

Review

## Metals and Metal Compounds in Cancer Treatment

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**Abstract.** *Metals and metal compounds have been used in medicine for several thousands of years. In this review we summarized the anti-cancer activities of the ten most active metals: arsenic, antimony, bismuth, gold, vanadium, iron, rhodium, titanium, gallium and platinum. The first reviewed metal, arsenic, presents the anomaly of displaying anti-cancer and oncogenic properties simultaneously. Some antimony derivatives, such as  $Sb_2O_3$ , salt (tartrate) and organic compounds, show interesting results. Bismuth directly affects *Helicobacter pylori* and gastric lymphoma; the effects of bismuth complexes of 6-mercaptopurine are promising. Gold(I) and (III) compounds show anti-tumour activities, although toxicity remains high. Research into the potential use of gold derivatives is still ongoing. Several derivatives of vanadium show anti-proliferative activity, but their toxicity must be overcome. Several pieces of evidence indicate that iron deprivation could be an excellent therapeutic approach; furthermore, it is synergistic with classic anti-cancer drugs. Rhodium belongs to the same group as platinum and it also presents interesting activity, but with the same nephrotoxicity. Several rhodium compounds have entered phase I clinical trials. In contrast to the platinum complexes, titanium derivatives showed no evidence of nephrotoxicity or myelotoxicity; titanocene dichloride is undergoing clinical trial. The anti-proliferative effect of gallium could be related to its competition with the iron atom; in addition a derivative appears to reverse the multidrug resistance. The last metal reviewed, platinum, has given some of the very best anti-cancer drugs. Four derivatives are used today in the clinic; their mechanism of action and of resistance are described.*

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*Key Words:* Anti-cancer drugs, arsenic, antimony, bismuth, gold, vanadium, iron, rhodium, titanium, gallium, platinum, review.

The importance of metal compounds in medicine is undisputed, as can be judged by the use of compounds of antimony (anti-protozoal), bismuth (anti-ulcer), gold (anti-arthritis), iron (anti-malarial), silver (anti-microbial) and platinum (anti-cancer) in the treatment of various diseases. In terms of anti-tumour activity, a wide range of compounds of both transition metals and main group elements have been investigated for efficacy (1). The earliest reports on the therapeutic use of metals or metal-containing compounds in cancer and leukemia date from the sixteenth century.

Superoxide dismutase inhibition leads to selective killing of cancer cells *in vitro* and *in vivo* (2,3). As a consequence, reactive oxygen species (ROS) may not only be involved in causing cancer, but could also be a specific treatment of tumours.

### Arsenic

Arsenic compounds are natural substances used in China for medical treatment for more than twenty-five centuries. Arsenic trioxide ( $As_2O_3$ ) has been used for ten years in patients with acute promyelocytic leukemia (APL). It is apparent that arsenic trioxide is a successful treatment for APL. Furthermore, arsenic trioxide is safe and effective not only in patients with leukemia, but also in patients with many other malignancies (4).

At the present, there are several possible explanations for the mechanism of action of arsenic trioxide. It induces a p53-dependent G1 or G2/M cell cycle arrest, through an activation of caspase 8 or caspase 9 (5). Others have also reported that arsenic trioxide induces cell cycle arrest or apoptosis (6). In the study of Li *et al.*, it induces apoptosis and differentiation in cancer cells (7), and the latter can be blocked by cAMP analogs (8).

The anti-carcinogenic effects of arsenic trioxide may be related to the induction of apoptosis (9). The up-regulation of p53 and down-regulation of bcl-2 may be an underlying mechanism (10). A simultaneous down-regulation of bcl-2 and up-regulation of bax was also reported by Zhao *et al.*

(11). However, it should be noted that, although a high concentration of arsenic trioxide (5  $\mu\text{mol/l}$ ) causes apoptosis, at low concentrations (0.5  $\mu\text{mol/l}$ ) it causes cell proliferation (9,12).

The induction of apoptosis could be also related to an increase in the production of ROS and a decrease in anti-oxidation capacity (9). As a matter of fact, toxic lipid peroxidation products, such as the polyunsaturated fatty acid docosahexaenoic acid (DHA), sensitize tumor cells to ROS-inducing anti-cancer agents (13). Arsenic trioxide induces an increase in GSH content as a response to oxidative stress (14). This mechanism of action provides a biochemical basis for developing new drug combination strategies, using arsenic trioxide to enhance the activity of anti-cancer agents by promoting the generation of free radicals (2).

Histone H3 phosphoacetylation associated with the Caspase-10 gene may play an important role in the induction of apoptosis (15).

According to Duyndam *et al.*, p38 mediates the induction of VEGF mRNA expression by sodium arsenite (12). On the other hand, the p38 MAP kinase pathway could be a negative regulator of the apoptosis and inhibition of malignant cell growth induced by arsenic trioxide (16,17)

Unfortunately, resistance to arsenic trioxide has been reported. It is accompanied by cross-resistance to ionidamine and oxaliplatin (18). This resistance does not appear to be related to the expression of P-glycoprotein (19). It has been reported that resistance to arsenic trioxide in primitive UCB (primary umbilical cord blood) CD34+ cells is most probably related to cell-cycle status (19).

Several cell lines are sensitive to arsenic trioxide: esophageal carcinoma cells (9), OVCAR-3 (12), renal cell carcinoma cells (6) and small cell lung cancer (NeI-H cells) (10). Arsenic trioxide has also been shown to be active against nasopharyngeal carcinoma xenografts in BALB/C nude (20,21).

Disappointingly, arsenic trioxide causes cardiotoxicity, with electrocardiographic abnormalities (22). Furthermore, it induces hyperleukocytosis and APL differentiation syndrome (21). These effects can be prevented or managed successfully with careful patient monitoring during treatment.

Interestingly, trisenox, the injectable formulation of arsenic trioxide, has no known cross-resistance with ATRA or other anticancer agents. In addition, trisenox does not cause hair loss and is not myelosuppressive in patients with acute promyelocytic leukemia (21).

There is promising evidence of the activity of arsenic trioxide plus ascorbic acid in refractory/relapsed myeloma. The mechanism of potentiation by ascorbic acid is due to intracellular glutathione depletion. This treatment has acceptable toxicity and ascorbic acid does not alter the pharmacokinetics of arsenic trioxide (23).

The regimen of daily intravenous administration for 4-8 weeks has its attendant drawbacks: inconvenience, risks and expense of maintaining suitable vascular access and hospitalization. Sturlan *et al.*, therefore, developed an oral formulation and determined the systemic bioavailability of arsenic in patients. Compared with *i.v.* dosing, the oral arsenic trioxide solution was more convenient and cost effective, with a similar systemic bioavailability of arsenic (24).

### Antimony

We have seen that  $\text{As}_2\text{O}_3$  has remarkable potential against promyelocytic leukaemia and, because of structural analogy,  $\text{Sb}_2\text{O}_3$  has also been tested on the same leukemia with interesting results (25). Although normally regarded as a poison,  $\text{Sb}_2\text{O}_3$  is less toxic than  $\text{As}_2\text{O}_3$ .

The most studied antimony(III) compounds are organometallic. A series of antimony(III) compounds with polydentate carboxylic acids have shown anti-tumour activity in mice inoculated with S180 solid tumours (26).

Four diphenylantimony(III) thiolates have been tested. Three of them had marginal activity (ratio of Tumour to Control, T/C < 125 %). A fourth compound was the most active, but increased doses were associated with increased toxicity, including potential mutagenicity (27).

Triphenylantimony(V) polyamines displayed some cytotoxicity against several cell lines. Triorganoantimony(V) disalicylates showed no significant activity and were not investigated further (28).

The anti-tumour activity of potassium antimony tartrate in lymphoid malignancies is interesting (29-30), but it is compromised by cross-resistance with cisplatin in human ovarian carcinoma and other cell lines (31). Also, exposure to potassium antimony tartrate has been associated with over-expression of the multi-drug resistance-associated protein (MRP1), a drug export pump (32).

### Bismuth

*Helicobacter pylori* is implicated in the causation of gastric lymphoma and treatment with bismuth results in regression of the disease or even a cure (33). Bismuth increases production of metallothionein, a property which may be used to reduce the toxic side-effects of cisplatin (34).

Bismuth complexes of 6-mercaptopurine were the first anti-tumour compounds tested. They yielded promising results, as compared to platinum(II) analogues. Organobismuth compounds were also tested, but no useful activity was observed (28).

The emission of  $\alpha$ -particle by bismuth compounds shows potential in the form of radio-therapeutic agents (35). Efficacy is increased when the compounds are attached to a monoclonal antibody which can specifically target tumour

cells (36). Bismuth nuclei offer advantages over other radio-pharmaceuticals (28). No bismuth(V) compounds have been evaluated for anti-tumour activity (28).

## Gold

The use of gold in medicine, to treat a number of diseases, dates back to Arabic and Chinese physicians. More recently it has been used as a bacteriostatic and subsequently for the treatment of rheumatoid arthritis. Later still, the anti-tumour activity and anti-HIV activity of gold has been considered.

Studies of the anti-tumour activity of gold compounds were stimulated by several observations. (i) Patients treated with gold for rheumatoid arthritis (chrysotherapy) had lower rates of malignancy than other patients (37). (ii) The efficacy of cisplatin against cancer stimulated great interest because gold in the +III oxidation state is isoelectronic with platinum(II) and forms similar square-planar complexes.

*Gold(I) compounds.* A large number of gold(I) compounds have been studied. Many phosphine-gold(I) thiolates, auranofin analogues, display significant cytotoxicity. In short, the most potent class of compounds among those screened was that containing both phosphine and thioglucose ligands (38).

A number of tetrahedral gold(I) compounds have also been tested. Several compounds were cytotoxic against a variety of tumour cell lines (39). The most active compound,  $[\text{Au}((\text{C}_6\text{H}_5)_2\text{P}(\text{CH}_2)_2\text{P}(\text{C}_6\text{H}_5)_2)]^+$  was then evaluated *in vivo* (40). Side-effects in beagle dogs involved toxicity to the heart, liver and lungs (41) and to the heart in rabbits (42). These effects were attributed to perturbations in the normal functioning of mitochondria (43).

Compounds with chiral phosphines showed some potency and were relatively non-toxic against healthy lymphocytes. Compounds with bidentate thiolates have significant anti-tumour activity, but toxicity was also significant (38).

The results obtained with compounds with biologically active thiols and analogues were of sufficient interest to warrant further investigation. Studies performed *in vivo* were designed to ascertain anti-tumour activity and also anti-arthritis activity. It was shown that toxicity would not be a problem and compounds with moderate to very high activity were identified (38).

Some compounds, when combined with ferrocene derivatives, showed *in vitro* activity superior to cisplatin. Further research with ligands containing the ferrocene backbone is warranted (38).

*Gold(III) compounds.* Several compounds, combined with biologically-active molecules, demonstrated interesting activities against cisplatin-resistant cell lines. Each gold(III)

compound was significantly more active than the corresponding cobalt(II), zinc(II), palladium(II) and platinum(II) analogues. There is evidence that these compounds bind to DNA (44).

Some compounds incorporating imine donors are more potent than carboplatin (45). Other derivatives demonstrated more cytotoxicity than cisplatin in cisplatin-resistant cell lines and, in some cases, more cytotoxicity in the cisplatin-sensitive cell lines (46).

A number of organometallic compounds showed a similar cytotoxicity profile to that of cisplatin. Cross-resistance with cisplatin was found for some of the compounds (47), but not all (48). Studies into the potential anti-tumour activity of organogold(III) compounds are potentially interesting (49).

In conclusion, research into the potential use of gold derivatives has not been completed. Work is proceeding into, for example, heterometallic species such as compounds containing ferrocene and those containing 'water-soluble' phosphines.

## Vanadium

Vanadium was initially found to be inactive in 1967 (50). Fifteen years later, research on vanadium was re-initiated by English *et al.* (51) and by Thompson *et al.* (52) after they reported that vanadium was an inhibitor of terminal differentiation of murine erythroleukemia cells. In addition, dietary administration inhibited chemically-induced mammary carcinogenesis (52).

Other biological effects of vanadium compounds have been discovered, such as an insulin-like action (53,54) and the reduction of hyperlipidemia and hypertension. Since it has few adverse effects (55), vanadium has a good therapeutic potential.

Vanadium complexes have been shown to exert either anti-proliferative or, in some cases, proliferative effects on various types of cells. Notably, vanadium salts at low concentrations stimulated and at higher concentrations inhibited colony formation in human tumours (56). The anti-proliferative effects of vanadium compounds on normal and malignant cell lines appear to be exerted mainly through cell cycle arrest. Zhang *et al.* demonstrated that vanadate induced G2/M-phase arrest in p53-deficient mouse embryo fibroblasts and promoted S-phase entry in the corresponding p53 wild-type cells (57).

The oxidation state of vanadium seems also to determine the various biological effects of vanadium compounds; for instance, the activation of intracellular signal transduction pathways which in turn regulate cytosolic protein tyrosine kinases (58,59). Concentrations of vanadium, estimated by X-ray energy fluorescence, were found to be significantly higher in cancerous breast tissue compared to normal breast tissue (60).

Concomitant treatment with vanadate (IV) and peroxide ( $H_2O_2$ ) enhances the biological effects of the metal in various cell lines, probably due to the formation of peroxovanadate. Treatment of cancer cell lines with vanadate(V) and hydrogen peroxide ( $H_2O_2$ ) markedly increased protein tyrosine phosphorylation and phosphoinositide breakdown, combined with probable selective inhibition of protein tyrosine phosphatase and phosphotyrosine phosphatase activities *in vitro* (61,62). Peroxovanadium complexes appear to be 100-1000 times more effective inhibitors of protein phosphotyrosines than sodium orthovanadate *in vitro* (63,64).

The organometallic complexes, with the vanadium(IV) linked to organic ligands by direct carbon metal bonds, exhibit significant *in vitro* and *in vivo* anti-tumoral properties (65). One of the most promising among the metallocenes is vanadocene dichloride (65,66). Its anti-tumour effects against human colon and lung carcinomas xenograft in athymic mice were due to the inhibition of DNA and RNA synthesis in tumour cells (66).

Fluorescence-activated cell sorting investigations revealed that peroxovanadates block the G2-M transition of the cell cycle (67), reversibly at low concentrations and irreversibly at higher concentrations. Cell cycle arrest induced by vanadium complexes appears to be mediated through the inhibition of phosphotyrosine phosphatase, which in turn dephosphorylates subunits of the cyclin-B complex. Cell cycle arrest by vanadium complexes may also be exerted through activation of mitogen-activated protein kinases (MAPKs superfamily) signalling pathway. V(IV) activates p38 MAPK and induces the transcription of NF- $\kappa$ B, a factor involved in both cell-cycle progression and apoptosis (68).

The protein tyrosine phosphorylation, induced by vanadium, changes the invasive and metastatic potential of tumour cells by modulating cell-substrate adhesion (69), cell-to-cell contact and the actin cytoskeleton (70,71).

The anti-cancer effects of vanadium compounds have been investigated on a large variety of malignant cell lines, including human B cell lymphoma and T cell leukemia, murine erythroleukemia, rat basophilic leukemia, the leukemic cell lines L1210, HL-60 and M07e, human and rat hepatoma, human ovarian carcinoma, testicular cancer, nasopharyngeal carcinoma, larynx carcinoma, osteosarcoma as well as Ehrlich ascites carcinoma, mouse and rat neuroblastoma, rat glioma, mouse epidermal JB6 P+, Lewis lung carcinoma and Hela (65).

The most common side-effect of vanadium is a mild gastrointestinal disturbance (72,73). With long-term treatment, almost half of the subjects (coronary artery disease patients) experienced anorexia, weight loss and abdominal pain. Oral administration of vanadyl sulfate in humans has no effect on blood cells, viscosity and biochemistry (74), but it may induce DNA strand breaks (75).

## Iron

Several pieces of evidence indicate that iron deprivation could be an excellent therapeutic approach: (i) dietary iron restriction markedly decreases tumour growth in rodents (76); (ii) antibodies which block transferrin-binding to cellular receptors inhibit cancer cell growth *in vitro* and *in vivo* (77), (iii) the anti-tumour effect of bleomycin, an anti-cancer drug, is mediated by chelation of iron or copper, to form a complex which degrades DNA (78).

Iron is involved in energy metabolism, respiration and DNA synthesis. Amongst iron-containing enzymes, ribonucleotide reductase is one of the most sensitive to iron depletion. This enzyme catalyses the conversion of ribonucleotides to deoxyribonucleotides for DNA synthesis. Amongst key enzymes, ribonucleotide reductase shows the greatest increase in activity in neoplastic tissues (79).

Iron chelators up-regulate TfR1, thus increasing the incorporation of gallium (see section on gallium) and so increasing its efficacy.

Desferrioxamine (DFO) is currently the best drug for the treatment of iron overload diseases. The molecule is a hexadentate siderophore possessing a very high affinity for iron. When it is bound, iron is metabolically inactive and does not produce ROS. Several *in vitro* and *in vivo* studies, as well as clinical trials, have demonstrated that neuroblastoma and leukemia were sensitive to chelation therapy with DFO (80,81). In contrast, there was little effect on normal tissues; for example, bone marrow cells were 10-fold less sensitive (82). DFO is also effective at inhibiting the growth of a number of other tumours, for instance, a refractory lymphoid leukemia responded to DFO with a decrease in blast cells and several-fold increase in normal hematopoietic progenitor cells (83).

Iron depletion results in decreased mitochondrial oxygen consumption and ATP formation and an increase in glycolysis with increased generation of lactate (84). Other targets of DFO include proteins involved in cell cycle control. DFO has some disadvantages, such as high cost, requirement for prolonged *s.c.* administration, short half-life and poor absorption from the gut (76).

The thiosemicarbazone Triapine markedly inhibits the growth of several xenograft tumours in mice (85). Triapine crosses the blood-brain barrier and inhibits the growth of brain graft tumours (85). The combination of Triapine with etoposide, cisplatin, doxorubicin, or hydroxyurea was synergistic. The mechanism of this synergistic effect was probably due to triapine preventing the repair of the DNA damage induced by the cytotoxic agents.

Aroylhydrazones are highly efficient iron chelators. They demonstrate distinct activity against mammary tumours and certain leukemias in mice. There is no toxicity in normal mice when administered for 5 months (86). The most

promising derivative, named 311, makes a complex with iron, but is not redox active (87). At very low levels this compound induced the expression of the p53-regulated genes WAF1 and GADD45, but *via* a p53-independent pathway(88); WAF1 and GADD45 are involved in cell cycle arrest. A wide variety of cell cycle control molecules are altered after treatment with iron chelators (89).

Tachpyridine appears to bind iron in a six-coordinate manner, forming a redox active complex. It inhibits ferritin synthesis and the proliferation of bladder cancer cells in culture (90), also inducing apoptosis.

O-Trensox reduces DNA synthesis with greater efficiency than DFO (91). Like many other chelators, it induces apoptosis (91). Relatively high concentrations of O-Trensox were required to inhibit the growth of neoplastic cells (92). Further studies in animal models are necessary to judge whether O-Trensox will be an effective anti-tumour agent.

## Rhodium

Rhodium belongs to the same group as platinum.

*Dimeric rhodium (II) compounds.* The dirhodium tetracarboxylate complexes have anti-tumour activity against Ehrlich ascites, L1210 and P388 tumour cells (93), but toxic effects have prevented their use. The dirhodium tetraacetate complex inhibits DNA polymerase I and RNA polymerase (94) and is cytotoxic against the Ehrlich ascites tumour, sarcoma 180 and P388 lymphocytic leukemia, but with little activity against L1210 and B16 melanoma (94). The effect of rhodium(II) acetate, propionate and methoxyacetate is to irreversibly inhibit all enzymes that have sulfhydryl groups in or near their active site (95).

Rhodium(II) carboxylates increase sensitivity to radiation, an action that is attributed to their ability to deplete intracellular thiols (93). The ability of rhodium(II) complexes to interact with thiols depends upon the nature of the ligand, in the following order butyrate > propionate > acetate > methoxyacetate. The differences of efficacy between the carboxylate complexes could be related to differences in cellular uptake. It is interesting to note that a combination of rhodium(II) complexes with misonidazole given to hypoxic cells irradiated *in vitro* gave an additive response. However, this effect was not demonstrated *in vivo* (96). Recent structural studies suggest that the anti-tumour activity of dirhodium(II) carboxylates may be by binding to adjacent guanines on DNA, in a similar manner to cisplatin (93).

*Rhodium(I) derivatives.* Anti-tumour rhodium(I) compounds, with *in vivo* activity, are organometallic neutral and square planar rhodium(I) cyclo-octadiene complexes, which are active against Ehrlich ascites tumours (97).

Neutral organometallic complexes were reported to have activity on Ehrlich and Landschutz ascitic tumours. The most active complex was  $[\text{Rh}(\text{I})(\text{COD})\text{L}_2]^+ [\text{B}(\text{C}_6\text{H}_5)_4]^-$  where L=benznidazole (98). Although many rhodium(I) compounds are not air stable, all the complexes reported above were air stable at room temperature.

*Rhodium(III) derivatives.* One of the N-substituted derivatives, the oxalyl homocysteine thiolactone, forms a complex with rhodium trichloride, which possesses antineoplastic activity in mice transplanted with a rhabdomyosarcoma (99).

A number of rhodium(III) analogues of ruthenium(III) complexes have also shown antineoplastic activity. Ruthenium(III) complexes are thought to be activated by reduction to ruthenium(II), however rhodium(III) complexes are unlikely to be activated by reduction and this may account for their generally lower activity.

The coordination compound of rhodium(III) with 2,6 diaminopyridine exhibited the same nephrotoxicity as cisplatin, as shown by effects on sodium and calcium retention in the whole kidney (99). Cationic complexes of rhodium (III) with antimalarial drugs of the type  $[\text{L}_4\text{Cl}_2\text{Rh}(\text{III})]^+\text{Cl}^-$  where L=primaquine, mepacrine, amodiaquine, lepidine, plasmoquine, pentaquine, isopentaquine, were studied for their anti-tumour effects on Ehrlich ascitic tumours and P388 leukemia. The most promising was the lepidine complex (93).

Rhodium(III) polypyridyl complexes were also studied for their potential use in phototherapy (93).

Some rhodium metallointercalators exhibit specific DNA binding, suggesting that these may be a new type of DNA-targeting agent. One of them binds specifically to destabilized regions near base pair mismatches and is able to recognize a single mismatch in 2725 base pair plasmid DNA. Such specificity may have applications in the detection of mutations (100), thus opening another avenue for transition metal anticancer drugs.

*Radioactive isotopes of rhodium.* Brachytherapy with  $\beta$ -ray  $^{106}\text{Ru}/^{106}\text{Rh}$  plaques can be recommended for small- and medium-sized choroidal melanomas with good results (101). In 1985 a new form of  $^{106}\text{Ru}/^{106}\text{Rh}$  applicator was described with which it was possible to treat ciliary body tumors while preserving the cornea. Such an approach was an alternative to local excision and radiation of ciliary body melanomas with protons and helium ions (102). The  $^{105}\text{rhodium}$ -bleomycin complex is suitable for a targeted radiotherapy, but its application appears to be limited by the renal clearance of this agent (103).

In conclusion, the most common side-effect of rhodium is nephrotoxicity and this prevents further investigation. The mechanism of action of rhodium compounds has not yet

been studied systematically. However the mode of inhibition of DNA synthesis *via* the inhibition of essential enzymes remains a possibility. Several rhodium compounds have entered phase I clinical trials.

## Titanium

The first non-platinum complex tested in clinical trials was a titanium complex, *cis*-[(CH<sub>3</sub>CH<sub>2</sub>O)<sub>2</sub>(bzac)<sub>2</sub>Ti(IV)], which was tested against a wide variety of ascites and solid tumours (104). However, the medicinal properties of transition metal organometallic complexes were not explored until 1979. The first metallocene shown to have anti-tumour activity was titanocene dichloride (105). In contrast to the platinum complexes, titanocene dichloride showed no evidence of nephrotoxicity or myelotoxicity (106). Based on these medicinal properties, titanocene dichloride is undergoing clinical trials (107).

*Bis(β-diketonato)titanium complexes.* These complexes exhibited anti-tumour properties against various animal tumours (104), although only marginal responses were observed with the leukemia cell lines P388 and L1210. One of these complexes, [Ti(bzac)<sub>2</sub>(OEt)<sub>2</sub>], is more effective than 5-fluorouracil against colon tumour and is currently in clinical trial (104). However, there are problems of both solubility and stability in aqueous solution. The limiting toxicities affect the liver and kidneys (108).

*Titanocene complexes. Diacido complexes.* As mentioned previously, in 1979 titanocene dichloride (Cp<sub>2</sub>TiCl<sub>2</sub>) was reported to possess anti-tumour activity (105). It has strong anti-proliferative activity against various cell lines *in vitro*. *In vivo* it is active against both allografts and xenografts (106), such as, interestingly, human adenocarcinomas of the stomach and of the colon which are resistant to common cytostatic drugs. It also showed anti-tumour activity in ovarian carcinoma cells resistant to both doxorubicin and cisplatin (109,110). Furthermore, in ovarian cancer xenografts, titanocene dichloride was as effective as paclitaxel and vinorelbine and showed higher activity than *cis*-platin, 5-fluorouracil and cyclophosphamide (110,111). Attempts aimed at improving the anti-tumour activity by modification of the cyclopentadienyl (Cp) ligand on titanocene are in progress.

*Ionic titanocene complexes.* The ionic titanocene acetonitrile complex exhibited comparable activity to that of titanocene dichloride in colon 38 adenocarcinoma and Lewis lung carcinoma, but was less effective in B16 melanoma. Furthermore, it was more active than the neutral species against gastrointestinal and breast carcinomas, as well as in head and neck carcinoma xenografts (112).

Other ionic titanocene complexes containing aminoacids and thionucleobases have been prepared (113). These complexes showed good to moderate anti-tumour activity against fluid Ehrlich ascite tumours, but were not as active as the parent neutral compound (113). Since these thionucleobases have their own specific anti-tumour activity, they could act synergistically with the parent neutral compound.

More research must still be done, since the mechanism of action has not been investigated the target is unknown. Most importantly, the exact chemical nature of the formulated solutions is still unknown. It is likely that the complexes hydrolyze extensively in water at pH > 5, to yield oligomeric [Ti(bzac)<sub>2</sub>O]<sub>2</sub> which is insoluble (112).

On the molecular level, we know that titanocene dichloride inhibits both protein kinase C, an enzyme that regulates cellular proliferation and human topoisomerase II, an enzyme that plays an important role in the DNA replication (114). However, these properties may not explain the mechanism of action, since inactive metallocene complexes also inhibited these enzymes

The iron-transport protein transferrin forms a strong complex with titanium(IV), with binding to specific iron(III) sites. Titanium(IV) is transferred at low pH from transferrin to ATP. Therefore, it has been proposed that transferrin acts as a mediator for the delivery of Ti(IV) to tumour cells (115).

## Gallium

Gallium (Ga) was discovered in 1875 by P.É. Lecoq de Boisbaudran. In most of its compounds gallium has an oxidation state of +3. The chemical behaviour of gallium is close to that of Fe<sup>+++</sup>, in terms of its electrical charge, ion diameter, coordination number and electron configuration (116). Anticancer properties were described for the first time in 1971 by Hart *et al.* (117,118).

The anionic component of the metal salt has no influence on toxicity. The chloride and sulphate have been tested and yield identical cytotoxicity (117,118).

At low gallium concentrations, gallium atoms are bound to the phosphates of DNA, forming a stable complex, but no interaction was seen between the metal and DNA bases. Gallium may also act as a competitor with magnesium for DNA binding, as it has an affinity for DNA 100 times higher than that of magnesium (119). According to Hedley *et al.*, gallium inhibits replicative DNA synthesis; the major gallium-specific target probably being ribonucleotide reductase (120).

In addition, Chitambar reported that gallium binds to transferrin with a lower affinity than that of iron. The transferrin-gallium complex inhibits DNA synthesis by acting on the M<sub>2</sub> subunit of ribonucleotide reductase (121).

Transferrin bound to gallium binds to specific receptors on the cell plasma membrane without modification. It is interesting to note that tumour cells have more transferrin receptors than normal cells. As with iron itself, after gallium enters the cell, it is transferred to cellular ferritin (122,123) in a process that is augmented by ATP (124,125). The addition of transferrin to culture medium markedly increases the toxicity of gallium (126).

Conversely, the addition of iron to the culture medium greatly reduces gallium toxicity (127-134) and, at a critical cellular concentration, iron induces resistance to gallium (135). When cells are depleted of iron, by addition of a chelator (desferrioxamine), their sensitivity to gallium is increased. However, excess of chelator in the culture medium could reduce gallium toxicity, since it also chelates gallium (136).

Within cells, gallium is found mostly as a phosphate salt in lysosomes, (137,138). For the processes of cellular metabolism, it appears that the trivalent  $Ga^{3+}$  ion acts as an antagonist to the actions of several divalent ions, including  $Mg^{2+}$ ,  $Fe^{2+}$ ,  $Zn^{2+}$  and  $Ca^{2+}$  (139).

A new compound, gallium-pyridoxal isonicotinoyl hydrazone (Ga-PIH), has anti-proliferative activity superior to that of gallium nitrate. Its mechanism of action seems to be different, since the addition of exogenous iron to the culture medium had only a minor effect on Ga-PIH toxicity (140).

The tris(8)quinolinolatoGa(III) compound has a powerful inhibitory effect on the growth of malignant cells, about 10 times higher *in vitro* than that of Ga chloride (141). It was very active in an experimental model, causing a reduction in tumour volume of more than 50%, without any significant toxicity (142). This compound circumvents both unicellular and multicellular-resistance. The  $ID_{50}$  against the parental A549 cells is identical to that of a subline resistant to etoposide, doxorubicin, cisplatin and vinblastine (143). So-called multicellular-resistance is obtained when cells are cultured as spheroids, instead of as a monolayer on the bottom of plastic flasks; *i.e.* this resistance appears as soon as cells have established contacts with other cells or with the extra-cellular matrix. This type of resistance is named "multicellular" since multiple cells are necessary for it to be observed, in contrast to resistance involving any mechanism which induces resistance in isolated cells and can therefore be named "unicellular resistance" (144). The most interesting point about tris(8-quinolinolato)Ga(III) is that its  $ID_{50}$  against a given parent cell line is not different when the cells are grown as an aggregate or as spheroids. In contrast, the concentration of etoposide had to be increased 163-fold, 35-fold for doxorubicin, 27-fold for cisplatin and 6625-fold for vinblastine, to overcome multicellular resistance.

A novel doxorubicin-gallium-transferrin conjugate has been formulated (Dox-Ga-Tf). It exhibited approximately

the same growth inhibitory effect as doxorubicin on MCF-7 cells. However, in resistant MCF-7 cells, Dox-Ga-Tf reversed resistance to free doxorubicin with a 100-fold decrease in  $IC_{50}$ . Compared to Ga-Tf, Dox-Ga-Tf was 500- and 3000-fold more inhibitory to MCF-7 and MCF-7-resistant cells, respectively. The reversal of resistance to doxorubicin by the Dox-Ga-Tf conjugate shows that it is mediated by (i) the transferrin receptor transmembrane transport mechanism, (ii) the redistribution of doxorubicin into the nucleus of doxorubicin-resistant MCF-7 cells and (iii) inhibition of MRP gene expression (145).

A modification of cell cycle distribution is observed after at least 24 hours incubation with gallium. The percentage of cells in S-phase was decreased, whereas the percentage of cells in the G0/1-phase was increased (146).

*Gallium nitrate i.v.* The action of gallium on bone metabolism was studied primarily because it decreases the hypercalcemia associated with cancer (147-151). Gallium inhibits osteoclastic activity and decreases hydroxyapatite crystal formation, with adsorption of gallium onto the surfaces of hydroxyapatite crystals. In addition, there is an increase of collagen synthesis related to the bone concentration of gallium and an increase in bone tissue formation *in vitro* (152-160). It has been reported that a protracted infusion was effective against cancer-associated hypercalcemia (147-151).

Preliminary studies in bladder carcinoma (161), carcinoma of the urothelium (162) and lymphomas (163-165) are also promising.

Another interesting schedule of subcutaneous injection with low doses of gallium nitrate has been proposed, especially for the treatment of bone metastases, but the definitive results have not yet been published (166).

*Oral gallium chloride.* Gallium uptake after oral administration as compared to intravenous injection is selective for tumour cells, especially metastases, compared to kidneys (167). Because of this aspect of tissue pharmacokinetics, oral administration of gallium has been suggested in order to potentiate radio- or chemotherapy in lung cancer.

It was reported that gallium potentiates the activity of cisplatin and etoposide (168). The percentage of partial responses was more significant in patients receiving gallium plus cisplatin and etoposide, as compared to those receiving only the two classical anticancer drugs. However, a delayed toxicity appeared after 3 courses of this triple chemotherapy. To avoid this cumulative toxicity, a dose adjustment has been proposed for cisplatin and for gallium. The purpose of the dose adjustment was to achieve a constant area under the curve (AUC) of total platinum concentration *versus* time from cycle to cycle. Several assays of total platinum plasma concentration were performed in

order to modify the cisplatin dosage to reach the target AUC. The result was an absence of major toxicity, with a clinical response comparable to that of the previous study (157,169-171). The dose adjustments can be performed during several courses without toxicity and thus permit an increase in the number of courses administered.

Preclinical studies have demonstrated synergy of gallium with paclitaxel (172), gemcitabine (173), vinorelbine (174), hydroxyurea (121), fludarabine (136) and interferon- $\alpha$  (175). This synergistic antineoplastic activity should be explored in clinical studies (176).

New gallium compounds, with a better bioavailability, are now under clinical investigations and could improve the anticancer activity first demonstrated with Ga nitrate or Ga chloride.

## Platinum

The first platinum-containing complex to be used in cancer treatment was cisplatin. It was first synthesised in 1844 and was known as Peyrone's chloride. One hundred and twenty years later Rosenberg reported the inhibitory activity of cisplatin on *E. coli* division (177). In the seventies efficacy in human cancer patients was established.

Since then, around three thousand platinum derivatives have been synthesised and tested against cancer cells; but, at most, only thirty compounds have reached clinical trials and more than half of those have already been rejected. Today, four are used clinically: cisplatin, available since 1978, and carboplatin, both being used world-wide, also oxaliplatin and nedaplatin. Some platinum complexes are still under clinical investigation, including those developed for oral administration (178).

In this review on metals in cancer treatment we will focus on the four compounds that are currently available on the market.

**Mechanism of action.** In fact, these four platinum drugs can be considered as pro-drugs, yielding after aquation the active diaquo-platinum compound. The main differences between these pro-drugs can be related to the different kinetics of activation. Hydrolysis of cisplatin is extremely rapid, whereas it is slower for carboplatin and nedaplatin.

The diaquo-platinum species react with the amine groups of proteins, RNA and DNA. The latter reaction yields platinum-DNA adducts, which appear to be associated with anti-tumour activity. Aquated platinum reacts preferentially with the N-7 position of guanine and adenine and produces cross-links between bases in the same strand (intrastrand) or opposite strands (interstrand). Interstrand adducts and DNA-protein binding represent only 1% of all adducts (179). Adduct formation can be as high as one for every  $1 \times 10^5$  bases, *i.e.* around 10,000 platinum atoms per cell.

The efficacy of platinum agents against cancer cells may be mediated to inhibition of DNA synthesis or to saturation of the cellular capacity to repair platinum adducts on DNA (180). Trans adducts are more easily repaired than cis adducts; for this reason, the cis configuration of the diaquo intermediate is 30 times more toxic than the trans configuration (181). Inhibition of DNA synthesis and repair could result from a modification of the three dimensional structure of DNA, which is induced by the metal adducts (182). Cells with enhanced DNA repair activity are resistant to cisplatin, confirming the importance of DNA repair inhibition (183). A direct relationship between the efficacy of cell-killing and the number of bound platinum atoms has been shown for nedaplatin (184).

Oxaliplatin is not as efficient at inducing adducts. However, due to the size of the diaminocyclohexane group and its greater hydrophobicity, the modification of the three dimensional structure of DNA could be more significant and more efficient in inhibiting DNA polymerisation and repair.

It is important to remember that the diaquo active metabolite can react with molecules other than DNA: *i.e.* with RNA and proteins as previously mentioned. It also has a high affinity for molecules containing a thiol group, for example cysteine, reduced glutathione, methionine, metallothioneins, thioredoxin, *etc.* Several enzymes, such as glutathione-S-transferase (GST), gamma glutamyl cysteine synthase ( $\gamma$ GCS) and gamma glutamyl transferase ( $\gamma$ GT), are involved in the activity of glutathione. Thus, when a platinum complex enters a tumour cell, cytotoxicity is not yet inevitable until the drug enters the nucleus and reacts with DNA.

After platinum has formed a large number of adducts on DNA, cell death may still be circumvented. As mentioned previously, cells with elevated activity of DNA repair enzymes may be resistant. On the other hand, cells with decreased DNA repair capacity, such as fibroblasts from patients with xeroderma pigmentosum, are more sensitive (185). Repair of DNA after platinum adduct formation involves nucleotide excision repair (NER) (186). By contrast, deficiency in mismatch repair (MMR) is associated with cisplatin and carboplatin resistance. MMR is also probably important in resistance to nedaplatin, since this drug yields the same active metabolite, but is not involved in oxaliplatin resistance (187,188).

In the early 90s, oncologists discovered that anti-cancer drugs kill cells through apoptosis and that modulation of apoptotic processes could induce resistance to cell death, *i.e.* a resistance to anti-cancer drugs (189). Overexpression of bcl-2 or bcl-XL blocks the release of cytochrome c and aborts the apoptotic response to cisplatin (190,191). Furthermore, it has been shown that the action of EGF-R on cisplatin resistance is mediated by an induction of bcl-XL expression (192).

In addition, modifications of expression of oncogenes (myc, ras, jun, fos, v-abl, Her/neu) or tumour suppressor genes (p53) also alter cell sensitivity to platinum complexes (193,194).

*Cisplatin (195-198)*. Evidence of activity was first reported against testicular and ovarian cancer, in terms of both objective response and of prolonged survival. Because of its marked renal toxicity, cisplatin was almost withdrawn from clinical trials. Interest was rekindled when hyperhydration with isotonic saline circumvented this problem. One of the attractions of cisplatin, besides its efficacy, is that its toxicity is different from that of other anticancer drugs, thus making it attractive for combination regimens.

In testis, bladder, head and neck, small cell lung cancer (SCLC) and in several paediatric malignancies, cisplatin remains superior to carboplatin. Conversely, carboplatin has tended to replace cisplatin in the treatment of other cancers.

*Carboplatin (195-198)*. After extensive preclinical screening, involving a large number of platinum derivatives, carboplatin was selected mainly because of its lower non-haematological toxicity. Unfortunately, cross-resistance occurs to both platinum agents. The dose-limiting toxicity is myelosuppression, chiefly thrombocytopenia.

Early in the development of carboplatin, dose adjustment methods were proposed to reach a targeted area under the plasma concentration-time curve (AUC). This results in more reproducible toxicity and facilitates the development of combinations with other drugs and/or with radiotherapy. It is lamentable that such an approach has not yet been possible for all new anticancer drugs.

Carboplatin has been widely tested, relative to the parent compound, in a large number of randomized clinical trials. Its anti-tumour activity was demonstrated in ovarian cancer, although, a meta-analysis of 11 clinical trials, including more than 2000 patients, indicated no superiority over cisplatin (199).

In practice, carboplatin has replaced cisplatin in a number of indications. Besides its efficacy and lower toxicity, the single intermittent bolus or short infusion schedule is more practical than the protracted infusion of cisplatin. Also, although carboplatin is more expensive than cisplatin, the complete cost of treatment is cheaper.

It must be added that cisplatin and carboplatin have substantial activity in sensitizing tumour cells to radiotherapy in head and neck, lung, oesophagus, cervix, bladder and rectal cancer.

*Oxaliplatin (188,195,196,200-203)*. This drug was synthesised by Kidani at the University of Nagoya, Japan, and developed primarily in France with the support of Roger Bellon Laboratories, Debiopharm Laboratories and now Sanofi-

Synthelabo Laboratories. This drug was selected for development because it has a higher efficacy and a lower toxicity than cisplatin in *in vivo* preclinical studies and, most importantly, it has no cross-resistance with cisplatin. The dose-limiting toxicity is significant sensory neuropathy, which cannot be predicted. Early in the drug development process, Levi and colleagues reported the superiority of the chronomodulation of infusions over constant-rate delivery in preclinical and in clinical studies (204,205,202). This particular mode of administration resulted in a different profile of toxicity: emesis and sensory neuropathy were more frequent with chronomodulation and stomatitis was more frequent with constant-rate infusion.

Oxaliplatin gave interesting results in ovarian, breast, head and neck cancer, in non-Hodgkin's lymphoma, malignant melanoma, glioblastoma and NSCLC. Its efficacy is more remarkable against cancer which is resistant to other platinum derivatives. The best results to date have been obtained in the treatment of colorectal cancer. Synergy was observed with leucovorin and fluorouracil in a so-called "de Gramont" or "FolFox" protocol which yielded an impressive objective response rate (206,207).

*Nedaplatin or 254-S (195,208,209)*. Shionogi Pharmaceutical, Osaka, Japan, are currently developing this platinum derivative. It was selected because it produced better results than cisplatin in preclinical studies. Unfortunately, nedaplatin is cross-resistant with cisplatin. Its main toxicity in humans is myelosuppression, with a delayed nadir and recovery. The official indications in Japan are head and neck, testicular, lung (NSCLC and SCLC), oesophageal, ovarian and cervical cancer.

A randomised clinical trial compared nedaplatin to cisplatin, both in combination with vindesine. Nedaplatin showed no advantage over cisplatin in objective response and overall survival, but was less toxic. More thrombocytopenia was observed, but there was less leucopenia, nephrotoxicity and gastrointestinal toxicity.

## Conclusion

Much work has been done on metals and metal compounds. The actions of these molecules are ambivalent: some can induce cancer (210) while others can treat cancer, some even have both properties. This is not a novel phenomenon, since we know that the majority of anti-cancer drugs used today in the clinic are mutagenic and thus potentially carcinogenic. When looking at the age of the references in this review, it appears that research on some metals, which had been abandoned for some time, is being reactivated. The efficacy of cisplatin has given an impetus to research for new metal compounds. The results are not yet satisfactory and a great deal of work remains to be done.

The second half of the past century, with the advent of chemotherapy, produced high hopes of curing cancer. What will happen in the third millennium? If humans lived for 150-200 years, it seems likely that 100% of people would develop some form of cancer, as the result of accumulated DNA mutations. In this case molecular therapy may be rationally the only really effective treatment. We are just beginning such therapies and it can only be concluded today that we will require a long time to achieve efficacy in the clinic.

An alternative approach might provide a solution. We now know that approximately two-thirds of cancers could be avoided by modifying our everyday life (cigarettes, alcohol, nutrition, sexual behaviour, *etc.*). Thus, it can be concluded that it is statistically easier to avoid a cancer than to cure it. Using the same approach, we could hypothesize that it would be more efficient to protect our cells from mutations, rather than focussing exclusively on curing cancer.

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Received November 4, 2003

Revised February 9, 2004

Accepted April 1, 2004