Renal Clearable Organic Nanocarriers for Bioimaging and Drug Delivery

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Chemotherapy has led medical oncology along with targeted therapy, radiation therapy, and hormonal therapy.[1] However, standard chemotherapy agents are cytotoxic and often cause serious side effects such as immunosuppression, myelosuppression, mucositis, and alopecia due to nonspecific uptake by the immune system and normal cells.[2] Therefore, enormous efforts have been made to develop ideal chemotherapeutic formulations, capable of delivering drugs selectively in cancerous regions without damaging healthy organs.[3]

Recently, theranostic nanocarriers (TNCs) have been developed for disease- and patient-specific diagnosis and treatment.[4–5] TNCs can control the distribution-mediated pharmacokinetics by overcoming several drawbacks of traditional chemotherapeutics including: (i) protection of drug from unwanted degradation, (ii) prevention of nonspecific interactions, and (iii) enhancement of drug absorption into the target tissue.[5] TNCs are composed of inorganic and organic nanomaterials such as silica-based nanoparticles,[6–8] hollow shells,[9–11] polymer micelles,[12,13] and liposomes.[14,15]

The ideal TNC, however, should actively target cancer cells and be safe for normal cells.[16] This generally requires rapid excretion from the body and/or an efficient degradation into nontoxic products.[17] Renal clearance, compared with the hepatobiliary excretion route, is preferred for TNCs because unwanted agents need to be rapidly eliminated from the body with limited cellular internalization/metabolism, thus effectively minimizing their exposure to the immune system.[18–20] To this end, it is necessary to understand the key design considerations for TNCs such as hydrodynamic diameter (HD), shape, composition, and surface characteristics based on the “Choi Criteria” that govern the fate of administered TNCs in the body.[17,18,21]

Therefore, clinical TNCs should be composed of biocompatible nanomaterials with an overall HD smaller than the threshold of kidney filtration (~5.5 nm) for preferable renal clearance and balanced nonnyslurry surface charges that can enhance active targeting by reducing nonspecific tissue uptake.[19,22,23]

Here, we report an ideal TNC (a.k.a., “H-Dots”), composed of biocompatible ε-polylysine (EPL) for various surface modifications, near-infrared (NIR) fluorophores for bioimaging, and β-cyclodextrins (β-CDs) for potential drug delivery (Figure 1a). H-Dots are designed to excrete from normal tissue/organ via renal excretion after complete targeting to the tumor site without nonspecific uptake by the immune system. β-CDs (1) were conjugated on the primary amine of EPL (4) via reductive amination of aldehyde β-CD (Ald-CD; 3) (Figure 1b).[24,25] After purification of resulting CDPLs (5) by membrane dialysis, the average number of β-CDs on the EPL backbone was determined to be 6.7 by measuring 1H-NMR (Figure S1, Supporting Information). Next, a zwiterionic NIR fluorophore ZW800-1C[26] was conjugated to the terminal amine of CDPL for real-time trafficking after intravenous administration (Figure 1c). Once the positively charged ZW800-CDPL (ZW800-CDPL+, 7) was obtained, the residual amine groups were further modified by using either succinic anhydride (SA) or acetic anhydride (AA), and the number of remaining amine groups was confirmed using ninhydrin test (Figure S2, Supporting Information). SA was used to convert the overall charges to be either zwiterionic (ZW800-CDPL, 8) or negative (ZW800-CDPL−, 9) by reacting with the partial or whole primary amines, respectively (Figure S3, Supporting Information). Capping with AA was used to render the CDPL to be noncharged (ZW800-CDPL−, 10). The final physicochemical properties of ZW800-CDPLs with varied charges were summarized in Table S1 (Supporting Information).

The size exclusion chromatogram of ZW800-CDPL+ revealed successful conjugation of ZW800-1C on the polymer backbone with >91% of reaction yield (Figure S4a, Supporting Information).
Information). The absorbance (solid line) and fluorescence (dotted line) spectra of ZW800-CDPL + ($\lambda_{\text{Abs}} = 769$ nm; $\lambda_{\text{FL}} = 790$ nm) represent no spectral changes compared with the control ZW800-1C (Figure S4b, Supporting Information).[26] Next, serum stability was confirmed by incubating ZW800-CDPLs with fetal bovine serum (FBS; 5 w/v% in saline) at 37°C (Figure S4c, Supporting Information). As a result, the intensities of absorption and fluorescence decreased slightly over 24 h postincubation (>81%), representing the stability of ZW800-CDPLs in the body without optical and physicochemical degradation. The HD of ZW800-CDPLs was calculated by both fluorescence correlation spectroscopy and intrinsic viscosity-based approximation. As shown in Table S1 (Supporting Information), all ZW800-CDPLs were smaller than 5.5 nm, indicating potential and preferable renal clearance.[19]

We then investigated the biodistribution, renal clearance, and pharmacokinetics of four different charged ZW800-CDPLs (+, ±, −, and Ac) in CD-1 mice. The initial distribution was continuously observed for 1 min by the real-time imaging system immediately after a single intravenous injection of each ZW800-CDPL. Overall, ZW800-CDPLs distributed rapidly in the blood, heart, lung, liver, and other major organs within 1 min postinjection, and then gradually accumulated into kidneys, followed by renal excretion to the bladder. The NIR fluorescence signals of ZW800-CDPLs were mainly located in the urinary system 4 h postinjection (Figure 2a). Interestingly,
ZW800-CDPL* showed relatively high fluorescence in liver and abdominal cavity because of electrostatic interactions with negatively charged cell membrane.[27] In contrast, all the other ZW800-CDPLs left no fluorescence signals in the liver, of which signal-to-background ratio (SBR; organs vs muscle) was calculated in Figure 2b,c along with other resected organs. These results indicate that zwitterionic, negative, or acetylated CDPLs can elude nonspecific uptake by the reticuloendothelial system (RES) and exclusively excrete (>80 %ID) to the bladder within 4 h postinjection (Figure 2d).

As shown in Table S2 (Supporting Information), the pharmacokinetic parameters of ZW800-CDPLs were summarized after a single intravenous injection. The blood concentration curves represent that ZW800-CDPLs exhibit a two-compartment profile of in vivo kinetics (Figure 3a). The rapid initial decay of blood concentration was reflected by the efficient

![Figure 2.](image-url)

**Figure 2.** In vivo biodistribution of various charged ZW800-CDPLs. Each ZW800-CDPL (10 nmol) was injected intravenously into CD-1 mice, and their NIR fluorescence images of A) abdominal cavity and B) resected organs 4 h postinjection. C) SBR of each organ against muscle (Mu). Abbreviations used are: Bl, bladder; Du, duodenum; He, heart; In, intestine; Ki, kidneys; Li, liver; Lu, lungs; Mu, muscle; Pa, pancreas; Sp, spleen. Exposure time, 25 ms; scale bar, 1 cm (n = 3, mean ± s.d., ***P < 0.001).
initial distribution into capillaries, and the final concentrations after 4 h postinjection reached close to 0 %ID g⁻¹ representing rapid elimination from the body by the systemic clearance. The half-life values of ZW800-CDPLs (Figure 3b) are ranging from 0.52 ± 0.12 (ZW800-CDPL⁺) to 2.86 ± 0.15 min (ZW800-CDPL⁻) during the distribution phase (t₁/₂d), and from 14.41 ± 1.25 (ZW800-CDPL⁻Ac) to 39.80 ± 4.17 (ZW800-CDPL⁺) for the terminal phase (t₁/₂t). Among them, ZW800-CDPL⁺ showed relatively longer blood half-lives than the other ZW800-CDPLs (⁎⁎P <0.01), which might be resulted from the nonspecific interaction associated with plasma proteins. In addition, urinary excretion of nonpositively charged ZW800-CDPLs was >80 %ID at 4 h postinjection (Figure 3c), while only ~45 %ID of ZW800-CDPL⁺ was found in the bladder (⁎⁎⁎P <0.01). The blood clearance and urinary excretion of CDPLs are similar to those of renal clearable small molecule fluorophores such as ZW800-1,[28] ZW800-1C,[26] and ZW700-1,[29] but much faster than those of previously reported renal clearable inorganic nanoparticles such as silica,[28] gold cluster,[30] and quantum dots.[17,18,21] The values for plasma clearance and volume of distribution were estimated based on the pharmacokinetics data depicted in Figure 3d. Despite the relatively short blood half-life, the plasma clearance value of zwitterionic ZW800-CDPL appeared to be 0.21 mL min⁻¹, which is 2.6-fold faster than that of negative or acetylated CDPLs. Interestingly, the volume of distribution for ZW800-CDPL⁻ also showed the highest value among the tested albeit no significant signals in the major organs except kidneys. To support these results, protein binding assay was carried out by incubating ZW800-CDPLs in 5% FBS for 4 h, and gel filtration chromatography was used to measure the changes in retention time. Consequently, the zwitterionic CDPL exhibited only minimum adsorption with serum proteins (14%), while charged CDPL⁺ and CDPL⁻ resulted in 23% and 26% of protein binding, respectively (Table S1, Supporting Information). This is well explained by the pharmacokinetics data including plasma clearance and volume of distribution for CDPL derivatives: ZW800-CDPL⁺ systemically circulated and distributed to the whole body without nonspecific uptake by the RES, then eliminated efficiently from the body.

To demonstrate efficient tumor targeting and drug delivery using zwitterionic CDPL, we additionally recruited both xenograft and genetically engineered gastrointestinal stromal tumor (GIST) mouse models.[31] Imatinib, a tyrosine-kinase inhibitor for treating GIST, was selected as a therapeutic drug because it forms a 1:1 stoichiometric host-guest complex with β-CD.[32] Imatinib was conjugated with a fluorescent dye, Cy3-N-hydroxysuccinimide (NHS) Ester (GE Healthcare), to track the distribution and clearance of imatinib in tumor-bearing mice (Figure S6a, Supporting Information). Prior to carrying out in vivo tumor targeting, the imatinib-CDPL⁺ inclusion complex was tested for pH-induced drug release by measuring the changes in absorbance spectra of Cy3 (Figure S6b,c, Supporting Information). While imatinib-loaded ZW800-CDPL⁺ was relatively stable at pH 7.4, up to 60% of imatinib was released from the CDPL delivery vehicle in 12 h postincubation at pH 5.0 due to the reduced hydrophobic interactions between imatinib and the apolar cavity of β-CD. This result suggests that the inclusion complex is stable at the physiological environment (pH 7.4), but releases the complexed drugs efficiently in the tumor microenvironment (pH 5.0).

Next, the imatinib-CDPL⁺ complex was administered intravenously into GIST-bearing xenograft mice, and real-time...
intraoperative NIR imaging was performed for 24 h postinjection (Figure 4a, and Figure S7 in the Supporting Information). The tumor-to-background ratio (TBR) increased significantly over the time course of 12 h postinjection, and remained constant up to 24 h. This result demonstrates that the imatinib-loaded CDPL successfully target the tumor region by the enhanced permeation and retention effect. We then sacrificed the xenograft mice at 24 h postinjection and observed their abdominal cavity to confirm biodistribution and clearance (Figure 4b). Almost no background signal was observed in the major organs except the urinary excretory system including kidneys and bladder where ZW800-CDPL is actively being eliminated. Tumors were resected subsequently along with other tissues and organs, and their NIR fluorescence signal was compared against to muscle, of which TBR marked over 8.0 (Figure 4b).

Furthermore, the drug delivery and tumor targeting efficiency of zwitterionic CDPL was demonstrated in genetically engineered GIST mice having tumors in the cecum area since birth.[31] The imatinib-CDPL complex was injected intravenously into the GIST-bearing mice 24 h prior to imaging, and their tumors were imaged along with duodenum, intestine, and muscle (Figure 4c). The complex successfully targeted tumors around the cecum, but showed partial uptake in liver and pancreas. This is mainly because of the lipophilicity of imatinib, which is not fully compensated by the formation of inclusion

![Figure 4](image)
complex with β-CD as well as by the zwitterionic property of CDPL (Figure S7c, Supporting Information). However, it cannot be overstated that the imatinib-loaded CDPL could avoid nonspecific uptake by lung and spleen, while the same dose of imatinib-conjugated with ZW800-1 showed relatively high nonspecific uptake by the RES because of interaction with macropores (data not shown). Tumors were then resected, and the intratumoral microdistribution of imatinib-loaded CDPL was investigated by H&E histology and fluorescence microscopy (Figure 4d). Fluorescence images by red (590–650 nm) and NIR (790–830 nm) filters were taken in order to detect Cy3-imatinib and ZW800-CDPL, respectively. Interestingly, ZW800-CDPL (pseudocolored in lime green) was predominantly observed in the boundary of tumoral regions and the signals of Cy3-imatinib (pseudocolored in red) were spread out intratumorally. This result indicates that zwitterionic CDPLs can successfully deliver hydrophobic drugs to the tumor site by forming a stable inclusion complex, and the anticancer drug is released from the delivery vehicle at the acidic pH generated by the tumor microenvironment.

In conclusion, we designed an innovative nanocarrier (a.k.a. H-Dots) that delivers anticancer drugs to the tumors without trapping into the immune system, followed by the rapid renal clearance of untargeted agents from the body. H-Dots allow early detection of target tumor due to rapid distribution and fast clearance, which drastically reduce nonspecific background uptake, thus, enhance target-to-background ratio. These nanocarriers are composed of biocompatible EPL and β-CD for delivering drugs and/or contrast agents to the target, and their global electric charges can be controlled from negative to neutral or positive. Among the tested four different ZW800-CDPL derivatives, zwitterionic CDPL is of particular interest since it presents (1) rapid systemic circulation and whole body distribution, (2) the lowest nonspecific capture by the RES, and (3) complete excretion to the bladder within 24 h. These precisely designed H-Dots could be used as promising theranostic nanoplatforms that potentially reduce the side effects of conventional chemotherapies when combined with appropriate anticancer drugs.

Experimental Section

Synthesis of ZW800-CDPL: To form the NHS-activated ester, ZW800-1CD(500 mg, 0.5 mmol) was dissolved in 50 mL of anhydrous DMSO. Then, 0.5 mL of N,N-diisopropylethylamine and dipyridilimino(N-succinimidylxyl)carnbium hexafluorophosphate (HSPyU; 410 mg, 1 mmol) were added to the solution. After stirring for 2 h at room temperature, the reaction mixture was poured in 250 mL of acetone/N,N,DMSO. Then, 0.5 mL of N,N-diisopylethylamine and dipyrrolidino(β-CD(20 mg, 0.1 mmol) were added into each ZW800-CDPL (Figure S7c, Supporting Information). However, it cannot be overstated that the imatinib-loaded CDPL could avoid nonspecific uptake by lung and spleen, while the same dose of imatinib-conjugated with ZW800-1 showed relatively high nonspecific uptake by the RES because of interaction with macropores (data not shown). Tumors were then resected, and the intratumoral microdistribution of imatinib-loaded CDPL was investigated by H&E histology and fluorescence microscopy (Figure 4d). Fluorescence images by red (590–650 nm) and NIR (790–830 nm) filters were taken in order to detect Cy3-imatinib and ZW800-CDPL, respectively. Interestingly, ZW800-CDPL (pseudocolored in lime green) was predominantly observed in the boundary of tumoral regions and the signals of Cy3-imatinib (pseudocolored in red) were spread out intratumorally. This result indicates that zwitterionic CDPLs can successfully deliver hydrophobic drugs to the tumor site by forming a stable inclusion complex, and the anticancer drug is released from the delivery vehicle at the acidic pH generated by the tumor microenvironment.

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Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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