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COMMUNICATION

Oral Delivery of Platinum Anticancer Drug Using Lipid Assisted Polymeric Nanoparticles

Cite this: DOI: 10.1039/x0xx00000x

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Received 00th January 2015,
Accepted 00th January 2015

DOI: 10.1039/x0xx00000x

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Self-assembled cholesterol-asplatin-incorporated nanoparticles (SCANs) were prepared for oral delivery of Pt(IV) prodrug. SCANs exhibit high gastrointestinal stability, sustained drug release and enhanced cell uptake. The oral bioavailability of SCANs was 4.32 fold higher than that of free Pt(IV) prodrugs. The oral administration of SCANs efficaciously inhibits tumor growth with negligible toxicity.

Platinum drugs, including cisplatin, carboplatin and oxaliplatin, are highly effective in the cancer chemotherapy.¹ However, their application is limited due to their severe toxicities and drug resistance.² All three platinum drugs are administrated with intravenous injection, which may lead to high peak of drug level in the plasma above the maximum tolerable concentration (MTC), causing severe side effects, and followed by the subsequent fast decay of drug concentrations below the minimum therapeutic level. Oral administration can maintain an optimum concentration of drugs in a prolonged circulation period, which could decrease the adverse effects and improve the drug efficacy.³ In addition, this is also a preferred route for chemotherapy because of the convenience and compliance of patients.⁴ Nevertheless, the drug stability and absorption in gastrointestinal tract are the big challenges for the oral bioavailability of drugs.

Pt(II) drugs, such as cisplatin, carboplatin and oxaliplatin, are not orally bioavailable due to their chemical reactivity, poor absorption and severe gastrointestinal side effects.⁴⁻⁵ On the other hand, Pt(IV) complexes exhibit high advantages for the oral administration because they are kinetically more inert in the coordination substitution. Satraplatin, the first orally administered Pt(IV) complex, has entered the phase III clinical trial for the hormone refractory prostate cancer.⁶ Although satraplatin was not approved by the FDA due to no significant difference in terms of overall survival,¹ this effort opens a new route for designing oral available drugs using Pt(IV) compounds. The clinical studies of satraplatin in combination with various drugs are still ongoing.⁷

Pt(IV) compounds are prodrugs; once entering cancer cells, they are reduced and activated by cellular reductants (such as glutathione and ascorbic acid) and form active Pt(II)

complexes. However, the undesired reduction of Pt(IV) complexes could occur during oral administration, which reduces the bioavailability of Pt(IV) drugs. For example, two Pt(IV) drugs, satraplatin and tetraplatin both suffered from the deficiency of being reduced prematurely in the blood plasma prior to cell entry.^{1,8} In addition, tetraplatin was abandoned in Phase I due to severe neurotoxicity.⁸ We rationalize that these challenges could be addressed by using a drug delivery strategy. Generally, nanocarriers can protect the drug molecule from the metabolic degradation within gastrointestinal tract and promote the controlled drug release and drug targeting.⁴ Thus nanomedicine could achieve improved efficacy with reduced toxic side effects.

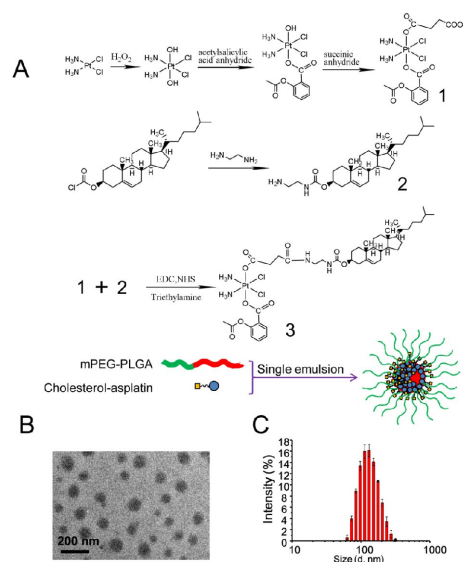


Figure 1. Preparation and characterization of SCANs. (A) The synthetic route for preparing cholesterol-asplatin conjugate. The schematic representation shows the preparation of SCANs by self-assembly from mPEG-PLGA and cholesterol-asplatin conjugate. (B) Transmission electron microscopic image of SCANs (Scale bar, 200 nm). (C) Size distribution of SCANs measured using dynamic light scattering.

In this work, we prepared self-assembled nano-particles for oral delivery of Pt(IV) prodrug using a methoxy-poly(ethylene glycol)-poly(lactide-co-glycolide) copolymer (mPEG-PLGA). PLGA is a biocompatible polymer and has been successfully used for the drug delivery.⁹ The PEGylation prolongs circulation time of the nanoparticle,¹⁰ and the mPEG-PLGA copolymer has been used to deliver Pt-based drugs.^{9a, 11} Asplatin, *c,c,t*-[PtCl₂(NH₃)₂(OH)](aspirin), a novel Pt(IV) prodrug of cisplatin, which demonstrated significant cytotoxicity to tumor cells with lower systemic toxicity compared to cisplatin, was chosen as an ideal platinum complex for the study.¹² To achieve supramolecular assembly, a cholesterol-tethered asplatin conjugate (compound **3**) was synthesized according to the literature method (Figure 1A, see SI for details).¹³ The complex was characterized using NMR and ESI-MS (Figure S1-7). It was loaded to mPEG-PLGA to form self-assembled cholesterol-asplatin-incorporated nanoparticles (SCANs) by a single emulsion method.¹⁴ (Figure 1A).

Transmission Electron Microscopy (TEM) images showed the spherical morphology of SCANs (Figure 1B), and dynamic light scattering (DLS) indicated the narrow size distribution of particles with the average hydrodynamic radius of 129.3±1.4 nm and a PDI of 0.175±0.05 (Figure 1C). The drug loading was analyzed by measuring platinum content in particles and in solution using ICP-MS. SCANs typically display a drug-loading of 10% with the encapsulated efficiency of ~90%. The fluorescence labeled nanoparticles, self-assembled cholesterol-rhodamine-incorporated nanoparticles (SCRNs) were prepared in the same method. The nanoparticles without the drug or fluorescence agent were also prepared and are labeled as vehicle.

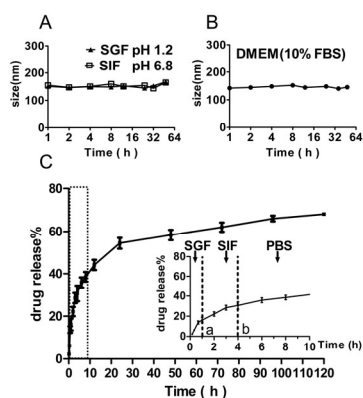


Figure 2. Stability and in vitro drug release of SCANs. (A) (B) Particle size variation of SCANs during the incubation in SGF, SIF (A), or in DMEM medium containing 10% FBS (B). (C) The platinum release profile of SCANs in SGF, SIF and PBS, respectively. The two horizontal dashed lines indicate the two time points of buffer changing: a (1 h) and b (4 h). The inset shows the expanded curve in the first 10 h. Data represent mean \pm s.d. ($n = 4$). All experiments were conducted at 37°C.

Gastrointestinal stability of nanocarriers is a major concern in the development of oral drug delivery system. The stability of SCANs was evaluated in the simulated gastric fluid (SGF) and the simulated intestinal fluid (SIF) at 37°C for a period of 48 h.¹⁵ No significant change in the particle size was observed during 48 h, indicating the high stability of nanoparticles in the gastrointestinal condition (Figure 2A). In addition, SCANs were found to remain stable in DMEM medium containing 10% fetal bovine serum (FBS) for 48 h at 37°C (Figure 2B). The drug release of SCANs was monitored in SGF and SIF in

accordance to the gastrointestinal transit time (1 h in stomach and 3 h in small intestine).¹⁶ The result showed that no initial burst release in acidic SGF or SIF, and a steady drug release from SCANs was observed over 5 days (Figure 2C). These data suggest that SCANs are suitable for the oral delivery with sustained release of the platinum drugs.

Upon oral administration, nanoparticles in the gastrointestinal tract need to be absorbed by the intestinal cells to function; otherwise they would be degraded/eliminated. Human epithelial colorectal adenocarcinoma Caco-2 cells are widely used for the evaluation of the absorption of orally administered drugs in the gastrointestinal barrier. In addition, human liver hepatocellular carcinoma HepG2 cells were used to assess the uptake of nanoparticles in cancer cells. The flow cytometry analysis showed that, with the load of cholesterol-rhodamine, SCRNs can be efficiently internalized in both Caco-2 and HepG2 cells upon 4 h incubation (Figure 3A,B). Consistent with this result, confocal laser scanning microscopy (CLSM) imaging on HepG2 cells showed that SCRNs dispersed in the cytoplasm (Figure 3D). On the contrary, free rhodamine dye could hardly enter cells. The quantification of cellular drug accumulations was analyzed using ICP-MS (Figure 3C). Results showed that the drug in SCANs is more efficiently accumulated than free drugs of cisplatin, asplatin and satraplatin.

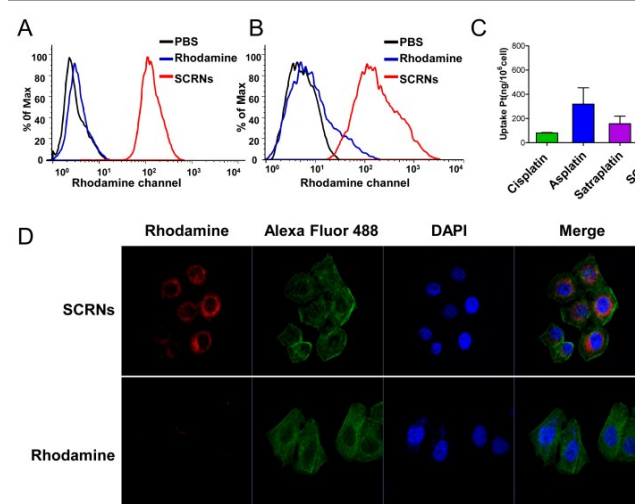


Figure 3. Cellular uptake measurements. (A) (B) Flow cytometric analyses of the fluorescence intensity in cells after the 4 h treatment of SCRNs or rhodamine (50 μg/ml rhodamine). (A) HepG2 cells; (B) Caco-2 cells. (C) Platinum content in HepG2 cells after 4 h treatment of 50 μM platinum formulations. (D) Confocal laser scanning microscopy images of HepG2 cells incubated with SCRNs or rhodamine for 4 h.

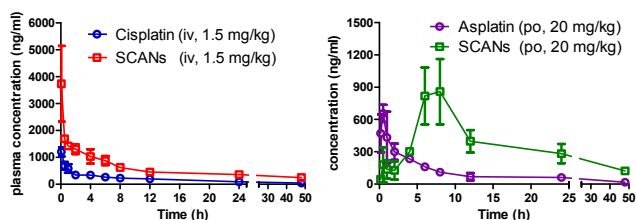
The in vitro antitumor activity of SCANs was evaluated using MTT assay on cervical cancer HeLa, breast carcinoma MDA-MB-231, hepatocellular carcinoma HepG2, lung carcinoma A549 and the cisplatin-resistant A549R cell line. Cell viability measurements showed that SCANs were more efficacious than cisplatin against all cell lines analyzed in this work (Table 1). In addition, the SCANs were found to circumvent the cisplatin resistance of A549R cells with an IC₅₀ value of 4.15 μM in comparison to 29.0 μM of cisplatin. These results indicate that the SCANs possess superior inhibitor effects on the viability of cancer cells, although the free asplatin shows higher cytotoxicity against HeLa and HepG2 cells.

Table 1. Inhibitory effect (IC_{50} in μM) of SCANs on cancer cells.

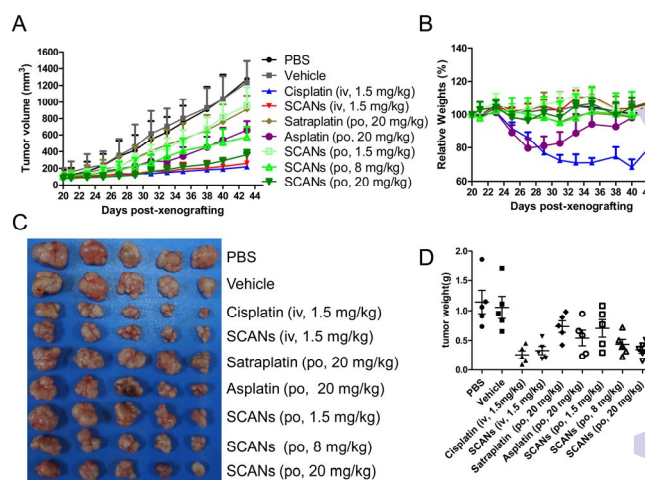
IC_{50} (μM)	Cisplatin	Asplatin	SCANs
HeLa	6.03 \pm 1.04	0.584 \pm 0.09	1.96 \pm 0.26
HepG2	6.44 \pm 0.65	0.849 \pm 0.134	4.31 \pm 0.45
MDA-MB-231	5.93 \pm 0.33	4.82 \pm 0.56	4.30 \pm 0.34
A549	4.75 \pm 0.62	4.20 \pm 0.32	2.53 \pm 0.02
A549/DDP	29.0 \pm 2.5	5.48 \pm 0.36	4.15 \pm 0.05

Flow cytometric analyses were conducted on HepG2 cells to assess the cell cycle arrest in response to the treatment of cisplatin, asplatin and SCANs (Figure S9A,B). SCANs caused a persistent S- and G_2 -phase arrest at 48 and 72 h, which is similar to that of asplatin. Equimolar cisplatin caused only a transient S- and G_2 phase arrest at 48 h; many of them proceed to G_1 -phase at 72 h. Hence, SCANs and asplatin induce the cell cycle arrest more efficiently than cisplatin. In addition, the apoptosis induced by platinum agents was quantified on HepG2 cells using a flow cytometric assay (Figure S9C,D). Results showed that SCANs induced apoptosis to 14.57% and 29.84% in 48 and 72 h, respectively, which is more effectively than cisplatin (8.24% and 18.79%). Consistent with the cell viability assay (Table 1), free asplatin induced the most significant apoptosis on HepG2 cells.

The *in vivo* pharmacokinetic study was conducted by measuring plasma drug concentration varying with the treatment time (Figure 4). Data in Table S1 show that the oral administration of SCANs lead to a 1.61 fold higher peak concentration (C_{max}) compared to that of free asplatin (1.06 versus 0.66 mg/L). The significant delay of peak time (T_{max}) was observed in oral treatment of SCANs compared to free asplatin (7.33 versus 0.36 h), which is probably associated with the sustained release of the nanoparticles and different absorption mechanisms compared to free drugs.⁴ The area under the plasma concentration-time curve (AUC), which is corresponding to the *in vivo* therapeutic effects, is 4.32 times higher by the oral administration of SCANs than that of free asplatin (19.57 versus 4.53 mg h/L). These data clearly indicate the improved bioavailability with the nanoparticulate systems. In comparison to the free cisplatin or asplatin, SCANs exhibit prolonged half-life of the drug in the plasma ($t_{1/2}$) in both intravenous (from 16.08 h to 33.92 h) and oral (from 4.80 h to 24.86 h) administrations. Additionally, the corresponding total body clearance (CL) decreased considerably in both intravenous (0.19 L/h/kg for cisplatin versus 0.05 L/h/kg for SCANs) and oral (4.42 L/h/kg for asplatin versus 1.03 L/h/kg for SCANs) administrations. The postponed clearance of platinum in plasma is probably associated with the long-circulating effect of PEGylation. These data indicate that the nano-delivery system significantly improves the pharmacokinetic properties of the Pt(IV) prodrug with the oral administration.

**Figure 4.** Plasma concentration-time profiles of platinum in mice. (A) Intravenous injection of cisplatin or SCANs; (B) oral administration of asplatin or SCANs. Data represents mean \pm s.d. (n = 4).

The antitumor effect the oral treatment of SCANs was assessed on the HepG2 tumor xenograft mice model. The intravenous injection of cisplatin and SCANs, and the oral treatment of satraplatin and asplatin were conducted. The mice were inoculated with HepG2 cancer cells and the tumor grew to a size of 100-150 mm³ after 18 days. The mice were randomly divided into nine groups with 5 mice in each group. All of drugs were given 5 times in 2-day intervals at the dose of 1.5, 8.0 or 20 mg Pt per kg mice. The tumor volumes were measured in every two days. The tumor growth profiles showed that the oral administration of SCANs efficiently inhibited the growth of HepG2 tumor in a dose-dependent manner (Figure 5A). With a reasonable dosage, the drug efficacy of SCANs (po, 20 mg/kg) is comparable to cisplatin with iv injection (p > 0.05). The measurement of tumor weights at the end of assay confirmed this result (Figure 5D). It is worth noting that SCANs exhibited significantly higher drug efficacy than free Pt(IV) agents (satraplatin and asplatin) with oral administration in the same dosage, although asplatin showed higher *in vitro* inhibitory effect than SCANs on HepG2 tumor cells. This result indicates that the drug carrier significantly improves the *in vivo* efficacy of Pt(IV) drugs in the oral administration.

**Figure 5.** *In vivo* antitumor effect of SCANs. Balb/c nude mice bearing HepG2 tumors were treated with different platinum agents (n = 5, q2d \times 5). PBS and vehicle were used as control. (A) Tumor growth curves show the effect of the different treatments on tumor volume as a function of time. (B) The body weight of mice during the treatments. (C) The image of tumors at the end of the experiment. (D) The tumor weight in each group at the end of the experiment. Error bars denote standard deviations.

While similar drug effectiveness was observed on SCANs (po) and cisplatin (iv), the oral administration of SCANs demonstrated significantly lower toxicity based on the weight measurement of mice (Figure 5B). Cisplatin caused continuous decrease of body weight in a period of 25-43 days; on the contrary, the treatment of SCANs nearly unaffected the weight of mice even in the high dosage group (po, 20 mg/kg). On the other hand, the oral treatment of free asplatin induced moderate toxicity with a short period (25-33 days) reduction of body weight. SCANs showed lower toxicity than asplatin in both intravenous and oral administration, probably associated with the sustained drug release that prevented the high concentration of free drug in plasma. These data indicate that the oral

treatment of SCANs exhibits both advantages of high drug efficacy and low toxicity in comparison with the intravenous treatment of cisplatin and the oral treatment of satraplatin and asplatin.

After the treatment of drugs, tumors in mice were taken for histological analyses (Figure S10-11). The cell apoptosis in tumors was analyzed with TUNEL staining (Figure S11). The oral administration of SCANs caused a dose-dependent apoptosis of tumor cells, and the high dosage group (20 mg Pt/kg) induced the apoptosis similar to those with the iv injection of cisplatin (1.5 mg/kg). Consistent with the tumor growth inhibition assay, much more cell apoptosis was observed in tumors with the treatment of SCANs than that of asplatin or satraplatin at the same dosage. In addition, the nephrotoxicity was evaluated by the TUNEL staining of kidney tissue (Figure S11). The intravenous injection of cisplatin clearly caused TUNEL-positive cells in kidney, indicating the severe nephrotoxicity. The oral treatment of asplatin and satraplatin induced a much lower nephrotoxicity than that of cisplatin. No detectable apoptotic cells were observed on the oral treatment of SCANs, which indicates the low nephrotoxicity of this drug delivery system.

Oral administration is an attractive strategy in the chemotherapy, especially for drugs with severe side-effects. The lower peak concentration with prolonged drug exposure of oral chemotherapy provides an effective route to reduce the side-effect with the improved drug effectiveness. However, the high chemical reactivity prevents the oral administration of Pt(II) drugs. Pt(IV) complexes are more kinetically inert in the coordination substitution. So far, three Pt(IV) complexes (satraplatin, iproplatin and tetraplatin) underwent clinical trials.^{1,8} However, both satraplatin and tetraplatin suffered from the disadvantage of being reduced prematurely in the blood prior to cell uptake,⁸ resulting in undesired binding to plasma protein molecules and thus limiting its effectiveness as a prodrug. On the other hand, iproplatin displayed poor drug efficacy because of its highly negative reduction potentials.¹ Here, we design a drug delivery system that protects the Pt(IV) prodrug with sustained release and improved gastrointestinal absorption. Asplatin was chosen because of its high cytotoxicity to tumor cells with lower systemic toxicity compared to cisplatin.^{12a} The lipophilic cholesterol-asplatin conjugate is loaded in the hydrophobic region of the nanocarrier, which could substantially reduce the metabolic degradation of the Pt(IV) prodrug. In addition, the long circulation of PEGylated nanoparticles also improves the in vivo pharmacokinetics. These properties improve oral bioavailability of Pt(IV) drugs and circumvent their disadvantages in oral administration.

In summary, a self-assembled cholesterol-asplatin-incorporated nanoparticles (SCANs) were prepared for oral delivery of the Pt(IV) prodrug for cancer therapy. SCANs exhibit high gastrointestinal stability, sustained drug release and enhanced uptake by intestinal Caco-2 cells as well as cancer cells. SCANs show prominent cytotoxicity compared with cisplatin in vitro, and are effective to cisplatin-resistant cells. In vivo assays show that the oral treatment of SCANs efficaciously inhibits the tumor growth, comparable to the intravenous injection of cisplatin; nevertheless, SCANs demonstrate significantly lower toxicity with negligible nephrotoxicity and unaffected body weight. On the other hand, the long circulation and sustained release improve the pharmacokinetics of SCANs, resulting in the enhanced oral bioavailability of SCANs with 4.32 fold increase in comparison

to free Pt(IV) prodrugs. Moreover, SCANs demonstrate the prolonged drug release and the postponed drug clearance. Hence, SCANs show significant therapeutic effect than satraplatin and asplatin in the oral administration, and cisplatin in the intravenous injection as well. This work provides a novel strategy to improve the oral bioavailability of Pt(IV) prodrugs using nano-delivery system.

This work was supported by the National Basic Research Program of China (973 Program, 2012CB93250, 2015CB932100), the National Science Foundation of China (U1332210, 21171156, 21573213) and the Collaborative Innovation Center of Suzhou Nano Science and Technology.

Notes and references

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Electronic Supplementary Information (ESI) available: [Experimental details, characterization of cholesterol-asplatin by ESI-MS, NMR, cytotoxicity assay, cell cycle and apoptosis analysis, H&E and TUNEL analyses, and pharmacokinetic data]. See DOI: 10.1039/c000000x/

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