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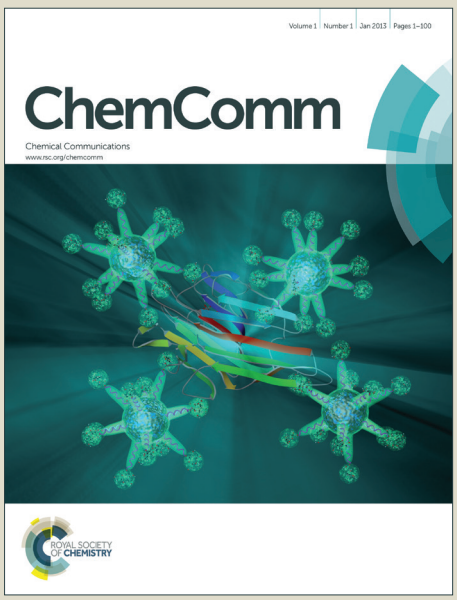

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ARTICLE TYPE

Delivering Photosensitive Transplatin Prodrug to Overcome Cisplatin Drug Resistance

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Clinic ineffective transplatin was converted into a photosensitive prodrug for drug delivery and triggered release to overcome cisplatin resistance.

Since its approval in 1978, cisplatin has been widely used for solid tumor chemotherapy. But its isomer transplatin is proved to be ineffective [1,2] due to its instability and rapid deactivation in blood circulation [3]. Introducing bulky ligand into trans-platinum compounds, people have discovered effective trans-compounds[4,5].Generally, platinum drugs in cis-configuration such as cisplatin, carboplatin and nedaplatin share the same resistance mechanism of reduced cellular accumulation [6]. However, this is not the case for trans-compounds, which provides a rationale for designing trans-geometry drugs to overcome drug resistance [7]. What's more, recent progress in nanotechnology paves the way for protection and delivery of a drug to the diseased site via enhanced permeation and retention (EPR) effect, avoiding the rapid deactivation and circumventing the conventional biological barriers for free drugs [8-11]. Therefore, to maximize the drug efficacy and minimize the side effects, triggered release of the drug on site is inevitable [11].

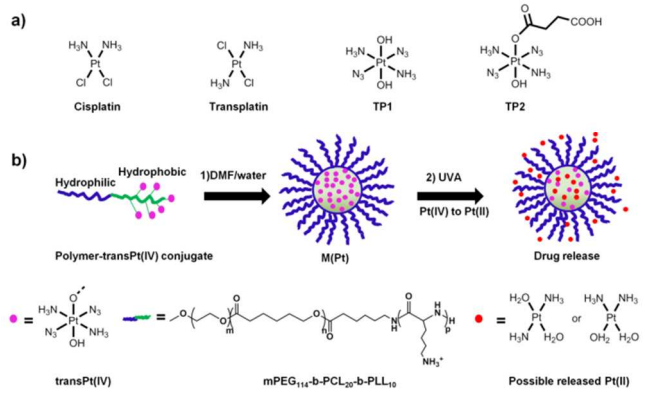

Figure 1. (a) Molecular structures of cisplatin, transplatin, TP1 and TP2 of transplatin with two N_3 ligands in the transpositions. (b) TP2 is attached to copolymer $mPEG_{114}$-b-PCL_{20}-PLL_{10} and the polymer-transPt(IV) conjugate self-assembled into micelles (M(Pt)). Upon UVA irradiation, the Pt(IV) prodrugs are rapidly reduced to Pt(II) and released[12,13].

Sadler et al. designed a series of photo-sensitive Pt(IV) prodrugs which can be triggered to concomitantly release cytotoxic Pt(II) and break down the azide ligands in a homolytic way to release nitrogen gas by light irradiation[12,13]. Their recent mechanistic study revealed the photo-reduction may include new platinum intermediates with one azide and one hydroxide ligand[14]. In a different way, Lippard et al showed a number of examples for delivering Pt(IV) prodrugs via nanoparticles to the cancer cells which can be directly chemically reduced to cytoxic Pt(II) drugs rather than triggered by light[15,16]. Combing these exemplary work, we synthesized a photosensitive transplatin(IV) prodrugs t,t,t-$[(NH_3)_2Pt(N_3)_2(OH)_2]$(TP1) and t,t,t--$[(NH_3)_2Pt(N_3)_2(OOCCH_2CH_2COOH)_2]$(TP2) starting from transplatin. (Scheme S1 and Figure 1a)[12-16]. TP2 was characterized by IR, ESI-MS and ^{1}H NMR (Fig.S1 to S3). TP2 in the presence of 5 mM glutathione(GSH) showed great stability during 24 h(FigS4), making it possible for photo-trigged release.Therefore, It was further attached to biodegradable polymer methoxy-poly(ethylene glycol)-block-poly(ε-caprolactone)-block-poly-L-lysine, $mPEG_{114}$-b-PCL_{20}-PLL_{10}) to prepare a polymer conjugate which self-assembles into micelles (M(Pt)) with the Pt species and the hydrophobic chain as the core and hydrophilic mPEG as corona, (Figure 1b and Scheme S2). In this way, the nanoparticle could protect the TP2 from deactivation in blood circulation. Moreover, M(Pt) could be

internalized via endocytosis and accumulated inside the cancer cells, which differs from cisplatin that has a resistance mechanism of reduced intracellular accumulation[17,18]. At last, as TP2 can be rapidly activated by mild UVA irradiation to release toxic active transplatin species on site, the unique drug activation pathway endows M(Pt) with good controllability for on-demand drug delivery[19,20].

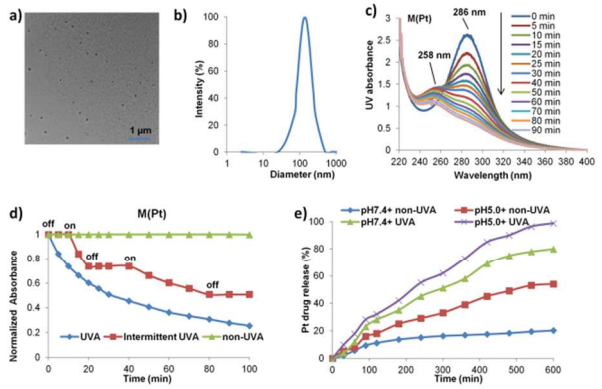

Figure 2. Characterization of M(Pt). (a) TEM of M(Pt) micelles.(b) DLS of M(Pt) micelles. (c) UV absorption of M(Pt) *v.s.* UVA irradiation time(0-90 min).(d) Normalized UV absorption at 286 nm of M(Pt) under UVA light off, on and in an intermittent manner. (e) Pt release under different pHs and UVA irradiation. The photo source is UV365 at1.8 mW/cm^2.

M(Pt) were spherical micelles with a mean diameter of 112 nm (determined by TEM, Fig. 2a) or 140 nm (by DLS, Fig. 2a),respectively, and a zeta potential of + 6.2 mV. Pt loading was 11.4 % w/w, corresponding to a loading of 17.5% Pt-drugs in each polymer chain. M(Pt) showed rapid response to UVA irradiation (UV365, 1.8 mW/cm^2). The UV peak at 286 nm (Fig. 2c, 0 min) which can be attributed to ligand to metal charge transfer band (LMCT, N_3 to Pt) displayed drastic decrease upon UVA irradiation, denoting the rapid breakdown of Pt-N_3 bond and simultaneous reduction of Pt(IV) to Pt(II)[19,20]. The transformation of trans-geometry of Pt(IV) to cis-geometry was also found, as a new peak centred at 258 nm which could be attributed to the platinum(IV) azide compound in cis-geometry appeared and reached its maximum after 10 min irradiation[21]. Both the peaks at 258 nm (cis-geometry) and 286 nm (trans-geometry) dropped gradually. This photo-reduction followed almost a first order kinetics in the first 80 min (Fig. S5). When M(Pt) were put in the dark (UVA off), the absorbance at 286 nm did not change at all (Fig. 2d). Continuous UVA irradiation resulted in drastic drop, while intermittent UVA irradiation caused synchronous change. The results here revealed the stability of M(Pt) in the dark and the UVA-triggered activation of M(Pt).

As M(Pt) showed direct response to UVA irradiation, the Pt drug release in buffered solution in the dark and upon UVA irradiation were conducted[22]. As shown in Fig. 2e, the Pt release rate of M(Pt) followed an order of "pH7.4+non-UVA< "pH5.0+non-UVA" < "pH7.4+UVA" < "pH5.0+UVA", indicating greater dependence on UVA light over pH values. This is due to the direct rapid breakdown of platinum(IV) to release platinum(II) species. To further prove DNA binding ability, TP2 were co-cultured with 5'-dGMP, a guanine which is the major biding base in DNA strand for Pt drugs, upon UVA irradiation. Bisadduct of 5'-dGMP and its fragments losing one and two $-NH_3$ can be found at m/z = 922.2, 905.2, 890.2, respectively, in both case (Scheme S3 and Fig. S6).

The greatest drawback of Pt drugs is the intrinsic or acquired resistance. To demonstrate the possibility of overcoming the drug-resistance by rapid release of transplatin, A2780 ovarian cancer cells (cisplatin sensitive) and their acquired cisplatin-resistant counterparts A2780DDP cells were treated with M(Pt) PT1, PT2, transplatin, and cisplatin under or without UVA irradiation, with the SKOV-3 cells (intrinsic cisplatin) as control. As shown in Fig. 3a, A2780DDP cells showed almost a 3-fold resistance to cisplatin (IC_{50} = 72.4 μM) compared to A2780 (IC_{50} = 23.5 μM) in the dark. UVA irradiation at 1.8 mV/cm^2 displayed negligible toxicity to the cells (Fig. S7). M(Pt) had very low cytotoxicity in the dark, but were very toxic under UVA irradiation to A2780 and A2780DDP cells (Fig. 3b and 3c, Fig.S8), indicating triggered release of Pt(II) species under UVA irradiation. Then, the IC_{50} values of the drugs were calculated and collected in Fig. 3d-3f. It is seen that IC_{50} values of cisplatin for the three cell lines were almost identical with UVA on or off, suggesting no irradiation dependence of cisplatin. Free transplatin was almost non-toxic to all three cell lines in the

dark (IC_{50} = ~100 to ~400 μM), but its toxicity was comparable to cisplatin under UVA irradiation. This observation was consistent with literature results and might be caused partly by the transformation of transplatin to cisplatin under UVA irradiation [23]. TP1 and TP2 showed limited toxicity both in the dark and upon UVA irradiation with little irradiation dependence. M(Pt) showed IC_{50} values of 157, 215 and 100.3 μM to A2780, A2780DDP and SKOV-3 cells in the dark, respectively, and of 46.5, 30.4, and 16.5 μM which were comparable to or even smaller than those of cisplatin, indicating the success of using UVA activation for treatment. Specifically, M(Pt) had shown a resistant fold of 0.65, which was less than that of cisplatin (3.1). This result clearly proved that photo-triggered release can help to overcome drug resistance.

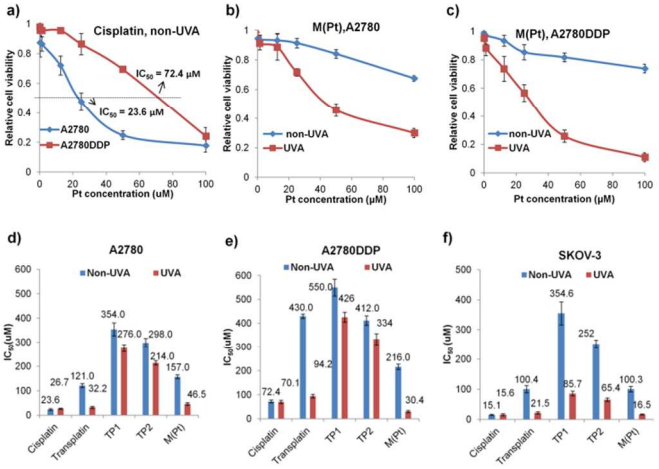

Figure 3. Viability vs. drug concentration curves and IC_{50} values. (a) A2780 and A2780DDP treated with cisplatin of various concentrations (equimolar based on Pt dissolved in RPMI-1640) in the dark. (b and c) Cells treated with M(Pt) with UVA on and off. (d–f) IC_{50} values of cisplatin, transplatin, TP1, TP2, and M(Pt) on A2780, A2780DDP, and Skov-3 cells in the dark and under UVA irradiation. The photo source is UV365 at1.8 mW/cm^2. (Data were shown as mean ± standard deviation, only mean value is presented above the bars, n=3)).

To explain the less resistance of A2780DDP against M(Pt), internalization of various drugs by A2780 and A2780DDP was examined and compared to each other. Firstly, M(Pt) were labelled with RhB to obtain fluorescent micelles M(Pt/RhB). Treatment of both A2780 and A2780DDP cells with M(Pt/RhB) resulted in no significant difference in red fluorescence in both cells (Fig.S9), suggesting the equal ability of M(Pt/RhB) entering both cells. In other words, the mechanism of drug-resistance of A2780DDP against cisplatin doesn't function for M(Pt/RhB).

Secondly, intracellular uptake of Pt drugs by cells was quantitatively determined by ICP-MS and expressed as "ng Pt per million cells". As shown in Fig. 4a and 4b, after treatment of the cells for 4 h and 8 h with drugs, much more amount of M(Pt) was internalized than all other free drugs ($p<0.05$) (400 to 900 ng Pt/million cells for M(Pt) vs. <100 ng Pt/million cells for other free Pt drugs), supporting the greater efficacy of M(Pt) than the others. For the sake of quantitative comparison, a parameter of "Pt uptake ratio" was defined as the ratio of Pt amount up-taken by A2780 cells after treatment of various drugs for a certain time to that by A2780DDP cells. As shown in Fig. 4c, typically, the Pt uptake ratios were 10.9 and 3.2, respectively, at 4 and 8 h for cisplatin, and 1.2 and 1.3 for M(Pt). Obviously, comparable cell uptakes are responsible for the reduced drug resistance against M(Pt).

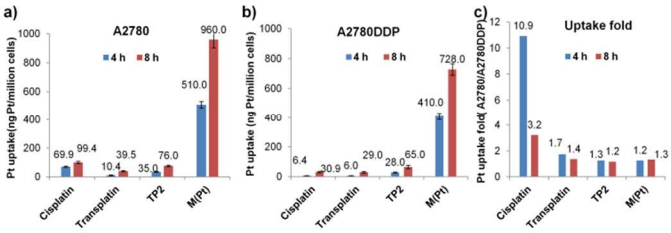

Figure 4.Uptake and uptake fold of drugs by A2780 and A2780DDP cells. (a,b) Uptake of Pt drugs by A2780 and A2780DDP at 4 h and 8 h respectively. (c) The Pt uptake ratios calculated from Fig. 4a and 4b. Drugs were dissolved in RPMI-1640 at an equal Pt concentration of 40 μM. Cells were dosed in triplicates. Data were shown as mean ± standard deviation (n=3).

In summary, ineffective transplatin was transformed into a photosensitive platinum(IV) prodrug and delivered into cancer cells and triggered rapid release in cancer cells. This drug system showed almost non-toxicity in the dark, but comparable efficacy with or even better efficacy than cisplatin upon UVA irradiation. Moreover, M(Pt) can help overcome cisplatin resistance. Therefore, the present study suggests a new strategy of overcoming cisplatin-

resistance by delivering and activating platinum drugs in trans-geometry.

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