Inorganica Chimica Acta 421 (2014) 513–518

Contents lists available at ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Analysis of montmorillonite clay as a vehicle in platinum anticancer drug delivery

Michael G. Apps, Alaina J. Ammit, Alice Gu, Nial J. Wheate *

Faculty of Pharmacy, The University of Sydney, NSW 2006, Australia

ARTICLE INFO

Article history:
Received 1 April 2014
Received in revised form 23 June 2014
Accepted 1 July 2014
Available online 17 July 2014

Keyword:
Clay
Montmorillonite
Phenanthroline
Platinum
Cancer
Cytotoxicity

ABSTRACT

As a proof-of-concept study, the platinum anticancer complex [(1,10-phenanthroline)(1*S*,2*S*-diaminocyclohexane)platinum(II)]chloride, PHENSS, was loaded into montmorillonite (MMT) clay to evaluate its utility as a drug delivery vehicle. Loading is complete within one hour and the total amount of PHENSS that can be loaded into the clay is based on the PHENSS solution concentration in which the MMT is suspended. From a PHENSS solution concentration of 30 mM, a maximum loading of 0.257 mmol per gram of MMT can be achieved. The pH of the solution also has an effect with a solution pH of 6 giving maximum loading of PHENSS. Metal complex release from the MMT was examined using the dialysis bag and dispersion methods. PHENSS is incompletely released from MMT; after 4 h just 47% has been released from the clay using the dialysis method and 30% using the dispersion method. The release is also very fast with a half-life of just 10–16 min. The MMT was shown to have a negative effect on the in vitro cytotoxicity of PHENSS in the human breast cancer cell lines MCF-7 and MDA-MB-231, presumably due to the incomplete release of the metal complex from the clay. Overall the results demonstrate that MMT is not a suitable slow release vehicle for PHENSS, although it may still be of use to other platinum complexes and drugs.

1. Introduction

The many limitations of platinum-based chemotherapy drugs, like their severe side effects [1] and the ability of cancers to develop drug resistance [2], may mean that they are eventually replaced in the clinic by more actively targeted and selective drugs [3]. To provide a future for platinum drugs, it is therefore important to develop ways to improve their effectiveness, safety and patient tolerability.

Recently a family of new phenanthroline-based anticancer complexes has been developed by Aldrich-Wright and group at the University of Western Sydney [4,5], Totaling over 70 in number, all contain a derivative of 1,10-phenanthroline and a chiral amine ancillary ligand. The complexes containing the 1*S*,2*S*-diaminocyclohexane ligand are the most cytotoxic with IC_{50} values up to 100-fold lower than cisplatin [6]. The complex [(1,10-phenathroline)(1*S*,2*S*-diaminocyclohexane)platinum(II)]chloride, abbreviated as PHENSS (Fig. 1), is one of the most potent complexes in this class and has displayed in vivo cytotoxicity using a PC3 human prostate xenograft [7]. Whilst this class of metal complex is able to bind to DNA via intercalation [8], their mechanism of action is not known and is thought to potentially be derived from binding to mitochondria and/or cell cycle proteins [9–12].

One of the major drawbacks to all platinum-based anticancer drugs is their very short blood plasma residence time. The highest concentration of cisplatin is achieved in a very short period of time after administration (potentially as short as just 5 min) after which the plasma concentration drops rapidly as it is cleared by the body [13,14]. As anticancer drugs are most effective when they are able to circulate in the blood stream for significantly longer periods (hours to days) then slow release delivery methods are of particular interest. Examples of slow release platinum formulations include: hydrogels [15], micelles and polymers [16], nanoparticles [17], carbon nanotubes [18] and macrocycles [13]. Whilst effective, these new types of delivery vehicles are not without their own pharmaceutical and regulatory drawbacks and the use of natural materials which are already generally regarded as safe, instead of new synthetic vehicles, provides an advantage in developing new delivery systems [19].

Clay has been used in medicine for thousands of years either to treat poisoning or infection and was administered orally or topically [20]. Currently, clays and other minerals are used in pharmaceutical formulations as lubricants, desiccants, disintegrants, diluents and binders, pigments and opacifiers, emulsifying, thick-

* Corresponding author. Fax: +61 2 9351 4391.
E-mail address: nial.wheate@sydney.edu.au (N.J. Wheate).

http://dx.doi.org/10.1016/j.ica.2014.07.009
0020-1693/

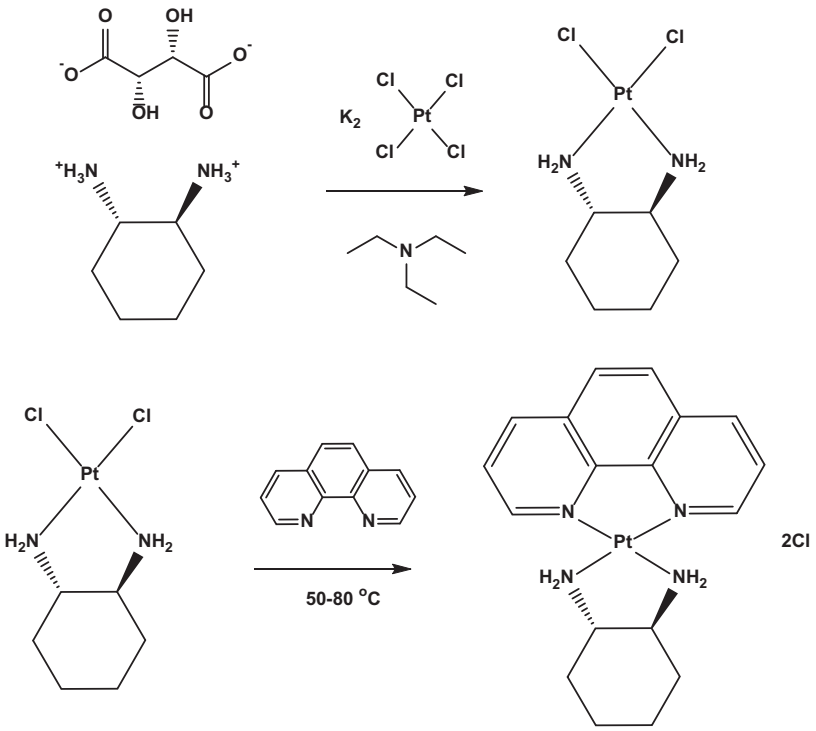

Fig. 1. The modified synthetic method for PHENSS using the *D*-tartrate salt of 1*S*,2*S*-diaminocyclohexane as the starting reagent. The ligand is converted to the free base by triethylamine and then reacted with $K_2[PtCl_4]$ to produce [$PtCl_2$(*S*,*S*-dach)]. PHENSS is synthesized by the reaction of 1,10-phenanthroline with [$PtCl_2$(*S*,*S*-dach)] in hot water.

ening and anticaking agents, flavor correctors and isotonic agents [21]. There has also been growing interest in the use of clays in drug delivery as slow release vehicles [22–28]. Montmorillonite (MMT) is a common clay named for the area in France where this smectite-type mineral is found. The clay is composed of $Al_2O_3 \cdot 4SiO_4 \cdot H_2O \cdot xH_2O$ which forms layers with negatively charged surface groups [29] and drugs can be intercalated between these anionic clay layers [23,30]. As a flat, dicationic drug, PHENSS is a perfect candidate for drug delivery using MMT. PHENSS can potentially intercalate between the layers of the clay where binding is stabilized by electrostatic interactions.

In this paper we report the first investigation of a naturally occurring clay as a slow release delivery vehicle for a platinum-based anticancer complex. Factors affecting the loading capacity of PHENSS into MMT including time, solution pH and metal complex concentration were investigated using UV–Vis spectrophotometry. The slow release properties of the PHENSS-MMT complex were examined using two methods: the dialysis bag and dispersion methods. Finally, the effect of the clay on the in vitro cytotoxicity of PHENSS was examined using the human breast cancer cell lines MCF-7 and MDA-MB-231.

2. Experiment

2.1. Materials

$K_2[PtCl_4]$ was purchased from Precious Metals Online. Cisplatin, MTT, montmorillonite, 1*S*,2*S*-diaminocyclohexane *D*-tartrate, 1,10-phenanthroline and triethylamine were purchased from Sigma Aldrich. C-18 reverse phase Sep-Paks (500 mg; 3,000 MWCO) were purchased from Waters. Simulated gastric fluid (SGF) was made according to the British Pharmacopeia 2013: 2.002 g NaCl, 3.228 g pepsin, 7 mL of 37% w/v HCl and water to a total volume of 1 L. The breast cancer cell lines MCF-7 and MDA-MB-231 were purchased from ATCC. All water was obtained from an SG Ultra clear water system.

2.2. Synthesis of PHENSS

The metal complex was made using a modification to a method previously reported [6]. Equimolar amounts of $K_2[PtCl_4]$ and the *D*-tartrate salt of 1*S*,2*S*-diaminocyclohexane (*S*,*S*-dach) were combined in water (10 mL per 100 mg of $K_2[PtCl_4]$). Four mole equivalents of triethylamine were added and the solution stirred at room temperature for 24 h to give [$PtCl_2$(*S*,*S*-dach)] as a yellow coloured precipitate. The precipitate was collected by vacuum filtration, then washed with water, methanol and allowed to air dry. The [$PtCl_2$(*S*,*S*-dach)] (400 mg) was stirred with one mole equivalent of 1,10-phenanthroline in 800 mL of water at 80 °C until all solid had dissolved. The solution was then allowed to cool to ~50 °C and stirred overnight at this temperature. For purification, the solution was rotary evaporated to near dryness and filtered to remove platinum metal. The remaining solution was eluted on a C-18 reverse phase Sep-Pak, the eluant collected and rotary evaporated to dryness to produce a yellow coloured powder. ^{1}H NMR (400 MHz, D_2O, ppm): 8.64, d, 2H; 8.49, d, 2H; 7.78, d, 2H; 7.76, d, 2H; 7.59, s, 2H; 2.66, dd, 2H; 2.13, d, 2H; 1.56, d, 2H; 1.43, q, 2H; 1.16, m, 2H. ^{13}C NMR (400 MHz, D_2O): 150.9, 145.8, 140.5, 130.0, 127.5, 126.4, 61.7, 32.0, 23.8 ppm. Electrospray ionization mass spectrometry, Expected [M] = 489.15 *m*/*z*; Found, $[M-H^+]^+$ 488.08 *m*/*z*. UV–Vis (H_2O, 100 μM): 227 nm, 0.884; 277 nm, 0.773; ε = 30 920 M^{-1} cm^{-1}.

2.3. Clay purification

MMT (10 g) was prepared by suspending the clay in 100 mL of water and stirring overnight. The clay was then collected by vacuum filtration and washed with water before it was resuspended

in a 200 mL 0.5 M NaCl solution and stirred overnight. Finally, the clay was filtered, washed with water to remove excess salt, air dried for 3 h and oven dried (80 °C) for a further 2 h.

2.4. UV–Visible spectrophotometry

UV–Vis was performed on a Shimadzu UV-1800 with paired 1 cm quartz cells. PHENSS calibration graphs were generated using 5 to 40 μM samples with maximum absorption measured at 277 nm. Sample analysis for intercalation kinetics, concentration and pH loading and drug release experiments were performed by centrifuging the PHENSS loaded MMT suspensions at 13 000 rpm for 3 min. The supernatant solutions were removed and the concentration of free PHENSS used to determine the amount of PHENSS loaded/remaining in the clay.

2.5. Intercalation kinetics

Loading of PHENSS into MMT was studied over time periods from 1 to 50 h. PHENSS (1 mL, 8.5 mM) was stirred with MMT (25 mg) in eppendorf tubes in water. At time intervals, the solutions from individual eppendorf tubes were centrifuged and the PHENSS loading was determined by UV–Vis spectrophotometry.

2.6. Effect of pH of PHENSS loading

Eight samples of MMT (25 mg) were suspended in 1 mL of PHENSS solution (8.5 mM) with pH values between 3 and 10. The solutions were stirred for several hours before PHENSS loading was examined by UV–Vis spectrophotometry.

2.7. Effect of PHENSS concentration on loading

PHENSS was dissolved in 1 mL of water at various concentrations (3, 5, 10, 19, 30 mM) with 25 mg of MMT in eppendorf tubes in triplicate and stirred overnight before PHENSS loading was examined by UV–Vis spectrophotometry.

2.8. PHENSS release study – dispersion method

The PHENSS loaded MMT (25 mg) was stirred in 60 mL of SGF solution at 37.5 °C. Aliquots (1 mL) were taken at time intervals of between 20 min and 5 h and PHENSS release was examined by UV–Vis spectrophotometry.

2.9. PHENSS release – dialysis bag method

Dialysis sacks were allowed to equilibrate in SGF before PHENSS loaded MMT (25 mg) suspended in 1 mL of SGF was added inside the dialysis sacks. Each dialysis sack was then placed in a reservoir of SGF (100 mL) which was maintained at 37 °C with stirring. At time intervals, 3 mL aliquots were taken from the SGF reservoir and PHENSS concentration determined by UV–Vis spectrophotometry.

2.10. Cytotoxicity

Cell respiration, an indicator of cell viability, was determined by the mitochondrial-dependent reduction of 3-(3,4-dimethylthiazol-2yl)-,5-diphenyl tetrazolium bromide (MTT) to formazan. MTT assays were performed according to Guh et al. [31]. The cytotoxicities of MMT, PHENSS and PHENSS loaded MMT were compared with cisplatin in the MCF-7 and MDA-MB-231 human breast cancer cell lines using 72 h drug exposure times.

3. Results and discussion

3.1. Metal complex synthesis

The synthetic method for PHENSS, and its derivatives, requires the use of enantiometrically pure 1*S*,2*S*-diaminocyclohexane (*S*,*S*-dach). In this form the ligand costs AUD$190 per gram (based on November 2013 prices). We have now modified this method to use the *D*-tartrate salt of the 1*S*,2*S*-diaminocyclohexane ligand which costs just AUD$20 per gram. As a tartrate salt the ligand is a dication, but can be converted to the free base by the addition of four equivalents of triethylamine (Fig. 1). At the same time, $K_2[PtCl_4]$ is also added to the *S*,*S*-dach solution; as the free base of *S*,*S*-dach is generated it reacts with the platinum to form [$PtCl_2$(*S*,*S*-dach)]. As a single step, one pot reaction the intermediate [$PtCl_2$(*S*,*S*-dach)] is produced in almost stoichiometric yield and at significantly lower cost compared with the old method. The [$PtCl_2$(*S*,*S*-dach)] crystallizes from solution and is collected by vacuum filtration at which point the triethylammonium chloride/tartrate and KCl are washed from the product. The [$PtCl_2$(*S*,*S*-dach)] is then reacted with 1,10-phenanthroline to yield PHENSS which is purified by elution through a C-18 reverse phase column and rotary evaporated to dryness. The characterisation data for the metal complex (^{1}H and ^{13}C NMR, ESI-MS and UV–Vis) are consistent with the proposed compound and identical to the values previously reported [6].

3.2. PHENSS loading as a function of time, concentration and solution pH

Prior to the loading of PHENSS, the MMT clay was washed with water and then washed with 0.5 M NaCl to ensure all cations within the structure were Na^+. Excess NaCl was removed by washing with more water before the clay was air and oven dried. A single batch of MMT was produced in this way and used for all subsequent experiments with PHENSS.

The loading of PHENSS into MMT was measured as a function of time. The clay was suspended in a solution of water and then PHENSS predissolved in water was added with continuous stirring. At intervals, an aliquot of the PHENSS loaded MMT suspension was removed and centrifuged. The amount of PHENSS loaded into the clay was determined by measuring the amount of unbound PHENSS. The results indicate that absorption of the PHENSS onto the clay is rapid and complete within 1 h, which is consistent with the results obtained for the drug timolol and MMT [30].

As PHENSS loading is a dynamic and reversible process the amount of metal complex loaded into the clay will, in part, be determined by the equilibrium between the free and bound species. The use of higher PHENSS solution concentrations should yield higher loading into the clay. Total loading in the clay is either limited by the maximum concentration of PHENSS in solution or the number of potential binding sites in the clay.

The loading of PHENSS was therefore examined by suspending MMT in solutions of PHENSS at metal complex concentrations between 3 and 30 mM. The results indicate a direct relationship between PHENSS concentration and loading of the metal complex into MMT. The loading of PHENSS increases with increasing metal complex concentration to the limit of PHENSS solubility: 30 mM (Fig. 2). At this highest concentration the loading of PHENSS is 0.257 mmol per gram of MMT. This result is significantly lower than the loading capacity for other drugs, like timolol maleate, which has almost double the loading capacity at 0.499 mmol per gram of MMT [30].

Finally, the loading of PHENSS into MMT was examined as a function of solution pH. Potentially, the pH of the solution has an

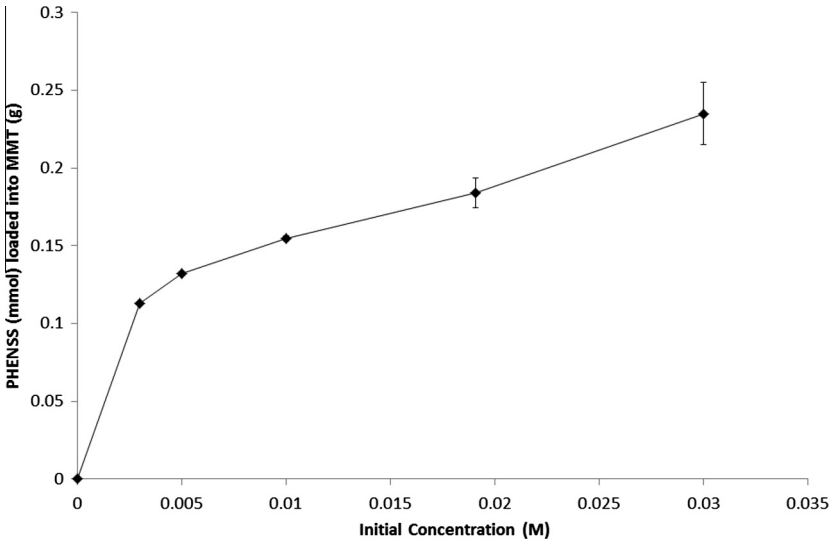

Fig. 2. The loading capacity of PHENSS in MMT (moles metal complex per gram of clay) as a function of PHENSS concentration. The results demonstrate increasing loading as concentration increases up to the limit of PHENSS solubility (30 mM).

ability to affect the metal complex loading due to competitive cation binding to the clay's anionic groups at low pH by H^+ and at high pH by Na^+ or K^+ depending on the base used to prepare the solution. As such, the loading of PHENSS was examined between pH 3 and 10. The results indicate that within the error of the experiment there is no significant difference in loading between pHs 4 to 10. Only at a very low pH of 3 has the loading capacity of the clay dropped; at this pH the loading is only 65% compared with the loading at pH 6.

Overall the results indicate that PHENSS can be loaded into MMT, but the extent of loading is dependent on both the PHENSS concentration and the pH of the solution in which the clay is dispersed.

3.3. Metal complex release rate

There are various ways in which drug release from a delivery vehicle can be measured and there is considerable debate as to which measures are the most reliable and representative of biological systems [32,33]. In this work we examined the rate of release of PHENSS from MMT using two different methods: the dialysis bag and the dispersion methods. Both experiments were completed using simulated gastric fluid (SGF) as the dissolution media as the particulate nature of clay makes it unsuitable as a delivery vehicle in intravenous injections. In traditional medical use, clay is orally administered, and as an excipient has been found suitable for the production of oral tablets [29].

In examining metal complex release using the dialysis bag method samples of PHENSS loaded MMT were placed into dialysis sacks as a suspension in SGF. The sacks were then further placed in reservoirs of SGF and the PHENSS concentration in the reservoir fluid measured at intervals using UV–Vis spectrophotometry (Fig. 3). From the data there is a very small, but statistically significant ($P < 0.05$), difference in the release half-lifes of free PHENSS (no clay; $t_{1/2} = 11.9 \pm 0.78$ min) compared with PHENSS loaded MMT ($t_{1/2} = 16.3 \pm 0.80$ min). However, these half-lifes are very much shorter compared with literature release rates for other drugs using the same clay; timolol release has a half-life of approximately 1.5 h and irinotecan has a half-life of approximately 1.25 h) [23,30]. Given that PHENSS and PHENSS loaded MMT have near identical half-lifes it demonstrates that the PHENSS is most likely not slowly released from the clay but instead enters solution via burst release. As such, we hypothesize that despite the apparent slow release shape of the graphs, the PHENSS release profile is rather a measurement of the diffusion of PHENSS out of the dialysis bag and not diffusion out of the clay.

The release of PHENSS out of the clay, as examined using the dialysis bag method, is also incomplete. The maximum percentage release of PHENSSS is 47% which is achieved after four hours. This result is consistent with the pH loading experiments completed in Section 3.2. At a pH of 3, the PHENSS loading was just 65% of the maximum loading observed at pH 6. Given that the SGF solution used in the release studies was pH 1.2, then the incomplete release is very similar to the results observed for the loading experiment. Release is not based on kinetics but on competitive binding with other solution cations; thus, burst release is observed.

In measuring PHENSS release using the dispersion method, the PHENSS-MMT complex was suspended in SGF with stirring. At regular time intervals, an aliquot of the solution was withdraw and centrifuged to determine the concentration of released metal complex. The results indicate PHENSS release from MMT using the dispersion method has similar release kinetics to the dialysis bag method. The PHENSS is burst released with a half-life of less than 10 min. The release of the metal complex is also incomplete by this method with only 30% of the PHENSS in solution after more than four hours.

Overall, the results indicate that PHENSS is rapidly released from the clay, most likely under burst kinetics. It is unclear why this is the case when other drugs demonstrate slow release. The results may indicate that the PHENSS is not intercalated between the clay layers but instead may only be absorbed onto the surface of the clay. If this is the case, then there is no barrier to metal complex release and the PHENSS dissolution into the SGF is dictated simply by its solubility, diffusion kinetics and competitive binding by solution cations.

3.4. In vitro cytotoxicity

Whilst platinum drugs are not used in the clinic for the treatment of breast cancers, PHENSS has shown high cytotoxicity in breast cancer cells lines. As such, the drug can potentially be developed as an alternative to current breast cancer chemotherapy drugs. The effect of MMT on the in vitro cytotoxicity of PHENSS was examined using the MCF-7 and MDA-MB-231 human breast

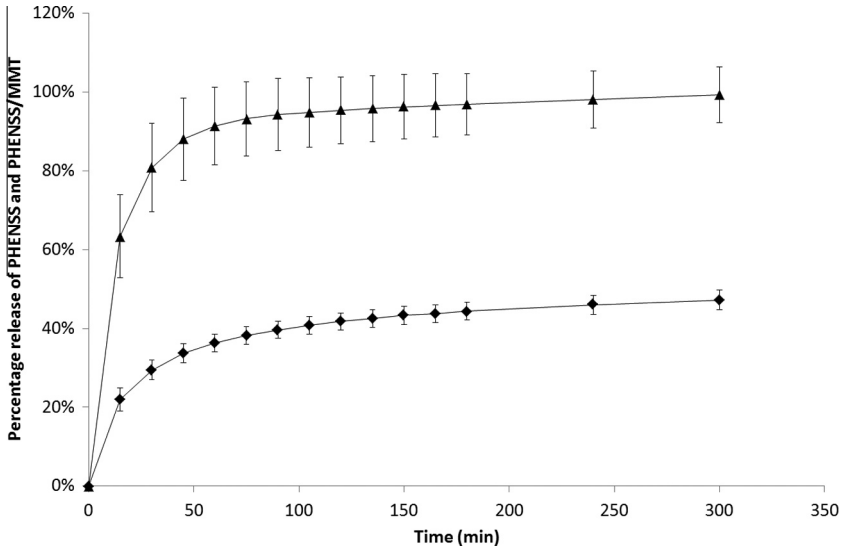

Fig. 3. The release profiles of free PHENSS (▲, no clay) and PHENSS loaded MMT (♦), showing the similar release half-life profiles for both formulations and the incomplete release of metal complex from the clay, even after four hours. The apparent slow release profiles are a function of the slow diffusion of PHENSS out of the dialysis sacks and not slow release of the metal complex from the clay.

Table 1
The effect of MMT on the in vitro cytotoxicity of PHENSS in the human breast cancer cell lines MCF-7 and MDA-MB-231. The values given are for inhibition concentration 50 (IC_{50}) which is defined as the concentration of drug or complex required to inhibit cell growth by 50% compared with untreated cells.

Drug/complex	IC_{50} (μM)	
	MCF-7	MDA-MB-231
Cisplatin[a]	13.3 ± 1.32	55 ± 4.8
MMT	>50	11.3 ± 0.65
PHENSS	0.31 ± 0.12	0.64 ± 0.23
PHENSS-MMT	1.73 ± 0.22	0.90 ± 0.002

[a] Data taken from Refs. [34,35].

cancer cell lines (Table 1). Free PHENSS is 4- to 85-fold more cytotoxic to these cell lines than cisplatin, which is consistent with the high PHENSS activity observed in other animal and human cancer cell lines, such as murine leukaemia L1210 (2.5-fold more cytotoxic) and human prostate PC3 (10-fold more cytotoxic) [7].

The results indicate that MMT has a measurable negative effect on the cytotoxicity of PHENSS. In the MCF-7 cell line the PHENSS cytotoxicity drops 5.5-fold and in the MCA-MB-231 cell line the cytotoxicity drops 1.4-fold. Given that PHENSS is burst released from the clay, the drop in cytotoxicity is not likely due to slower release and uptake of the metal complex, but more likely it can be explained in part by the incomplete release of PHENSS from the clay. With just 30–47% PHENSS being released from the clay this would be expected to affect significantly the metal complex's measured cytotoxicity.

Interestingly, the MMT appears to have some inherent cytotoxicity to the MDA-MB-231 cells with an IC_{50} nearly 4-fold lower than cisplatin. It is unclear by what mechanism the MMT could inhibit cell growth and cytotoxicity of MMT has not been previously reported. The intrinsic cytotoxicity of MMT to the MDA-MB-231 may also explain the better PHENSS cytotoxicity compared with the MCF-7 cells.

4. Conclusions

In this work a proof-of-concept study was undertaken to determine the suitability of montmorillonite as a slow release delivery vehicle for the phenanthroline-based platinum complex PHENSS. Even though PHENSS could be loaded into the clay in a solution concentration and pH dependent manner, the metal complex does not appear to intercalate between the clay layers and is burst released upon suspension in simulated gastric fluid. The results indicate that MMT is unsuitable as a slow release vehicle for PHENSS and most likely for other 1,10-phenathroline based drugs; however, MMT clay may still have application for other platinum based drugs. For instance, MMT may be useful for the delivery of the mono-aquated form of cisplatin, which is cationic. It may also be useful for highly cationic drugs, like BBR3464 [36], where binding into MMT may be facilitated by the multiple electrostatic bonds that can be formed between the drug and the clay. Alternatively, other clays may provide better drug absorption between their layers. Examples of other clays which can be examined include: laponite, beidellite, nontronite, saponite, kaolinite, halloysite and hisingerite [25].

References

[1] A.-M. Florea, D. Büsselberg, Cancers 3 (2011) 1351.
[2] R.D. Baird, S.B. Kaye, Eur. J. Cancer 39 (2003) 2450.
[3] W.D. Joo, I. Visintin, G. Mor, Maturitas 76 (2013) 308.
[4] D.M. Fisher, P.J. Bednarski, R. Grunert, P. Turner, R.R. Fenton, J.R. Aldrich-Wright, ChemMedChem 2 (2007) 488.
[5] K.B. Garbutcheon-Singh, P. Leverett, S. Myers, J.R. Aldrich-Wright, Dalton Trans. 42 (2013) 918.
[6] N.J. Wheate, R.I. Taleb, A.M. Krause-Heuer, R.L. Cook, S. Wang, V.J. Higgins, J.R. Aldrich-Wright, Dalton Trans. (2007) 5055.
[7] D.M. Fisher, R.R. Fenton, J.R. Aldrich-Wright, Chem. Commun. (2008) 5613.
[8] S. Kemp, N.J. Wheate, D.P. Buck, M. Nikac, J.G. Collins, J.R. Aldrich-Wright, J. Inorg. Biochem. 101 (2007) 1049.
[9] K.B. Garbutcheon-Singh, S. Myers, B.W.J. Harper, N.S. Ng, Q. Dong, C. Xie, J.R. Aldrich-Wright, Metallomics 5 (2013) 1061.
[10] K.J. Davis, J.A. Carrall, B. Lai, J.R. Aldrich-Wright, S.F. Ralph, C.T. Dillon, Dalton Trans. 41 (2012) 9417.
[11] S. Wang, M.J. Wu, V.J. Higgins, J.R. Aldrich-Wrigh, Metallomics 4 (2012) 950.
[12] S. Kemp, N.J. Wheate, M.P. Pisani, J.R. Aldrich-Wright, J. Med. Chem. 51 (2008) 2787.
[13] J.A. Plumb, B. Venugopal, R. Oun, N. Gomez-Roman, Y. Kawazoe, N.S. Venkataramanan, N.J. Wheate, Metallomics 4 (2012) 561.
[14] P.M. Specenier, T. Ciuleanu, J.E. Latz, L.C. Musib, L.S.D. Christelle, J.B. Vermorken, Cancer Chemother. Pharmacol. 64 (2009) 233.
[15] N.V. Nukolova, H.S. Oberoi, Y. Zhao, V.P. Chekhonin, A. Kabanov, T.K. Bronich, Mol. Pharm. 10 (2013) 3913.
[16] G.P. Stathopoulos and T. Boulikas, J. Drug Delivery, 2012, Article ID 581363, 10 pages.
[17] S. Dhar, W.L. Daniel, D.A. Giljohann, C.A. Mirkin, S.J. Lippard, J. Am. Chem. Soc. 131 (2009) 14652.

[18] S. Dhar, Z. Liu, J. Thomale, H. Dai, S.J. Lippard, J. Am. Chem. Soc. 130 (2008) 11467.
[19] N.J. Wheate, Nanomedicine 7 (2012) 1285.
[20] M.I. Carretero, G. Lagaly, Appl. Clay Sci. 36 (2007) 1.
[21] M.I. Carretero, M. Pozo, Appl. Clay Sci. 46 (2009) 73.
[22] C. Aguzzi, P. Cerezo, C. Viseras, C. Caramella, Appl. Clay Sci. (2007) 36.
[23] R.I. Iliescu, E. Andronescu, C.D. Ghitulica, G. Voicu, A. Ficai, M. Hoteteu, Int. J. Pharm. 463 (2014) 184.
[24] W. Chrzanowski, A. Khademhosseini, Adv. Drug Del. Rev. 65 (2013) 403.
[25] W. Chrzanowski, S.Y. Kim, N.E.A. Abou, Aust. J. Chem. 66 (2013) 1315.
[26] M. Ghadiri, H. Hau, W. Chrzanowski, H. Agus, R. Rohanizadeh, RSC Adv. 3 (2013) 20193.
[27] H. Hau, R. Rohanizadeh, M. Ghadiri, W. Chrzanowski, Drug Deliv. Transl. Res. 4 (2014) 295.
[28] M. Ghadiri, W. Chrzanowski, W.H. Lee, A. Fathi, F. Dehghani, R. Rohanizadeh, Appl. Clay Sci. 85 (2013) 64.
[29] K.-N. Wai, H.G. DeKay, G.S. Banker, J. Pharm. Sci. 55 (1966) 1244.
[30] G.V. Joshi, B.D. Kevadiya, H.A. Patel, H.C. Bajaj, R.V. Jasra, Int. J. Pharm. 374 (2009) 53.
[31] J.-H. Guh, W.-L. Chang, J. Yang, S.-L. Lee, S. Wei, D. Wang, S.K. Kulp, C.-S. Chen, J. Med. Chem. 53 (2010) 2552.
[32] Y. Zambito, E. Pedreschi, G. Di Colo, Int. J. Pharm. 434 (2012) 28.
[33] G. Shazly, T. Nawroth, P. Langguth, Dissolut. Tech. 15 (2008) 7.
[34] X. Liao, J. Lu, P. Ying, P. Zhao, Y. Bai, W. Li, M. Liu, J. Biol. Inorg. Chem. 18 (2013) 975.
[35] A.J. Pope, C. Bruce, B. Kysela, M.J. Hannon, Dalton Trans. 39 (2010) 2772.
[36] N.P. Farrell, Curr. Top. Med. Chem. 11 (2011) 2623.