Ruthenium Phthalocyanines

NOVEL WATER SOLUBLE AGENTS FOR PHOTODYNAMIC CANCER THERAPY

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Photodynamic therapy is a clinical technique employing the combination of light, oxygen and a sensitising compound to induce the photochemical destruction of unwanted tissue. Light therapy has been known for some time, but it was not until the earlier part of this century that the first clinical work was performed using sensitisers and light. More recently a sensitising compound of a complex mixture of porphyrins has been tested on various cancers, and been awarded regulatory approval for its use. In this paper the synthesis and properties of some novel and easily prepared water soluble ruthenium phthalocyanine complexes are reported. One of these complexes, JM 2929, has been extensively studied and has photosensitising properties which when used in combination with light and oxygen in vitro and in vivo during photodynamic therapy displays remarkable cytotoxic effects.

Although photodynamic therapy (PDT) as an innovative cancer treatment has received much attention recently, the basic concept is not new. The healing aspects of light were described by the Greek historian Herodotus in the 5th Century BC, and the first use of a combination of a sensitiser (eosin) and light to treat skin cancer took place in 1903 (1). In recent years, clinical work in PDT has been focused on Photofrin™, a complex mixture of porphyrins. Photofrin™ has been extensively studied in the treatment of bladder, oesophageal and lung cancers and has recently received regulatory approvals in Canada, Japan and also the Netherlands.

However, there is great interest in the development of "second-generation" PDT agents to overcome some of the drawbacks of treatment with Photofrin™. Some of the issues which need to be considered in the development of new PDT agents include:

[a] The need for a pure compound: the heterogeneous nature of Photofrin™ makes its production and analysis very difficult. Additionally, the use of a mixture renders preclinical and clinical results harder to interpret.

[b] Reduction of normal skin photosensitivity: patients receiving Photofrin™ must stay out of bright sunlight for many weeks.

[c] Light penetration into tumours can be improved by using photosensitisers that have their maximal light absorption in the 650–750 nm range of the spectrum.

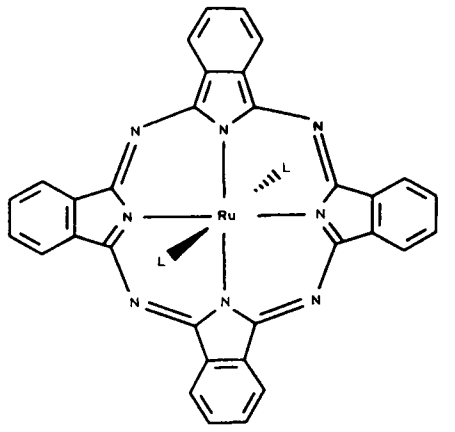

Fig. 1 General structure of a six-co-ordinate ruthenium phthalocyanine complex

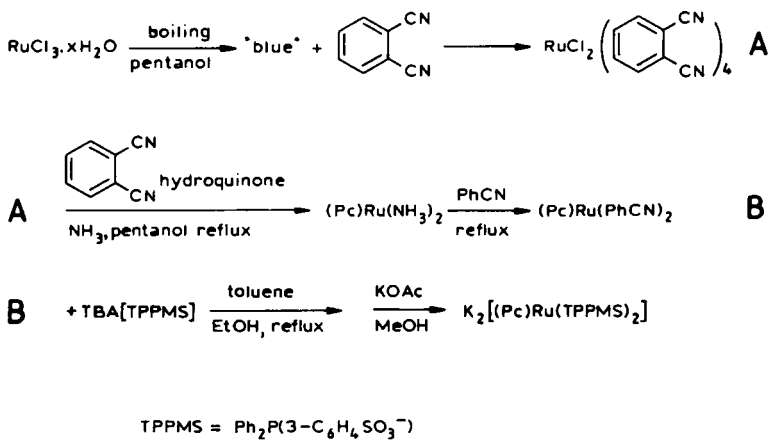

Fig. 2 The synthesis of ruthenium phthalocyanine $K_2[(Pc)Ru(TPPMS)_2]$, designated JM 2929

As a class of dyes, phthalocyanines (Pcs) and metallophthalocyanines (MPcs) generally have strong Q-absorption bands in the 600–750 nm range. Therefore, Pcs and MPcs have been extensively studied as potential PDT agents, but unfortunately, many of these compounds are insoluble in water (2). Water soluble derivatives of Pcs and MPcs have been prepared by the addition of sulphonic acid or carboxylate moieties to the periphery of the macrocyclic ring, often giving complex isomeric mixtures.

An alternative approach is to confer improved solubility to an MPc via axial co-ordination of solubilising groups to the central metal atom, see Figure 1. Since six-co-ordinate ruthenium(II) Pcs are well established in the literature (3) we synthesised a variety of water soluble ruthenium phthalocyanine derivatives with the goal of studying their photosensitising properties.

Synthesis

In order to prepare a variety of water soluble ruthenium phthalocyanine, RuPc, complexes, a general starting material was needed of the type $PcRuL_2$, where L is an easily replaced ligand. Previous syntheses of pure RuPcs have been difficult, tedious and suffered from low yields (4, 5). The synthetic pathway that we developed for the RuPcs is summarised here in Figure 2.

A template reaction for the formation of ruthenium phthalocyanine occurred in pentanol in the presence of a base. Under these conditions, but without a metal, phthalocyanine did not form. Using $RuCl_3.xH_2O$ directly as the metal template resulted in low yields of highly contaminated products.

When an anhydrous "ruthenium blue" solution, (made by boiling hydrated ruthenium chloride, $RuCl_3.xH_2O$, in pentanol until it was completely blue and no water remained) was used as the ruthenium source, the reactions for the formation of phthalocyanine were essentially quantitative. Insoluble $RuCl_2$(phthalonitrile)$_4$, **A**, was formed initially when the "ruthenium blue" solution was treated with phthalonitrile, and could be isolated in good yield. This was then converted into a RuPc over a period of several days.

The base used in these reactions was often co-ordinated to the ruthenium in the final product. With ammonia this resulted in the formation of the previously unreported bis(ammine) complex, $(Pc)Ru(NH_3)_2$, as a blue insoluble solid. The formation of this insoluble $(Pc)Ru(NH_3)_2$ was an essential ingredient to the success of the reaction shown in Figure 2. It allowed the bulk of the impurities to be easily washed away after the formation of RuPc. The

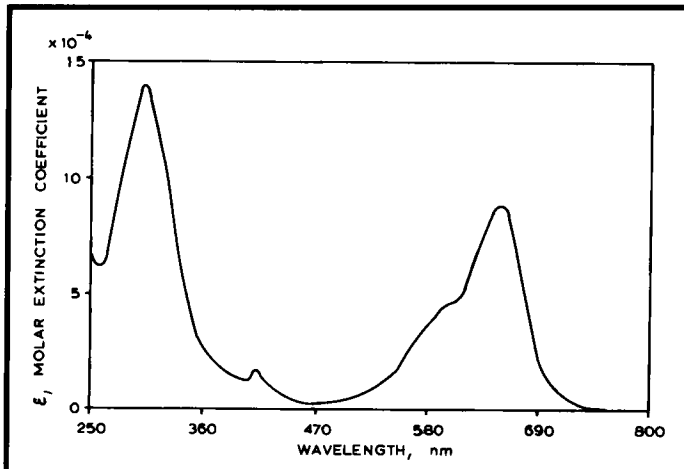

Fig. 3 The UV-visible spectrum of JM 2929 in water

reaction of $(Pc)Ru(NH_3)_2$ with neat benzonitrile at reflux temperature gave the new complex $(Pc)Ru(PhCN)_2$, **B**, in high yield. The high solubility, high purity, and the relative lability of the axial ligands of $(Pc)Ru(PhCN)_2$ made it particularly suitable as a starting material for the synthesis of water soluble $(Pc)RuL_2$ complexes.

The first water soluble RuPc complex which was synthesised employed the water soluble phosphine ligand, triphenylphosphinemonosulphonate (TPPMS). The reaction of the tetrabutylammonium (TBA) salt of TPPMS with $(Pc)Ru(PhCN)_2$ resulted in a high yield of $(TBA)_2[(Pc)Ru(TPPMS)_2]$, which was converted to water soluble $K_2[(Pc)Ru(TPPMS)_2]$, JM 2929.

JM 2929 has a typical optical metal phthalocyanine spectrum, which is shown in Figure 3. The Q-band absorbance, important for PDT work, is at 652 nm with an extinction coefficient of 9.0×10^4/M cm. The compound has been characterised by elemental analysis, ^1H and ^{31}P NMR spectroscopies, field desorption mass spectroscopy and high-performance liquid chromatography (6).

Solution studies of JM 2929 have shown that the complex forms a five-co-ordinate species, [(Pc)Ru(TPPMS)], in aqueous solution. Thus a wide variety of different water solubilising ligands, including those of sulphonate and carboxylate substituted pyridines, nitriles, amino acids and other water soluble phosphines, have subsequently been evaluated (6).

Biological Studies

Our first indication that JM 2929 possessed interesting photosensitising properties came from a study in which its cytotoxicity, in the absence of light, was compared with its phototoxicity to HeLa (HeLa cells are a standard tumour cell line commonly used in cancer research) cells *in vitro* (7), as shown in Figure 4. Whereas concentrations of more than 10^{-5} M of JM 2929 are non-toxic to HeLa cells, a 10^{-6} M concentration of the compound, in combination with light of wavelength 650 nm killed 50 per cent of the cells. Identical results were obtained with the isolated five-co-ordinate complex.

Subsequent studies showed that the light mediated cytotoxic effects of JM 2929 were dependent upon the presence of oxygen, as is the case with most PDT agents. Preliminary studies of the toxicity indicated that the compound could be safely administered to animals at a sufficiently high dose to ensure a photodynamic effect on the cancerous irradiated tissue. Moreover, using the RIF-1 tumour model in mice, biodistribution studies showed ratios for the tumour:surrounding muscle of approximately 7, indicating there is selective uptake in the target tissue.

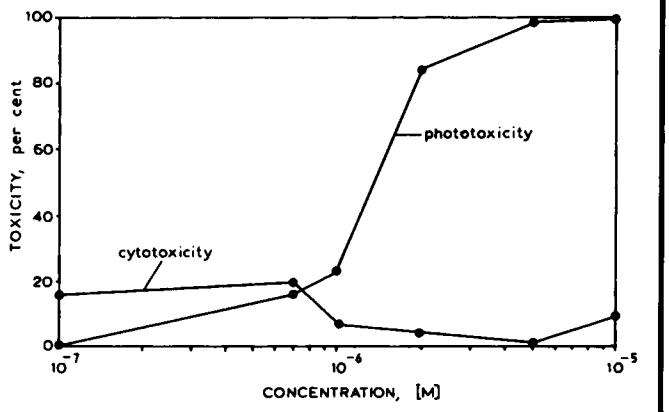

Fig. 4 Phototoxicity and cytotoxicity of JM 2929 to HeLa cells, the standard human tumour cell line

Actual PDT experiments were conducted using the RIF-1 tumour model in mice. It was found that a dose of 20 mg/kg of JM 2929 administered intraperitoneally, followed by 150 J/cm^2 of 650 nm laser irradiation at 4, 24, 72 or 168 hours after treatment with the drug resulted in the complete destruction of the tumour, with no regrowth, see Figure 5. Neither light nor drug alone had any effect on the tumour. Preliminary experiments indicate that the photosensitivity of skin due to JM 2929 is much less pronounced than it is due to Photofrin™.

Mechanism of the Action

The mechanism by which PDT agents cause tumour destruction is most likely to be a combination of the direct killing of tumour cells and a shut-down in the vascular supply to the tumour (8). Cytotoxicity resulting from PDT treatment is most probably the result of photo-oxidation reactions. The excited sensitiser can induce photochemical damage by two major pathways (9). Type I photo-oxidation involves the reaction of photoexcited sensitiser with a substrate molecule, by a mechanism which involves hydrogen or electron transfer, to yield radical species that react further with oxygen. In the Type II photo-oxidation reaction the triplet excited state of the sensitiser transfers energy to molecular oxygen to produce singlet oxygen, $^1O_2$, which is a highly reactive species that can oxidise biological molecules. Recent work has shown that JM 2929 does not produce singlet oxygen upon irradiation in aqueous solution (10). This is in contrast

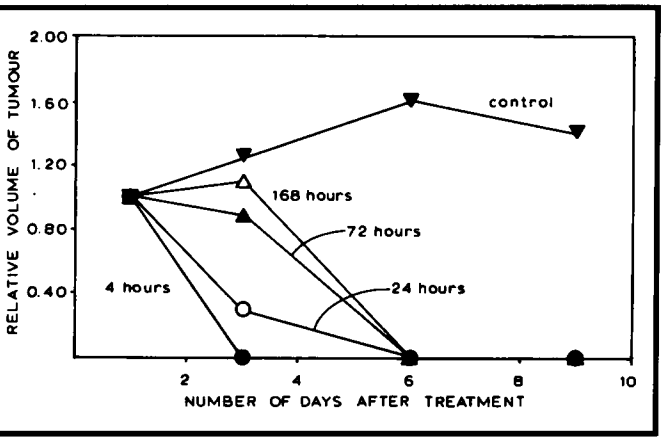

Fig. 5 The effect of JM 2929 photodynamic therapy on the RIF-1 tumour in mice at various times between drug dosing and visible light irradiation

to the large number of Pc and MPc PDT agents studied to date, all of which produce $^1O_2$ in solution. JM 2929 is, to our knowledge, the first transition metal MPc to show PDT activity. The exact nature of the Type I reaction which is involved in the JM 2929 mediated phototoxicity is currently being investigated.

Conclusions

Novel water soluble RuPcs sensitisers have been synthesised using the versatile precursor (Pc)Ru(PhCN)$_2$. These new complexes can be easily prepared in high yield as non-isomeric pure compounds. The biological properties of one of these complexes, JM 2929, have been extensively studied and it has been found to be a potent PDT agent both *in vitro* and *in vivo*. The development of JM 2929 and other ruthenium based sensitisers is continuing towards clinical evaluation.

Acknowledgements

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The Published Platinum Metal Alloy Systems

Phase Diagrams of Precious Metal Alloys, First Supplement

COMPILED BY HE CHUNXIAO, ZHOU YUEHAU AND WANG WENNA

The Metallurgical Industry Press, People's Republic of China, 1993, 340 pages, U.S.$60

Some ten years ago, an important compilation of phase diagrams of precious metals alloy systems which had been published prior to 1976, was brought to the attention of readers (*Platinum Metals Rev.*, 1984, **28**, (3), 108). During the years 1976 to 1985 there were significant developments in the study of precious metal alloy phase diagrams, and by the end of 1985 the number of known systems had reached 754, mainly ternary and quaternary systems. In order to meet the needs of researchers, a First Supplement to the former book – which was published in 1983 – has now been compiled (mainly in Chinese but supported where necessary in English). This supplement collects together information on 380 systems of 641 phase diagrams published from 1976 to 1985, including 150 binary, 212 ternary and 15 quaternary systems that contain a precious metal. Of these over 85 binary, 160 ternary and eight quarternary systems involve the platinum group metals.

It is worth noting that the systems occurring in both the earlier book and this supplement are listed in the contents section of the latter. In addition to over 1765 binary and ternary compounds of the precious metals, their structures and crystal lattice constants are given, together with supporting references.

This First Supplement will be a valuable reference book for people working in the field of precious metal alloys. Both it and the earlier 1983 publication can be purchased from The Metallurgical Industry Press, Mr. Zhang Wei, Beijing 100009, People's Republic of China, or contact direct Prof. He Chunxiao, Institute of Precious Metals, Kunming 650221, People's Republic of China. L.G.-F.