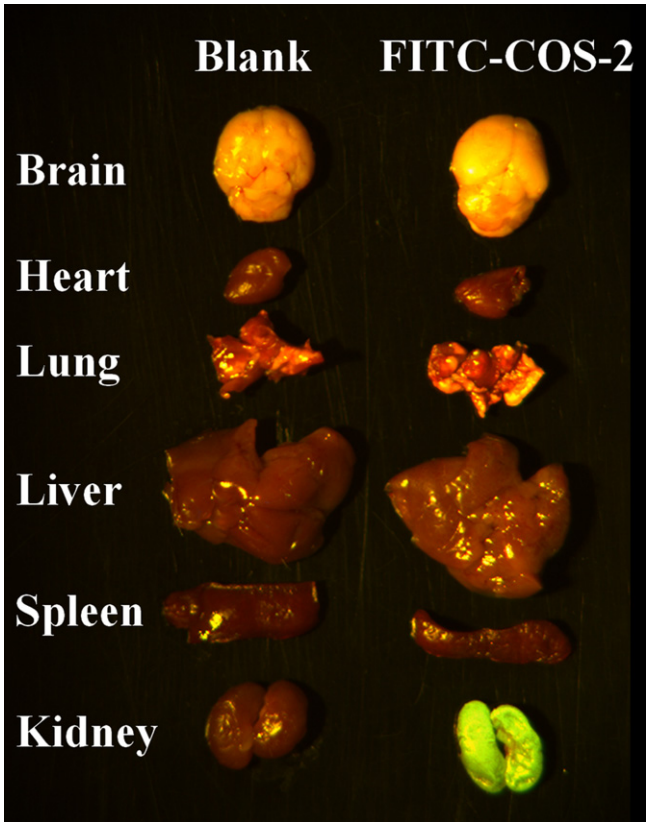


Fig. 1. Fluorescence imaging of the tissue distribution of FITC-COS-2. The samples of FITC-COS-2 were collected at 90 min after *i.v.* injection. FITC-COS-2 accumulated in nowhere but kidney.

Onishi and Machida (1999) firstly reported the renal accumulation of randomly 50% N-acetylated chitosan, a water-soluble substitute of chitosan, with a MW of 110 kDa. However, since the MW of the water-soluble chitosan is higher than the barrier of the glomerular filtration (≤70 kDa), there was no explanation for the reason of its renal accumulation. To verify the hypothesis of the accumulation of the water-soluble chitosan with lower MW in kidney, Yuan et al. (2007) synthesized LMWC with relative low MW from 3.8 to 31 kDa. The FITC labeled LMWC (19 kDa) was uptaken

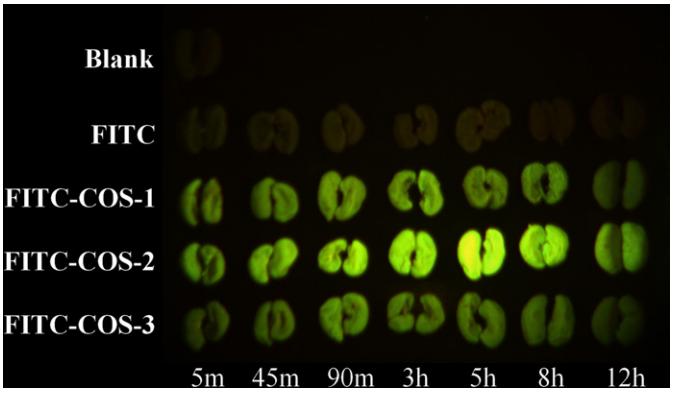
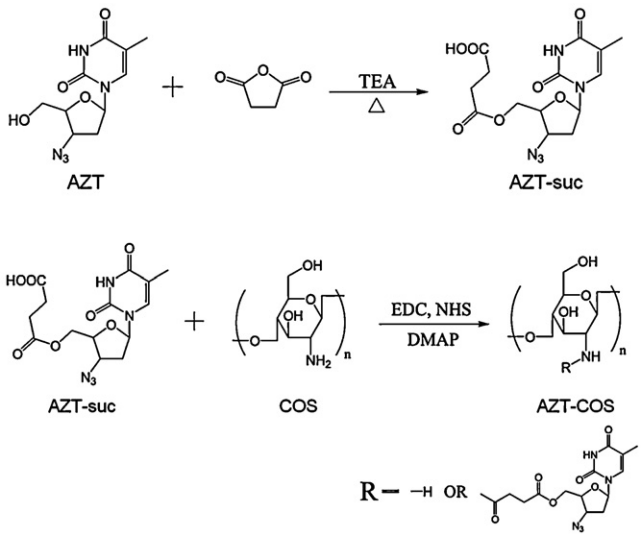


Fig. 2. Fluorescence imaging of renal accumulation of control FITC and various FITC-COS. Three kinds of FITC-COS accumulated in kidney much more than FITC throughout the whole course after *i.v.* injection, whereas FITC-COS-2 exhibited the highest renal accumulation among three test groups.



Scheme 1. Synthesis routes of AZT-COS conjugates.

immediately and accumulated in kidney until fading off after 2 h after *i.v.* administration. Fluorescence images showed that FITC-LMWC accumulated in renal proximal tubules, and the renal uptake was further verified to be mediated by the megalin receptors (Yuan et al., 2009). Recently Yuan et al. (2011) reported that the renal accumulation of chitosan was suppressed by N-acetylation. The DD of chitosan can significantly influence the affinity between chitosan and the megalin receptors in renal proximal tubules. Our high DD and low MW COS added strong evidences that the degree of N-acetylation of COS might overweight the size of the polymer carrier when it is within 31 kDa.

3.2. Synthesis and characterization of AZT-COS

To covalently bond AZT with COS, AZT-COS was synthesized by a two-step reaction (Scheme 1). Firstly, AZT was reacted with succinic anhydride in the presence of triethylamine, which is a well established method to prepare the 5′-O-succinoylated AZT (AZT-suc) (Giammona et al., 1998; Neeraj et al., 2011; Wannachaiyasit et al., 2008). The obtained AZT-suc was covalently coupled with various COS via a amide bond to form AZT-COS conjugates using EDC, NHS and DMAP as coupling agents. The absence of the reagents and uncoupled AZT-suc within the conjugates was confirmed by TLC and HPLC analysis.

The coupling of AZT with COS was verified via FTIR spectra (Fig. 3). In addition to the characteristic peaks found in the spectrum of COS, there was a sharp peak at 2110.57 cm^{-1} in the spectrum of AZT-COS, indicating the characteristic of $-N_3$ group of AZT. Which demonstrated the successful coupling of AZT with COS. After alkaline hydrolysis, there was no AZT-suc but AZT released from AZT-COS, which probably due to the significant difference between the stability of ester bond (AZT-suc) and amide bond (suc-COS) in the conjugates. The average contents of AZT coupled to COS-1, COS-2 and COS-3 were 12.04%, 12.50% and 12.22% (w/w), respectively, determined by HPLC after alkaline hydrolysis. All the conjugates were easily dissolved in water and physiological saline with the solubility of more than 50 mg/ml.

3.3. In vitro drug release of AZT-COS

AZT has a very short plasma half-life (approximately 1 h) (Aggarwal, Gogu, Rangan, & Agrawal, 1990). *In vitro* hydrolysis of various esters in human plasma indicated that esterised AZT were

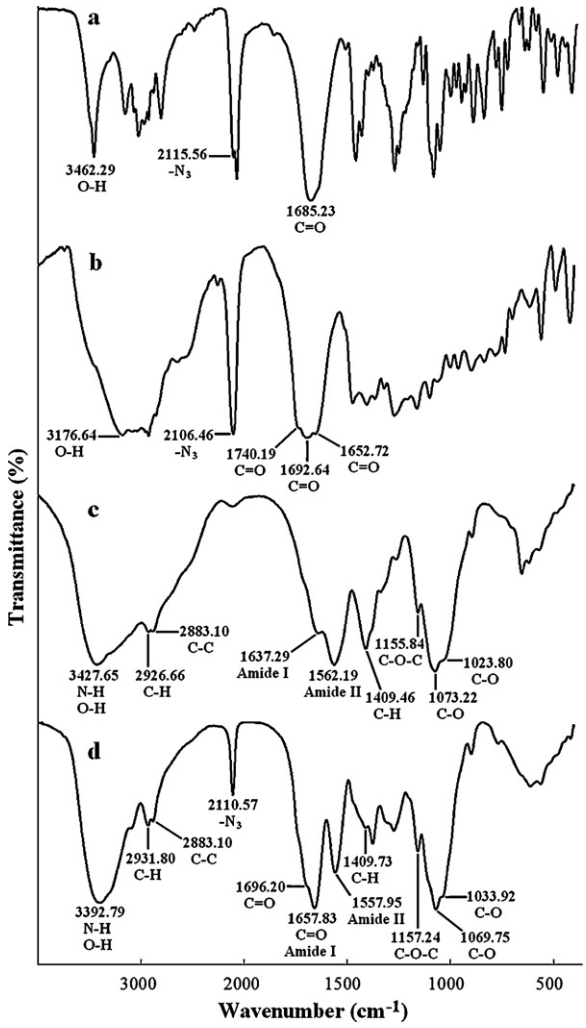


Fig. 3. FTIR spectra of AZT (a), AZT-suc (b), COS (c) and AZT-COS (d). The characteristic peak of $-N_3$ group (2110.57 cm^{-1}) in the spectrum of AZT-COS verified the coupling of AZT with COS.

relatively stable towards plasma esterases with the *t*1/2 ranging from 10 to 240 min. Under the influence of enzymatic removal of AZT, the AZT content released from AZT-COS conjugates could not be directly measured. In this study, the AZT contents of residual AZT-COS instead of released AZT was detected at each time point to investigate the release profiles of AZT-COS in mouse plasma and renal homogenate (Fig. 4). The removal of AZT in mouse plasma was faster than in renal homogenate. There was 37.4–43.6% of AZT released from AZT-COS in mouse plasma after 6 h, whereas only 13.6–16.5% of AZT released in renal homogenate. It might correspond to the enzymatic removal related the plasma esterases. The slow and steady removal of AZT in renal homogenate might attribute to the lack of esterases in renal homogenate. Due to the low MW of COS, there was no significant difference in AZT removal among the three types of AZT-COS conjugates.

AZT-COS conjugates are first reported here. The release profiles could be explained as the cleavage of the ester bond of AZT with the succinic spacer. The sustained release of AZT in AZT-COS conjugates could be due to the conjugation with COS. The slow removal of AZT in renal homogenate together with its quick accumulation in kidney was in line with the sustained fluorescence imaging effect in 5 h. Therefore, AZT removal in AZT-COS was COS dependent. Wannachaiyasit et al. (2008) prepared a dextrin-AZT conjugate via a succinic spacer. The release of AZT and AZT-suc at

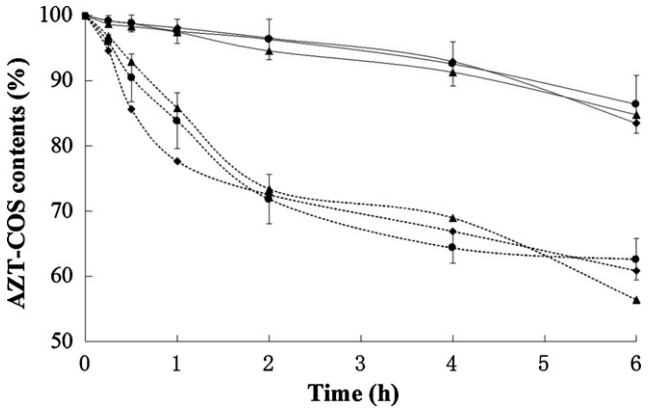


Fig. 4. Drug release profiles of various conjugates. AZT-COS-1 (♦), AZT-COS-2 (●) and AZT-COS-3 (▲) could release AZT both in mouse plasma (---) and in renal homogenate (—), whereas the release rates in mouse plasma were faster than in renal homogenate. There was no obvious difference been noticed among three kinds of AZT-COS. For clarity, the error (±SD) bars are drawn for AZT-COS-2 only ($n = 3$).

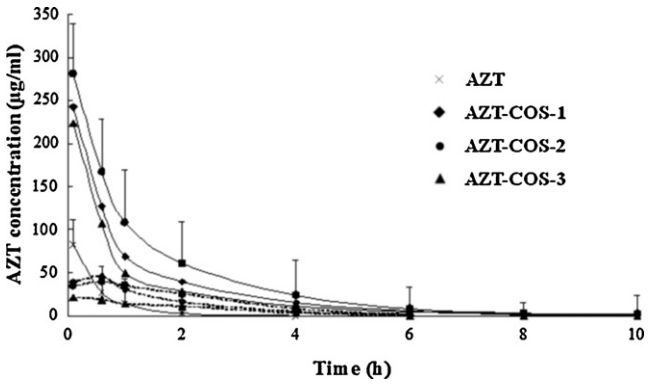


Fig. 5. Pharmacokinetics of control AZT and various AZT-COS after *i.v.* injection. The plasma concentration of released AZT (---) and total AZT (both released and unreleased, —) of the conjugates were stated separately. For clarity, the error (+SD) bars are drawn for the control group and total AZT of AZT-COS-2 only ($n = 5$).

buffer pH 7.4 showed a steady slow release (10–30%) in 24 h. However, the release of AZT and AZT-suc at human plasma was faster (~40%) in 24 h. Our results in mice plasma and renal homogenate were similar to these found in human plasma and buffer pH 7.4 as they reported. Neeraj et al. (2011) prepared a sustained release prodrug-poly(HEMA-AZT) conjugate. Sustained release of AZT was found in AZT-suc and poly(HEMA-AZT) in human plasma, 30% of AZT from AZT-suc and less than 10% of AZT from poly(HEMA-AZT) were detected released against ~60% of AZT from AZT control in 24 h. Li et al. (2010) prepared a sustained release prodrug-mPEG-AZT. The release of AZT from mPEG-AZT was approximately 60% in simulated intestinal fluid at pH 6.8 in 24 h. AZT-COS conjugates in our study could achieve similar results *in vitro* and have no biphase AZT and AZT-suc released.

3.4. Pharmacokinetics of AZT-COS

The pharmacokinetics of AZT-COS conjugates was studied after *i.v.* administration to mice. The concentration of AZT in plasma was significantly higher in the test groups when the conjugates were administrated than when AZT control was administrated (Fig. 5), indicating the difference of *in vivo* behaviors between AZT and the conjugates. The mean plasma concentration of AZT-COS-1, AZT-COS-2 and AZT-COS-3 at 5 min after administration were 242.8, 280.9 and 223.8 μg/ml, respectively, which were 2.7–3.4 times than 81.9 μg/ml of the control group. The mean retention time (MRT) of AZT-COS-1, AZT-COS-2 and AZT-COS-3 were significantly higher than that of control AZT (0.59 h), with the value of 1.49, 1.64 and 1.39 h, respectively. The plasma concentration of dissociated AZT from the conjugates was also investigated. The initial concentration of dissociated AZT of various conjugates ranged from 19.1 to 45.7 μg/ml, which were notable lower than that of the control group, indicating the advantage of lower dose-dependent toxicity over controlled AZT. The plasma clearance in mice was comparable to the results of prednisolone-LMWC conjugates (Yuan et al., 2007). The clearance of prednisolone-LMWC conjugates from blood decreased as the molecular weight of LMWC increased. This was due to the cleavage of drug from the conjugates and the size of the polymer carrier.

There were several attempts been reported to prolong the blood retention of AZT through the conjugation of AZT with various macromolecules (Li et al., 2010; Neeraj et al., 2011; Wannachaiyasit et al., 2008). The sustained release of AZT was achieved through polymer conjugation by changing the nature of polymers, the size of polymer carriers, the nature of the covalent bond to AZT. The nature of the polymers includes the DD of COS, which indicated certain level of control in sustained release of AZT from AZT-COS conjugates.

3.5. Biodistribution studies

After intravenous administration, the controlled AZT quickly distributed to all organs tested without obvious specificity, and

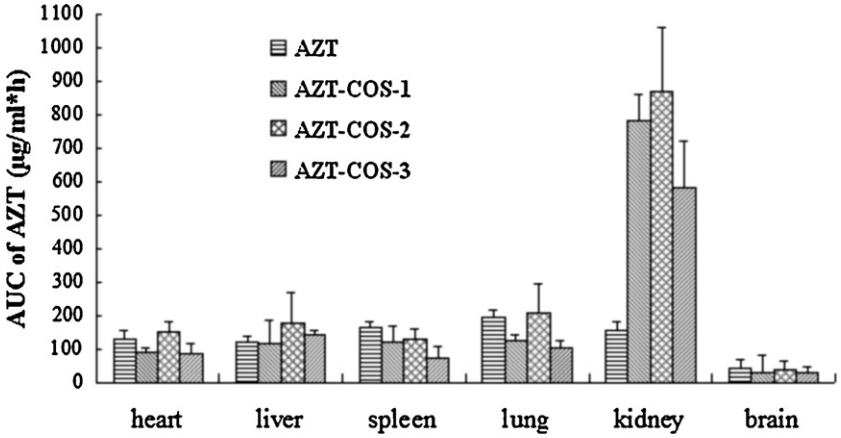


Fig. 6. Tissue distribution of control AZT and various AZT-COS after *i.v.* injection. The conjugates significantly distributed to kidney without notable increase of accumulation in the other organs, compared with control AZT ($n = 5$).

eliminated rapidly from all tissues including kidney within 4 h. In contrast with AZT control, the conjugates dramatically accumulated in kidney after administration, whereas no notable accumulation in the other organs was observed (Fig. 6). The AZT concentration of the three test groups in all tissues except kidney were lower than that of the control group within 1 h, but slightly higher afterwards, which might owing to the rather longer retention of AZT-COS in blood circulation than that of control AZT. As for kidney, the concentrations of various conjugates were significantly higher than that of the control group throughout the whole course. The renal area under the curve ($AUC_{0\to t}$) of AZT-COS-1, AZT-COS-2 and AZT-COS-3 were 781.2 ± 78.0, 868.5 ± 193.8 and 580.7 ± 141.3 μg h/ml, respectively, which were 4.99, 5.55 and 3.71 times than 156.4 ± 28.4 μg h/ml of the controlled AZT. The data indicated that the conjugates could be dramatically uptaken by kidney, and the degree of polymerization might influence the renal targeting efficiency to some extent. This result demonstrated that COS could delivery AZT to kidney effectively and has the potential to be developed to a renal-targeting drug delivery carrier.

To improve the therapeutic effect in various HIV associated complications, some drug targeting delivery strategies had been employed to alter the *in vivo* distribution of AZT (Kaur, Nahar, & Jain, 2008; Mishra et al., 2006; Mizrachi et al., 1995). Although AZT has been proved to be beneficial to patients with HIVAN, it suffers considerable renal excretion and metabolism after administration (Patel, Chu, & Boudinot, 1989), which severely limited its clinical outcomes. To our best knowledge, there was no report on the renal targeting delivery of AZT. Based on the results described above, AZT-COS conjugates could be the potential prodrugs targeted to kidney.

4. Conclusion

The renal targeting delivery of AZT was achieved in preparation of AZT-COS conjugates. The sustained release of AZT was confirmed through *in vitro* and *in vivo* studies. The renal targeting was confirmed through fluorescence imaging study of the kidney. In mouse plasma and renal homogenate, the removal of AZT achieved approximately 40% and 15% in 6 h, respectively. The sustained release of AZT in AZT-COS conjugates was verified with evidences of a longer MRT compared to control AZT group in pharmacokinetic study. The accumulation of AZT in kidney was indicated by the fact that the renal $AUC_{0\to t}$ of various conjugates were much higher than that of control AZT without notable increase of accumulation in the other tissues, in agreement with the results that the fluorescence images demonstrated that COS, especially COS-2, could accumulate in kidney for more than 12 h without notable distribution in the other organs. In that case, the conclusion can be drawn that COS could be uptaken by kidney effectively and has the potential to be used as drug carriers for renal targeting delivery of AZT to improve the therapeutic effect of treating HIVAN.

Acknowledgements

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