Platinum Drug Distribution in Cancer Cells and Tumors

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Contents

1. Introduction

The era of platinum-based anticancer drugs was heralded by the clinical introduction of cisplatin (**1**), a square-planar platinum(II) complex whose antitumor properties were first reported by Rosenberg in 1969.[1] The success of cisplatin paved the way for the second- and third-generation platinum(II) drugs, carboplatin (**2**) and oxaliplatin (**3**), while the platinum(IV) complex satraplatin (**4**) has recently undergone phase III trials and was considered for FDA approval.[2] Platinum drugs continue to play a central role in the treatment of cancer and are used in the chemotherapeutic regimes of around half of all cancer patients.[3,4] The search for new platinum anticancer drugs is driven by the need to overcome the side effects and limited tumor penetration of existing platinum agents, as well as intrinsic and acquired resistance. To achieve such advances, an understanding of the mechanisms by which tumors and cells distribute and process platinum drugs is crucial.

A comprehensive understanding of the cellular processing of platinum drugs remains elusive, with often seemingly contradictory reports appearing in the literature. For example, the relative contributions of passive diffusion, and active and facilitated transport to the cellular uptake, and accumulation of platinum drugs have generated an ongoing debate, while the existence of an active efflux mechanism is also a contentious topic and both have been the subject of recent and extensive review.[5] [It should be noted that "uptake" and "accumulation" are distinct terms. Accumulation is a steady-state phenomenon resulting from

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Alice Klein obtained her undergraduate Chemistry degree at the University of Sydney in 2008, completing an Honours project on the subject of platinum anticancer complexes. She is now undertaking a Ph.D. at the University of Sydney and is pursuing her interest in platinum chemistry under the supervision of Professor Trevor Hambley. In particular, her research focuses on developing novel platinum(IV)-intercalator hybrid complexes and investigating their potential as antitumour agents in cells.

Trevor Hambley received his B.Sc. (Hons) degree from the University of Western Australia in 1977 and then moved to Adelaide, where he undertook his Ph.D. work on molecular modelling of metal complexes with Dr. Michael Snow. Following postdoctoral studies at the Australian National University in 1982, he moved to CSIRO Energy Chemistry, Lucas Heights. His move east across Australia was completed in 1984 when he took up a position at the University of Sydney, where he is currently a Professor in the School of Chemistry and Director of Research in the Faculties of Science. His scientific interests are in the area of medicinal inorganic chemistry with emphases on platinum anticancer agents, hypoxia selective metal complexes, MMP binding agents, and metal-based anti-inflammatory drugs. He has won awards for research and for postgraduate teaching and has published more than 460 books, reviews, and papers.

simultaneous influx and efflux of platinum and, thus, refers to the cellular platinum concentration at any given point in time. Consequently, it is a property that can be measured quantitatively. Uptake, conversely, is the dynamic process

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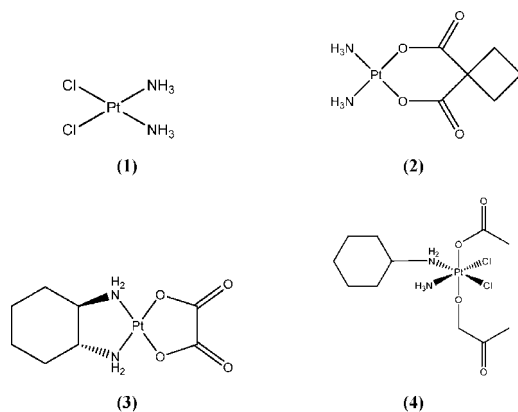
of platinum *influx*.] There is overwhelming evidence that platinum drugs exert their cytotoxic effects via the formation of platinum−DNA adducts;[6−9] however, the relative contributions of the different types of adducts is still unknown.[2] Additionally, the genetic and cellular changes responsible for tumor resistance have become a major focal point of current research.[10,11]

The vast majority of our knowledge of platinum drug accumulation and distribution has been derived from studies involving cell lines in vitro, and this technique has borne many valuable insights. However, this approach neglects the fact that cells in monolayer culture are generally exposed to uniform conditions and concentration of drug,[12] while in contrast, the tumor microenvironment is heterogeneous by nature and the constituent cells are exposed to a large concentration gradient as the drug diffuses from the blood vessels.[12] Platinum(IV) drugs, for instance, have demonstrated advantages over their platinum(II) relatives in monolayer culture including increased stability[13−15] and lipophilicity.[13,16] However, these benefits in vitro have thus far failed to translate to benefits in vivo, and few platinum(IV) agents have advanced to clinical trials.

A detailed understanding of the in vivo behavior of platinum drugs and their distribution is expected to provide a biological rationale for the design of new agents with improved properties and fewer side effects. Studies of the cellular processing of platinum complexes allow specific targets in cellular pathways to be determined and manipulated. For example, Barnes and co-workers used the discovery that estrogen sensitizes estrogen receptor-positive (ER⁺) breast cancer cells to cisplatin[17] as inspiration for developing an active estrogen-tethered platinum(IV) cisplatin analogue (**5**).[18] Equally, knowledge of how the unique tumor environment affects the penetration of platinum drugs is important for optimizing the effectiveness of these agents at a macroscopic level. An example of this approach is to exploit the EPR (enhanced permeability and retention) effect, a phenomenon whereby tumors are characterized by vascular permeability, allowing enhanced penetration and retention of macromolecules.[19−21] This was the basis for the design of AP5280 (**6**),[19] a macromolecular platinum drug that comprised a water-soluble HPMA (*N*-(2-hydroxypropyl)-methacrylamide) copolymer coupled to a cisplatin analogue via pH-sensitive peptide linkers, which has recently undergone phase I trials.[22]
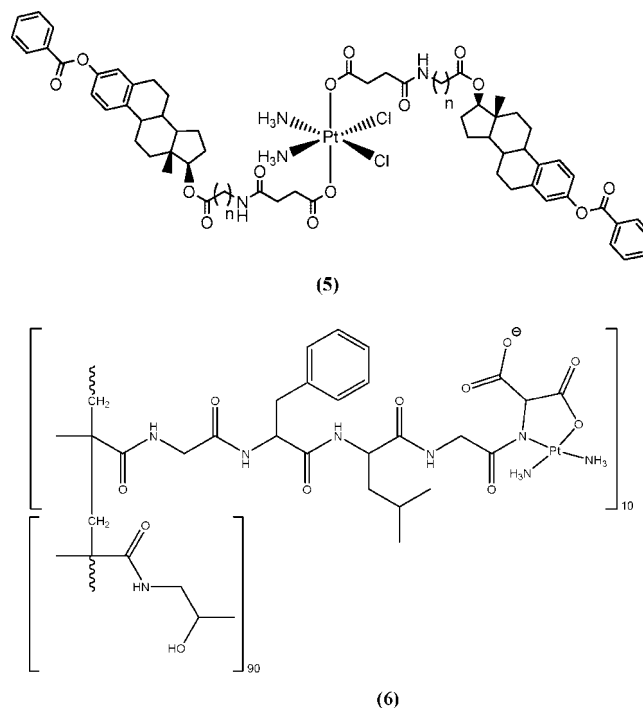
The purpose of this review is to highlight the recent advances made in our understanding of how platinum drugs enter and exit cells, their intracellular behavior and distribution, and how these considerations translate to the context of tumors. In parallel, the growing number of techniques for monitoring platinum drug accumulation and distribution are discussed.

2. Cells

### 2.1. How Do Platinum Drugs Enter Cells?

Cisplatin is highly polar, and cellular accumulation generally occurs at a slower rate than that of other small-molecule anticancer drugs.[2] The complex is believed to exist primarily in its neutral, intact form in blood plasma, due to suppression of aquation by the high concentration of chloride ions (∼100 mM).[23,24] Anticipated aquation and hydrolysis products of cisplatin are shown in Figure 1, with calculations revealing that, at a chloride concentration mimicking the extracellular environment, 68% of the complex remains in its original
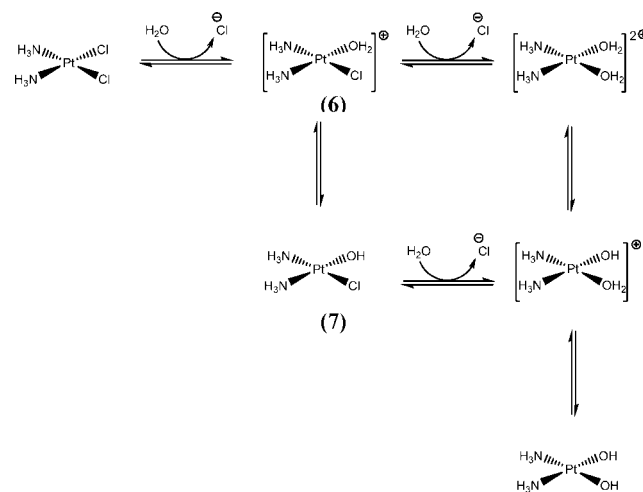
Figure 1. Possible aquation and hydrolysis products of cisplatin in aqueous solution.

form, while 24% exists as the chloridohydroxido species (**6**).[24] The lipophilicity of these two species is similar (log $P_{oct} = -2.4$ and -2.7, respectively),[25] and both are suitable for passive diffusion across the lipid bilayer of cell membranes because of their neutrality.

The traditional view has been that neutrality is required for platinum drug uptake; however, this was recently challenged by the work of Farrell and colleagues, who demonstrated increasing cellular accumulation of multi-nuclear platinum complexes with increasing positive charge.[26] In addition, the cationic platinum−acridine hybrid (**8**) has been found to be ∼100 times more potent than cisplatin in non-small-cell lung cancer cells,[27] providing further confirmation of the efficacy of charged platinum complexes. It is possible that the transporters currently being implicated in facilitated platinum drug uptake do not require neutrality for transportation of platinum complexes into cells, and this notion is supported by a recent study revealing that $[Pt(NH_3)_2(pyridine)Cl]^+$ is an excellent substrate for the organic cation transporters OCT1 and OCT2.[28]

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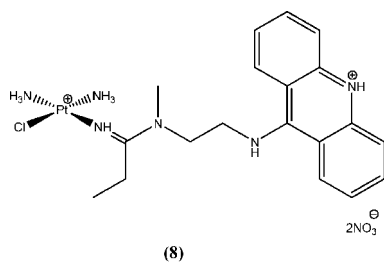
The uptake of platinum drugs has conventionally been attributed to passive diffusion down a concentration gradient, based on early observations that increasing concentrations of cisplatin and its analogues have a linear accumulation that cannot be saturated up to a concentration of 1 mM.[29−31] Additionally, cisplatin accumulation was not reduced by competitive inhibition with structural analogues,[32,33] as would be expected if active transportation was the dominant mechanism of uptake. Finally, other compounds believed to rely wholly on passive diffusion such as mannitol displayed similar accumulation behavior to cisplatin in cisplatin-sensitive and -resistant cells.[34]

However, the case for a facilitated transport mechanism is steadily building, with the copper transporter-1 CTR1 being increasingly implicated in cisplatin influx.[35,36] CTR1 is the major plasma-membrane transporter responsible for copper homeostasis, and mutation or deletion of the CTR1 gene has been shown to increase resistance to cisplatin and reduce its accumulation in both mouse and yeast cells.[37] While wild-type (CTR1 expressing) mouse embryonic cells accumulate cisplatin and carboplatin readily compared to knockout strains, oxaliplatin and satraplatin do not appear to have the same dependence on the transporter, indicating that CTR1 is structurally discriminative.[38,39] An unresolved mystery relates to the phenomenon whereby elevated platinum accumulation in cells with increased CTR1 expression does not appear to translate into increased cisplatin−DNA adduct formation and, hence, cytotoxicity.[40] Since the terminus of CTR1 contains a methionine- and histidine-rich domain, it is speculated that these residues may bind to cisplatin and its analogues during the transport process, displacing ammine ligands and thus deactivating the platinum center.[5,40]

The organic cation transporters (OCTs) constitute another class of transporters that are believed to play a role in platinum drug uptake.[41,42] OCTs mediate the uptake of cationic species having $M_r < 400$ Da[28] and are expressed in tissues associated with the toxic side effects of cisplatin such as the liver and kidneys.[43−45] Cisplatin has been shown to compete for uptake with the OCT substrate tetraethylammonium,[45] and OCT-mediated uptake of oxaliplatin is believed to be a major contributor to its activity.[46]

Thus, it appears that platinum drug accumulation is likely to be due to a combination of passive, active, and facilitated transport mechanisms. The idea of multiple pathways is supported by findings that the use of inhibitors or genetic techniques (such as CTR1 knockouts), despite reducing accumulation, does not abolish it entirely. For example, while several aldehydes are known to inhibit the uptake of cisplatin, they afford a maximum inhibition of only 50%.[47]

2.2. What Happens to Platinum Drugs in the Intracellular Environment?

Since cancer is characterized by rapid and uncontrolled cell proliferation, the principal biological target of many antitumor drugs, including platinum agents, is nuclear DNA (Figure 2). There is overwhelming evidence that, in response to the significantly lowered chloride ion concentration found inside cells (3−20 mM), cisplatin is activated intracellularly by the aquation of one of its two chlorido leaving groups.[2] In its activated monoaqua form (**7**), cisplatin is able to bind to the N7 position of the purine bases (particularly guanine), to form either monofunctional or bifunctional adducts, depending on the displacement of either one or both leaving groups.[6] Bifunctional adducts are most prevalent, with cross-links usually occurring between adjacent bases on the same DNA strand, although cross-links may also form between guanines that are separated by another base or between opposite strands.[48] These adducts cause distortions in the DNA structure, including unwinding and bending, and are recognized by a number of cellular proteins that ultimately trigger apoptotic cell death.[48,49]

In the case of platinum(II) cisplatin analogues, the nature of the leaving group(s) affects the biodistribution and toxicity of the complex. For instance, the cyclobutane-1,1-dicarboxylate leaving group in carboplatin confers greater stability and thus reduces side effects.[3] The nonleaving group(s) (usually ammine ligands), on the other hand, dictate the nature of the DNA adducts formed. In the case of platinum(IV) complexes, in vivo reduction is accompanied by the loss of the two additional axial ligands, releasing the cytotoxic platinum(II) species, which is then able to participate in DNA-platination.[14]
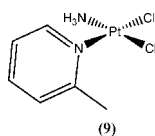
2.3. Active Efflux of Platinum Drugs

While the primary intracellular target of platinum drugs is DNA, cisplatin and its analogues are capable of binding to cytoplasmic glutathione, metallothioneins, and other thiol-containing biomolecules because of the tendency of sulfur to coordinate to platinum. These sulfur compounds deactivate platinum drugs and, thus, provide a primary detoxification mechanism in cells.[5,50,51] Elevated levels of these sulfur compounds are correlated with increased resistance to cisplatin and carboplatin,[51−53] the observation of which contributed to the design of ZD0473 (**9**), whose steric bulk reduces its reactivity with glutathione.[5,54] The coordination
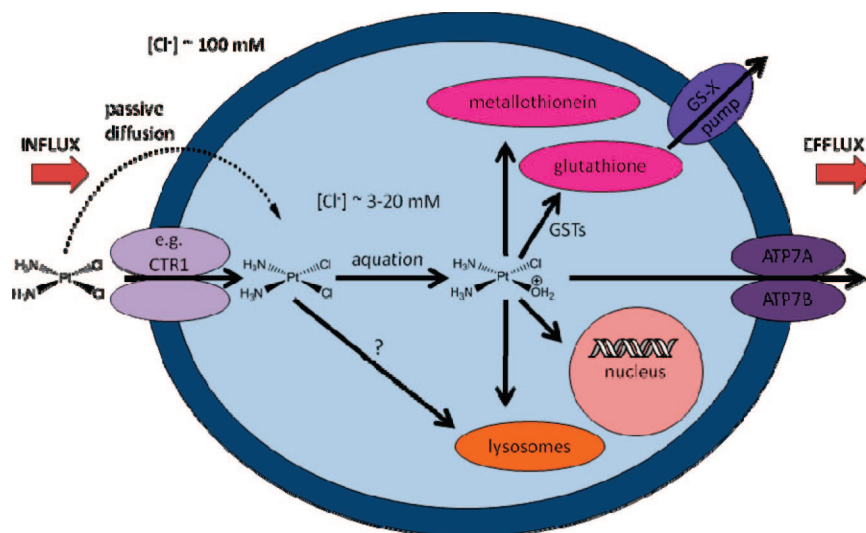
Figure 2. It is believed that platinum drugs enter cells using a combination of passive diffusion and active and facilitated transport. The low chloride ion concentration found inside cells enables water to displace chlorido ligands, yielding the activated aqua species. The activated species is able to react with thiol-containing biomolecules such as glutathione and metallothioneins, as well as its pharmacological target—DNA. Any remaining drug that fails to reach the nucleus is believed to be sequestered into the lysosomes, most likely in the aquated form, and it is known that some platinum drugs containing fluorophores primarily end up in these vesicles. Active export from the cell is mediated by the copper exporters ATP7A and ATP7B as well as GS-X efflux pumps, particularly MRP2. GSTs: glutathione S-transferases. Developed from Figure 1 of reference 2.

of glutathione to platinum drugs may be catalyzed by glutathione S-transferases, giving rise to platinum—glutathione complexes that are more anionic. Consequently, these complexes may be readily exported from the cell by GS-X efflux pumps, a family of ATP-dependent organic anion transporters, particularly MRP1 and MRP2.[5,55]

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Echoing the link between the copper influx transporter CTR1 and platinum uptake, the copper efflux transporters ATP7A and ATP7B have been implicated in the active efflux of platinum,[36,56] suggesting a mutual interference in the cellular processing of platinum and copper. Cells transfected with ATP7B have been found to accumulate less cisplatin and carboplatin,[57] while cells transfected with ATP7A were found to be resistant to cisplatin, carboplatin, and oxaliplatin as a result of increased sequestration into vesicles.[58]

2.4. Methods for Studying Platinum Distribution in Cells

Knowledge of the distribution of platinum drugs within cells is invaluable for several reasons. Primarily, it is important to confirm that these agents reach their desired target, that is, nuclear DNA. Moreover, information regarding the other subcellular compartments, transporters, and receptors with which platinum drugs are associated is useful, since it may help to explain deactivation and/or resistance mechanisms, why different types of tumor cells respond differently, and toxicity. Overall cellular platinum concentrations following treatment with platinum complexes can be readily measured using elemental spectroscopy techniques such as graphite furnace atomic absorption spectrometry (GF-AAS)[59] and inductively coupled plasma mass spectrometry (ICP-MS),[60] allowing the extent of drug accumulation to be

determined. However, monitoring the pathways traveled by these agents once they enter cells and the subcellular compartments in which they accumulate has proven more challenging. Several techniques have been employed, with the main two classes being those that monitor elemental distributions and those that track fluorescent labels.

2.4.1. Atom-Based Analytical Techniques

Elemental imaging techniques such as electron microscopy,[61,62] X-ray microanalysis,[63–65] synchotron radiation-induced X-ray emission (SRIXE),[66–68] micro-X-ray absorption near-edge structure spectroscopy (micro-XANES),[69] and X-ray fluorescence[70] allow the cellular distribution of platinum and other types of atoms to be mapped with high accuracy. The two major drawbacks of atom-based imaging methods are (a) they cannot be performed on live samples and (b) they are limited by their capacity to monitor only elemental concentrations, rather than levels of intact drug.

The electron-dense nature of platinum makes it highly suitable for imaging by electron microscopy, with Beretta and co-workers using the technique to reveal the presence of platinum in contact with the plasma membrane and the nuclear envelope, as well as in the nuclear matrices and cytoplasm of ovarian carcinoma cells.[61] While the resolution of this technique is excellent, the platinum-treated cells must be fixed with glutaraldehyde, extracted with organic solvents, and imbedded in plastic prior to analysis, which is a procedure known to cause redistribution of cellular contents.[65]

SRIXE has the advantage of being able to map elemental distributions within cells at submicrometer resolution.[67] For example, Hall and co-workers employed this approach to map platinum distributions in cisplatin-treated A2780 ovarian carcinoma cells, revealing almost exclusive localization of platinum in the nucleus after 24 h, with a small amount present in the surrounding cytoplasm.[67] A subsequent study used the same technique to determine the distribution of the bromine-labeled platinum complexes, cis-[PtCl₂(3-Brpyr)-(NH₃)] (**10**) and cis,trans,cis-[PtCl₂(OAcBr)₂(NH₃)₂] (**11**), in

order to study the intracellular behavior of both platinum(II) and platinum(IV) complexes.[66] Both complexes showed platinum to be localized in the nucleus, with the platinum(II) complex generating complementary platinum and bromine maps.[66] Since the bromine label was attached to an amine group, this result confirmed the conventional wisdom that amine ligands are nonleaving groups.[66] A less intense bromine map resulted for the platinum(IV) complex, suggesting that extensive reduction had occurred prior to cellular uptake, thereby displacing the bromine-labeled axial ligands, or that the bromoacetate had been removed from the cells following intracellular reduction.[66]


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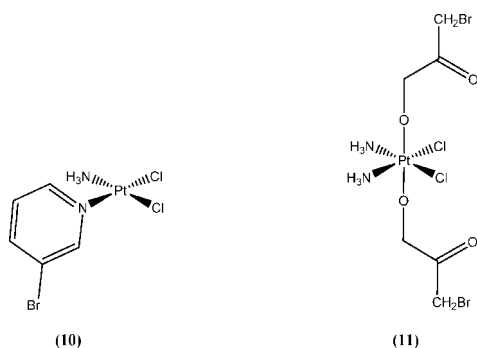
2.4.2. Fluorescent-Tagged Compounds

The synthetic flexibility of platinum complexes offers the potential to incorporate various ligands whose properties can be exploited to facilitate imaging studies. A popular use of this approach is the tethering of fluorophores to platinum centers and subsequent mapping of the localization of drug fluorescence in a relatively noninvasive procedure that can be performed on live cells. This area was first investigated by Reedijk and colleagues, who covalently linked carboxyfluoresceindiacetate to [Pt(en)Cl$_2$], producing the complex CFDA−Pt (**12**).[71] The fluorescence of this species is activated by acetate hydrolysis by esterases inside living cells.[71] Using digital fluorescence microscopy, it was found that the complex was readily taken up by U2-OS human osteosarcoma cells and accumulated in the nucleus after 2−3 h. Fluorescence became increasingly visible in the cytoplasm after 6−8 h, displaying a punctate pattern that appeared to colocalize with a Golgi-specific stain. No fluorescence was observed in the nucleus after 24 h, and the overall fluorescence of the cell diminished over time, consistent with gradual efflux.[71] However, the ability of CFDA−Pt to adequately imitate cisplatin is questionable, since the potential for the fluorophore to be detached from the platinum center has not been investigated, the cytotoxicity of the complex is unknown, and no differences in localization were found for resistant and sensitive cells.

A variant of CFDA−Pt, FDDP (**13**), has contributed significantly to our understanding of the intracellular behavior of platinum drugs.[72] Deconvoluting digital microscopy provides a means of tracking the fluorescent complex, with Safaei and co-workers using this technique to monitor FDDP in ovarian carcinoma cells stained with organelle-specific markers.[72] The complex was found to be initially sequestered into the lysosomes, consistent with the purported role of the lysosomes in the sensitivity of tumor cells to cisplatin and the association between cisplatin-resistance and lysosomal dysfunction.[73,74] The near absence of FDDP in early endosomes was interpreted as a strong indication that the sequestration of FDDP into lysosomes is not mediated by


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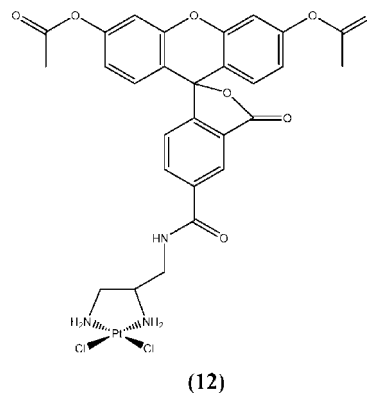
endocytic pathways[72] (the process by which cells absorb external substances by engulfing them in their cell membrane), and thus, further work is required to elucidate the mechanism of drug sequestration into the lysosomes. Finally, the exclusive localization of FDDP in vesicles constitutes evidence that the drug does not diffuse freely through the cytoplasm.[72]

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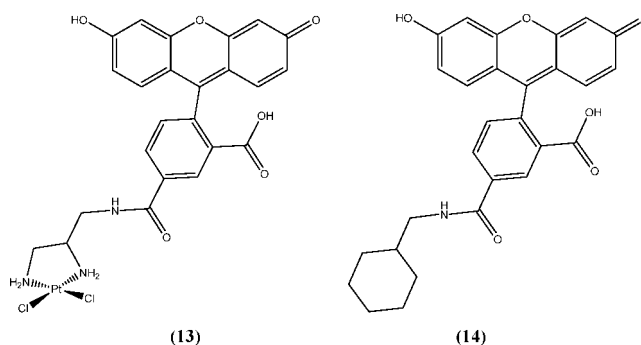
Following lysosomal accumulation, FDDP was observed in Golgi-derived vesicles expressing golgin97, a protein thought to be involved in vesicle-docking.[72] Since the vesicles that are exported from cells via secretory pathways expressing MRP2 are believed to be derived from the Golgi, the observation that FDDP colocalized with vesicles expressing golgin97 and MRP2 supports the notion that FDDP passes through the Golgi before being directed to vesicles involved in the secretory pathway.[72] The contribution of this pathway to the removal of cisplatin was confirmed by showing that its disruption by both wortmannin and H89 increased total cellular levels of both FDDP and unmodified cisplatin.[72]

An interesting result to emerge from this study was the extensive colocalization of FDDP and the copper efflux transporter described previously, ATP7A.[72] This observation provides further evidence of the role of copper transporters in the cellular management of cisplatin (and presumably other platinum drugs), and is reinforced by a similar report describing the colocalization of FDDP with the other major copper efflux transporter, ATP7B.[75]

The major advantage of FDDP compared to CFDA−Pt is that it mimics the properties of cisplatin in terms of cytotoxicity, profile of accumulation, and colocalization with ATP7A/7B.[72] Consistent with cisplatin, FDDP accumulation is reduced in resistant cells, and importantly, it shows a different distribution pattern to that of the free fluorophore, CHMA−F (**14**).[72] This suggests that the distribution of the fluorophore is dictated at least in part by that of the platinum moiety to which it is bound.
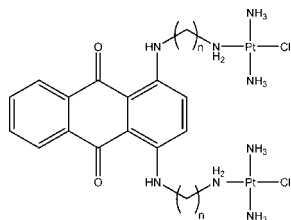
Figure 3. Dinuclear platinum complexes with *N*,*N*′-bis(aminoalkyl)-1,4-diaminoanthraquinones as linking ligands.

A number of platinum complexes incorporating fluorescent intercalators have been developed not simply to mimic the behavior of cisplatin, but rather they have been designed as a class of potentially chemotherapeutic platinum drugs in their own right with the aim of combining the benefits of intercalating agents with those of platinum complexes. Several intercalators such as daunorubicin and doxorubicin are known to be effective anticancer agents per se due to their high affinity for DNA.[76] There is evidence that, when coadministered with cisplatin, intercalators often generate a synergistic effect,[77] providing the rationale for coupling the two types of anticancer agents. A major benefit of these hybrids is that the innate fluorescence of intercalators allows the subcellular localization of their platinum complexes to be monitored using fluorescence microscopy.

The value of this type of approach was exemplified by Reedijk and colleagues, who synthesized dinuclear platinum complexes incorporating fluorescent anthraquinone intercalators (Figure 3).[78−80] Fluorescence microscopy revealed rapid accumulation of these complexes in U2-OS human osteosarcoma cells, followed by accumulation in the nucleus,[79] thereby reaching the biological target of both the platinum and intercalating moieties—DNA. Similarly to FDDP, the dinuclear platinum—anthraquinone complexes were found to be expelled from the cell via a Golgi-mediated mechanism.[79] The distribution of each complex was compared with that of the free ligands to confirm their distinctiveness, since the possibility that any observed fluorescence may belong to cleaved fluorophores must be excluded.

An interesting feature of these complexes is that their cellular processing was found to be similar in cisplatin-resistant and -sensitive US-O2 cell lines, most likely due to formation of structurally different DNA-adducts (resulting from the intercalator inclusion) being able to evade the DNA repair mechanisms responsible for removing cisplatin adducts.[79] However, contrasting results were found in A2780 ovarian carcinoma cells,[78] highlighting the fact that different cell lines are likely to process platinum drugs differently. In cisplatin-resistant A2780 cells, the platinum complexes were sequestered into lysosomes and displayed cross-resistance with cisplatin.[78] This cross-resistance was attributed to the high levels of glutathione present in cisplatin-resistant A2780 cells, which are known to deactivate platinum drugs.[78]

Liang and co-workers investigated the trafficking and localization of Alexa Fluor 546-cisplatin (**15**), a fluorescent platinum(II) complex synthesized and patented by Kreatech Biotechnology BV.[81] The behavior of this complex is believed to be closely related to that of cisplatin, since its accumulation was significantly reduced in cisplatin-resistant KB-3-1 cells, with only a punctate cytoplasmic staining and little fluorescence in the nucleus being observed by confocal

microscopy.[81] Live-cell fluorescence microscopy revealed that, upon entry into KB-3-1 cells, Alexa Fluor 546-cisplatin accumulated in the Golgi after binding to a membrane protein or proteins. Following penetration into the nuclei, the complex displayed a punctate distribution suggesting nucleosomal localization rather than diffuse DNA-binding throughout the entire nucleus.[81]
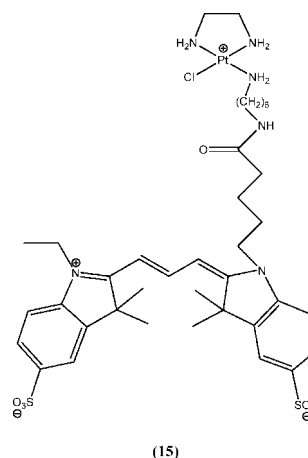
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A final platinum-intercalator complex of interest is the platinum—anthraquinone hybrid Pt−1C3 (**16**), whose cellular distribution has been investigated by Hambley and co-workers.[82] Interestingly, Pt−1C3 was found to have a higher cellular accumulation and cytotoxicity than cisplatin after a 24 h incubation period in A2780 cells, despite confocal microscopy studies failing to detect any nuclear-localized fluorescence.[82] Instead, fluorescence from both Pt−1C3 and the free ligand 1C3 was localized almost exclusively in the lysosomes.[82]

However, there is now mounting evidence that Pt−1C3 does in fact target nuclear DNA. It was revealed using Hoechst 33342 staining techniques that Pt−1C3-treated cells had misshapen nuclei, with a concentration of Hoechst fluorescence at the inner face of the nuclear membrane.[83] This mimics the results for cisplatin, suggesting that the mechanism by which Pt−1C3 induces cytotoxicity is similar to that of cisplatin. Furthermore, Whan observed that cells treated with Pt−1C3 had approximately 50-fold more nuclear-localized platinum than the cisplatin-treated cells.[83] DNA intercalation can lead to the damping of fluorescence,[78−80] and this could be responsible for the lack of observable fluorescence in the nucleus. Another possible explanation for these findings is that the platinum moiety is cleaved from the 1C3 component in the intracellular environment, allowing the platinum component to enter the nucleus, bind with DNA, and trigger apoptosis, while the fluorescent 1C3 ligand is localized within the lysosomes. Consequently, care must be taken when mapping platinum—fluorophore complexes to ensure that the platinum complex as a whole is being probed, rather than the cleaved fluorophore alone.
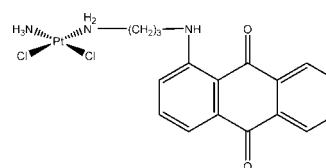

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A number of strategies have been investigated recently for increasing the cellular accumulation of platinum com-

plexes and/or targeting the complexes to tumor cells.[84] For example, the attachment of carbon nanotubes (long boats) has been found to increase the efficacy of a platinum(IV) compound when tethered via an axial site.[84] The accumulation and subcellular distribution of nanotube/Pt conjugates was visualized by attaching fluorophores to the nanotubes. The fluorescence was found to be concentrated in small vesicles, consistent with uptake being primarily via endocytosis.[84]

2.4.3. Fluorophores as Indicators of Complex Metabolism

An understanding of the fate of platinum complexes inside cells and of the rate of processing is crucial for the rational development of more effective agents. The reduction of platinum(IV) to platinum(II) has been investigated by XANES studies of bulk whole cells[69] and microXANES studies of cell sections.[67] Similarly, the fate of the platinum ligands in platinum(II) and platinum(IV) complexes has been monitored using bromine labeling and SRIXE[66] as described above. However, these methods require extensive sample preparation and long data collection times, and therefore, it is not possible to monitor drug metabolism in real time or in live cells. Changes in ligand fluorescence associated with metabolism have the potential to provide such real time information and have been used successfully to monitor the loss of ligands from cobalt(III) complexes.[85] Similar approaches have recently been described for a platinum(IV) complex having a fluorescent ligand in one of the nonleaving group positions (**17**).[86] Reduction from platinum(IV) to platinum(II) to give (**18**) was shown to result in a substantial increase in the fluorescence of the coumarin ligand, and differences between the subcellular distributions for cells treated with the platinum(II) and platinum(IV) complexes were observed.[86]
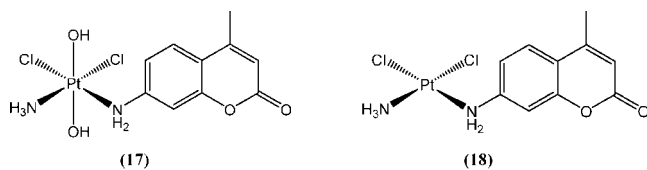
These complexes are cytotoxic and, therefore, reasonable models of platinum anticancer agents. However, aniline complexes are not as stable as ammine or aliphatic amine complexes, and therefore, the cellular processing is expected to be different in some respects.

Platinum complexes with a trans arrangement of the amine ligands almost certainly have a different mode of action than their cis analogues,[87] and therefore, any differences in the cellular processing of these two classes of cytotoxic complexes will be of great interest. Cis and trans complexes of the fluorescent ligand 7-azaindole, (**19**) and (**20**), respectively, are excellent models of other active cis and trans pairs in that they have almost identical activity to those complexes where the 7-azaindole is replaced by quinoline.[88] When bound to platinum, the 7-azaindole is not fluorescent, but it recovers its fluorescence when displaced. Reaction with thiols results in loss of the 7-azaindole for the cis complex, but not for the trans, consistent with the expected trans effect of the thiol ligand. However, fluorescence is observed in cells treated with either (**19**) and (**20**), showing that cellular processing can result in ligand displacement even when the amines are trans to one another.[88]
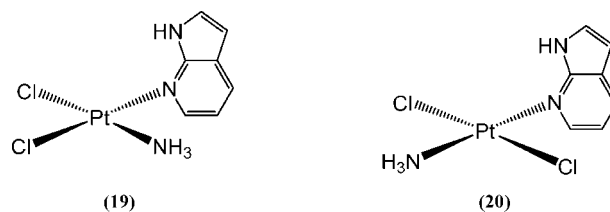
3. Multicellular In Vitro 3-D Tumor Models

Traditionally, the major focus of drug development has been cellular uptake and, more recently, molecular targeting. However, there is now a growing body of evidence to suggest that cells in monolayer culture do not adequately mimic the microenvironment of solid tumors and are, thus, unrealistic models in which to investigate the behavior of platinum-based and other anticancer drugs.[12,89] A major cause of the limited efficacy of anticancer agents is believed to be multicellular resistance associated with poor penetration into regions of solid tumors more than 40−100 μM from the vasculature.[90] These isolated regions evolve as a result of tumor cells proliferating at a faster rate than the development of new blood vessels, and are characterized by oxygen-deprivation (hypoxia) and low pH resulting from the buildup of metabolic products such as lactic acid and carbonic acid.[12,68,89] Chemotherapy is believed to encounter resistance from these regions due to the harsh conditions and the greater distance that drugs must diffuse from the vasculature to reach them.[12,91,92]

An important consideration for platinum drug development lies in the fact that optimizing properties to improve cellular uptake and accumulation may reduce the efficacy of a drug's penetration, since effective cellular uptake is likely to lead to exclusive accumulation in the peripheral cells of tumors and reduce access to central regions. One means of overcoming this dichotomy is the use of prodrug forms that are poorly taken up by cells but that undergo changes in the tumor microenvironment in response to the altered environmental conditions. For example, platinum complexes containing *O*-alkyldithiocarbonato ligands have exhibited enhanced cytotoxicity in slightly acidic conditions as a result of activation by ring-opening (Figure 4), and are thus potential candidates for targeting acidic tumor regions.[93]

In order to study the processing and distribution of platinum drugs on a macroscopic level, direct in vivo measurements in real tumors are advantageous, since they are the most faithful replicates of the clinical environment. However, 3-dimensional in vitro models such as the multi-cellular layer (MCL) model[12,91,92] and the multicellular spheroid (MCS) model[12,89,90] avoid complicating factors such as the often altered pharmacokinetics and hepatic metabolism in humans and other species, and do not require any animal-sacrifice.

Multicellular spheroids are spherical aggregates of tumor cells that mimic solid tumors in terms of (a) the ability to form an extracellular matrix (ECM), (b) an inner layer of quiescent cells surrounded by an outer layer of proliferating cells, (c) the development of a hypoxic region, and (d) the buildup of metabolic waste products in the interior.[12] The ability of platinum(II) and (IV) complexes to penetrate DLD-1 human colon carcinoma spheroids has been investigated using X-ray fluorescence microtomography, revealing uniform platinum distribution for all compounds throughout the core regions, with almost double the platinum content being found in the exterior regions.[90] Studies using [14]C[ethane-
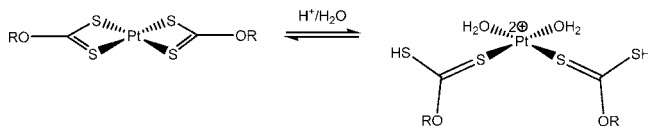
Figure 4. Activation of platinum xanthate complexes in slightly acidic medium.

1,2-diamine] complexes indicated that the majority of this observed platinum was bound to DNA and intracellular proteins, with unbound platinum most likely being washed out during spheroid preparation.[90]

The MCL system has the advantage of allowing the distribution and flux parameters of platinum drugs to be determined quantitatively and is widely accepted as a useful model of solid tumors.[12,68,91,92] Tumour cells are grown on microporous plastic support membranes, and a mathematical model has been proposed by Hicks and colleagues for analyzing the time-dependent penetration of anticancer drugs though the resulting MCLs.[94] Modok and co-workers implemented this model in their examination of the distribution and fluxes of [^{14}C]-labeled [PtCl$_2$(en)] and cis,trans-[PtCl$_2$(OH)$_2$(en)] complexes.[68] The flux through colon cancer MCLs, accumulation, and diffusion constants were found to be similar for the platinum(II) and (IV) compounds, with diffusion constants being found to be almost 10-fold higher than that of the anticancer drug vinblastine.[68] Using elemental imaging analysis by SRIXE, the mathematical prediction of a platinum concentration gradient decreasing from the surface of the MCL to the plastic membrane was verified.[68]

4. Tumors

Only a handful of studies have investigated the accumulation of platinum drugs in tumors themselves, with the first of these using flameless AAS to determine platinum concentrations in human autopsy tumor samples obtained from patients who had undergone cisplatin treatment.[95] This work represents the first hard evidence supporting the role of platinum accumulation in the clinical efficacy of platinum drugs, since tumors belonging to patients who had responded to cisplatin contained significantly higher levels of platinum that those belonging to patients who did not.[95] Liver metastases were found to accrue the highest levels of platinum, consistent with the known hepatic toxicity of the drug.[95]

Nuclear microprobe microanalysis utilizing micro-particle induced X-ray emission (PIXE) and micro-rutherford back-scattering spectrometry (RBS) analyses is one of the few available methods for determining platinum content in drug-treated tissues, with Ortega and co-workers using the technique to compare postmortem platinum levels in rat brain tumors following intratumoral injection of either cisplatin or carboplatin.[96] Platinum accumulation by tumors treated with carboplatin was higher than those treated with cisplatin. While both drugs produced higher concentrations of platinum in tumors rather than the surrounding healthy tissue, diffusion into this neighboring tissue was clearly observed.[96]

The same technique was adopted by a study investigating the effect of the type of local administration of cisplatin on resulting tumor platinum concentrations.[97] Rats with induced-tumor nodules were treated with cisplatin via either intravenous, intratumoral, or peritumoral administration, before determining the distribution of platinum across the diameter of the nodules.[97] The highest platinum levels were accumulated following intratumoral treatment, with platinum concentrations decreasing markedly from the core of the tumor to the peripheral regions. The lowest platinum concentration was achieved by intravenous injection, whereas an intermediate concentration was observed for peritumoral administration. The latter gave rise to a uniform platinum concentration throughout the entire tumor, with only a small enrichment at the periphery.[97]

The main limitation common to the aforementioned studies is that they are measuring the concentration of platinum, which does not necessarily reflect levels of the active drug as a whole. Similarly, no discrimination can be made between the intact drug and its metabolites, nor between the unbound drug and platinated DNA. It would be extremely useful to be able to measure platinum concentrations in the tumors of human patients; however, as yet there are few noninvasive procedures for achieving this means. Gamma camera imaging has been used to visualize the accumulation of ^{191}Pt-cisplatin in the tumors and tissues of patients,[98] but the resolution of this technique is only sufficient to identify the organs in which platinum is localized, providing no information on the distribution within tumors.

5. Concluding Remarks

Platinum drugs continue to be one of the most successful types of anticancer agents available today; however, there is still vast room for improvement in terms of optimizing tumor-selectivity and reducing side effects. A detailed understanding of how cells and tumors process platinum compounds is hoped to provide the basis for more rational design of novel compounds with fine-tuned properties. A large body of information on the distribution of platinum agents in cells has emerged in recent years, providing many valuable insights into mechanisms of cellular uptake, resistance, platinum−DNA adduct formation, and efflux, which may provide the inspiration for the development of new platinum-based drugs in the future.

Screening techniques for platinum drugs that are based on multicellular models are becoming increasingly popular, in response to growing evidence that the potency of drugs in monolayer culture does not necessarily translate to effectiveness in the tumor context. Spheroids and MCLs are more realistic models of solid tumors that are increasingly used for studying the distribution and behavior of platinum compounds in the tumor microenvironment.

Finally, a great deal of work is being directed at developing ways of mapping the distribution of intact platinum drug molecules in cells and tumors, in order to build on the information derived from elemental imaging techniques. Fluorescent labeling has emerged as an useful technique, although it is important to ensure that the fluorescent tags remain bound to the platinum component in the intracellular environment. The intercalating properties of some fluorophores improve the DNA-targeting capabilities of platinum drugs, opening up a new class of platinum drugs with therapeutic potential that may be readily monitored using fluorescence microscopy. However, in general it is important that a fluorescent complex is an appropriate model for anticancer active complexes, and this is an area in need of further development.

6. References

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