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Cucurbit[7]uril encapsulated cisplatin overcomes cisplatin resistance *via* a pharmacokinetic effect

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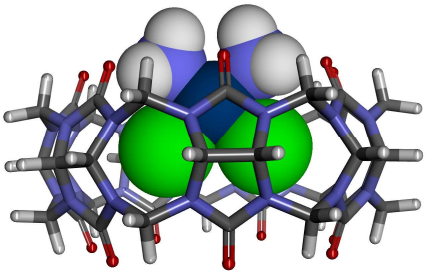


The encapsulation of the anticancer drug cisplatin within the cavity of the macrocycle cucurbit[7]uril affects the drug's pharmacokinetics, resulting in higher plasma and organ concentrations.

Abstract

The cucurbit[*n*]uril (CB[*n*]) family of macrocycles has been shown to have potential in drug delivery where they are able to provide physical and chemical stability to drugs, improve drug solubility, control drug release and mask the taste of drugs. Cisplatin is a small molecule platinum-based anticancer drug that has severe dose-limiting side-effects. Cisplatin forms a host-guest complex with cucurbit[7]uril (cisplatin@CB[7]) with the platinum atom and both chlorido ligands located inside the macrocycle, with binding stabilised by four hydrogen bonds (2.15-2.44 Å). Whilst CB[7] has no effect on the *in vitro* cytotoxicity of cisplatin in the human ovarian carcinoma cell line A2780 and its cisplatin-resistant sub-lines A2780/cp70 and MCP1, there is a significant effect on *in vivo* cytotoxicity using human tumour xenografts. Cisplatin@CB[7] is just as effective on A2780 tumours compared with free cisplatin, and in the cisplatin-resistant A2780/cp70 tumours cisplatin@CB[7] markedly slows tumour growth. The ability of cisplatin@CB[7] to overcome resistance *in vivo* appears to be a pharmacokinetic effect. Whilst the peak plasma level and tissue distribution are the same for cisplatin@CB[7] and free cisplatin, the total concentration of circulating cisplatin@CB[7] over a period of 24 hours is significantly higher than for free cisplatin when administered at the equivalent dose. The results provide the first example of overcoming drug resistance via a purely pharmacokinetic effect rather than drug design or better tumour targeting, and demonstrate that *in vitro* assays are no longer as important in screening advanced systems of drug delivery.

Introduction

Platinum-based drugs represent the major class of agents in chemotherapy for the treatment of a range of human cancers including: testicular, head and neck, colorectal, bladder, lung and ovarian.[1, 2] Cisplatin was the first drug approved in this class and after 40 years remains in use, but clinical activity is limited by systemic toxicity and tumour drug resistance (Figure 1).[1] A number of platinum analogues have been developed in an attempt to improve the therapeutic efficacy of cisplatin.[1] The introduction of carboplatin resulted in a significant reduction in the nephrotoxicity associated with platinum-based chemotherapy.[2] Oxaliplatin, a recently approved platinum based drug is used primarily in the treatment of colorectal cancer; a tumour type previously resistant to cisplatin treatment.[2] New drugs continue to be developed, such as the multinuclear drug BBR3464,[3, 4] orally active drugs like satraplatin and sterically hindered drugs like picoplatin.[1, 2]

Advances in drug delivery, however, can also be exploited to improve the clinical efficacy of anticancer drugs. The delivery of platinum drugs can be improved through their encapsulation in macrocycles, polymers or liposomes. Use of these vehicles protects the drugs from binding to serum proteins whilst in circulation, and allows the drugs to be better targeted to tumours through the enhanced permeability and retention effect.[5]

Cucurbit[*n*]urils (CB[*n*], Figure 1) are a family of rigid macrocycles made from the acid condensation of glycoluril and formaldehyde.[6, 7] They have a hydrophobic cavity, accessible through two hydrophilic oxygen lined portals, and are capable of storing and releasing small molecules.[8, 9] Encapsulation of a drug molecule by cucurbituril can provide a range of benefits including: chemical[10-12] and thermal stability,[13-15] improved drug solubility,[16, 17] controlled drug release,[18, 19] and potential taste masking of some drugs.[14] Cucurbiturils of all

sizes have been shown to be non-cytotoxic and non-toxic,[10, 20] and can be formulated into dosage forms suitable for human drug administration.[9, 21]

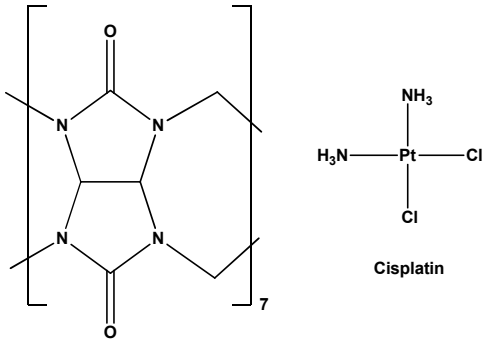


Figure 1. The chemical structures of cucurbit[7]uril (CB[7]) and cisplatin.

In this paper we report for the first time the use of cucurbiturils to enhance the cytotoxicity, and overcome drug resistance, of an platinum anticancer agent *via* a purely pharmacokinetic effect. The mode of cisplatin encapsulation by CB[7] has been investigated using molecular modeling and the effect of the macrocycle on the drug's *in vitro* and *in vivo* cytotoxicity determined using matched human ovarian carcinoma cell lines. The whole body pharmacokinetic effect of CB[7] has also been examined *in vivo* and assessed to determine peak drug serum concentration times and uptake of the drug into different vital organs.

Results and Discussion

Molecular modelling

Cucurbiturils form a range of host-guest complexes with drugs by two possible complementary modes utilising hydrophobic interactions between the cavity of the

112 macrocycle and drug and/or ion-dipole or dipole-dipole (hydrogen bonding) between the
113 cucurbituril carbonyl groups and drug am(m)ine groups.[9] For platinum-based drugs that have
114 organic ligands, like oxaliplatin or multinuclear drugs, the association constant of the host-
115 guest complex can be relatively high (10^5 M^{-1}), although the strength of binding and the rate
116 of drug release can be controlled by varying the size of the cucurbituril used.[10] Whilst we
117 have previously shown that cisplatin can form host-guest complexes with CB[7],[22] the nature
118 of the binding has not been examined. How the drug binds to cucurbiturils is important as
119 cisplatin has no organic ligand with which it can utilise hydrophobic interactions with the
120 macrocycle's cavity. As such, binding may be quite weak and the drug easily dissociated
121 when dissolved at pharmaceutically relevant concentrations.
122
123 Molecular models of cisplatin with CB[7] were generated, with the cisplatin positioned
124 pointing into the macrocycle, and alternatively, with cisplatin positioned at the edge of the
125 cucurbituril pointing out from the macrocycle (Figure 2a); two modes that have been
126 predicted from ^{1}H and ^{195}Pt NMR spectra.[22] In the *pointing in* position the platinum atom and
127 the two chlorido ligands of the drug are located within the CB[7] cavity, where steric
128 hindrance provides protection of the drug from attack from potential biological nucleophiles,
129 like glutathione, and proteins containing accessible cysteine and methionine residues.[12, 23, 24]
130 In this case, binding into the cavity is stabilised by four hydrogen bonds, with lengths of
131 between 2.15 and 2.44 Å (Figure 2b).

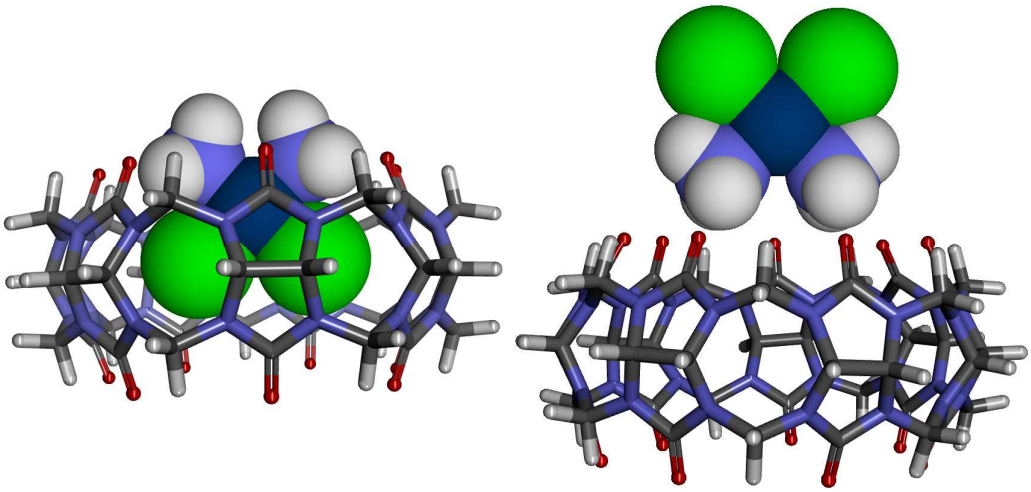


Figure 2a. Molecular models of the host-guest complexes of the anticancer drug cisplatin with cucurbit[7]uril, showing the two potential modes of binding: *pointing in*, where the platinum atom and chlorido ligands are located within the macrocycle's cavity and *pointing out*, where binding occurs only at the CB[7] portals and is less energetically favourable.

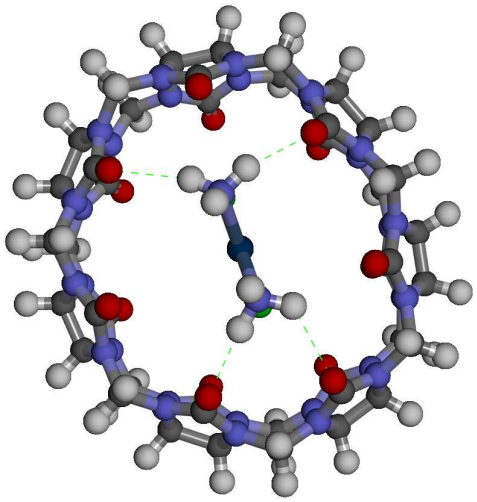


Figure 2b. A molecular model of the *pointing in* mode of binding of cisplatin to CB[7] showing the four hydrogen bonds from the drug's ammine hydrogen atoms to the macrocycle's carbonyl oxygen atoms (bond lengths: 2.15, 2.22, 2.38 and 2.44 Å) that stabilise the host-guest complex.

In the *pointing out* mode of cisplatin binding, the distance between the drug ammine hydrogen atoms and the CB[7] carbonyl oxygen atoms is too great to form hydrogen bonds properly; 2.60 to 3.40 Å. Binding in this manner is also less energetically favourable compared with the *pointing in* mode of binding by 0.961 kJ/mol. Attempts to measure the association constant of cisplatin to CB[7] using fluorescent displacement assays of methylene-blue were unsuccessful and indicate that the K_b is less than 10^4 M^{-1}. The results therefore clearly indicate a preferred mode of binding by the drug in which it is pointing in to the cavity of CB[7], which is potentially useful in drug delivery.

In vitro cytotoxicity

The ovarian cell line A2780 is relatively sensitive to cisplatin. It has a functional wild type p53 gene and expresses the MLH1 component of the DNA mismatch repair pathway. This pathway has been shown to be involved in the recognition of cisplatin-DNA adducts and induction of apoptosis.[25-27] Loss of mismatch repair (MMR) enzyme function results in resistance *in vitro* to a number of clinically important anticancer drugs, including cisplatin and doxorubicin,[28-30] and has been associated with selection for drug-resistant breast and ovarian tumours during chemotherapy.[29, 31] A2780/cp70 and MCP1 are cisplatin resistant cell lines derived from A2780 that show a 27- and 3-fold resistance to cisplatin *in vitro*, respectively. Re-expression of MLH1sensitises xenografts of A2780/cp70 to cisplatin.[32]

The *in vitro* growth inhibition assay is the gold standard as a first screening tool when evaluating new drug candidates. A compound which has a high IC_{50} (the concentration of drug required to inhibit cell growth by 50%) is not generally further developed. The IC_{50} of cisplatin is dependent on the cell line used and the length of exposure of the drug to the cells, but is usually somewhere between 0.1 and 10 μM. Therefore a new platinum drug candidate

in the past has needed an IC_{50} in the sub-micromolar concentration range to warrant further development.

Encapsulation of cisplatin in CB[7] (cisplatin@CB[7]) had no effect on the cytotoxicity of the drug in the A2780 cell line and had no effect on the resistance of A2780/cp70 and MCP1 (Table 1). Similarly, p53 was induced 24 hours after treatment of cells with either free cisplatin or cisplatin@CB[7] and showed the same dose dependent increase in the two cell lines with wild type p53 (A2780 and MCP1; Fig 3a). The induction of apoptosis, as measured by the appearance of an 85 kDa cleavage product of poly ADP ribose polymerase, also showed the same dose dependence for free cisplatin and cisplatin@CB[7] (Fig 3b).

Table 1. *In vitro* cytotoxicity of free cisplatin and cisplatin@CB[7] in the human ovarian cancer cell line A2780 and its cisplatin-resistant derivatives: A2780/cp70 and MCP1. IC_{50} is defined as the concentration of drug required to inhibit cell growth by 50%.

Cell line	IC_{50} (μM)	
	cisplatin	cisplatin@CB[7]
A2780	0.11 ± 0.01	0.09 ± 0.01
A2780/cp70	3.01 ± 0.09	2.73 ± 0.21
MCP1	0.34 ± 0.01	0.35 ± 0.08

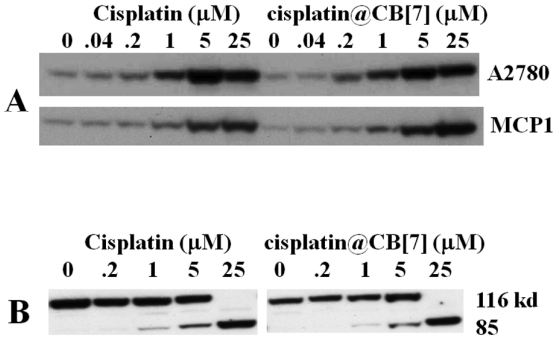


Figure 3. (A) The induction of p53 expression and (B) PARP cleavage by free cisplatin and cisplatin@CB[7] in A2780 cells demonstrating no difference in the action of either drug.

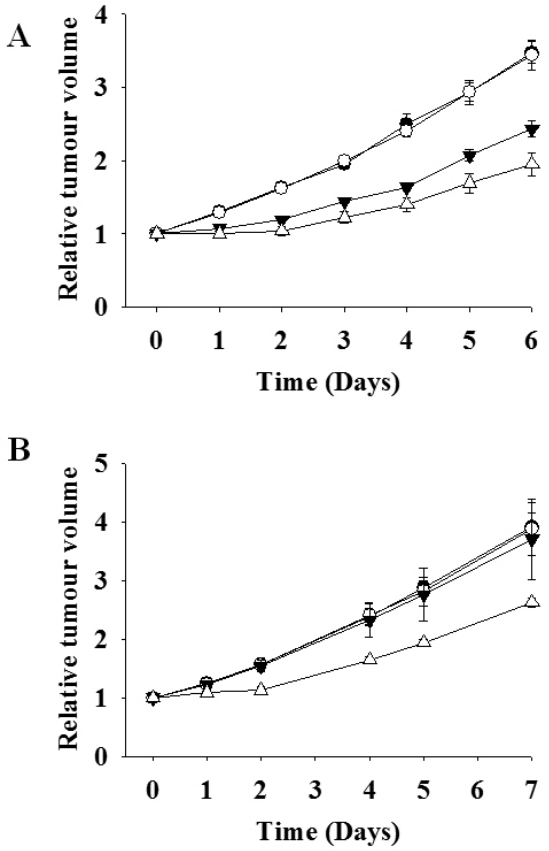
In some instances, encapsulation of platinum drugs within different sized CB[*n*]s has led to large increase in IC_{50}, or complete loss of *in vitro* cytotoxicity.[10, 24] Previously we and others have speculated that the decrease in *in vitro* cytotoxicity of some platinum drugs upon encapsulation in CB[*n*]s was due to either decreased cell uptake or because the drugs were too strongly bound by the CB[*n*] and could not go on to bind DNA at a sufficiently fast rate.[10, 33, 34] In only a few instances has encapsulation by CB[6] increased the cytotoxicity some platinum(II)-based DNA intercalator drugs.[34, 35] Ordinarily, the lack of change in *in vitro* cytotoxicity of cisplatin upon encapsulation within CB[7] would not warrant further testing, although recent research with other drug delivery vehicles have demonstrated a lack of correlation between *in vitro* and *in vivo* results when testing drug delivery systems.[36] On this basis free cisplatin and cisplatin@CB[7] were also examined using *in vivo* models.

***In vivo* cytotoxicity**

Intraperitoneal injection (i.p.) of CB[7] alone is well tolerated in nude mice and a dose of 250 mg/kg had no effect on the tumour growth rates of either A2780 or A2780/cp70 xenografts nor on the weight of the animals. Tumours of A2780 are sensitive to cisplatin (i.p.) and show a significant growth delay when treated with cisplatin ($P < 0.001$, Table 2 and Fig 4a). Treatment with cisplatin@CB[7] (i.p.) at an equivalent dose resulted in a slightly increased growth delay ($P < 0.005$).

Surprisingly, the xenografts of A2780/cp70, which are resistant to the maximum tolerated dose (MTD) of cisplatin (6 mg/kg), are sensitive to cisplatin@CB[7] (34 mg/kg; which yields

209 6 mg/kg of cisplatin) with a tumour doubling time 1.6-fold that of free cisplatin ($P < 0.001$,
210 Table 2 and Fig 4b). Neither of the platinum treatments had any significant affect on the body
211 weight of the mice (results not shown).
212
213



214

215 **Figure 4.** Growth of (A) cisplatin sensitive A2780 and (B) cisplatin resistant A2780/cp70
216 human ovarian tumour xenografts following intraperitoneal injection on day 0 of saline (●),
217 CB[7] at 250 mg/kg (○), free cisplatin at 6 mg/kg (▼), and cisplatin@CB[7] at 34 mg/kg (Δ,
218 equivalent cisplatin dose of 6 mg/kg). Results are the mean ± SEM of six mice.
219

Table 2. The amount of time required for the human tumour xenografts in nude mice to double in volume following treatment on day 0 by intraperitoneal injection with either control (saline), CB[7], free cisplatin or cisplatin@CB[7].

Treatment	Tumour doubling time (days)	
	A2780	**A2780/cp70**
control	3.1 ± 0.1	3.2 ± 0.3
CB[7]	2.9 ± 0.2	3.2 ± 0.4
cisplatin	4.9 ± 0.2	3.8 ± 0.6
cisplatin@CB[7]	6.3 ± 0.5	5.3 ± 0.2

Since CB[7] encapsulation had no effect on the *in vitro* cytotoxicity of cisplatin the increased activity in the resistant xenograft model suggests that encapsulation has altered the bioavailability of the drug. Previously, we hypothesised that the main benefit of CB[*n*] encapsulation of platinum drugs would be from steric hindrance that prevents degradation and deactivation by thiols.[12, 23, 33] As increased glutathione levels are not a major mechanism of resistance in A2780/cp70 cells, and the fact that encapsulation did not result in a higher MTD of cisplatin (as would have been expected if serum protein binding was reduced) then the results imply some other pharmacokinetic effect, such as altered drug distribution to the tumour, is responsible for the enhanced *in vivo* activity.

Plasma and tissue pharmacokinetics

Plasma levels of platinum were measured at various times after a single i.p. dose of either cisplatin (6 mg/kg) or cisplatin@CB[7] (34 mg/kg, Fig 5a). The peak plasma level was observed 5 minutes after injection and this level was higher following injection of free cisplatin than for cisplatin@CB[7]. Plasma platinum levels decreased rapidly, but the decline

was slower for cisplatin@CB[7] such that after 15 minutes, plasma levels of platinum were higher for cisplatin@CB[7] compared with free cisplatin. This difference was maintained for up to 24 hours to the extent that the total area under the curve (AUC) was significantly lower for cisplatin (16.3 h.µg/mL) than for cisplatin@CB[7] (28.8 h.µg/mL). Injection of cisplatin at 8 mg/kg resulted in a higher peak plasma level compared to a 6 mg/kg dose of free cisplatin (Fig 5b). The AUC for the first hour after injection ($AUC_{0\text{-}1\ h}$) was 4.2 h.µg/mL for free cisplatin at a dose of 6 mg/kg which increased to 4.9 h.µg/mL at a dose of 8 mg/kg, which was similar to that obtained for cisplatin@CB[7] (4.8 h.µg/mL). The AUC over the first 6 hours after injection was higher for cisplatin@CB[7] (13.2 h.µg/mL) than for cisplatin at either 6 mg/kg (7.6 h.µg/mL) or 8 mg/kg (10.6 h.µg/mL).

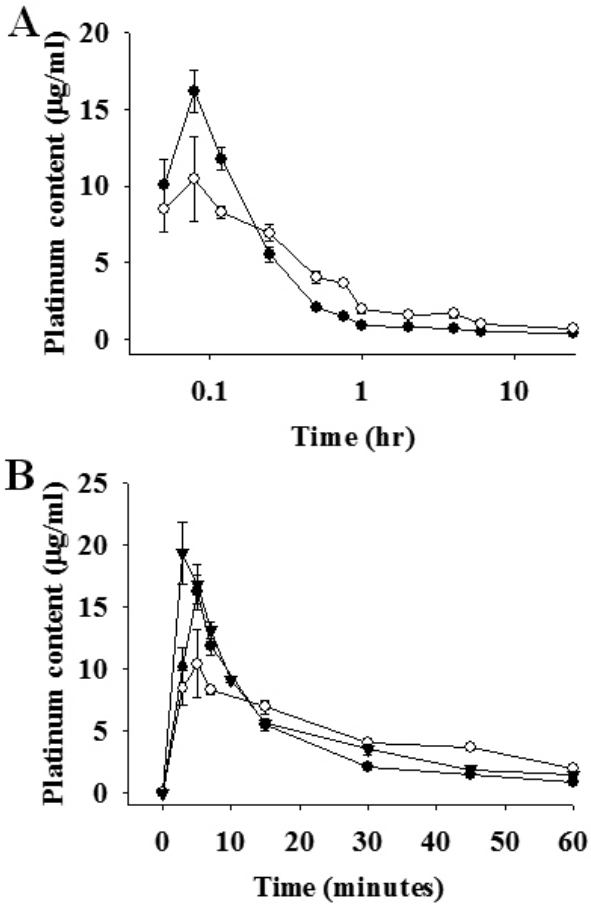


Figure 5. (A) Levels of platinum measured in mouse plasma collected at various times up to 24 hours after a single i.p. bolus dose of either free cisplatin (6 mg/kg; ●) or cisplatin@CB[7] at 34 mg/kg (○). (B) Levels of platinum in mouse plasma as in (A) over the first hour after drug administration and also including results for cisplatin administered at 8 mg/kg (▼).

The dose limiting toxicity of cisplatin is associated with the peak plasma drug level. For the drug sensitive A2780 xenograft a clear dose response to treatment is observed,[37] but the MTD of cisplatin is 6 mg/kg in our mice. A comparison of plasma platinum levels shows that the peak plasma level is increased (from 16.2 to 19.3 μg/mL) when the dose is increased from 6 to 8 mg/kg (Fig 5B). The peak plasma platinum level observed following treatment with cisplatin@CB[7] (10.4 h.μg/mL) is lower than that for the free drug (16.2) but the $AUC_{0\text{-}24}$, a measure of the drug exposure over the first 24 hour after treatment, for cisplatin@CB[7] was 28.8 h.μg/mL, nearly double that for free cisplatin (16.3 h.μg/mL). Thus, plasma pharmacokinetics show that cisplatin is retained in the circulation for longer when administered as cisplatin@CB[7] rather than as the free drug, supporting the suggestion that CB[7] protects the drug from degradation. This increased exposure could explain the increased cytotoxic activity observed *in vivo* in the cisplatin resistant tumour xenograft.

Measurement of tissue and tumour levels of platinum show that the increased exposure increases the platinum levels in general and that there is no improved tumour selectivity upon encapsulation within CB[7] (Fig 6). This is not unexpected since the encapsulation does not incorporate a targeting moiety and CB[7] is probably too small (< 1 nm in diameter) to exploit the enhanced permeability and retention effect. We were not able to increase the dose of cisplatin@CB[7] beyond 34 mg/kg. This may be explained by the observation that the $AUC_{0\text{-}1}$, a measure of the drug exposure during the first hour after administration, is similar

for cisplatin at 8 mg/kg (4.9 h.µg/mL) and cisplatin@CB[7] (4.8 h.µg/mL), compared to that of free drug at 6 mg/kg (4.2 h.µg/mL).

Table 3. Comparative pharmacokinetic parameters of intraperitoneal injection of free cisplatin or cisplatin@CB[7] over a period of 24 hours.

Pharmacokinetics parameter	**cisplatin**	**cisplatin@CB[7]**
C_{max} (µg/mL)	16.2	10.4
T_{max} (min)	5	5
AUC_{0-24} (hr.µg/mL)	16.3	28.8

Table 4. Short and midterm comparative pharmacokinetic parameters of intraperitoneal injection of free cisplatin, at both high and normal dose, or cisplatin@CB[7].

Pharmacokinetics parameter	**cisplatin (6 mg/kg)**	**cisplatin (8 mg/kg)**	**cisplatin@CB[7] (34 mg/kg)**
C_{max} (µg/mL)	16.2	19.3	10.4
T_{max} (min)	5	3	5
AUC_{0-1} (h.µg/mL)	4.2	4.9	4.8
AUC_{0-6} (h.µg/mL)	7.6	10.6	13.2

Platinum levels were also measured in tissues taken from tumour bearing mice at one, four and six hours after injection of either free cisplatin (6 mg/kg) or cisplatin@CB[7] (34mg/kg). Levels in the liver, kidneys and tumours (A2780 and A2780/cp70) were consistently higher after injection of cisplatin@CB[7] than for free cisplatin, but this difference did not always reach statistical significance (Figure 6). Although liver platinum levels were higher after

injection of cisplatin@CB[7] compared to free cisplatin at one hour, they were similar after four hours and significantly higher after free cisplatin at six hours (Fig 6A).

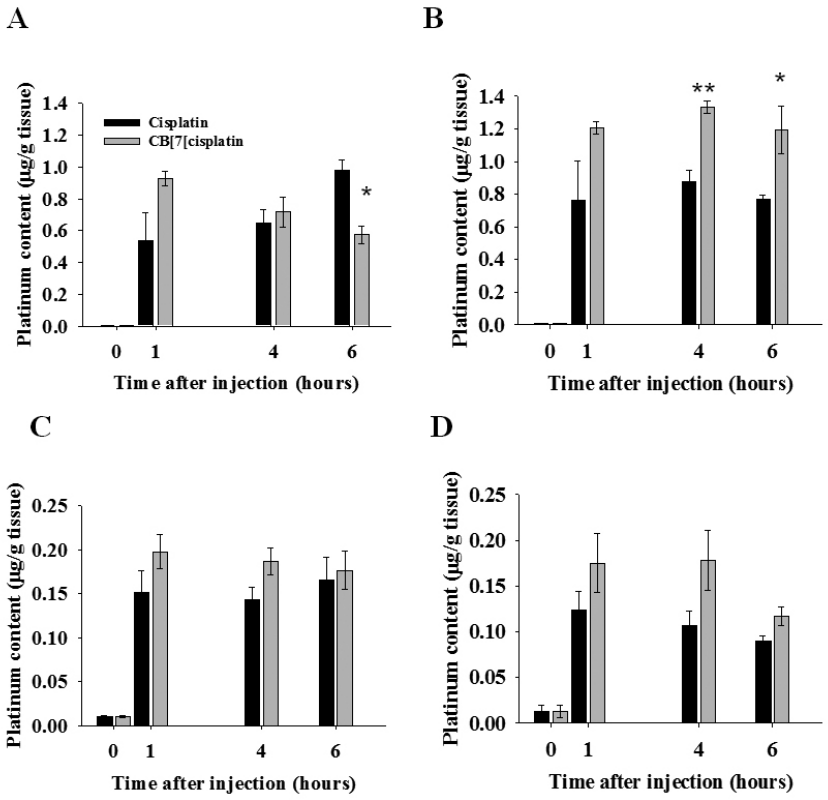


Figure 6. Levels of platinum measured in (A) liver, (B) kidney, (C) A2780 tumours and (D) A2780/cp70 tumours, collected at one, four and six hours after a single i.p. bolus dose of either free cisplatin (6 mg/kg; black bars) or cisplatin@CB[7] (34 mg/kg; grey bars). Significant differences between free cisplatin and cisplatin@CB[7] are shown (* $P<0.01$, ** $P<0.004$).

Conclusions

Regardless of the mechanism of action, this positive *in vivo* result has implications for the further testing and evaluation of not just cucurbituril-based drug delivery vehicles, but for other macrocycles and polymers as well. Previously our group and others have concluded that when no change in the *in vitro* cytotoxicity is observed upon encapsulation of a platinum-

303 based drug or attachment of a platinum drug to a nanoparticle, then the host-guest complexes
304 formed are probably not going to have better *in vivo* activity compared with the free drug.[38, 39]
305 Our results here demonstrate otherwise and indicate that *in vitro* results, whether good, bad
306 and unchanged from that of the free drug may not be sufficient to determine whether the
307 vehicle will improve the delivery of the platinum drug in question. Overall, our results
308 demonstrate that CB[7], and possibly other sized cucurbit[*n*]urils, may have utility in the
309 treatment of drug-resistant human cancers and warrant further investigation. One area for
310 further development is to attempt to reduce the rate of release of the encapsulated drug into
311 circulation in order to reduce the initial drug exposure and thus allow increased doses of the
312 drug.
313
314 **Methods**
315 **Preparation of cisplatin@CB[7]**. Cisplatin (Sigma-Aldrich) and CB[7][40] were stirred
316 together in hot water until dissolved, then stirred for a further 3 h before being either freeze
317 dried or rotary evaporated to dryness. The water content of the cisplatin@C[7] complex was
318 then determined by elemental analysis and found to be between 5 and 13 water molecules per
319 batch. These waters of crystallisation were taken into account when calculating the molecular
320 mass of cisplatin@CB[7] and the subsequent concentrations of each batch in solution before
321 administration.
322
323 **Molecular modeling.** The geometry optimisations were performed by using the spin-
324 polarised DFT implemented in the Dmol3 package. The package is for an accurate and
325 efficient density functional calculation where a rapidly convergent 3D numerical integration
326 scheme for molecules is used. The exchange–correlation interaction was treated within the
327 generalised gradient approximation (GGA) in which the Becke exchange functional and the

Lee–Yang–Parr correlation functional (BLYP) were used. In the electronic structure calculations, effective core potential treatment with a double-numerical basis plus polarised functions (DNPs) was chosen.

Cell lines. A2780/cp70 is an *in vitro* derived cisplatin resistant variant of the ovarian cancer cell line A2780 originally obtained from Dr R.F. Ozols (Fox Chase Cancer Centre, Philadelphia, PA). A second *in vitro* derived cisplatin resistant variant, MCP1, was derived in house.[41] Cells were grown in RPMI1640 supplemented with glutamine (2mm) and FCS (10%). A2780/cp70 and MCP1 are mismatch repair deficient and do not express MLH1 due to hypermethylation of the *hmlh1* gene promoter.[41]

Drug sensitivity *in vitro.* Drug sensitivity was determined by a tetrazolium dye-based microtitration assay.[42] Cells were plated out in 96 well plates at a density of 300 – 1000 cells/well and allowed to attach and grow for 2 days. Cells were exposed to the drug at a range of concentrations for 24 hours and then the medium was replaced with drug free medium for a further 3 days. On the final day MTT (50 µL of a 5mg/ml solution) was added to the 200 µL of medium in each well and plates were incubated at 37^{o}C for 4 h in the dark. Medium and MTT was then removed and the MTT-formazan crystals dissolved in 200 µL DMSO. Glycine buffer (25 µL per well, 0.1 M, pH 10.5) was added and the absorbance measured at 570 nm in a multiwell plate reader. A typical dose-response curve consisted of 8 drug concentrations and 4 wells were used per drug concentration. Results are expressed in terms of the drug concentration required to kill 50% of the cells (IC_{50}) estimated as the absorbance value equal to 50% of that of the control untreated wells.

Induction of p53 and apoptosis. Cells were plated at a density of 10^5 cells in a 25 cm^2 flask and allowed to attach and grow for 48 h. Drug was added at a range of concentrations for 24 h. Both adherent cells and those in the medium were harvested and washed twice with ice cold PBS. They were resuspended in 200 μL of lysis buffer (50 mM Hepes pH 7.0, 250 mM NaCl, 0.5% NP-40) supplemented with protease inhibitors ('Complete' from Roche Diagnostics Ltd, Lewes, UK) and incubated on ice for 20 min. Samples were centrifuged at 12,000 g for 5 min at 4 ^oC to remove debris. Proteins were separated on 4-12% Bis-Tris gels with MOPS SDS running buffer. The "Novex Xcell II" blotting apparatus (Invitrogen) was used to transfer proteins onto Immobilon PVDF membrane (Millipore). The membrane was blocked for 1 h in Tris-buffered saline containing 0.02% Tween 20 and 5% powdered milk and then incubated overnight at 4 ^oC with the primary antibody (anti-p53, Novocastra clone D-01) from Leica Biosystems Ltd and anti-PARP, BD Biosciences). The membrane was then washed and incubated for 1 hour at room temperature with the secondary antibody (sheep anti-mouse HRP, Amersham). After washing protein bands were visualised by enhanced chemiluminescence (ECL, Amersham).

Human tumour xenografts. Animal studies were carried out under an appropriate United Kingdom Home Office Project License and all work conformed to the UKCCR Guidelines for the welfare of animals in experimental neoplasia. Monolayer cultures were harvested with trypsin/EDTA and resuspended in PBS. For the A2780 and A2780/cp70 xenografts about 10^7 cells were injected subcutaneously into the right flank of athymic nude mice (CD1 *nu/nu* mice from Charles River). After 7 to 10 days when the mean tumour diameter was at ≥ 0.5 cm, animals were randomized in groups of 6 for experiments. A standard sterile clinical formulation of cisplatin was used (Western Infirmary Pharmacy, Glasgow). Mice were

376 treated i.p. with CB[7] (250 mg/kg), cisplatin (6 mg/kg) or cisplatin@CB[7] (34 mg/kg
377 equivalent to 6 mg/kg cisplatin). Mice were weighed daily and tumour volumes were
378 estimated by calliper measurements assuming spherical geometry (volume = d^3 x $\pi/6$).
379
380 **Pharmacokinetics.** Tumour bearing mice were treated with either cisplatin or CB[7]cisplatin
381 as above. Blood, liver, kidney and tumour were sampled at various times. Blood was
382 collected by cardiac puncture and samples placed into ice cold EDTA tubes and centrifuged
383 at 1500 g for 10 min at 4 ^{o}C. Plasma was removed and stored at -70 ^{o}C until analysis. Tissues
384 were dissected rapidly and snap frozen in liquid nitrogen and stored at -70 ^{o}C until analysis.
385 They were then thawed, weighed and homogenised in PBS (1 mg tissue/mL PBS). Tissue and
386 plasma samples were incubated overnight at 65 ^{o}C with nitric acid (1 mL homogenate + 9 mL
387 nitric acid (OPTIMA 68%); 1 volume plasma: 1 volume nitric acid). The samples were then
388 diluted with water/0.1% Triton-X100 to a final concentration of 1% acid. The platinum
389 content of samples was determined by ICP-MS. Pharmacokinetic parameters were
390 determined by non-compartmental analysis (WinNonLin Version 4.0 software, Pharsight,
391 Mountain View, USA).
392
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399 **References**

1. N. J. Wheate, S. Walker, G. E. Craig and R. Oun, *Dalton Trans.*, 2010, **39**, 8113-8127.
2. L. Kelland, *Nat. Rev. Cancer*, 2007, **7**, 573-584.
3. N. J. Wheate and J. G. Collins, *Coord. Chem. Rev.*, 2003, **241**, 133-145.
4. N. J. Wheate and J. G. Collins, *Curr. Med. Chem. - Anti-Cancer Agents*, 2005, **5**, 267-279.
5. F. Kratz, I. A. Müller, C. Ryppa and A. Warnecke, *ChemMedChem*, 2008, **3**, 20-53.
6. J. Lagona, P. Mukhopadhyay, S. Chakrabarti and L. Isaacs, *Angew. Chem. Int. Ed.*, 2005, **44**, 4844-4870.
7. J. Kim, I.-S. Jung, S.-Y. Kim, E. Lee, J.-K. Kang, S. Sakamoto, K. Yamaguchi and K. Kim, *J. Am. Chem. Soc.*, 2000, **122**, 540-541.
8. K. Kim, N. Selvapalam, Y. H. Ko, K. M. Park, D. Kim and J. Kim, *Chem. Soc. Rev.*, 2007, **36**, 267-279.
9. S. Walker, R. Oun, F. J. McInnes and N. J. Wheate, *Isr. J. Chem.*, 2011, **51**, 616-624.
10. N. J. Wheate, *J. Inorg. Biochem.*, 2008, **102**, 2060-2066.
11. N. i. Saleh, A. L. Koner and W. M. Nau, *Angew. Chem. Int. Ed.*, 2008, **47**, 5398-5401.
12. S. Kemp, N. J. Wheate, M. P. Pisani and J. R. Aldrich-Wright, *J. Med. Chem.*, 2008, **51**, 2787-2794.
13. R. Wang and D. H. Macartney, *Org. Biomol. Chem.*, 2008, **6**, 1955-1960.
14. F. J. McInnes, N. G. Anthony, A. R. Kennedy and N. J. Wheate, *Org. Biomol. Chem.*, 2010, **8**, 765-773.
15. A. R. Kennedy, A. F. Florence, F. J. McInnes and N. J. Wheate, *Dalton Trans.*, 2009, 7695-7700.
16. Y. Zhao, M. H. Pourgholami, D. L. Morris, J. G. Collins and A. I. Day, *Org. Biomol. Chem.*, 2010, **8**, 3328-3337.
17. Y. Zhao, D. P. Buck, D. L. Morris, M. H. Pourgholami, A. I. Day and J. G. Collins, *Org. Biomol. Chem.*, 2008, **6**, 4509-4515.
18. M. J. Pisani, Y. Zhao, L. Wallace, C. E. Woodward, F. R. Keene, A. I. Day and J. G. Collins, *Dalton Trans.*, 2010, **39**, 2078-2086.
19. K. M. Park, K. Suh, H. Jung, D.-W. Lee, Y. Ahn, J. Kim, K. Baek and K. Kim, *Chem. Commun.*, 2009, 71-73.
20. V. D. Uzunova, C. Cullinane, K. Brix, W. M. Nau and A. I. Day, *Org. Biomol. Chem.*, 2010, **8**, 2037-2042.
21. S. Walker, R. Kaur, F. J. McInnes and N. J. Wheate, *Mol. Pharm.*, 2010, **7**, 2166-2172.
22. N. J. Wheate, D. P. Buck, A. I. Day and J. G. Collins, *Dalton Trans.*, 2006, 451-458.
23. M. S. Bali, D. P. Buck, A. J. Coe, A. I. Day and J. G. Collins, *Dalton Trans.*, 2006, 5337-5344.
24. Y. J. Jeon, S.-Y. Kim, Y. H. Ko, S. Sakamoto, K. Yamaguchi and K. Kim, *Org. Biomol. Chem.*, 2005, **3**, 2122-2125.
25. D. A. Anthoney, A. J. McIlwrath, W. M. Gallagher, A. R. M. Edlin and R. Brown, *Cancer Res.*, 1996, **56**, 1374-1381.
26. D. R. Duckett, S. M. Bronstein, Y. Taya and P. Modrich, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 12384-12388.
27. J. Gong, A. Costanzo, H.-Q. Yang, G. Melino, W. G. Kaelin, M. Levrero and J. Y. J. Wang, *Nature*, 1999, **399**, 806-809.
28. S. Aebi, D. Fink, R. Gordon, H. K. Kim, H. Zheng, J. L. Fink and S. B. Howell, *Clin. Cancer Res.*, 1997, **3**, 1763-1767.
29. R. Brown, G. L. Hirst, W. M. Gallagher, A. J. McIlwrath, G. P. Margison, A. G. J. van der Zee and D. A. Anthoney, *Oncogene*, 1997, **15**, 45-52.

30. N. J. Toft, D. J. Winton, J. Kelly, L. A. Howard, M. Dekker, H. T. Riele, M. J. Arends, A. H. Wyllie, G. P. Margison and A. R. Clarke, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 3911-3914.
31. H. J. Mackay, D. Cameron, M. Rahilly, M. J. MacKean, J. Paul, S. B. Kaye and R. Brown, *J. Clin. Oncol.*, 2000, **18**, 87-93.
32. J. A. Plumb, G. Strathdee, J. Sludden, S. B. Kaye and R. Brown, *Clin. Cancer Res.*, 2000, **60**, *6039-6044*.
33. N. J. Wheate, A. I. Day, R. J. Blanch, A. P. Arnold, C. Cullinane and J. G. Collins, *Chem. Commun.*, 2004, 1424-1425.
34. S. Kemp, N. J. Wheate, S. Wang, J. G. Collins, S. F. Ralph, A. I. Day, V. J. Higgins and J. R. Aldrich-Wright, *J. Biol. Inorg. Chem.*, 2007, **12**, 969-979.
35. N. J. Wheate, R. I. Taleb, A. M. Krause-Heuer, R. L. Cook, S. Wang, V. J. Higgins and J. R. Aldrich-Wright, *Dalton Trans.*, 2007, 5055-5064.
36. G. J. Kirkpatrick, J. A. Plumb, O. B. Sutcliffe, D. J. Flint and N. J. Wheate, *J. Inorg. Biochem.*, 2011, **105**, 1115-1122.
37. J. A. Plumb, Strathdee, G., Sludden, J., Kaye, S.B. & Brown, R., *Cancer Res.*, 2000, **60**, 6039-6044.
38. A. M. Krause-Heuer, N. J. Wheate, M. J. Tilby, D. Pearson, C. J. Ottley and J. R. Aldrich-Wright, *Inorg. Chem.*, 2008, **47**, 6880-6888.
39. N. J. Wheate, G. M. Abbott, R. J. Tate, C. J. Clements, R. Edrada-Ebel and B. F. Johnston, *J. Inorg. Biochem*, 2009, **103**, 448-454.
40. A. Day, A. P. Arnold, R. J. Blanch and B. Snushall, *J. Org. Chem.*, 2001, **66**, 8094-8100.
41. G. Strathdee, MacKeen, M.J., Illand, M. & Brown, R., *Oncogene*, 1999, **18**, 2335-2341.
42. J. A. Plumb, Milroy, R. & Kaye, S.B., *Cancer Res.*, 1989, **49**, 4435-4440.