Platinum-Based Chemotherapeutic Drugs

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28 Apr 2008

Table of Contents

Introduction

Cancer is one of the most well known maladies of the human body in the modern world. It strikes the young as well as the old, the healthy as well as the ill. Unlike sicknesses caused by external factors such as viruses or bacteria, cancer is characterized by internal factors, specifically, an irregular cell called the tumor cell. Cancerous tumors are conglomerations of cells that have lost the ability to discontinue growth. They frequently continue to grow until they impede bodily functions, which can lead to death of the host in the case of most cancers.

One of the most successful treatments for cancer since the World War II era has been chemotherapy. Because of the pivotal position of chemotherapy in cancer treatment, chemotherapy is and has been extensively researched. To date, about five major classes of chemotherapy drugs have been substantially developed.[1] One of these classes, the platinum-based drugs, has been extremely successful. Cisplatin is the oldest and most widely used of the platinum-based drugs. The success of these drugs in the fight against cancer has been due to the high initial effectiveness of cisplatin and the further development of platinum-based drugs over the years.

Medical Use of Heavy Metals before 1965

Cisplatin is notable for being the first of the platinum drugs to be discovered and used for treating cancer. Also, it is the first drug to be used that contains a heavy metal after a period in medicine during which heavy metals were considered to be poisonous and carcinogenic. Although heavy metals in medicine were used in many ways during the turn of the century, they were largely ignored later on because of the success of other medicinal compounds in the 1930s.[2,3] The avoidance of heavy metals in medicine was a reaction to the numerous toxicities of the first few that were well characterized.

Mercury played a part in bringing about the conviction that heavy metals are more toxic than therapeutic. It was primarily used before 1905 to treat syphilis as well as skin diseases among others. It is converted in the body to a chemical, methylmercury, that has toxic side effects, including stomatitis, dysentery, nephritis, and brain degeneration.[4] Mercury was soon phased out by better drugs, such as penicillin and the sulfa drugs, due to the negative effects of its toxicity.

Another metal used in medicine early on that later proved to be toxic was arsenic. Although it is not considered a heavy metal, it shared many biological properties in common with the group. The primary use of arsenic was as an antisyphilitic compound called arsphenamine, which was an organic compound of arsenic. During the first few decades of this century arsphenamine was very useful to patients, as it had few side effects and high efficacy.[5] Later, however, it was replaced with other less toxic agents that did not contain arsenic, such as penicillin. Arsenic, considered a metal for medical purposes, was later left out of the search for compounds of medical value until much later. The toxicity of arsenic shed doubt on the possibility for successful medical treatments using metal compounds.

Discovery and Early Successes of Cisplatin

Given that the testing of heavy metals as chemotherapeutic agents was initially so unenthusiastic, cisplatin, the first platinum-based drug, was recognized to be effective as a result of an accident.[6,7] In 1964, Rosenberg and VanCamp conducted an important experiment that explored the interaction between bacteria and an electric field. The reasoning upon which the experiment was based was faulty to say the least. They thought that the lines of mitotic spindles of bacteria resembled the lines of an electric or magnetic field. If there was such a field, something interesting might happen if a real electric field were applied at a resonant frequency. They created a chamber for the cell growth containing two platinum electrodes. They intended ultimately to use mammalian cells, but wanted to test the device using the common bacteria *Escherichia coli*. They immersed the bacteria in a standard growth medium containing a number of solutes for the bacteria to feed upon.

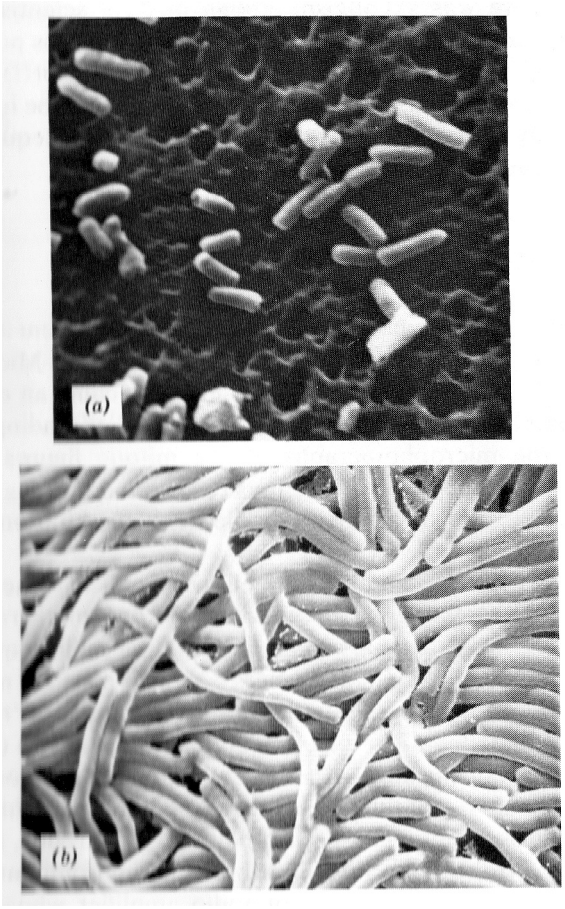
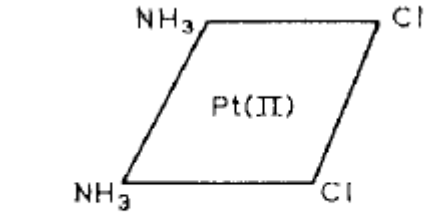


Figure 1: On the top are the *E. coli* in a normal state, and on the bottom are the bacteria in the filamentous form induced by platinum.

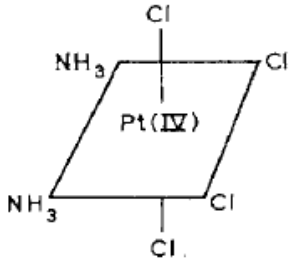
After the experimenters turned on the apparatus, they noted that the bacteria in the chamber had grown into filaments about 300 times the length of the normal bacterium. Cell growth was not inhibited, but cell division was. Surprised, Rosenberg, VanCamp and Krigas, the chemist, narrowed down the possible causes by testing and ruling out possible reagents with control reactions. They found that ammonium (NH_4^+) and chloride (Cl^-) containing media seemed to produce the same results as the medium they originally used. Suspecting that the platinum electrodes might have contributed to the bacterial filamentation, they tested ammonium hexachloroplatinate ($(NH_4)_2PtCl_6$) as a medium in the chamber. This time, they did not electrolyze the medium. The

bacterial elongation proceeded just as it had originally. They later determined that the platinum concentrations in this trial were similar to that in the electrolyzed medium. This indicated that the platinum complex was causing the elongation of the bacteria. This was a previously undocumented effect of a heavy metal and aroused the curiosity of the scientists involved. The identity of the platinum complex remained uncertain, however. At the time, the scientists thought it was ammonium hexachloroplatinate, but this would prove untrue.

Later studies determined that while pure hexaplatinate solely killed the bacteria, if it was exposed to UV, it would convert photochemically to an electrically neutral agent that caused the bacteria to elongate.[8] Leaving the hexachloroplatinate on the shelf for a few days exposed it to enough light to cause the photochemical changes needed for it to cause filamentous growth.[9] Since ammonium was the other ion required to induce filamentous growth, it was speculated that the active chemical contained an ammoniated chloroplatinum compound. In each hexaplatinate ion, the platinum ion has a charge of +4, while each of the six chloride ligands has a charge of -1, leaving a net ionic charge of -2. Since the ammonium ion gives away a hydrogen ion when binding to platinum as an ammine group, then when an ammonium group displaces a chloride, it decreases the negative charge on the ion by one. Therefore, if two ammine groups are bound to the ion, the molecule is neutral, as in diamminetetrachloroplatinum, or $(NH_3)_2(Cl^-)_4Pt$. This was tested for activity and found to be the active ion.[10] Along with diamminetetrachloroplatinum, another chemical, diamminedichloroplatinum, or $(NH_4)_2Cl_2Pt$ was reasoned to be have a similar effect on bacteria because of its similar structure, except it had the platinum in a +2 oxidation state and did not have two of the original chlorides opposite from each other to leave a square planar complex.



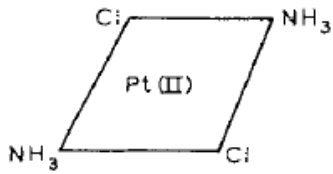
cis-dichloro-
diammineplatinum(II)



cis-tetrachloro-
diammineplatinum(IV)

Figure 2. The structures of diamminedichloroplatinum and diamminetetrachloroplatinum. The Roman numerals stand for the charge on the platinum ion, which in these neutral complexes cancels out each single negative charge on the chloride groups.

Interestingly enough, when the chemical was created synthetically, it seemed to do nothing. In fact, the *trans* isomer had been created, while only the *cis* isomer was effective. In coordination chemistry, *cis* means that two similar ligands are adjacent to each other, while *trans* means that they are across from each other.[11] When the *cis* isomer was finally created, the compound was effective in eliciting filament formation.



trans-dichloro-
diammineplatinum(II)

Figure 3. *trans*-diamminedichloroplatinum has no elongating effect on bacteria, and later would prove to have no effect against cancer.

Since the *cis*-diamminedichloroplatinum inhibited cell division without directly killing rapidly dividing bacteria, scientist thought that the chemical was worth trying against cancer, or rapidly dividing eukaryotic cells.[12] The first test was conducted in 1968 using mice.[13] Sarcoma 180 and Leukemia L1210, a common tumor and common cancer cell line for testing anti-cancer compounds, were used. The trial was very successful. The tumors and leukemias generally regressed, and sometimes did not recur for many months after the experiment. This study was the first in decades to establish a solid link between a heavy metal complex and biological processes that was not entirely damaging. The success was followed by a plethora of other trials to test in what ways the compound would be most effective. Rosenberg himself noted a number of important observations about the drug's activity, among these that it acted against many different types of tumors, it worked against drug-resistant tumors as well as more sensitive ones, it worked against fast- and slow-growing tumors, it regressed normal tumors as well as those caused by viruses, and it could regress tumors that are on the verge of killing the host. The animal trials were successful enough that the drug went into Phase I trials. Phase I trials went successfully, identifying important side effects. Later trials were equally successful, and the FDA approved what had by then been named cisplatin in 1978.

DNA Adduct of Cisplatin

The incredible success of cisplatin prompted many to wonder how exactly the drug worked. Knowing how cisplatin worked would help it to be administered more effectively as well as lead to the development of new drugs. The first thing to determine was which part of the cancer cell it was inhibiting. An early radiotracer study by Renshaw and Thomson (1967) found that platinum compounds in general bound primarily to cytoplasmic proteins[14]. It bound second most to nucleic acids, including DNA. However, these data were found to be misleading as to the mechanism of action, because 96% of the hexachloroplatinate ion (also tested) was found to bind to cytoplasmic proteins, yet it had no effect on tumor cells or bacteria. Cisplatin, a specific platinum compound that was known to be effective, only formed 45% of the total adducts on the cytoplasmic proteins. Most of the rest of the cisplatin bound to DNA. This study therefore proved inconclusive.

A later study by Harder and Rosenberg in 1970 showed DNA to be the active site of cisplatin, not the cytoplasmic proteins.[15] They reasoned that since UV light and hydroxyurea also caused filamentation in bacteria, the mechanism of action might be similar between those two agents and platinum. UV and hydroxyurea inhibited DNA synthesis. They thought cisplatin might do the same. They tested this hypothesis by observing the incorporation of radiolabeled precursors to DNA, RNA, and protein synthesis. Respectively, these were ^{3}H-thymidine, ^{3}H-uridine, and ^{3}H-L-leucine. They found that DNA synthesis was blocked by cisplatin at concentrations that had no effect on RNA or protein synthesis. This seemed to indicate that DNA synthesis was the active lesion, while inhibition of RNA and protein synthesis was an unhelpful side effect.

These inconclusive results were backed up by the simultaneous studies of Howle and Gale in 1970.[16] They found that all DNA, RNA, and protein synthesis were all inhibited in cell suspensions for a few hours, but after about 8

hours, RNA and protein synthesis returned to normal while DNA synthesis remained low. From these studies, it was concluded that DNA synthesis was inhibited, presumably by cisplatin.

Further studies also pointed to DNA being the target of cisplatin. Scarlett Reslová conducted studies in 1971 in which she figured out that cisplatin, when given to lysogenic bacteria, caused them to lyse (i.e., to disintegrate and die).[17]. This phenomenon had been shown in previous studies to distinguish antitumor agents.[18] Later, a study was done in which the chromosome of F' bacteria was exposed to cisplatin. The cells were allowed to conjugate with F- cells, a process in which only DNA can be transferred to the exclusion of RNA and proteins. The cells lysed. This indicated that DNA contained the active lesion that caused lysis of lysogenic bacteria, and therefore, the DNA contained the lesion responsible for the antitumor activity of cisplatin.[19]

After these studies, a number of other studies were designed to ascertain the nature of the DNA adduct. For a while, DNA interstrand crosslinks were believed to be the active crosslink that gave cisplatin its antitumor effect. DNA has two separate strands, and an interstrand crosslink is a chemical connection between these two strands.[20]

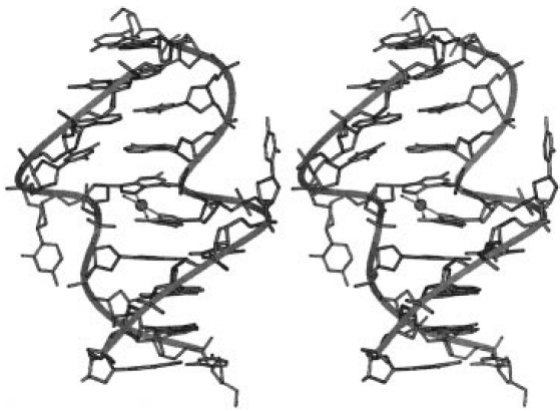


Figure 4. A stereoimage of the interstrand crosslink of cisplatin.

Indeed, many other anticancer compounds did exactly that. For example, the alkylating agents were known to crosslink DNA.[21] Therefore, when scientists were searching for the cause of the efficacy of cisplatin, they first looked to interstrand crosslinks. This type of crosslink was much easier to measure given the techniques available in the late 70s than the intrastrand, so the former was assumed to be the active adduct.[22] For a long time, scientists would believe that interstrand crosslinks on DNA were the cause of the anticancer activity of cisplatin.

This theory was flawed, however. The inactive *trans* isomer caused interstrand crosslinking too, while it had no activity against cancer.[23] This was explained by assuming that the *trans,* being more chemically labile, did not reach the DNA in sufficient amounts to cause an antitumor effect.[24]. As time went on, however, evidence amassed that the cause was an intrastrand crosslink. While some studies showed that interstrand crosslinks were indeed related to the effect of cisplatin, others showed no effect. In many of these early studies that suggested that the interstrand adducts were active, the intrastrand crosslinks were not even measured.[25] Also, less than 1% of the adducts formed by cisplatin were interstrand.[26] In addition, other studies showed that it only took on average five adducts for a DNA strand to become unable to function properly.[27] Therefore, the interstrand, which would be very unlikely to appear in only five adducts, was too rare to be a candidate for the active adduct.

The active adduct was found to be between two adjacent nucleotides on the same strand. This was the second adduct extensively studied after the interstrand crosslink. Scientists determined that the adduct was probably between two adjacent guanine residues (bases found on DNA) on the N7 nitrogen atoms.[28] Among the many ways that scientists ascertained this was through biochemical studies. For example, buoyant density studies were used to directly measure the frequency of adducts on DNA. Also important were studies using restriction enzymes, which were affected by the number and location of platinum adducts. Exonucleases were also preferentially disabled

near platinum adducts, and it was found that the same places near which they were disabled were close to numerous guanine residues. NMR studies and stereochemical considerations were also important. In these studies, modern tools were used to analyze the actual shape of the adduct. Chromatographic methods and ELISAs (Enzyme-Linked Immunosorbent Assays) were used. Chromatographic methods involved separating enzymatically digested DNA components containing platinum from those that had no platinum. ELISAs, which allowed scientists to analyze the platinum adducts based on their affinity to enzymes, confirmed that the same adducts that were being discovered *in vitro* were relevant *in vivo*. Thus, the structure of the active DNA adduct gradually became better defined.

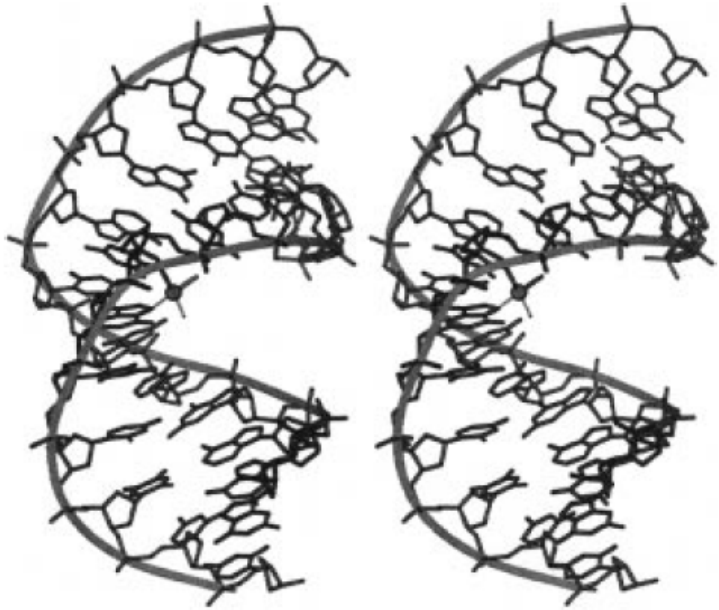


Figure 5. Stereoimage of the active intrastrand adduct of adjacent guanines.

To confirm many of these results, Pinto and Lippard conducted a study in 1985 in which a batch of viral DNA was treated with cisplatin and another with its *trans* counterpart.[29] The sequence of the DNA was already known. After this, DNA polymerase, the enzyme that duplicates DNA strands, was incubated with the platinated DNA. This showed that cisplatin was forming an

adduct at these locations, and this somehow prevented the DNA synthesis by stopping the polymerase as it unzipped DNA. The places on DNA where this occurred were generally close to numerous guanine residues. The *trans* formed adducts more on (GpNpG), where N is any nucleotide, more often than the *cis*, and this adduct also stopped polymerase. The main conclusions of this experiment were that cisplatin adducts stopped DNA synthesis by bringing the polymerase (DNA-splitting enzyme) to a halt, and, to confirm previous studies, that the *trans* version did not form (GpG) adducts. These experiments suggested how cisplatin interacted with DNA. They also suggested that the reason the *trans* was ineffective had nothing to do with inability to stop the polymerase, at least when the polymerase was able to reach it at all.

The precise details of how cisplatin interacted with DNA and how different types of adducts did so differently remained unknown. The development of the proper electrophoresis methods to determine the structural perturbation of DNA induced by cisplatin had to wait until 1988, by which time other methods had been used to give scientists the idea that cisplatin caused some sort of bend in the DNA. Finally, in 1988, Pinto, Lippard, Rice, and Cruthers conducted a study in which they used gel electrophoresis to determine the angle at which DNA bends.[30] Gel electrophoresis is a procedure used to separate biomolecules, usually by mass, but in this case according to friction of the body of the molecule through gel against electromotive force. Strands of DNA 22 bases long of known base content were synthesized, and then they were randomly ligated, or attached, to one other. Each 22-base piece had one pair of guanines to which the cisplatin could bind and no more. These strands were then reacted with cisplatin, so each 22-base section of the resulting DNA strands would have a single bend in it. From this, there were 22-base strands with one nick, 44-base strands with two, 66-base strands with three, and so on. A similar procedure was carried out with 27-base strands of DNA. Finally, a few of the same 22-base strands of the DNA were not platinated so as to be controls.

The results were clear: the heavier 22-base multimers had as little as half the mobility of 27-base-mers as well as the controls. This phenomenon had been seen in previous studies,[31] and was therefore attributed to in-phase bending and out-of-phase bending of the 22- and 27-based multimers respectively. In other words, with the 22-base multimers, the DNA always bent in the same direction, so that the bends added up. With the 27-base multimers, each bend was arranged in opposite direction to its neighbor so that the DNA was a zigzag and therefore had nearly normal mobility. The controls had normal mobility.

Alone, all that these results indicated was that there was a bend conditioned by the cisplatin adduct in the DNA. Fortunately, studies had been done using similar methods on bends induced by adenine tracts, which are bends in DNA conditioned by the presence of a series of consecutive adenine residues.[32] Certain adenine tracts had caused similar changes in mobility with twice the as many bends, so therefore the angle of the bend of the cisplatin adduct was reasoned to be about 40°. This allowed scientists to understand further the perturbation caused in DNA by cisplatin.

The most important study that would help determine the mechanism of action of cisplatin came not long after the previously mentioned study. In 1988, Naser *et al.* published a study in which a synthetic oligonucleotide (a single strand of DNA) of 12 bases was platinated with cisplatin and then subjected to spectroscopic analysis to ascertain the three-dimensional structure of the platinum-DNA adduct.[33] A d(GpG) intrastrand crosslink was found. After this, the platinated oligonucleotide was inserted into a plasmid (a circular strand of DNA easily inserted into living bacteria). The plasmid was then introduced into bacterial cells. The d(GpG) crosslink completely stopped the DNA polymerase, which is a processive enzyme, starting at an origin and proceeding in a linear fashion. Such enzymes were expected to be sensitive to a small number of adducts that would block one-dimensional motion along the chain. This demonstrated that platinum adducts can stop processive enzymes that

process DNA. The question remained: how did this preferentially affect cancer cells?

Role of Differential Repair of DNA on the Efficacy of Cisplatin

While many researchers were ascertaining how cisplatin bound to DNA, still others wanted to know why this adduct was so effective against cancer. Any chemotherapy drugs must be selective to cancer cells. A great majority of tentative drugs prove either too toxic to ordinary cells or not toxic enough to cancerous cells. Therefore, many researchers devoted their studies to understand the causes of the selective toxicity of cisplatin.

Early studies showed that cisplatin is not selective in migrating to tumor cells; instead, it diffuses throughout the body.[34] Because cisplatin circulates through the blood stream, it reaches all organs. Cisplatin binds to DNA in cells throughout the body, but most cells are not as heavily affected as tumor cells. This is not due to increased DNA binding in tumor cells, as studies have shown that there is no selective uptake of cisplatin into tumor cells. Instead, certain studies showed that DNA repair enzymes were crucial in differentiating cancer cells from normal cells.[35,36] The lack of DNA repair in cancer cells was suspected to be the cause of their reaction to cisplatin. An important study used bacteria with missing repair genes to see how they reacted with cisplatin. The repair-deficient bacteria lost the ability to form colonies and were more sensitive to cisplatin. The second study used people with a condition called Xeroderma pigmentosum (XP). People with this condition have trouble repairing DNA damage caused by ultraviolet light because of the lack of an enzyme that normally repairs this enzyme, part of a process known as nucleotide excision repair (NER). The study showed that the same cells that could not repair UV damage also were unable to repair cisplatin adducts, suggesting that cell sensitivity might be related to lack of repair processes.

Evidence leads us to believe that while cisplatin binds equally to DNA from all cells, it is less efficiently repaired in tumor cells than in healthy cells. This differential repair is thought to be responsible for the clinical efficacy of

platinum-based chemotherapeutic agents.[37,38] This is because most DNA repair happens during the rest phase. Tumor cells have an abbreviated rest phase so they do not repair cisplatin adducts as efficiently.[39] The cells are more likely to enter the apoptotic pathway and die when they contain platinum. Therefore, cisplatin is more toxic to tumor cells and kills the cancer before it can kill the patient.[40] In fact, this balance of being just prominent enough to cause problems in a cancerous cell yet being subtle enough not to be picked up by the repair enzymes in is what makes cisplatin so much more effective than similar chemicals. For example, its *trans* isomer is has a prominent enough adduct to cause problems in both cancerous and noncancerous cells, but it causes so marked a shift in the structure of DNA that it is recognized by repair enzymes in cancerous cells and is repaired before it can do damage. [41]

Side Effects of Cisplatin

Although cisplatin is thus one of the most successful chemotherapeutic drugs on the market, like all cancer drugs, it has significant side effects. The dose-limiting primary toxicity of cisplatin is nephrotoxicity, or kidney toxicity.[42] Dose-limiting means that the dose of the drug under consideration can be increased until a specified toxicity prevents it from being increased further. The dose-limiting toxicity prevents higher doses. For cisplatin, this means that renal failure is the foremost concern when one is treated with cisplatin. This toxicity was surprising to early oncologists because earlier chemotherapeutic drugs did not hurt the kidneys to such a degree.[43]

The reason for the kidney toxicity of cisplatin is complex,[44] but it is related to the high level of sulfur-containing molecules in the kidney, such as glutathione. Platinum has a high affinity to sulfur. Platinum undergoes a series of reactions that turn glutathione into reactive thiols, which are organic molecules that are particularly toxic to the kidney. Kidneys also have a higher concentration of cisplatin than other organs because of transporter-mediated uptake. This process involves proteins only found on renal cell walls that burn ATP to pull cisplatin into the cell. This contrasts with most cells, including tumor cells, where passive diffusion, in which cisplatin enters the cell by random chance, is the primary means of cell entrance for cisplatin. The cell death also results in the creation of reactive oxygen species such as superoxide, hydrogen peroxide, and hydroxyl radicals within renal cells. The kidney toxicity, if unchecked, can lead to renal failure.

Kidney toxicity of cisplatin was at first so bad that it nearly led researchers to abandon it in early trials.[45] The doses of cisplatin allowed before kidney toxicity became preventative were lower than other drugs. Many methods were tried to undo the toxicity. The first success was with mannitol diuresis. Diuresis is the process of causing the body to excrete copious amounts of urine. This method was first tested on dogs,[46] and then on

humans.[47] Since high concentrations of cisplatin within the kidneys were causing the toxicity, mannitol, along with extensive prehydration, helped by flushing water through the kidney and causing cisplatin to be continually taken from the kidneys into the urine, thus maintaining a lower concentration of cisplatin in the kidneys.[48] As kidney toxicity was the dose-limiting toxicity, this allowed more cisplatin to be administered before the toxicity became preventative, effectively allowing more cisplatin to be administered. Cancers that required higher doses now were treatable with cisplatin.

The other side effects of cisplatin are not as harmful, yet they are nonetheless important for patients to take into consideration[49]. An important one of these is gastrointestinal toxicity, including persistent nausea and vomiting. This can be ameliorated by various antiemetics. Also, anemia, or blood cell deficiency, can result from cisplatin. An idiosyncratic side effect of cisplatin is its ototoxcity, or toxicity to the sense of hearing, which in this case consists of tinnitus (ringing sounds) and often some loss of hearing in the higher frequency range.

Many of these toxicities are caused by a specific aspect of the action of cisplatin. Cisplatin tends to inhibit DNA synthesis in fast growing cells such as tumors.[50,51] This is good because it means that the cisplatin selectively damages tumor cells while generally leaving other cells alone. Unfortunately, tumor cells are not the only cells in the body that grow rapidly. Cells in the epithelium of the alimentary canal constantly replace themselves, and grow rapidly to do so. This toxicity to the lining of the digestive tract is the cause of the nausea associated with cisplatin treatment. In addition, the cells within hair follicles reproduce rapidly, causing cisplatin to act specifically on them. This accounts for the hair loss associated with cisplatin chemotherapy.[52] Cells in the bone marrow that create blood cells also must divide rapidly to produce the blood cells, and so these are suppressed by cisplatin. Differential repair of cisplatin adducts thus accounts for a great number of known toxicities.

Clinical Use of Cisplatin

Many studies on cisplatin have been done because it plays a huge role in modern chemotherapy. Cisplatin is among the most widely used cancer drugs. It is most effective against testicular cancer. Testicular cancer, however, is relatively uncommon, so cisplatin more often is used to treat small-cell lung cancer. This is one of the most common cancers in the world today, partially due to the prevalence of smoking.[53] Cisplatin is also used frequently for head and neck cancers, skin cancers, and cancers of the bladder.

Although cisplatin is widely used, it does not work well alone. Like most cancer drugs, it is far more effective when used in combination chemotherapy (i.e., the use of multiple drugs together to have an additive effect). Finding the drugs that complement cisplatin well has been an important step to bringing it into general use. Cisplatin was first used for testicular cancer. One of the earlier combination therapies for testicular cancer was PVB, or platinum vinblastine bleomycin. However, a study in 1985 indicated that replacing vinblastine with etoposide resulted in a more effective combination.[54] BEP (bleomycin etoposide platinum) is now the standard treatment for testicular cancer. Another combination therapy using cisplatin that is commonly used for small-cell lung cancer is ICE, or ifosfamide cisplatin etoposide.[55] Cisplatin has found use in so many combination chemotherapy regimens that it is now one of the more common drugs used to fight cancer.

Platinum-based Drugs beyond Cisplatin

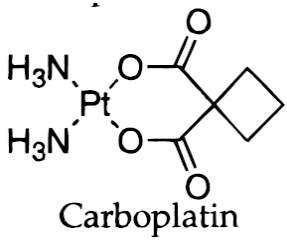
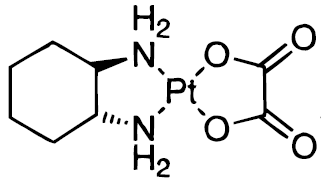


Figure 6. Chemical structure of carboplatin. The only difference between carboplatin and cisplatin is the cyclobutane dicarboxylate group on the right. As soon as the carboplatin enters the cell, that group is stripped off and replaced with aqua (H_2O) ligands and hydroxy (OH^-) ligands, just as with cisplatin.

A few new drugs have entered testing and the market since cisplatin first came into usage. The first of these was *cis*-diammine-1,1-cyclobutane dicarboxylate platinum (II), or carboplatin. Early efforts to find a drug superior to cisplatin were driven by the hope of finding a less nephrotoxic or even less emetic or nauseating alternative to cisplatin.[56] Hundreds of platinum compounds were studied, but many proved too toxic or to ineffective for use.[57] Carboplatin, however, proved to have less kidney toxicity and to cause less vomiting. The first clinical study showed it to be very effective.[58] The kidney toxicity was low, and the dose-limiting toxicity was blood platelet synthesis suppression. Because of this, carboplatin is used when the kidney toxicity of cisplatin prevents its further use. Carboplatin is also a slower-acting drug and is used in certain cancers in which it is more effective, such as non-small-cell lung cancer and ovarian cancer.[59] All of these factors are taken into consideration when deciding whether to use carboplatin.

In many ways, carboplatin is the same drug as cisplatin and they are generally substitutable.[60] The only difference chemically is the cyclobutane dicarboxylate group in place of the chlorides of cisplatin. In the same way that the chlorides are replaced by aqua groups inside the cell, the cyclobutane

carboxylate group is replaced by water inside the cell. Thus the active species in both drugs is the aquated complex, and the DNA adducts are identical. The two drugs have a different side effect profile because in the bloodstream, the leaving groups (chloride and cyclobutane dicarboxylate) are still bound to the platinum ion.[61] Because the drugs are still chemically different in the bloodstream, they have different effects on the various organs. The same reactions in the kidney that make cisplatin so toxic do not happen with carboplatin, but the leaving group of carboplatin makes it toxic to the bone marrow.



Oxaliplatin

Figure 7. The chemical structure of oxaliplatin. The diaminocyclohexane group is to the left of platinum, and the oxalato group is to the right.

After carboplatin, research into new platinum drugs moved in a different direction.[62] Instead of looking for drugs that would avoid serious side effects, researchers began to look for drugs that would work for a growing group of people that were considered to be platinum-resistant. A number of people (especially those on whom platinum chemotherapy was already used) became unresponsive to the drug for cancers that usually responded. These people often had to stop taking the drug. Oxaliplatin was discovered in the search for a platinum drug that would be effective against cisplatin- and carboplatin-resistant cancers. Its chemical name is *trans*-diaminocyclohexaneoxalatoplatinum. Carboplatin was not useful for cisplatin-resistant tumors because it had the same active structure as cisplatin. As it turned out, oxaliplatin was not best at regressing cancers that were normally nonresistant to cisplatin or carboplatin. Instead, oxaliplatin was found to be useful for colorectal cancer, a cancer for which both cisplatin and carboplatin

were useless. Therefore, there was much clinical interest, as platinum had never been seriously considered for colorectal cancer treatment before.

Like most chemotherapeutic drugs, oxaliplatin was not very effective on its own. The combination therapy that was used most often for colorectal cancer in the US before oxaliplatin came into use was known as FOLFIRI, or IFL, both of which contain irinotecan, 5-fluorouracil, and leucovorin.[63] In 2002, the FDA approved oxaliplatin to be used for colorectal cancer in a regimen already in use in Europe known as FOLFOX, containing 5-fluorouracil, leucovorin, and oxaliplatin. This today is still the primary and best known use of oxaliplatin.

The causes for the success of oxaliplatin in cancers that proved refractory to the other two earlier platinum cancer drugs lies in its chemical structure and the DNA adducts that the molecule forms. Whereas carboplatin only differs from cisplatin in its leaving group, namely the cyclobutanedicarboxylato group in place of the dichloro group, oxaliplatin differs from both in its permanent group, i.e. the group that is permanently fixed to the molecule even in the DNA adduct. While cisplatin and carboplatin both have two ammine groups attached to the platinum ion in this location, oxaliplatin has a much bulkier diaminocyclohexane group here. This causes stearic crowding in the DNA adduct, creating a slightly different shape and distortion of DNA.[64] Therefore, oxaliplatin reacts with the cell differently. Exactly how this difference in distortion of DNA results in its effectiveness for previously refractory cancers such as colorectal cancer is currently unknown. Still, the cause is importantly related to the nonleaving groups of oxaliplatin.

Like any chemotherapy drug, there is inevitable toxicity associated with oxaliplatin. The dose-limiting toxicity is sensory neuropathy, which is a tingling of the extremities.[65] Unlike many of the toxicities of cisplatin, the neuropathy induced by oxaliplatin is generally reversible after completion of chemotherapy. Unlike cisplatin, it causes no toxicity to the hearing organs or to the kidneys.

The gastrointestinal toxicity is manageable with antiemetics, and bone marrow suppression is not common.

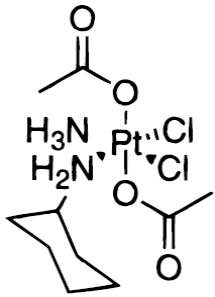


Figure 8. Satraplatin may be the first oral platinum-based drug approved. It is very different from the other three platinum-drugs because it has octahedral instead of planar geometry around the platinum atom, which means it has a ligand bond along every axis of the Cartesian space.

The FDA has approved these three platinum drugs in the US for chemotherapy: cisplatin, carboplatin, and oxaliplatin. Hundreds are being tested for anticancer activity today. One prominent chemical that shows promise is satraplatin, or bis-acetato-ammine-dichloro-cyclohexylamine-platinum (IV). This drug is currently undergoing clinical trials worldwide for hormone-refractory prostate cancer. It first came into clinical trials in 1994[66] and is currently in the final stages of testing before approval by the FDA.[67] Some of the major advantages of this drug are that it can be taken orally as opposed to intravenously (reducing the burden of chemotherapy), and it is effective against prostate cancer. The most common side effects are low blood platelet counts and low white blood cell counts, both as a result of bone marrow toxicity.

The ability of satraplatin to be taken orally is thought to be a result of its octahedral structure. The extra ligands around the platinum ion make it less reactive within the alimentary canal than the earlier platinum (II) drugs, so the previously problematic gastrointestinal toxicity does not prevent satraplatin from being taken orally. Due to the two acetate ligands, satraplatin is more attracted to fat, and therefore has an easier time than other platinum-based drugs being absorbed into the blood stream.[68] Similar to oxaliplatin, satraplatin

has different nonleaving groups than previous drugs, so its adduct with DNA and therefore its scope of action are different. This is only partially true, however, because the extra ligands are sometimes removed *in vivo* by reducing agents in hypoxic tumor tissue.[69] Thus, satraplatin is the first platinum drug that is effective against prostate cancer.

Future Prospects and Conclusion

The future holds no less potential for platinum-based drugs than the past. Not only are many drugs in development, but modern researchers anticipate finding new ways of delivering the drugs so they reach their targets without such unpleasant side effects. In addition, further research into the mechanisms of platinum-based drug resistance will help us find ways to circumvent it.

Platinum-based drugs have been some of the most beneficial to cancer patients over the last 37 years. Chances are they will continue to be central to chemotherapy regimens, perhaps more so as new drugs enter the market. New drugs will be able to target more types of cancer and avoid preventative side effects. More research into the mechanism of action will help us circumvent resistance in new drugs and new methods of drug delivery. For example, we may be able to modulate the causes of platinum resistance within cells with other drugs. One concern that has not been thoroughly investigated is that while it is clear that they are carcinostatic (cancer-stopping) in the short term, they may be carcinogenic (cancer-causing) in the long term. Most tests of platinum drugs have only been done over a time period of no more than two years. Yet many heavy metals, if present in the body for long periods of time (as mercury or lead), can themselves cause cancer. According to this line of reasoning, patients who used platinum drugs years ago might survive the cancer for which they were treated, but be at higher risk for developing cancer later because of the platinum remaining in their body. To further examine this possibility, a number of studies have been done both on animals and humans to determine whether cisplatin is carcinogenic or not.[70] The animal studies showed alone that cisplatin was a mutagen to mammalian DNA, and that it could induce cellular transformation. Human data was harder to interpret, because cisplatin is usually given to humans in combination chemotherapy, and many of the things that work with cisplatin are also carcinogenic. However, it is

notable that cancer has recurred in many patients that have been given cisplatin. However, the causality between the two is at least uncertain.

Cisplatin and its analogues have been crucial to chemotherapy both when it was first applied to cancer in the 70s and in the present. This is because it is extremely effective at regressing cancers and has been developed extensively in recent years. Cisplatin brought the sights of the scientific and pharmaceutical communities back on inorganic chemicals after inorganic chemicals had been in disfavor as drugs for decades. In fact, it took a totally serendipitous experiment to bring platinum complexes into the spotlight. Nevertheless, the genius of Rosenberg and other scientists led ultimately to the clinical testing of platinum. While it was being clinically tested, a number of pointed nonclinical studies were underway attempting to deduce the mechanism of cisplatin and its reaction with DNA. They have helped us come closer to developing more closely targeted therapies for cancers. Although cisplatin and its analogues have serious side effects, they can be mollified by techniques such as prehydration and use of mannitol. Cisplatin has led to the development of a number of new drugs that have important advantages over cisplatin. Overall, cisplatin has been extremely successful.

Yet the story of cisplatin is not only useful for what it has led to. It is also a great example of the scientific method at work. Rosenberg's careful analysis of his experiment in 1964 was a result of a shrewd analysis of what seemed to be faulty data. The going back on assumptions and checking them against the data exemplify how science should work. In this way, it was discovered that the byproduct of the platinum electrodes were causing the filamentation. In this way, the story of the development of platinum complexes as chemotherapeutic agents is one that shows how science should be conducted.

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