Ruthenium in Medicine: Current Clinical Uses and Future Prospects

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There is no doubt about the success of precious metals in the clinic, with, for example, platinum compounds being widely used in the treatment of cancer, silver compounds being useful antimicrobial agents and gold compounds used routinely in the treatment of rheumatoid arthritis. The medicinal properties of the other platinum group metals are now being recognised and of these a ruthenium anticancer agent has recently entered the clinic, showing promising activity on otherwise resistant tumours. Like all metal drugs, the activity of the ruthenium compounds depends on both the oxidation state and the ligands. By manipulating these features ruthenium-centred antimalarial, antibiotic and immunosuppressive drugs have been made. In addition, ruthenium has unique properties which make it particularly useful in drug design. In this review we discuss ruthenium from a clinical stance and outline the medicinal uses of ruthenium-based compounds.

Precious metals have been used for medicinal purposes for at least 3500 years, when records show that gold was included in a variety of medicines in Arabia and China. At that time precious metals were believed to benefit health – because of their rarity – but research has now linked the medicinal properties of inorganic drugs to specific biological properties. The elucidation of a drug mechanism is however complex and the exact route of activity for many drugs remains unknown. The biological targets or mechanism of action of many metal drugs are now being resolved step by step, and this information is then used to design improved drugs with increased potency and reduced side effects.

Metals in the Clinic

Perhaps the most well known and best studied platinum metals drugs are the anticancer compounds of platinum itself which, after the fortuitous discovery of anticancer properties of cisplatin in the 1960s, heralded research into other platinum compounds and founded a revolution in cancer therapy. Today, many platinum drugs are used in the clinic and even more are being evaluated in clinical trials, not just to treat cancer but to fight a range of diseases, including parasitic and bacterial infections. Other metals of the platinum group and gold and silver have been used in medicine and these are listed in the Table.

Ruthenium Properties Suited to Biological Applications

There are three main properties that make ruthenium compounds well suited to medicinal application:

(i) rate of ligand exchange
(ii) the range of accessible oxidation states and
(iii) the ability of ruthenium to mimic iron in binding to certain biological molecules. These will be briefly described in turn.

(i) Ligand Exchange

Many ruthenium complexes have been evaluated for clinical applications, particularly in the treatment of cancer, due in part, to Ru(II) and Ru(III) complexes having similar ligand exchange kinetics to those of Pt(II) complexes. Ligand exchange is an important determinant of biological activity, as very few metal drugs reach the biological target without being modified. Most undergo interactions with macromolecules, such as proteins, or small S-donor compounds and/or water. Some interactions are essential for inducing the

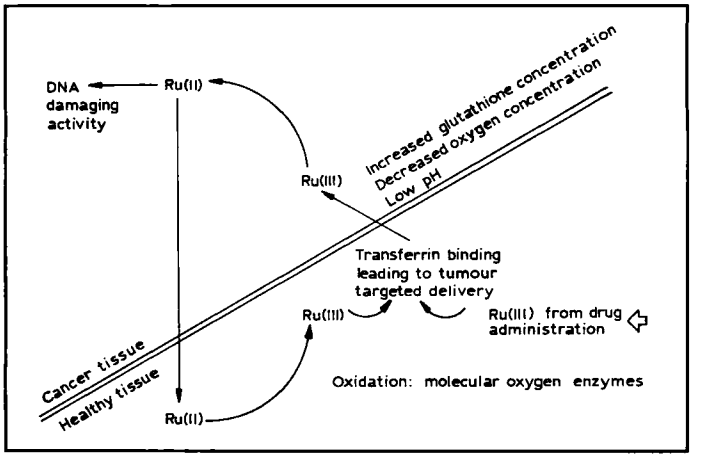

Fig. 1 The oxidation state changes of ruthenium in cancer and healthy cells. The reductive environment of cancer cells favours Ru(II), which is more biologically active than Ru(III). Hence Ru(III) compounds are essentially prodrugs that become activated by reduction on reaching the cancer cell

desired therapeutic properties of the complexes. As the rate of ligand exchange is dependent on the concentration of the exchanging ligands in the surrounding solution, diseases that alter these concentrations in cells or in the surrounding tissues can have an effect on the activity of the drug.

(ii) Oxidation State

Ruthenium is unique amongst the platinum group in that the oxidation states Ru(II), Ru(III) and Ru(IV) are all accessible under physiological conditions. In these oxidation states the ruthenium centre is predominantly hexacoordinate with essentially octahedral geometry, and Ru(III) complexes tend to be more biologically inert than related Ru(II) and (IV) complexes. The redox potential of a complex can be modified by varying the ligands. In biological systems glutathione, ascorbate and single electron transfer proteins are able to reduce Ru(III) and Ru(IV), while molecular oxygen and cytochrome oxidase readily oxidise Ru(II).

The redox potential of ruthenium compounds can be exploited to improve the effectiveness of drugs in the clinic. For example, the drug can be administered as relatively inert Ru(III) complexes, which are activated by reduction in diseased tissues. In many cases the altered metabolism associated with cancer and microbial infection results in a lower oxygen concentration in these tissues, compared to healthy ones, and this promotes a reductive environment. Cancer cells are known to have higher levels of glutathione and a lower pH than healthy tissues, creating a strongly reducing environment. If the active Ru(II) complex leaves the low oxygen environment, it may be converted back to Ru(III) by a variety of biological oxidants, see Figure 1.

Proteins that can catalyse the reduction of Ru(III) to Ru(II) include mitochondrial and microsomal single electron transfer proteins. The mitochondrial proteins are of particular interest in drug design as apoptosis, the desired mechanism for cell death, can be initiated in the mitochondria, as well as by other pathways, for instance, by the Fas/FasL pathway. Transmembrane electron-transport systems can also reduce Ru(III) complexes outside of the cell and this is highly relevant to the mechanism of action of a ruthenium-based drug in clinical use which has anticancer activity independent of cell entry (*vide infra*).

(iii) Iron Mimicking

The low toxicity of ruthenium drugs is also believed to be due to the ability of ruthenium to mimic iron in binding to many biomolecules, including serum transferrin and albumin. These two proteins are used by mammals to solubilise and transport iron, thereby reducing its toxicity. Since rapidly dividing cells, for example microbially infected cells or cancer cells, have a greater requirement for iron, they increase the number of transferrin receptors located on their cell surfaces, thereby sequestering more of the circulating metal-loaded transferrin. *In vivo*, the exact increase in

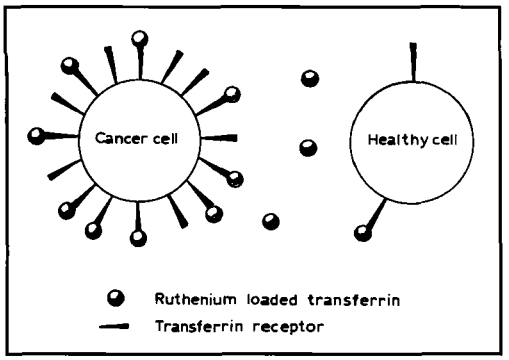

Fig. 2 Schematic representation of the selective uptake of transferrin by cancer cells. Ruthenium can mimic iron in binding to transferrin. Metal-loaded transferrin is delivered to cells according to the number of transferrin receptors on their surfaces. As most cancer cells have a higher number of transferrin receptors (left), compared to healthy cells (right), ruthenium is targeted to cancer cells

radio-labelled ruthenium compounds in cancer cells, compared to healthy cells, has been shown to range from 2–12 fold, depending on the cell type. As the drug is targeted to cancer cells, its toxicity is reduced because less of it will reach healthy cells, see Figure 2.

Current Uses of Ruthenium-Based Drugs

The array of clinical applications for some platinum group metals, see the Table, illustrates the versatility of metallodrugs in the clinic. The activity of each compound is a function of the oxidation state of the metal and the nature of the attached ligands. These features dictate not only how the drug interacts with the disease target but also the biological transformations that occur en route. By manipulating these features activity can be fine-tuned to maximise the potency but minimise the general toxicity of the drugs.

Immunosuppressants

Immunosuppressants, for example, cyclosporin A, are important clinical agents used in the treatment of a broad range of diseases, including aplastic anaemia, severe eczema, glomerulonephritis, psoriasis, systematic sclerosis and psoriatic arthritis. Cyclosporin A has a number of side effects including renal disease, hypertension and nausea, hence there is a continual drive to develop a more effective drug.

Ru(III) complexes with N-donor ligands were tested as immunosuppressants, with *cis*-$[Ru(NH_3)_4(Im)_2]$, Im is imidazole, see Figure 3, being particularly effective. These compounds are very stable and have been shown to inhibit the antigen-independent phase of T-cell proliferation at nanomolar concentrations – a marked improvement on cyclosporin – and hence have much potential for clinical development.

Antimicrobial

Ruthenium complexes are active against a wide range of parasitic diseases including malaria and Chagas' disease. The activity of organic antimicrobial drugs has been enhanced by binding the organic molecule to a ruthenium centre. The enhancement is due, in part, to the ruthenium complex overcoming resistance that the parasite has developed to the organic compound alone. Malaria threatens over 40 per cent of the world's population and the malaria-causing *Plasmodium* parasite is becoming resistant to some of the most successful drugs, such as chloroquine. The resistance mechanism of *Plasmodium* parasites to chloroquine involves a reduced rate of uptake of the drug. However, it has been found that metal complexes of chloroquine partially restore the antimalarial activity. The Ru(II)-chloroquine complex, $[RuCl_2(chloroquine)]_2$, is 2–5 fold more effective than chloroquine alone, suggesting that the ruthenium complex is taken up by a different mechanism to the chloroquine. The structure of $[RuCl_2(chloroquine)]_2$, see Figure 3, has not been fully established and some uncertainty remains as to its precise structure. It is thought that, once in the *Plasmodium* parasite, the ruthenium complex behaves in a similar way to chloroquine, altering the pH of the digestive vacuole of the parasite and inhibiting the polymerisation of heme (the heme is toxic to the parasite which therefore dies).

The *Trypanosoma cruzi* parasite causes Chagas' disease. Chagas' disease affects millions of people in Latin America and is incurable. The current treatment involves noxious organic drugs, the dose being limited by the toxicity of the therapy. Once

Some Medicinal Properties of the Precious Metals (Not all of these compounds have been clinically approved)			
Ru Immunosuppressant Cancer Dental alloys Microbial (malaria and Chagas' disease) Antibiotic Septic shock	**Rh** Cancer Radiosensitisers Dental alloys Microbial (malaria and Leischmaniasis) Bacterial infections	**Pd** Viral (leukaemia and HIV) Cancer Dental alloys	**Ag** Dental alloys Smoking Microbial
Os Dental alloys Microbial (Leishmaniasis) Rheumatoid arthritis	**Ir** Radioisotopes in cancer	**Pt** Cancer Dental alloys Microbial Anti-HIV	**Au** Cancer Viral (HIV/AIDS) Bronchial asthma Microbial (malaria) Rheumatoid arthritis

again, the effectiveness of these organic drugs can be dramatically enhanced by coordination to ruthenium centres.

Antibiotics

Coordinating ruthenium to organic antibiotic compounds often results in higher *in vitro* activity. A good example is the ruthenium(III) derivative of thiosemicarbazone; this exhibits a 70 per cent increase in antibiotic activity against the Gram negative bacteria *Salmonella typhi* and *Enterobacteria faecalis*. It is postulated that the improved activity arises from the delocalisation of the positive charge between the organic moiety and the metal ion, which favours the drug entering the normal cellular processes of the bacteria. However, it is also possible that the difference in activity stems from the ability of Ru(III) to bind to biological molecules, in a similar way as iron. It is believed that siderophore compounds are secreted by microorganisms to sequester iron from their surrounding environment. Therefore, if the ruthenium(III) complexes also bind to siderophores they will be more readily taken up by the cell.

Nitric Oxide Scavengers

Nitric oxide (NO) plays a central role in many physiological processes including signalling, regulation of cardiovascular function and immunological response to microorganisms and tumour cells. Consequently, malfunction of NO production results in many physiological symptoms.

Some ruthenium(III) polyaminocarboxylates, such as AMD6245 and AMD1226, see Figure 3, have been shown to enhance the activity of vasoconstrictor drugs and are proposed for the treatment of diseases that involve overproduction of NO including stroke, septic shock, arthritis, inflammatory bowel disease, epilepsy and diabetes. The edta complexes K[Ru(Hedta)OH_2] and K[Ru(Hedta)Cl] exist in equilibrium in aqueous solution. Both species bind NO rapidly and strongly, resulting in the reduction of the Ru(III) ion to form a linear Ru(II)-NO adduct.

Anticancer Activity

Despite the success of platinum-based anticancer compounds in the clinic, there is still a need for new and improved metal-based anticancer drugs. The need for new drugs is fuelled by the inability of platinum compounds to tackle some types of cancer of high social incidence and by the associated toxic side effects of the current platinum compounds in clinical use.

Platinum anticancer drugs bind DNA, causing damage that prevents protein synthesis and replication causing cell death. The success of platinum anticancer drugs has biased the screening of new

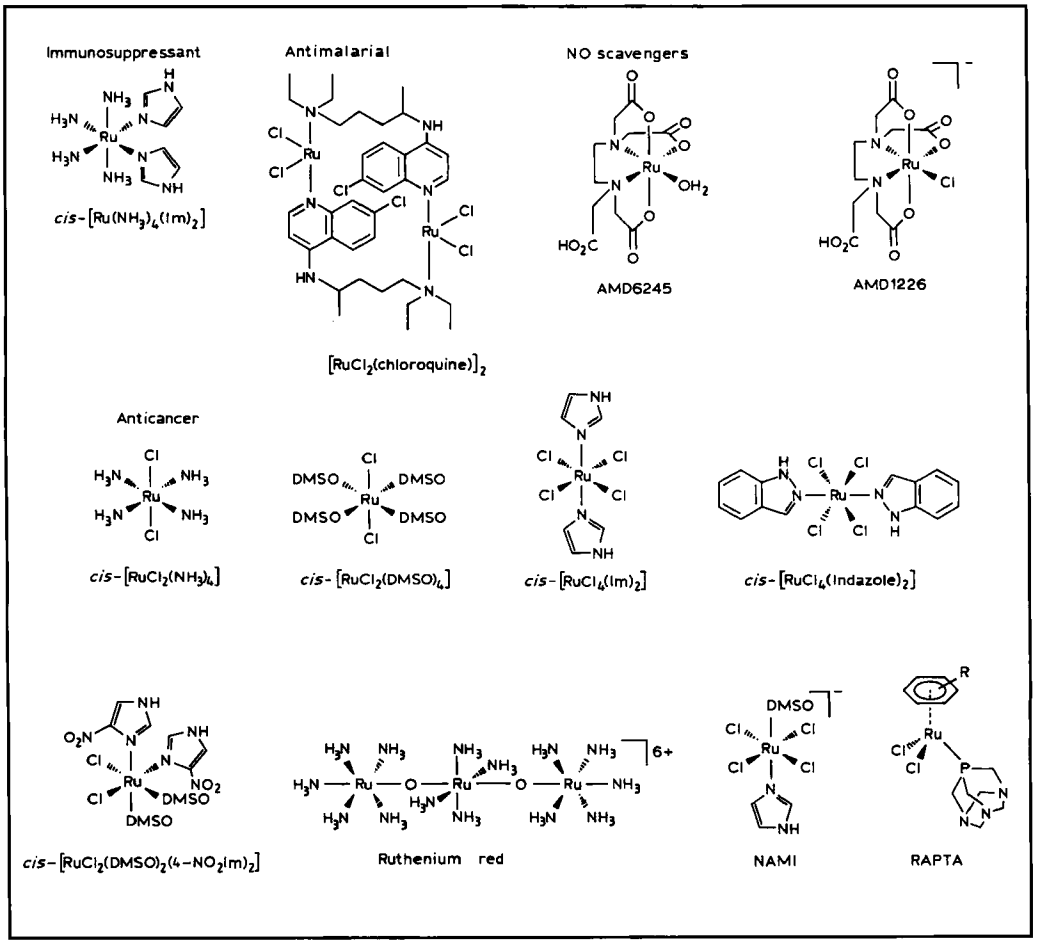

Fig. 3 The structures of various medicinal ruthenium complexes. The structure of the chloroquine complex [RuCl2(chloroquine)]2 remains uncertain. NAMI is the Na+ salt and NAMI-A is the imidazolium salt. RAPTA compounds, with C6H6, C6H5Me, C6H4MeiPr-1,4 and C6Me6, have been made

metal-based anticancer compounds towards looking for damage caused to DNA. Many Ru(II), Ru(III) and Ru(IV) complexes with amine, dimethylsulfoxide, imine, polyaminopolycarboxylate, and N-heterocyclic ligands have been found to bind to DNA, see Figure 3. However, many of these compounds are barely soluble in aqueous solution, which is necessary to allow efficient administration and transport. Solubility has been increased by using dialkyl sulfoxide derivatives, such as [*trans*-RuCl4(DMSO)Im][ImH], NAMI-A, see Figure 3, which is now recognised as the most successful ruthenium-based anticancer compound.

Interestingly, although NAMI-A can bind DNA, *in vivo* DNA damage does not appear to be part of its anticancer mechanism.

In general, anticancer activity hinges on the ability of a drug to bring about apoptosis (programmed cell death) of the tumour cells. Apoptosis is a complicated process by which the cell 'commits suicide' in a controlled manner such that there is no cell debris or damage done to surrounding cells. This process is perhaps best illustrated on a cellular level using neuroblastoma cell lines: growing cells are semi-differentiated, forming irregular shapes that can be clearly distinguished from the spherical apoptotic bodies, see Figure 4. Alternatively cells can die by a process called necrosis, which is less controlled, and causes inflammation and damage to adjacent cells.

There are many mechanisms by which apoptosis can be initiated involving interactions of drugs with both DNA and proteins. Some ruthenium complexes have been shown to damage DNA, either directly or indirectly, for example, by positioning radiosensitisers close to the DNA. In addition to DNA binding, ruthenium compounds interact with proteins, and it is likely that both activities contribute to the anticancer properties of the compounds.

(a) DNA Damaging Agents

A number of ruthenium compounds have been shown to bind DNA *in vitro* and there is a direct correlation between this activity and the cytotoxicity of Ru(III) am(m)ine complexes in tissue culture. The mechanism of DNA binding has been probed and certain ruthenium complexes form cross-links between DNA strands – possibly favoured due to the steric restrictions imposed by the octahedral geometry of the complexes. This binding mechanism differs from the intrastrand cross-links favoured by cisplatin, and consequently the cancer cell lines that have developed resistance to cisplatin by accelerating the rate of repair of intrastrand cross-links are still susceptible to ruthenium anticancer drugs.

Interestingly, it has been demonstrated that Ru(II) complexes are far more reactive towards DNA than Ru(III) AND Ru(IV) and it is therefore possible that the anticancer activity of Ru(III) involves initial reduction to Ru(II) at the tumour site, promoted by the altered physicochemical environment in tumour cells (*vide supra*). If this hypothesis is correct then Ru(III) complexes are essentially prodrugs. However, there is growing evidence to show that protein interactions are also extremely important in the anticancer activity of ruthenium compounds and these interactions could occur with the ruthenium in either oxidation state.

(b) Radiosensitisers

Radiation therapy is routinely used against some types of cancer. This treatment can be enhanced by using nitroimidazoles and halogenated pyrimidine radiosensitisers (compounds that increase the irradiation sensitivity of the target cells). As the activity of these compounds depends on their proximity to DNA, coordinating radiosensitisers to metals that are able to bind to DNA, for example platinum and ruthenium, enhances the radiosensitising properties.

The two key features of an effective radiosensitiser are the ability to bind DNA and the redox potential of the bound complex. Strong DNA binding affinity is a feature of many ruthenium compounds, although not all have radiosensitising activity. This activity depends upon the compound having a high reduction potential, which can be optimised by the use of appropriate ligands. For example, the nitroimidazole complex, $RuCl_2(DMSO)_2(4\text{-}NO_2Im)_2$, see Figure 3, is one of the most effective radiosensitisers having both a higher activity and a lower toxicity than 4-NO_2-imidazole alone.

(c) Photodynamic Therapy

As with radiotherapy, photodynamic therapy uses chemicals targeted to diseased cells. The chemicals become cytotoxic when exposed to electromagnetic radiation. For example, nitrosylruthenium(II) complexes release NO on reduction; the reduction may be triggered using photodynamic methods. Until recently, the application of photodynamic therapy was restricted by the poor accessibility to cancer cells, but a unique approach using ruthenium complexes has been developed which overcomes these restrictions. This new method of therapy centres on the Mössbauer absorption of γ-rays by ruthenium; this can induce emission of Auger electrons which damage the DNA to which the Ru is bound.

(d) Antimitochondrial

Apoptosis can be initiated by more than one pathway, one being via the mitochondria (the subcellular compartments associated with energy and heat generation). Consequently any compounds that target these structures are of great interest as anticancer drugs. Ruthenium red, see Figure 3, is routinely used by biologists to stain mitochondria selectively, as it binds to the calcium channels on their surfaces and has long been known to inhibit tumour cell growth, but its toxicity is too great for

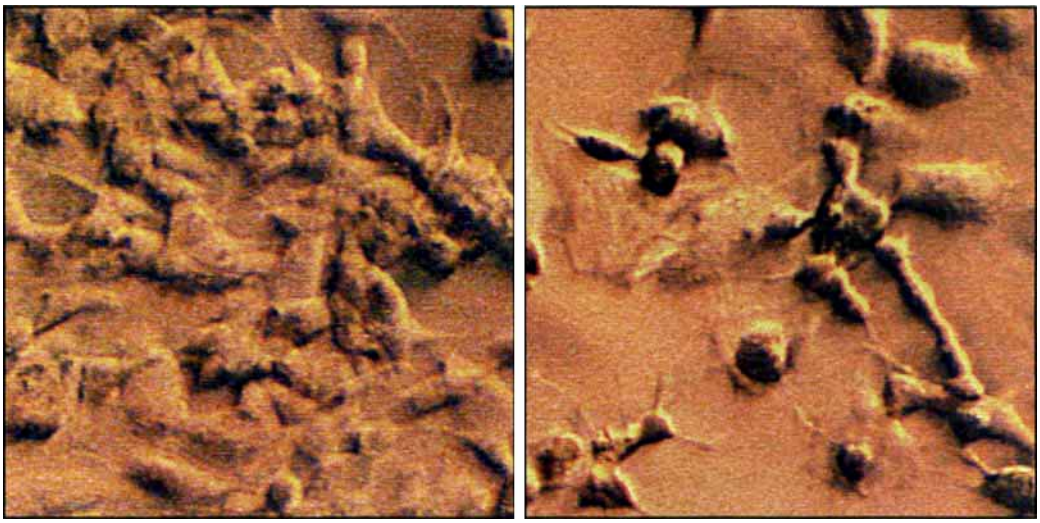

Fig. 4 Photographs of human neuroblastoma cells in culture grow to a high density and have an irregular shape (left). After incubation with 1.3 μM of the ruthenium drug RAPTA-C there are fewer cells in the culture (right). The smooth, more spherical appearance of the remaining cells is characteristic of apoptotic bodies, indicating cell death is occurring by apoptosis. Each cell has the average dimensions of 20–40 μm

use in the clinic. It is possible that the anticancer mechanism of some other ruthenium compounds may involve mitochondrial interactions. However, many ruthenium compounds with putative antitumour activity are unlikely to act like ruthenium red.

(e) Antimetastases

A particularly challenging area of cancer therapy is the treatment of metastases. Metastasis occurs at a late stage of the disease and involves the escape of cells from the primary tumour and their reestablishment at distinct secondary locations. The metastases are normally dormant and are often suppressed by hormones secreted from the primary tumour. However, if the primary tumour is removed or there are further genetic changes in the metastases, growth can begin. Tumour growth beyond about 1 mm^3 requires a blood supply, and the formation of the necessary blood vessels is termed angiogenesis. Some antimetastases drugs restrict angiogenesis, for example angiostatin, throbospondin and batimastat, but statistics show that once this process has occurred the chance of five-year survival drops by about 50 per cent, depending upon the type of cancer.

The first ruthenium anticancer drug to progress through clinical trials, [*trans*-$RuCl_4$(DMSO)Im][ImH], NAMI-A, see Figure 3, is strongly active against tumour metastases. NAMI-A appears to alter protein expression, either by binding to proteins or to RNA, causing thickening of the protein layer surrounding tumours and metastases. As a result the tumour becomes isolated, preventing escape of metastasing cells and reducing the blood flow, which ultimately suffocates it. Only a very small portion of the drug reaches the tumour target and its activity appears to be independent of its concentration in tumour cells. Rather, it appears that NAMI-A has an extracellular mode of activity centring on interactions with proteins.

In our own laboratory we have investigated the role of the ligands attached to ruthenium in the passive diffusion of the drug across cell membranes, facilitating the movement of the drug into and within cells. A series of drugs of formula [Ru(arene)Cl_2PTA], RAPTA, see Figure 3, have been developed that interact with proteins and DNA *in vitro*, triggering apoptosis in human cancer cell lines. The *p*-cymene derivative, RAPTA-C, has activity against, for example, SK-N-SH neuroblastoma cells, inducing apoptosis in nanomolar concentrations, see Figure 4. This cell line was derived from the metastases of a human neuro-

blastoma that had metastasised to the bone marrow. Figure 4 shows structurally distinct forms. In tissue culture, cells grow in the S-form, which is partially differentiated and characterised by the irregular shapes of the cells. These cells undergo two responses to stress: full differentiation or cell death. Full differentiation is observed by the more 'dendritic' appearance of the cells, which occurs 1–3 hours after the stress. Subsequently cells either dedifferentiate back to the S phase – if the stress can be overcome – or die.

Concluding Remarks

Ruthenium drugs are particularly important in the clinic due to their low toxicity. This is in part due to the ability of ruthenium to mimic the binding of iron to biomolecules, exploiting the mechanisms that the body has evolved for non-toxic transport of iron. In addition, the redox potential between the different accessible oxidation states occupied by ruthenium enables the body to catalyse oxidation and reduction reactions, depending on the physiological environment. As demonstrated for cancer tissues, but also true in other diseased states, the biochemical changes that accompany disease alter the physiological environment, enabling ruthenium compounds to be selectively activated in diseased tissues. These two features combine to give ruthenium drugs a remarkably low toxicity compared to other platinum group metal compounds and therefore make ruthenium compounds promising in the clinic.

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Bibliography

M. J. Clarke, F. Zhu and D. R. Frasca, 'Non-platinum chemotherapeutic metallopharmaceuticals', *Chem. Rev.*, 1999, **99**, 2511–2534

Z. Guo and P. J. Sadler, 'Medicinal inorganic chemistry', *Adv. Inorg. Chem.*, 1999, **49**, 183–306

G. Sava, E. Alessio, A. Bergamo and G. Mestroni, 'Sulfoxide ruthenium complexes: non-toxic tools for the selective treatment of solid tumor metastases', in "Metallopharmaceuticals I, DNA Interactions", eds. M. J. Clarke and P. J. Sadler, Springer-Verlag, Berlin, 1999, pp. 143–169

A. Eastman, 'The mechanism of action of cisplatin: from adducts to apoptosis', in "Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug", ed. B. Lippert, Wiley-VCH, Weinheim, 1999, pp. 111–134

M. J. Abrams and B. A. Murrer, *Science*, 1993, **261**, 725–730 (A general reference on metals in medicine)

S. P. Fricker, 'Nitrogen monoxide-related disease and nitrogen monoxide scavengers as potential drugs', in "Metal Ions in Biological Systems", Volume 36, eds. A. Siegel and H. Siegel, Marcel Dekker Inc., New York, 1999, pp. 665–721 (A review on metal compounds and nitric oxide focusing on ruthenium compounds)

M. J. Abrams, 'Ruthenium Phthalocyanines', *Platinum Metals Rev.*, 1995, **39**, (1), 14–18

S. P. Fricker, 'Ruthenium, Nitric Oxide and Disease', *Platinum Metals Rev.*, 1995, **39**, (4), 150–159

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Thiazepinones Synthesis with Rhodium

Thiazepinones are pharmaceuticals with potential use in the treatment of heart and inflammatory diseases. The 7-membered heterocycles have been prepared in multistep syntheses in which chirality is introduced before or during a transformation.

Researchers at the University of Ottawa, Canada, now report the synthesis of thiazepinones using cyclohydrocarbonylative ring expansion of acetylenic thiazoles in the presence of CO and H_2, via the zwitterionic rhodium complex catalyst (η^6-$C_6H_5BPh_3$)$^-$Rh$^+$(1,5-COD) with added triphenyl phosphite (B. G. Van den Hoven and H. Alper, *J. Am. Chem. Soc.*, 2001, **123**, (6), 1017–1022).

The transformation of the simple and functionalised 5-membered acetylenic thiazoles with CO and H_2 to 7-membered 2-(*Z*)-6-(*E*)-4*H*-[1,4]-thiazepin-5-ones occurred in 61 to 90 per cent yields with good chemo- and regioselectivities, at 70–110°C, after 18 to 36 hours. A model substrate of 2-hex-1-ynylthiazole was used to optimise the cyclohydrocarbonylation and ring expansion of 2-acetylenic thiazoles. The acetylenic unit can have various substituents in positions 4 and 5 of the thiazole ring as well as alkyl-, ether-, ester-, vinyl-, and aryl-substituted alkynes at position 2. The process is general and may be pharmaceutically interesting.