

Ruthenium, Nitric Oxide and Disease

A NOVEL INORGANIC CHEMISTRY APPROACH TO DRUG DESIGN

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Recent discoveries in the biological sciences have revealed that nitric oxide has a number of important physiological functions and is also implicated in a number of diseases. This realisation has provided a stimulus for nitric oxide related drug research and development. In this review new work aimed at the development of potential therapeutic use of ruthenium compounds, particularly ruthenium(III) polyaminocarboxylate, JM1226, is presented and discussed in the context of the chemistry and biology of nitric oxide. Based on these studies, prospects for the further development of JM1226 appear promising.

Nitric oxide (nitrogen monoxide, NO) is commonly viewed as being a toxic environmental pollutant. This has been the major motivation for research into both its chemical reactivity and biological activity. Nitric oxide is one of the major emissions from car exhausts and for many scientists the co-ordination chemistry of the platinum group metals and nitric oxide is synonymous with the development of the automobile catalytic converter. However, new developments in the biological sciences have now allowed us to look at some of the well known chemistry of transition metals and nitric oxide from a different perspective.

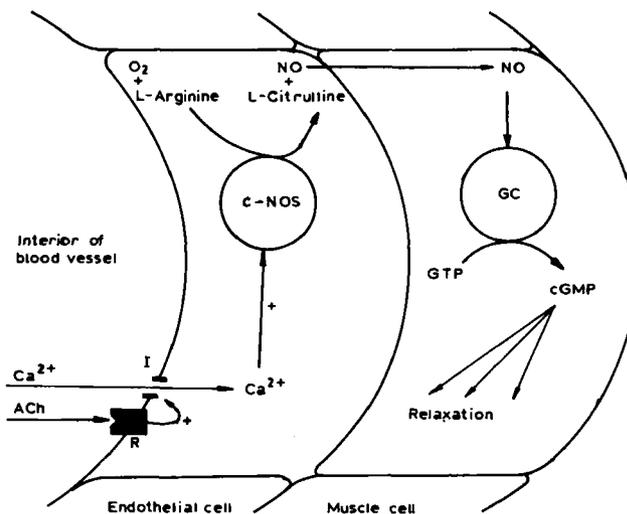
Nitric Oxide in Mammalian Cells

A major conceptual change has taken place in the biological sciences following the recent discovery that nitric oxide is synthesised and secreted by a number of mammalian cells (1, 2). Nitric oxide has been found to be an essential component of many physiological processes, such as the regulation of cardiovascular function, signalling between nerves in both the peripheral and central nervous system and mediating host defence against microorganisms and tumour cells. The biological activity of nitric oxide has been shown to be mediated by the nitrosylation of iron in iron-containing proteins. Many fundamental biological processes, such as regulation of blood pressure, learning and

memory, defence against microorganisms and tumours, and penile erection, are now known to be dependent upon the formation of metal-nitrosyl complexes. A breakdown in the regulation of nitric oxide metabolism has been implicated in a number of diseases, including hypertension, epilepsy, diabetes, arthritis and septic shock.

The Biomedical Technology Group at Johnson Matthey has made a major contribution to the research and development of metal-based drugs, most notably in the field of the platinum anticancer drugs (3, 4). Recently, we have adopted a novel approach to the problem of nitric oxide-mediated disease, by viewing nitric oxide as a ligand for transition metals, in particular ruthenium. This paper presents the background to this approach, together with a brief review of the chemistry of nitric oxide and an historical account of the discovery of the biological action of nitric oxide. The role of nitric oxide both in maintaining normal function and in the pathogenesis of disease is also reviewed. The mechanism by which nitric oxide exerts its biological effects is discussed, based upon our knowledge of its chemical reactions in a biological environment, and the application of this knowledge in the search for new drugs to treat nitric oxide-mediated diseases is described. New data from our laboratory are presented, demonstrating the potential therapeutic use of ruthenium

Fig. 1 A cross-section through an artery, showing the processes occurring in an internal endothelial cell and an adjacent muscle cell. Nitric oxide is synthesised in response to stimuli such as the neurotransmitter acetylcholine (ACh). Acetylcholine binds to a receptor (R) on the surface of an endothelial cell. This opens an ion channel (I) to allow calcium to enter the cell. This causes an increase in intracellular calcium which activates the constitutive nitric oxide synthase (c-NOS). The nitric oxide produced diffuses out of the endothelial cell into the muscle cell. Nitric oxide activates the enzyme guanylate cyclase (GC) which synthesises cyclic guanosine monophosphate (cGMP). The cGMP in turn activates a series of events leading to muscle relaxation, vasodilation of the blood vessel and a lowering of blood pressure



complexes as nitric oxide scavengers in septic shock, and possible future developments are discussed in the light of progress made to date.

The Chemistry of Nitric Oxide

Nitric oxide is a colourless gas at room temperature and is soluble in water. It has an electronic configuration of $(\sigma_1)^2(\sigma_1^*)^2(\sigma_2, \pi)^6(\pi^*)$ (5). The unpaired electron means that nitric oxide is formally a radical and is responsible for the unique properties of that molecule. Unlike most radicals, nitric oxide does not readily form dimers. This is because the unpaired electron cancels out the effect of the bonding electrons, giving a bond order of 2.5, which in a dimer would remain unchanged.

Nitric oxide is very reactive and in the gas phase will react very rapidly with oxygen to form nitrogen dioxide (NO_2). The mixture of nitrogen oxides (NO_x) emitted from the exhaust of internal combustion engines which are not cleaned up by catalytic converters, is of course, a major contribution to environmental pollution. Nitric oxide is paramagnetic but does not have an electron paramagnetic resonance (EPR) spectrum in solution. However, it does form paramagnetic

metal complexes and this property has been used to study haem iron in haemoglobin and more recently the interaction of nitric oxide with metalloproteins in biological environments (6).

The co-ordination chemistry of nitric oxide is similar to that of carbon monoxide, since both are π -acceptor ligands; however, nitric oxide has one more electron than carbon monoxide and this occupies a π^* orbital. The existence of metal nitrosyl complexes has been known for over a century but they have not been so extensively studied as their carbonyl counterparts. One reason for this is that the M-NO bond (M is metal) is very stable in most cases, which results in the metal nitrosyls being generally unreactive compared with M-CO complexes. Nitric oxide can form three types of metal-nitrosyl complex with:

- (i) linear, terminal, M-N-O groups
- (ii) bent, terminal, M-N-O groups and
- (iii) bridging NO groups.

Nitric oxide can lose the electron in the π^* orbital to form the nitrosonium ion, NO^+ . This will form linear NO complexes where the ligand is formally NO^+ .

In the bent, terminal complexes, having bond angles of 120 to 140°, the ligand can be

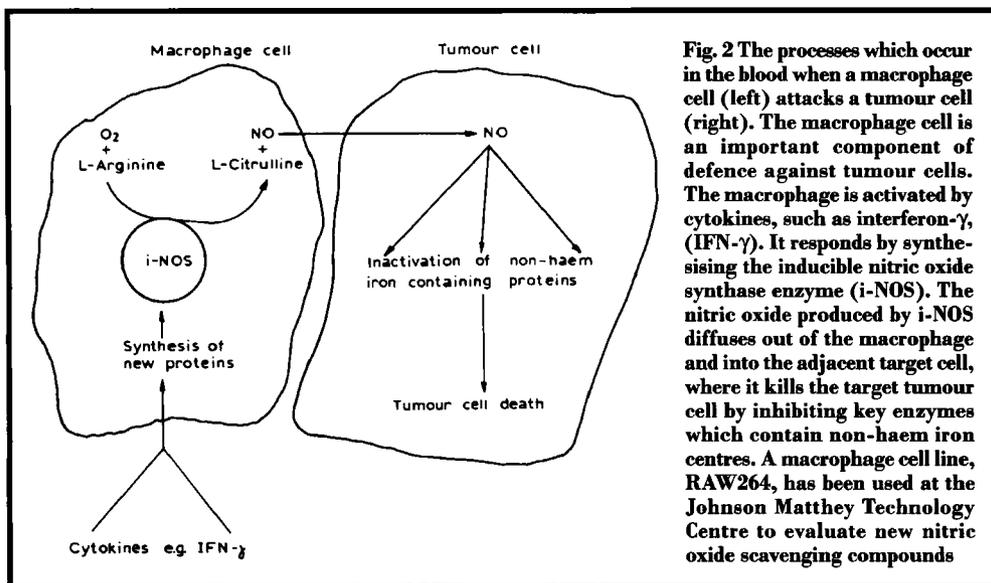


Fig. 2 The processes which occur in the blood when a macrophage cell (left) attacks a tumour cell (right). The macrophage cell is an important component of defence against tumour cells. The macrophage is activated by cytokines, such as interferon- γ , (IFN- γ). It responds by synthesising the inducible nitric oxide synthase enzyme (i-NOS). The nitric oxide produced by i-NOS diffuses out of the macrophage and into the adjacent target cell, where it kills the target tumour cell by inhibiting key enzymes which contain non-haem iron centres. A macrophage cell line, RAW264, has been used at the Johnson Matthey Technology Centre to evaluate new nitric oxide scavenging compounds

represented as NO⁻. This is an oversimplification of the situation (7, 8) but it provides us with a useful way of discussing the reactivity of nitric oxide with metals.

In subsequent sections we will look at the mechanism by which nitric oxide exerts its biological effects, by the nitrosylation of iron centres in proteins, and at the application of ruthenium compounds as scavengers of nitric oxide in disease.

The Biology of Nitric Oxide

The realisation that nitric oxide could have a positive biological function resulted from several convergent lines of research carried out in laboratories throughout the world (2).

Organonitrate compounds such as glyceryl trinitrate (nitroglycerin) have long been used in the treatment of angina. These compounds cause relaxation of arterial blood vessels (vasodilation) and act by releasing nitric oxide. A number of compounds which occur naturally in the body, such as the neurotransmitter acetylcholine (a chemical signalling molecule), also cause vasodilation. Arteries can be thought of as two concentric tubes, see Figure 1. The outer tube consists of muscle which is responsible for constriction and dilation of the artery. The inner

tube, the endothelium, consists of a layer of cells called endothelial cells.

In 1980 Furchgott demonstrated that for acetylcholine to function as a vasodilator the artery must have an intact endothelium (9). The implication was that acetylcholine acted on the endothelial cells, these in turn produced a second chemical signal which triggered the relaxation of the muscle layer. This chemical was called endothelium derived relaxing factor (EDRF). Two independent groups (those of Furchgott and Ignarro) proposed that EDRF was actually nitric oxide (2) and in 1987 Ignarro's laboratory and a research group at the Wellcome laboratories confirmed this hypothesis (10, 11).

Functions of Nitric Oxide Within the Body

It is now known that nitric oxide plays a key part in the regulation of blood pressure as a component of an integrated control mechanism.

In 1988 it was found that nitric oxide could also function as a neurotransmitter (1, 2). The two major neurotransmitters in the peripheral nervous system are acetylcholine and noradrenaline. These chemicals act as messengers at nerve/nerve and nerve/muscle junctions. However, some peripheral nerves were known

to use neither noradrenaline (nonadrenergic, NA) nor acetylcholine (noncholinergic, NC), and these were named NANC nerves.

NANC nerves are found in many of the bodily tissues including gastrointestinal tract, respiratory tract, cardiovascular muscle and the urogenital system. Nitric oxide was discovered to be the neurotransmitter for NANC nerves.

The involvement of nitric oxide in penile erection has received much attention. This is mediated by NANC nerves which release nitric oxide causing relaxation of the smooth muscle of the corpus cavernosum and increased blood flow leading to penile erection.

Nitric oxide is also a neurotransmitter in the brain. Its exact function in the brain is unknown but one hypothesis is that it may be involved in long-term experience and memory. All previously known chemical signalling molecules, such as neurotransmitters and hormones, were either small organic molecules like acetylcholine, or peptides and proteins. The discovery that a small, diatomic, inorganic molecule is a biological signalling agent has completely changed the way in which we now think about the mechanism of regulation of many vital bodily functions.

The study of the immune response (the body's defence against foreign cells such as bacteria, viruses and tumours) led to the discovery of yet another role for nitric oxide (12). It was known that the urinary excretion of nitrite and nitrate, which are the end products of the oxidation of nitric oxide in an aqueous biological envi-

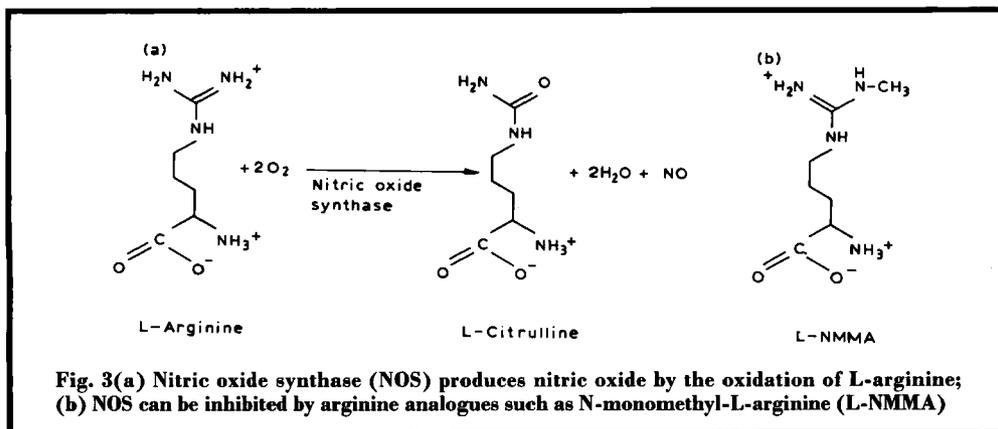
ronment, increased during infection. Other studies had demonstrated that the mechanism by which macrophages (one of the cells of the immune system) killed cells was by targeting intracellular iron. This effect was dependent upon the presence of L-arginine (an essential amino acid) but it could be reproduced by nitric oxide. It was concluded that the macrophages were using nitric oxide to kill the target cells, see Figure 2.

Nitric Oxide Synthase (NOS)

These findings also enable us to account for the biological source of nitric oxide. Nitric oxide is synthesised in cells by the enzyme nitric oxide synthase (NOS) which catalyses the oxidation of L-arginine to L-citrulline and nitric oxide, see Figure 3(a). There are a number of known inhibitors of nitric oxide synthase, many of which are substrate analogues of L-arginine, for example N⁰-monomethyl-L-arginine (L-NMMA), see Figure 3(b). These inhibitors have been used extensively as tools in the study of the biological action of nitric oxide, as discussed below.

The unique enzyme, NOS, has a complex reaction mechanism and many of its details are still unknown (13, 14). There are a number of isoforms of nitric oxide synthase and the simplest classification divides them into two main classes: the constitutive and the inducible forms.

The constitutive enzyme, c-NOS, as its name suggests, is always present in the cell. It is dependent upon calcium for its activity and it is



regulated by intracellular calcium concentrations. As an example, the neurotransmitter acetylcholine causes a transient increase in the calcium concentration in endothelial cells which activates c-NOS, leading to a burst of nitric oxide production. The local concentration of nitric oxide produced by c-NOS is low (of the order of nM), which is compatible with the function of nitric oxide as a chemical messenger.

The second form of the enzyme is the inducible form, i-NOS. This isoform is calcium independent and is synthesised by cells, for example macrophages, in response to external stimuli. It is regulated by turning on protein synthesis (transcription). The synthesis of i-NOS by macrophages is stimulated by components of the bacterial cell wall (lipopolysaccharide and endotoxin) and by cytokines (peptides that regulate the immune response) such as interferon- γ . Once i-NOS has begun to produce nitric oxide it will continue to do so for several hours and at concentrations (of the order of μ M) that are high enough to be toxic to the target cell.

Diseases Caused by Dysfunction in Nitric Oxide Metabolism

Since it is now known that nitric oxide has this multifunctional role in the human body, it is not surprising that dysfunctions in nitric oxide metabolism have been found in a number of diseases (1, 2). For example, it has been proposed that an impairment in c-NOS activity in endothelial cells is a contributory factor in essential hypertension.

Hypertension can be treated clinically with nitric oxide donor drugs, such as the organonitrates. An inorganic nitric oxide donor drug, sodium nitroprusside, is used in emergency cases. However, the overproduction of nitric oxide is toxic to the body. The production of excessive, toxic quantities of nitric oxide is a major contributory factor in diseases such as diabetes, arthritis, inflammation, epilepsy and septic shock.

The disease known as septic shock is caused by very high levels of bacteria circulating in the blood. This triggers the immune response, including the stimulation of macrophages and

other cells to synthesise i-NOS, which leads to an increase in the concentration of nitric oxide. This has a two-fold effect. First, the toxic levels of nitric oxide can cause tissue damage. Second, and more important, the nitric oxide also causes vasodilation, which leads to a severe drop in blood pressure and vascular collapse. This situation is made even worse by the fact that such patients also fail to respond to the vasoconstrictor drugs which are normally used to raise blood pressure. The lack of response to these drugs is also believed to be due to nitric oxide. Septic shock is fatal in more than 50 per cent of cases, therefore the search for new drugs that can reduce nitric oxide levels in this and in other diseases is a major challenge for medicinal chemists and is a potentially profitable area for the pharmaceutical industry.

The Biological Chemistry of Nitric Oxide

Nitric oxide mediates these multiple and diverse biological events by the nitrosylation of iron. However, the control of biological systems at the molecular level is worth considering, in order to put our understanding of the biological actions of nitric oxide into a wider context.

A mammalian cell receives an external signal, such as a hormone, for example adrenaline, and the result of this is seen as a biological event, in this case an increase in the metabolism of carbohydrate to provide energy for the "fright, fight or flight" response. There is a sequence, or pathway, of intracellular biochemical events between receipt of the external signal and the response. The study of the biochemistry of cell-signalling pathways is an area of intense activity, but in spite of this only some of the steps in the sequence are known.

A key step in a number of cell-signalling pathways is the formation of the second messenger molecule, cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP); cGMP then goes on to activate other biochemical actions in the signalling sequence.

This biochemical reaction (GTP to cGMP) is catalysed by the enzyme guanylate cyclase (GC)

see Figure 1. One of the significant observations made in the early stages of the research into nitric oxide was that nitric oxide increased the activity of guanylate cyclase in the arterial smooth muscle cells.

It is notable that this enzyme contains haem (1, 2). Haem is a complex of the tetradentate protoporphyrin ligand with Fe(II) and is found in a number of enzymes and the oxygen carrier protein haemoglobin.

Nitric Oxide Reactions in the Body

Nitric oxide will form complexes with Fe(II) haem and Fe(III) haem (6, 15). In haemoglobin (Hb) the normal oxidation state is Fe(II) (d^6) and in this case nitric oxide acts as a two electron donor to form Hb(Fe(II))NO. The remaining unpaired electron makes the molecule paramagnetic, which allows it to be detected by EPR. This has been exploited in the investigation of the biological properties of nitric oxide. The HbNO can be further oxidised in the presence of oxygen to methaemoglobin (HbFe(III)). Nitric oxide will also bind loosely to HbFe(III) (d^5) and reduce it slowly to Hb(Fe(II))NO.

Haem-ligand interactions are influenced by the local environment provided by the host protein. The important conditions are hydrophobicity/hydrophilicity of the surrounding amino acids, steric effects and the axial ligands. The latter may be particularly important for the activation of guanylate cyclase. It is known that both oxygen and carbon monoxide bind more strongly to haem when a basic ligand, histidine in haemoglobin, occupies the fifth co-ordination position, whereas nitric oxide binding to haem is enhanced when this position is empty. One possible explanation for the activation of guanylate cyclase is that when nitric oxide binds to the iron haem the result is an out-of-plane movement of the iron to give a haem core similar to that of a free porphyrin (15). There are still many questions to be answered concerning the mechanism of activation of guanylate cyclase by nitric oxide, not least the oxidation state of the iron in the haem group of guanylate cyclase.

A knowledge of the interaction between nitric oxide and haemoproteins is important for our

understanding of the aqueous chemistry of nitric oxide in a biological environment. The gas phase chemistry of nitric oxide is well documented but extrapolation to the aqueous phase is misleading. It is now known that nitric oxide reacts with oxygen in an aqueous environment to give nitrite (16). Nitric oxide will also react with oxyhaemoglobin to give methaemoglobin and nitrate.

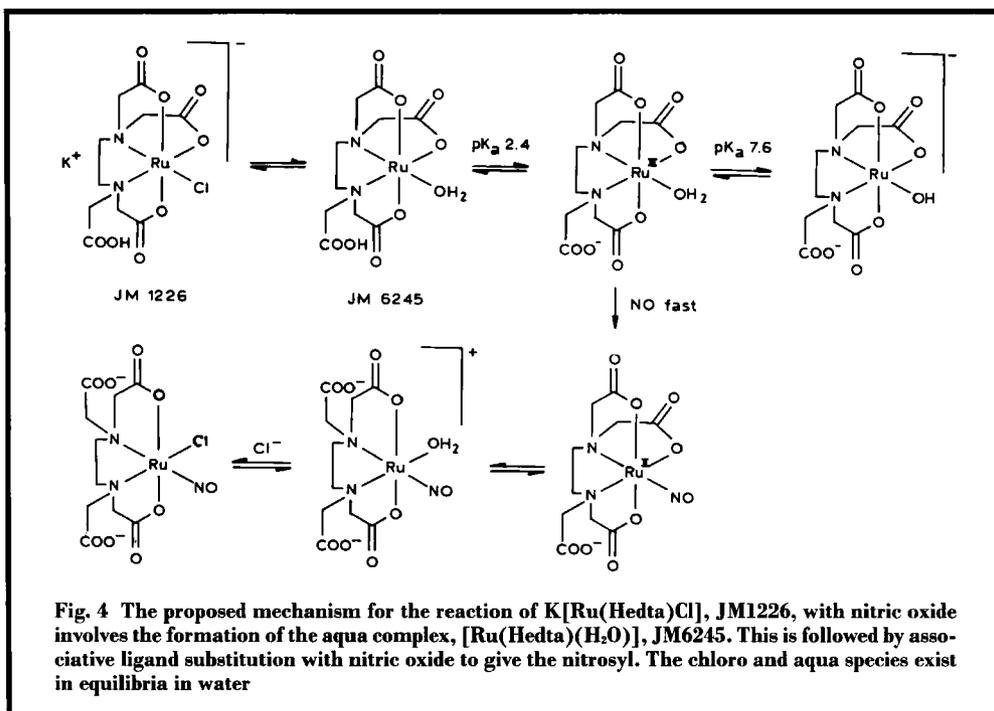
The nature of the oxidation products of nitric oxide is therefore dependent upon the oxygen status of the environment (6). In venous blood nitric oxide is converted primarily to NO_2^- , whereas in arterial blood (where oxyhaemoglobin is present) the main product is NO_3^- . These reactions of nitric oxide account for the raised levels of nitrate and nitrite seen in patients with bacterial infections (due to the elevated levels of nitric oxide produced by cells, such as macrophages).

Nitric Oxide Effects on Tumours

The toxicity of nitric oxide towards bacteria and tumours can also be explained by the formation of metal-nitrosyl bonds. In tumour cells the intracellular targets that macrophages attack have been identified (12). Three key proteins involved in energy metabolism were shown to be selectively inhibited; these were: aconitase, complex I and complex II. All contain non-haem iron as iron-sulphur clusters, for example Fe_4S_4 in aconitase. Nitrosylation of the iron in these enzymes has been demonstrated by EPR. Ribonucleotide reductase, a controlling enzyme in DNA synthesis, was also inhibited. This enzyme contains an Fe_2O centre, and the nitrosylation of this iron centre may be one mechanism by which nitric oxide inhibits this enzyme.

Ruthenium Complexes as Nitric Oxide Scavengers

The realisation that nitric oxide is involved in disease has provided a new focus for pharmaceutical research. The traditional approach to research by a medicinal chemist is via organic chemistry, and many researchers are at present engaged in investigating the use of inhibitors of the nitric oxide synthase enzyme.



Septic shock is being studied in a number of laboratories and the arginine analogue inhibitors of NOS have been studied clinically against this disease (17). However, they have a number of side effects, mainly affecting the local blood circulation within organs, particularly the lungs (18). This is because in addition to inhibiting the formation of the undesirable excess quantities of nitric oxide produced by i-NOS, they also inhibit the production of nitric oxide by c-NOS of endothelial cells. Many commercial and academic laboratories have attempted to produce selective inhibitors of i-NOS but to date there has been little success reported.

However, in the Biomedical Technology Group at Johnson Matthey we have pursued a different approach to the problem by exploiting our knowledge and expertise in co-ordination chemistry and inorganic pharmaceuticals. Armed with the knowledge that nitric oxide can act as a ligand for transition metals, we have examined metal complexes as possible scavengers for the therapy of nitric oxide-mediated disease.

Ruthenium has a rich co-ordination chemistry

(19) and complexes containing nitric oxide have been known for well over a hundred years; $K_2[Ru(NO)Cl_5]$, for instance, was isolated in the mid-nineteenth century. Ruthenium readily forms nitrosyls and there are more known nitrosyl complexes of ruthenium than of any other metal (7). The Ru-NO bond is generally very stable and as a ligand nitric oxide is not easily displaced, persisting through a variety of substitution and redox reactions.

Ruthenium (III) (d^5) will react with nitric oxide to form six co-ordinate mononitrosyl complexes with a linear Ru-N-O group. The Ru(III) is reduced to Ru(II) with the ligand being formally NO^+ (as mentioned earlier this is an oversimplification and ignores the structural problems associated with nitrosyl chemistry). Therefore ruthenium appeared to be a good candidate as the basis of a nitric oxide scavenger.

Drug Design

When designing drugs a number of factors have to be taken into consideration (20). These include the necessity that the chosen compounds

must be of low toxicity and that the distribution of the drug within the body and its excretion from the body must be controllable (pharmacokinetics). Toxicity and pharmacokinetics are interrelated and both depend upon the chemical properties of the compound, such as its solubility and its chemical reactivity.

The chemical properties of the transition metals are well suited to deal with the problems of drug design: transition metals have variable oxidation states, ligands are co-ordinated in a precise spatial arrangement and can be chemically modified so enabling the properties of the complex to be fine-tuned to control the pharmacological interactions in a biological system. For a nitric oxide scavenger we require a ligand set which is itself stable but which promotes specific binding of nitric oxide. Generally ligands around ruthenium(III) are kinetically inert and there is another benefit in that the formation of the Ru-NO bond further stabilises the *trans* ligand in the resulting ruthenium(II) complex.

Ruthenium(III)polyaminocarboxylates

One class of ligand that satisfies most of these requirements is the polyaminocarboxylates (21). The parent compound $K[Ru(Hedta)Cl]$ (JM1226) has emerged as the potential front runner in the search for a metal-based nitric oxide scavenger, see Figure 4. The ligand, ethylenediaminetetraacetic acid (edta), is pentadentate leaving one co-ordination position available for substitution by nitric oxide. The complex is water soluble and should therefore remain in the blood and not cross lipophilic cell membranes.

This has two important consequences: it should have a good pharmacokinetic profile, such as: be rapidly excreted in the urine, only have access to the excess nitric oxide in the bloodstream, and not be available to the nitric oxide produced by endothelial cells responsible for regulating blood pressure.

Initial evidence for the nitric oxide binding ability of this compound was provided by a chemical study in which nitric oxide gas was introduced above a stirred solution of the test compound in a closed apparatus. A decrease in

pressure was measured and taken to indicate that nitric oxide had been removed. At the end of the experiment the reaction mixture was freeze dried and analysed by infrared spectroscopy. A peak at 1897/cm, which is characteristic of the linear Ru-N-O bond, was indicative that nitric oxide had been bound and that the ruthenium(II) nitrosyl compound had been formed.

The proposed mechanism for this reaction involves the formation of the aqua species $[Ru(Hedta)(H_2O)]$, JM6245, which takes place readily at physiological pH (22), see Figure 4. This is followed by associative ligand substitution with nitric oxide to give the nitrosyl. The pendant carboxylate group may facilitate this substitution (23), since if the carboxylate is replaced by an alcohol group there is a reduction in the activity which is observed during the biological tests (see below).

Kinetic studies performed at the University of Essex, using stopped-flow equipment, indicate that the rate of formation of the nitrosyl is so fast that the reaction takes place within the dead time of the instrument (> 2 ms) at room temperature. Measurements made at 6.5°C give a rate constant of $2.6 \times 10^7/Ms$.

JM1226 Pharmacological Activity

The next step was to investigate the ruthenium compound in a simple biological system. We exploited the fact that macrophage cells produce nitric oxide in response to the bacterial cell wall component, lipopolysaccharide, and to interferon- γ . We used a macrophage cell designated RAW264 which can be cultured in the laboratory (24). The nitrite in the culture medium was assayed as a measure of the amount of nitric oxide produced by the cells. It was found that the level of nitric oxide could be reduced by the i-NOS enzyme inhibitor L-NMMA (the control experiment) and also by JM1226, see Figure 5(a).

In order to confirm the binding properties of JM1226 for nitric oxide in our biological system, we also made use of the ability of macrophages to kill tumour cells. We demonstrated that RAW264 cells could kill the mouse tumour cell P815 and that this was mediated by

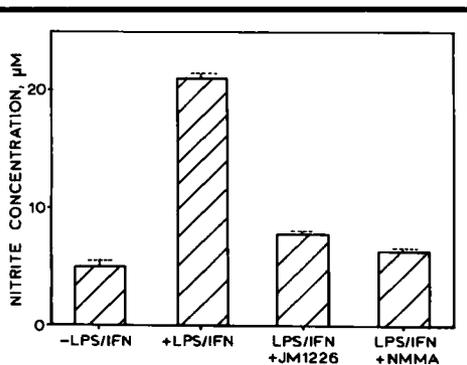
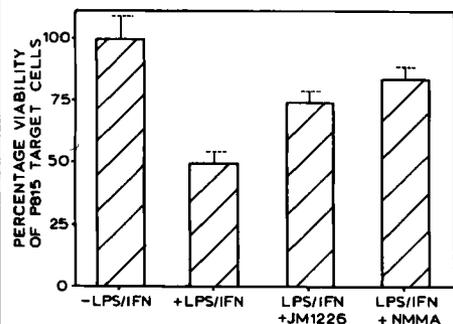


Fig. 5 The RAW264 macrophage cell line used as a biological system for testing the ability of ruthenium(III) compounds to scavenge nitric oxide. RAW264 cells produce nitric oxide when activated with lipopolysaccharide and interferon- γ (+LPS/IFN).

(a) JM1226, $K[Ru(Hedta)Cl]$, at a concentration of $100 \mu M$ can reduce the level of measurable nitrite, produced by oxidation of nitric oxide in the cell culture medium, showing the potential to scavenge nitric oxide in a biological environment. Background levels were determined in the absence of activators (-LPS/IFN). The nitric oxide synthase inhibitor, L-NMMA, a positive control at a concentration of $250 \mu M$, also reduced nitrite levels. Error bars represent a standard error of mean $n = 3$



(b) JM1226, $K[Ru(Hedta)Cl]$, can protect against nitric oxide-mediated cell killing. The nitric oxide generator cell was cocultured with the P815 tumour cell. Activated RAW264 cells killed 50 per cent of the P815 cells. Viability in the presence of $100 \mu M$ JM1226 was raised to 75 per cent. Error bars represent a standard error of mean $n = 6$

nitric oxide. The removal of nitric oxide should have a protective effect, and in the control experiment the enzyme inhibitor L-NMMA inhibited cell killing. More importantly JM1226 was

also able to protect the P815 cells against RAW264 nitric oxide-mediated cell killing, see Figure 5(b). Similar results were obtained with the aqua species $[Ru(Hedta)(H_2O)]$.

Having demonstrated that we had a potential nitric oxide scavenger we then needed to get closer to the real life situation. Collaborators were using isolated rat tail arteries to investigate the vasodilatory properties of nitric oxide donors (25). One commonly used nitric oxide donor is the nitrosothiol S-nitroso-N-acetylpenicillamine (SNAP). This compound will break down in water to release nitric oxide. The addition of SNAP to an isolated artery preparation was found to cause it to relax or vasodilate in a dose-dependent manner with an ED50 (the concentration giving a 50 per cent relaxation) of $6.0 \mu M$. Haemoglobin, which forms an iron-nitric oxide complex, will attenuate this response.

When JM6245, the aqua derivative of JM1226, was tested at a concentration of $10^{-4} M$ in the isolated artery model the SNAP response was attenuated, giving an ED50 of $1.8 mM$, which is a 300-fold inhibition in the response of the artery. Using the isolated artery model, it has also been shown that JM6245 will reverse the poor response of the artery to vasoconstrictor drugs, which is a major clinical problem when trying to treat patients with septic shock. We have gone further with this work, demonstrating that JM1226 has a positive benefit in two models of nitric oxide-mediated septic shock (26). In these models of disease the activity displayed by JM1226 is indicative of the potential therapeutic efficacy of the ruthenium(III) polyaminocarboxylates.

Conclusion

The use of inorganic nitric oxide scavengers is a novel approach to the therapy of diseases mediated by excess production of nitric oxide. We have discovered a class of compounds, the ruthenium(III) polyaminocarboxylate complexes, which have pharmacological activity in biological systems ranging from cultured cells through to sophisticated disease models. The project has progressed rapidly through the early stages of pre-clinical research and development

since our patent was filed in 1993 (21). There are many obstacles still to be overcome on the road to clinical success but the indications are positive.

Preliminary studies suggest that these compounds possess low toxicity and many of the desired pharmacokinetic properties. There is plenty of scope for ligand modification and an extensive synthetic programme is enabling new compounds with the potential for better activity to be identified.

Minor chemical modifications are also known to affect activity, thus giving scope for the design of further ruthenium compounds which might be used in the many diverse nitric oxide-medi-

ated diseases. Indeed inorganic medicinal chemistry may provide the key to success in this field of study, where the traditional organic chemistry approach to drug design appears to be struggling; and future prospects appear very encouraging for this potential new addition to the inorganic pharmacopoeia.

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Platinum and Palladium Convert Biofuel By-product

The search for non-polluting automotive fuels has encouraged a growth in alternative fuels, including biofuels such as ethanol and biodiesel. Several countries already use biofuels and in Europe biodiesel is produced from rapeseed oils, yielding up to 14 per cent of potentially valuable glycerol. Now, R. Garcia, M. Besson and P. Gallezot (*Appl. Catal. A: Gen.*, 1995, **127**, (1–2),

165–176) report that they can orientate the selectivity of glycerol oxidation to oxidation of the primary or secondary alcohol function. A 77 per cent selectivity at 90 per cent conversion to glyceric acid on palladium catalysts, and a 55 per cent selectivity to glyceric acid on platinum catalyst were achieved. A bismuth-platinum catalyst achieved a 37 per cent yield in dihydroxyacetone.