

# Novel norcantharidin-loaded liver targeting chitosan nanoparticles to enhance intestinal absorption

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**Abstract:** In this paper, two novel liver-targeting nanoparticles, norcantharidin-loaded chitosan nanoparticles (NCTD-CS-NPs) and norcantharidin-associated galactosylated chitosan nanoparticles (NCTD-GC-NPs), were prepared using ionic cross-linkage. The physical properties, particle size, encapsulation efficiency, and drug release characteristics of the nanoparticles were investigated in vitro. To investigate the intestinal absorption mechanisms of the two preparations, a series of experiments was carried out, including in situ circulation method, in vitro everted gut sacs, and Ussing chamber perfusion technique. The absorption rate constants ( $K_a$ ) of NCTD at different segments were found to be duodenum > jejunum > ileum > colon. The concentration had no distinctive effect on absorption kinetics, suggesting that drug absorption is not dose-dependent. The transport of NCTD was found to be inhibited by P-glycoprotein (P-gp) inhibitor, indicating that NCTD might be the substrate of P-gp. The order of the absorption enhancer effects were as follows: low molecular weight chitosan (CS-8kDa) > high molecular weight chitosan (CS-30kDa) > Poloxamer > sodium dodecyl sulfate (SDS) > sodium deoxycholate (SDCh). The results indicate that the chitosan nanoparticles can improve intestinal absorption of NCTD.

**Keywords:** P-glycoprotein, absorption enhancers

## Introduction

Norcantharidin (NCTD), a demethylation derivative of cantharidin obtained from the dried body of the Chinese blister beetle (*mylabris*), is used as a traditional medicine in China.<sup>1</sup> Clinical studies have shown that NCTD is effective against the primary carcinoma of liver as an inhibitor of protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A).<sup>2</sup> Recent studies have also indicated that NCTD administered orally or intravenously has potential applications in cancer chemotherapy.<sup>3-7</sup> However, the clinical use of NCTD is limited because of its significant side effects, including irritation of the urinary organs.<sup>8</sup> Moreover, NCTD usually has to be administered in high doses to strengthen its tumor inhibition effects due to its low intestinal permeability. The administration of high doses causes more intense irritation at the injection site.

Chitosan (CS), as a nontoxic and biodegradable polycationic polymer with low immunogenicity, has been investigated extensively as a formula carrier and delivery system for therapeutic molecules, such as gene, protein molecule, vaccine, and ammonium glycyrrhizinate.<sup>9,10</sup> Among the various mentions of chitosan derivatives in literature,<sup>11,12</sup> one can differentiate specific reactions involving the free  $-NH_2$  group at the C-2 position. Lactosaminated N-succinylchitosan was found to be a good drug carrier in the treatment of liver diseases,<sup>13,14</sup> because the lactose base can

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bind with asialoglycoprotein receptors in the liver surface to achieve active liver targeting. To improve the therapeutic efficacy of NCTD and decrease its side effects, two novel norcantharidin-loaded liver targeting chitosan nanoparticles (NCTD-NPs), including norcantharidin-loaded chitosan nanoparticles (NCTD-CS-NPs) and norcantharidin-associated galactosylated chitosan nanoparticles (NCTD-GC-NPs), were successfully developed in our previous studies.<sup>15,16</sup> By virtue of their unique uptake mechanisms and liver-specific targeting properties, NCTD nanoparticles have received much attention as a suitable alternative drug carrier system.

Although the physical properties and numerous oral dosage forms of NCTD have been studied, the understanding of NCTD absorption is very limited. Drug absorption is a key process governing in vivo bioavailability of drugs. It is affected by the physicochemical properties of the drug, dosage form, biological state of the gastrointestinal (GI) tract, and co-administered food components.<sup>17</sup> Various physicochemical parameters, such as molecular size and shape, lipophilicity, hydrogen bonding capacity, and molecular surface properties, appear to play crucial roles in the absorption of drugs that cross the intestinal barrier through passive diffusion, either through the transcellular or paracellular pathway.<sup>18–21</sup> In recent years, several studies have been devoted to the assessment of relationships between such parameters and drug absorption in various model systems.<sup>22–25</sup>

In our present study, the absorption mechanisms of two nanoparticles of NCTD, NCTD-CS-NPs and NCTD-GC-NPs, were investigated in comparison with that of NCTD. The absorption characteristics of NCTD-NPs in the intestinal barrier were studied using in vivo transport experiments, in vitro everted sac experiments, and Ussing chamber perfusion technique.

## Materials and methods

### Materials

Norcantharidin (lot number 20100515) with a purity of 99.21% was purchased from Surui Medicine Chemical Co, Ltd (Suzhou, Jiangsu, China). Chitosan with a deacetylation of 90.9% and a molecular weight (Mw) from 8 to 30 kDa was supplied by Xingcheng Biochemical Co, Ltd (Nantong, China). Galactosylated chitosan (GC) was synthesized as previously described by Chung et al.<sup>26</sup> Sodium tripolyphosphate (TPP) was obtained from the National Drug Group (Shanghai, China) and Pluronic F68 was purchased from Fluka Chemika (Buchs, Switzerland). Other chemicals and solvents were of analytical grade. Deionized twice-distilled water was used throughout the study.

Male Sprague–Dawley (SD) rats weighing from 220 to 270 g were obtained from the Laboratory Animals Center of the Medical College, Soochow University (Suzhou, China). All animal procedures were conducted in accordance with the guidelines for the care and use of animals of the National Institutes of Health.

### Samples preparation

NCTD-NPs were prepared according to a procedure<sup>15,16</sup> based on the ionic gelation of CS/GC with TPP anions, which was slightly modified. Briefly, 200 mg CS/GC was dissolved in 100 mL acetic acid aqueous solution containing 0.4 mg/mL NCTD. TPP aqueous solution was then added to the chitosan solution drop by drop under magnetic stirring, resulting in cross-linkage. After reaction for 10 minutes, 125 mg Pluronic F68 was dissolved in the solution as a stabilizer and 2% acetic acid aqueous solution concentration containing chitosan:TPP (50:20 V/V) was stirred at a constant rate of 500 rpm for 2 hours.

In the method above, NCTD gelata was prepared using equivalent distilled water instead of TPP.

The perfusion solution was a Krebs-Ringer buffer solution, which contained 7.8 g NaCl, 0.35 g KCl, 1.37 g NaHCO<sub>3</sub>, 0.02 g MgCl<sub>2</sub>, 0.22 g NaH<sub>2</sub>PO<sub>4</sub>, and glucose in 1.48 g/1000 mL purified water.

### Particle size analysis and morphological characteristics

Particle size analysis of the NPs was performed by dynamic light-scattering measurement using the Zetasizer Nanoparticle Analyser (Malvern Instruments Ltd, Malvern, UK) with a range from 0.6 to 600 nm. Morphological characteristics of the nanoparticles were studied through transmission electron microscopy (TEM; JEM-1230, JEOL, Japan). Colloid samples of the NPs were condensed using a Vivaflow 50 Ultrafilter (30,000 Da polysulfone resin filter membrane; Sartorius AG, Göttingen, Germany). A drop of nanoparticle suspension was placed onto a carbon film coated on a copper grid for TEM. Negative staining was performed using phosphotungstic acid (0.05%, w/w). Observation was performed at 80 kV.<sup>27</sup>

### Evaluation of drug entrapment efficiency and loading capacity

The concentration of NCTD in the nanoparticles was determined by a reversed-phase HPLC method. The HPLC system was composed of two pumps (LC-10AT VP, Shimadzu, Japan) and a UV-vis detector (SPD-M10A VP, Shimadzu) set at 210 nm. The chromatographic column was a Luna

C18 (4.6 × 250 mm; Hypersil ODS2, Elite, Dalian, China) maintained at 30°C. The mobile phase was a mixture of acetonitrile: H<sub>2</sub>O = 10:90 (adjusted to pH 3.1 by adding phosphoric acid), and the flow rate was 0.8 mL/min.

An appropriate volume of NPs colloid was filtered through a 0.45 μm filter to remove nonsoluble aggregate residues following the method in the previous report.<sup>28,29</sup> The filtered colloid was then ultracentrifuged at 40,000 rpm for 45 minutes using the Optima MAX Centrifuge (Berkman Co, Ltd, USA). The supernatant was sampled and the NCTD concentration in the supernatant was determined by HPLC.

Encapsulation efficiency (*EE%*) and drug loading efficiency (*DL%*) were calculated using the following equations:

$$EE = \frac{T - F}{T} \times 100\% \quad \text{and} \quad (1)$$

$$DL = \frac{T - F}{W} \times 100\% \quad (2)$$

where *T* is the total NCTD in the colloid, *F* is the free NCTD in the supernatant, and *W* is the weight of NPs.

### In vitro release studies

Drug release from NCTD- NPs in vitro was measured using a dialysis method and compared with that of the NCTD solution. Briefly, 6 mL NCTD-NPs was placed into a dialysis tube (8000–14,000 MWCO) and sealed at both ends with clips. The dialysis tube loaded with nanoparticles was then placed into a beaker containing 30 mL deionized twice-distilled water and incubated with stirring for 3 hours at 37°C ± 0.5°C. At various time points, 0.5 ml aliquots were withdrawn from the beaker and replaced with an equal volume of media. The NCTD concentrations were then measured by HPLC. The cumulative percentage of NCTD released was calculated as the amount of NCTD released from the solution or nanoparticles relative to the total amount of NCTD present in the formulation.

### In vivo transport studies

An in vivo recirculation perfusion technique was used and performed as reported previously.<sup>30–32</sup> This method was used to determine absorption characteristics at four different segments:<sup>33</sup> duodenum, jejunum, ileum, and colon. Three different concentrations were studied: 140 μg/mL, 160 μg/mL, and 180 μg/mL. The perfusate was prepared by dispersing NCTD/NCTD-NPs with different drug concentrations in

the Krebs–Ringer BH solution (100 mL) containing phenol red (20 μg/mL) for the purpose of correcting the perfusion volume. Next, 0.5 mL of the sample solution was used to detect the phenol red concentration by UV detection at 559 nm. Another 0.5 mL was digested by 88% methanoic acid (0.33 mL) and then centrifuged for 10 minutes at 4000 rpm. Next, 100 μL of the supernatant was separated and placed into another centrifuge tube, into which 200 μL methanol was added. After vortexing for 3 minutes, the extraction was centrifuged for 3 minutes at 10,000 rpm. Afterward, 20 μL of the supernatant was injected onto the HPLC for determination of NCTD using the same HPLC method described previously in “Evaluation of drug entrapment efficiency and loading capacity.” The absorption percentage (*P<sub>A</sub>*), absorption rate constant (*K<sub>a</sub>*) and absorption half-life (*t<sub>1/2a</sub>*) for the NCTD solution/NCTD-NPs were calculated.

### In vitro transport studies

Everted sacs of the rats were prepared using the method described previously,<sup>34</sup> which was also used to determine the absorption characteristics at different segments and with different concentrations. All samples were treated using the method described in “In vivo transport studies.”

### P-gp dependency of NCTD

To examine the saturation potential of the P-gp function, the transport of NCTD and NCTD-NPs at the duodenum was investigated using the everted sacs method. The P-gp inhibitor, Cyclosporin A (CyA), a well-known P-gp substrate, was used as the positive control group at concentrations of 5, 10, and 20 μg/mL.

### Effects of absorption enhancers

The effects of improving absorption of NCTD and NCTD-NPs used as enhancers at the duodenum were investigated using the chamber method. Five enhancers, namely, low molecular weight chitosan (CS-8kDa), high molecular weight chitosan (CS-30kDa), Poloxamer, sodium dodecyl sulfate (SDS), and Sodium deoxycholate (SDCh), were used with concentrations of 0.1%, 0.5%, and 1.0%. Apparent permeability coefficients (*P<sub>app</sub>*) were calculated according to the equation below:

$$P_{app} = \frac{dQ}{dt} \left( \frac{1}{AC_0} \right) \quad (3)$$

where *dQ/dt* is the permeability rate, *A* is the diffusion area, and *C<sub>0</sub>* is the initial donor solution concentration.

**Table 1** Physicochemical characteristics of NCTD nanoparticles (n = 3)

NCTD nanoparticles	Mean particle size (nm)	Encapsulation efficiency (%)	Drug loading efficiency (%)	Yield (%)
NCTD-CS-NPs	131 ± 11	45.12 ± 0.33	7.3 ± 0.13	99.62 ± 0.15
NCTD-GC-NPs	119 ± 9	57.92 ± 0.40	10.38 ± 0.06	98.71 ± 0.22

**Abbreviations:** NCTD-CS-NPs nanoparticles, norcantharidin-loaded chitosan nanoparticles; NCTD-GC-NPs, norcantharidin-associated galactosylated chitosan nanoparticles.

## Statistics

Values are expressed as  $\bar{x} \pm s$  (mean  $\pm$  SD). Data were analyzed by one-way ANOVA with the post-hoc Tukey's test applied for paired comparisons (SPSS 16, SPSS Inc, Chicago, USA). Moreover, the criterion of statistical significance was taken as \* $P < 0.05$ , \*\* $P < 0.01$ , or # $P < 0.05$ , ## $P < 0.01$ .

## Results and discussion

### Physicochemical properties of NCTD-NPs

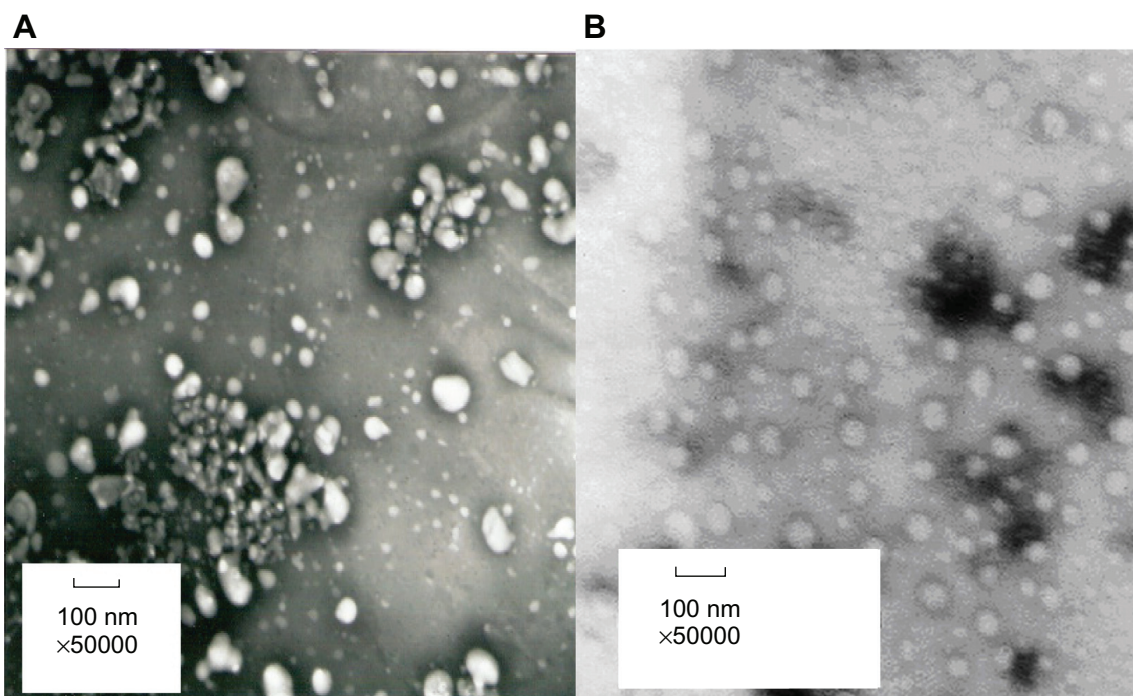
The physicochemical characteristics of NCTD-NPs, including particle size and yield, drug loading, and encapsulation efficiency, are summarized in Table 1. The two liver-targeting NCTD-loaded nanoparticles, NCTD-CS-NPs and NCTD-GC-NPs, had small particle sizes of about 100 nm and displayed an encapsulation efficiency of about 50%,

leading to the final NCTD loading values of about 10%. The values of the NCTD-GC-NPs were higher than those of the NCTD-CS-NPs.

The majority of the fenestrate of the liver sinusoid was reported to be typically less than 200 nm in diameter.<sup>35</sup> Permeability of the vasculum endothelium existing in the liver tumor was also enhanced greatly.<sup>36</sup> Thus, the novel NCTD-CS-NPs could pass through the fenestrate sinusoid and accumulate in the tumor sites of the liver. The small particle size could reduce the adverse reaction of NCTD-NPs on normal hepatic cells and reduce passive liver-targeting. The two NCTD-NPs appeared spherical in shape, as shown in Figure 1.

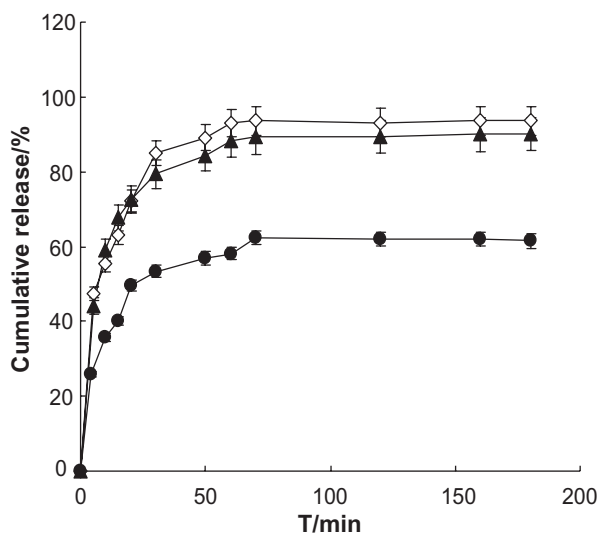
### In vitro drug release of NCTD-NPs

The data results of in vitro NCTD release characteristics are presented in Figure 2. The release curve conforms to the



**Figure 1** TEM photograph of NCTD-CS-NPs (A) and NCTD-GC-NPs (B).

**Abbreviations:** NCTD-CS-NPs nanoparticles, norcantharidin-loaded chitosan nanoparticles; NCTD-GC-NPs, norcantharidin-associated galactosylated chitosan nanoparticles; TEM, transmission electron microscope.



**Figure 2** In vitro drug release of NCTD-CS-NPs (◇) and NCTD-GC-NPs (▲) compared with NCTD gelata (●).

**Abbreviations:** NCTD-CS-NPs nanoparticles, norcantharidin-loaded chitosan nanoparticles; NCTD-GC-NPs, norcantharidin-associated galactosylated chitosan nanoparticles.

Weibull model; the soluble equation is plotted using the data and a good fit is achieved.  $T_d$ ,  $T_{50}$  and other parameters are calculated according to the soluble equation; the results are shown in Table 2. Analysis of the data shows that  $T_d$ ,  $T_{50}$  of the two formulations are significantly different ( $P < 0.01$ ) and the parameters of NCTD-NPs are significantly less than the gelata, which means that NCTD-NPs are more conducive for the release of the drugs than the gelata.<sup>37</sup> In conclusion, the in vitro release of nanoparticles was found to follow the Weibull equation (data not shown) by data fitting ( $r > 0.9500$ ). The drug release of NCTD-NPs was more complete than that of NCTD gelata. It was also argued that NCTD-NPs were more conducive to drug delivery than NCTD gelata.<sup>38</sup>

## Absorption properties of NCTD-NPs in rat intestines

### Absorption of NCTD-NPs in various intestinal segments of rats

Regional differences in intestinal drug permeability have been attributed to the difference in epithelial surface area,

pore radius of the paracellular pathway, mucus layer, and regional membrane fluidity.<sup>39</sup> In this NCTD-NPs study, the absorption tests were performed on four different intestinal segments to test whether the intestinal absorption of NCTD-NPs exhibited site-dependent changes. The intestinal absorption kinetics of NCTD-NPs and NCTD solution at a dose of 160  $\mu\text{g/mL}$  were investigated. The results are shown in Table 3, from which it could be concluded that NCTD absorption through the duodenum and the jejunum were much higher than those through the ileum and colon. The  $K_a$  of NCTD absorption was duodenum  $>$  jejunum  $>$  ileum  $>$  colon. In addition, drug absorption is affected mainly by the physicochemical properties of drugs, drug metabolism in the gastrointestinal mucosa, lipophilicity, unionized fraction of drugs, molecular size, and drug solubility.<sup>40</sup> Changing these absorption parameters along the gastrointestinal tract may contribute to the differences in site-specific absorption of melatonin. Regional differences in the functional expression of P-gp might be one of the possible factors for site-dependent absorption. Compared to the in vitro everted sac method,  $K_a$  of the in vivo method was much higher while  $t_{1/2a}$  was much lower. This is because during the in vitro method, the mesentery and blood vessels were cut off, which leads to less absorption. Among the three formulations, the order of  $K_a$  was NCTD-GC-NPs  $>$  NCTD-CS-NPs  $>$  NCTD solution. It has been suggested that chitosan nanoparticles could improve the intestinal absorption of NCTD because chitosan can trigger the opening of tight junctions.<sup>41</sup> In addition, there are plenty of Peyer's patches and M cells in rat intestines, and absorption via the lymphatic tissues is an important route for drugs with low oral bioavailability.<sup>42-44</sup> Nanoparticles, as compositions of intestine mucous membrane, have an important effect on the enhancement of absorption via the lymphatic route.<sup>45</sup>

### Absorption of NCTD-NPs in various concentrations in rats

As shown in Figure 3, the intestinal absorption percentages of NCTD at higher concentration were greater than those at lower concentrations in vivo or in vitro. However, the

**Table 2** Soluble equation and parameters (n = 5)

Group	Weibull equation	r	$T_d/\text{min}$	$T_{50}/\text{min}$
NCTD-CS-NPs	$\ln\ln[1/1-F(t)] = 0.532\ln t - 1.3334$	0.97379	$12.25 \pm 0.3$	$6.15 \pm 0.2$
NCTD-GC-NPs	$\ln\ln[1/1-F(t)] = 0.3775\ln t - 0.9438$	0.95917	$12.17 \pm 0.5$	$4.61 \pm 0.3$
NCTD gelata	$\ln\ln[1/1-F(t)] = 0.370\ln t - 1.6412$	0.97528	$84.33 \pm 0.7$	$31.35 \pm 0.6$

**Abbreviations:** NCTD-CS-NPs nanoparticles, norcantharidin-loaded chitosan nanoparticles; NCTD-GC-NPs, norcantharidin-associated galactosylated chitosan nanoparticles.

**Table 3** Absorption parameters at various segments in rat intestines (n = 3)

Segment	Groups	$P_A/\%$		$K_a/h^{-1} \times 100$		$t_{1/2a}/h$	
		In vivo (6 hours)	In vitro (2 hours)	In vivo	In vitro	In vivo	In vitro
Duodenum	NCTD solution	15.69 ± 1.12	4.76 ± 0.22	3.06 ± 0.33	2.81 ± 0.21	22.67 ± 2.44	24.70 ± 1.85
	NCTD-CS-NPs	29.16 ± 1.87	13.21 ± 1.13	6.89 ± 0.21	5.98 ± 0.37	10.06 ± 0.31	11.59 ± 0.72
	NCTD-GC-NPs	35.79 ± 0.34	16.48 ± 0.75	6.97 ± 0.42	9.05 ± 0.51	9.95 ± 0.15	7.66 ± 0.37
Jejunum	NCTD solution	12.49 ± 0.89	4.70 ± 0.15	2.78 ± 0.26	2.60 ± 0.13	24.93 ± 2.33	26.61 ± 1.33
	NCTD-CS-NPs	24.34 ± 1.01	10.88 ± 0.34	5.92 ± 0.35	4.24 ± 0.23	11.71 ± 0.69	16.36 ± 0.89
	NCTD-GC-NPs	29.43 ± 0.15	16.65 ± 1.21	6.16 ± 0.52	9.10 ± 0.36	11.25 ± 0.51	7.62 ± 0.53
Ileum	NCTD solution	7.73 ± 0.25	2.76 ± 0.12	1.51 ± 0.14	1.54 ± 0.17	45.89 ± 4.25	44.90 ± 4.96
	NCTD-CS-NPs	13.98 ± 0.97	8.20 ± 0.27	3.53 ± 0.17	2.76 ± 0.07	19.63 ± 0.95	25.13 ± 0.64
	NCTD-GC-NPs	17.67 ± 0.27	8.32 ± 0.13	4.09 ± 0.25	4.08 ± 0.11	16.94 ± 0.77	17.00 ± 0.75
Colon	NCTD solution	2.20 ± 0.33	1.18 ± 0.08	0.44 ± 0.01	0.71 ± 0.02	157.5 ± 3.58	97.54 ± 2.75
	NCTD-CS-NPs	3.54 ± 1.23	2.28 ± 0.06	0.95 ± 0.01	0.80 ± 0.01	72.95 ± 0.78	86.59 ± 1.08
	NCTD-GC-NPs	9.59 ± 0.23	3.61 ± 0.03	1.36 ± 0.23	1.74 ± 0.15	50.90 ± 1.23	39.73 ± 2.41

**Abbreviations:** NCTD-CS-NPs nanoparticles, norcantharidin-loaded chitosan nanoparticles; NCTD-GC-NPs, norcantharidin-associated galactosylated chitosan nanoparticles.

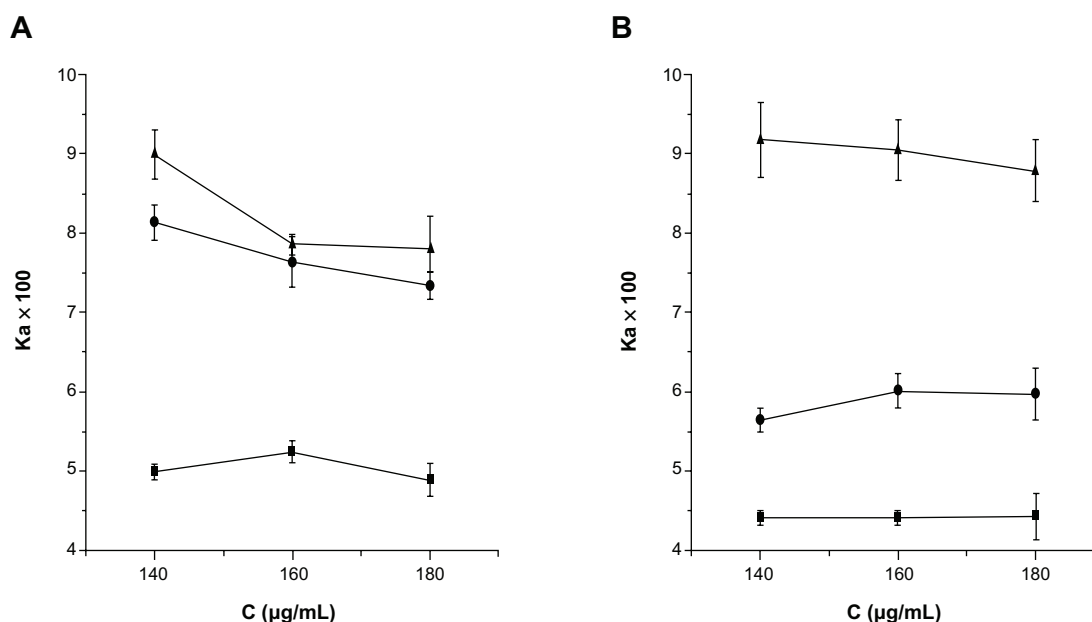
different concentrations did not induce a difference in the transport of NCTD ( $P > 0.05$ ). It has been suggested that the concentration has no distinctive effect on absorption kinetics. A comparison of absorption kinetics among different concentrations demonstrated a non-concentration-dependent change. Results indicated that the intestinal absorption of NCTD-NPs is via passive transfer by diffusion across the intestinal membranes.<sup>46</sup>

### P-gp dependency of NCTD

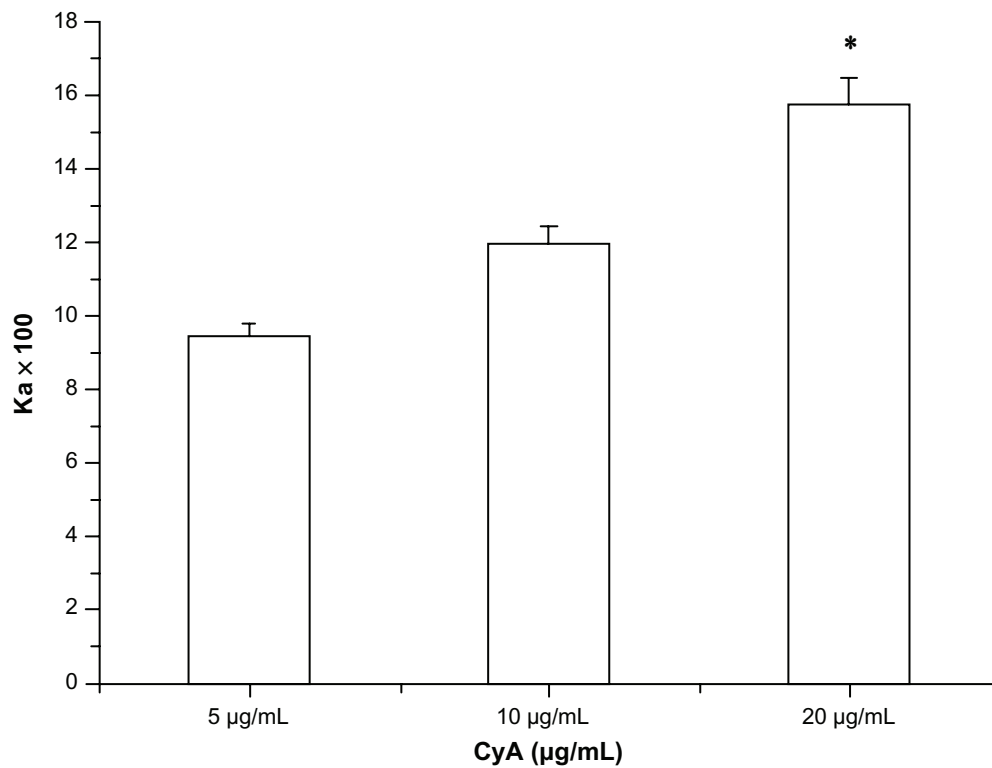
P-glycoprotein efflux pumps are located predominantly in the apical membranes of the epithelia (eg, small

intestine, colon) and significantly limit oral absorption of a number of drugs.<sup>47</sup>

The transport of NCTD across everted gut sacs in solutions with added 5, 10, and 20  $\mu\text{g/mL}$  CyA is shown in Figure 4. Results showed that the NCTD cumulative percentage in the duodenum at 120 minutes was significantly enhanced by CyA ( $P < 0.05$ ). As the CyA concentration increased, the cumulative concentration of NCTD gradually increased. When the concentration of CyA was 20  $\mu\text{g/mL}$ , the accumulative concentration of NCTD at 120 min was more than 200% of the control (in the absence of CyA).



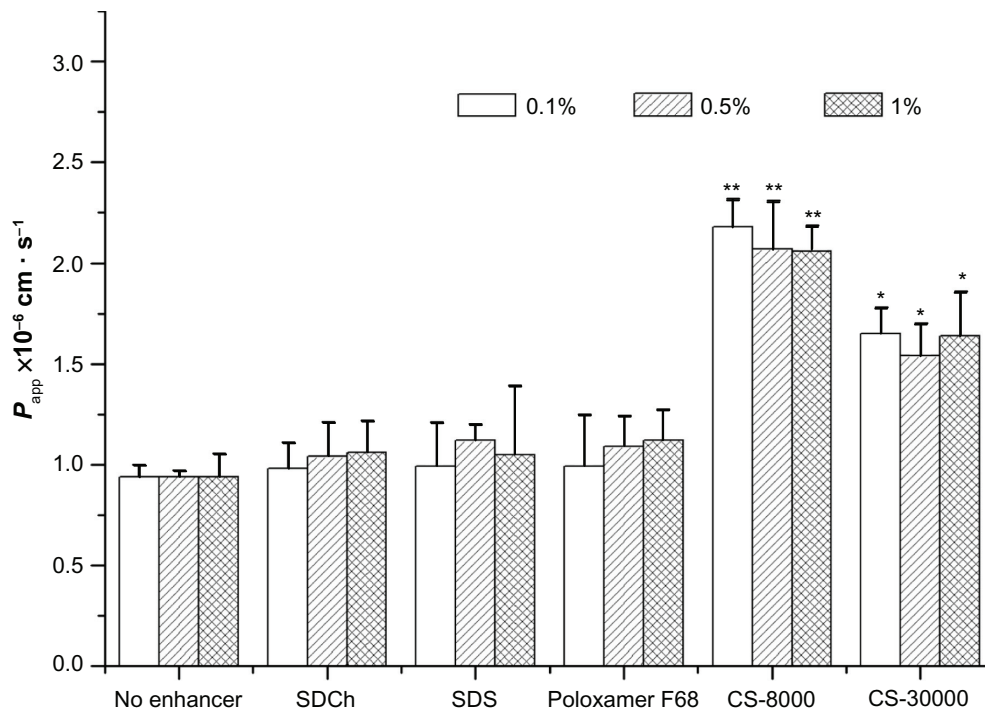
**Figure 3** Intestinal absorption of NCTD-CS-NPs (x) and NCTD-GC-NPs (o) compared with that of NCTD solution (▲) at different concentrations in vivo (A) and in vitro (B). **Abbreviations:** NCTD-CS-NPs nanoparticles, norcantharidin-loaded chitosan nanoparticles; NCTD-GC-NPs, norcantharidin-associated galactosylated chitosan nanoparticles.



**Figure 4** Intestinal absorption in vitro of NCTD with different concentrations of CyA.

**Note:** \* $P < 0.05$  versus 5 µg/mL group.

**Abbreviations:** NCTD-CS-NPs nanoparticles, norcantharidin-loaded chitosan nanoparticles; NCTD-GC-NPs, norcantharidin-associated galactosylated chitosan nanoparticles.



**Figure 5** Effects of absorption enhancers on the apparent permeability coefficients ( $P_{app}$ ) for different concentrations.

**Notes:** \* $P < 0.05$  versus no enhancer group; \*\* $P < 0.01$  versus no enhancer group.

**Abbreviations:** CS, chitosan;  $P_{app}$ , permeability coefficients; SDCh, sodium deoxycholate; SDS, sodium dodecyl sulfate.

## Effects of absorption enhancers

The results are shown in Figure 5. Compared with the NCTD solution, absorption enhancers can enhance the absorption rate of the drug. The order of cross-mucosal permeability is CS-8-KDa > CS-30KDa > Poloxamer > SDS > SDCh. Among them, 0.1% CS-8KDa showed the most significant absorption promotion effect. With different concentrations of absorption enhancers to promote absorption between the different degrees, SDCh and Poloxamer absorption increased as the concentration was increased. SDS as an anionic surfactant promotes the role of low concentration; upon reaching a certain concentration, its effect decreased. The effect of CS-8KDa on absorption decreased as the concentration was increased. Thus it is proven that drugs containing low molecular weight chitosan as a carrier can help to promote intestinal absorption. The reason may be as follows: on the one hand, chitosan has a biological adhesive function, which can extend drug residence time in the intestines; on the other hand, because of its positively charged nature, it can connect with a variety of negatively charged protein bindings in the intestinal mucosa effectively, thereby inhibiting the physiological functions of certain proteins.<sup>48</sup>

## Conclusion

Three intestinal absorption models in vivo and in vitro were used. They are simple, easy, and effective for drug absorption studies. The intestinal absorption mechanisms of NCTD solution and NCTD-NPs were investigated. Results of the study showed that NCTD-NPs significantly enhance drug absorption compared with the NCTD solution. Intestinal absorption kinetics provides methods for a more profound study of the process in vivo and in the development of new oral drugs.

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## Disclosure

The authors report no conflicts of interest in this work.

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