The therapeutic application of lanthanides

Simon P. Fricker

Received 11th April 2006
First published as an Advance Article on the web 28th April 2006
DOI: 10.1039/b509608c

The biological properties of the lanthanides, based on their similarity to calcium, have stimulated research into their therapeutic application. Historical medical uses of the lanthanides and recent advances and successes will be described in the context of the biological chemistry of lanthanides, including a new metal-based drug, lanthanum carbonate, which has recently been approved as a phosphate binder for the treatment of hyperphosphatemia. This *tutorial review* will be of interest to those working on metal-based drugs, including inorganic chemists, and biological scientists.

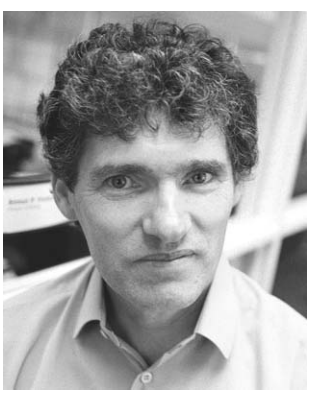
Introduction

The biological properties of the lanthanides, primarily based on their similarity to calcium, have been the basis for research into potential therapeutic applications of lanthanides since the early part of the twentieth century.[1,2] The lanthanides (for convenience abbreviated to Ln) are the group of elements from lanthanum (Z = 57) to lutetium (Z = 71). Though originally described as ''rare earths'' because of their natural occurrence as metal oxides, they are not particularly rare. With similar ionic radii to calcium, but a higher charge, the Ln^{3+} ions have a high affinity for Ca^{2+} sites on biological molecules and hence can act as either Ca^{2+} inhibitors or probes.

One of the earliest therapeutic applications of a lanthanide was the use of cerium oxalate as an anti-emetic.[3] First described in the mid-nineteenth century, formulations of cerium oxalate were available until the mid-twentieth century. The mechanism of action has never been defined, but as cerium oxalate is very insoluble the most likely effect is at a local level in the gastrointestinal tract. Antihistamine drugs eventually replaced cerium oxalate as a treatment for emesis. In the early twentieth century rare earth salts were being used for the treatment of tuberculosis. Later the lanthanides were found to have anticoagulant properties, but development for this indication was hampered by severe side effects. The observation that agents that reduce excessive calcium deposition retard the process of atherosclerotic plaque formation led to the investigation of the anti-atherosclerotic properties of lanthanum chloride. The interaction of the lanthanides with the immune system provided the stimulus for the investigation of a variety of further potential applications.[2]

Drug development is a challenging business and many of these applications have been unsuccessful, with the notable exception of the use of cerium nitrate as a topical cream with silver sulfadiazene for the treatment of burn wounds.[3] However, recently a new application for lanthanum carbonate (Fosrenol) as a phosphate binder for the treatment of hyperphosphatemia in renal dialysis patients has been approved in both the USA and Europe.[4] Fosrenol is the latest metal-based drug to reach the market place, and its approval is particularly significant as it provides an alternative approach to controlling the intake of dietary phosphate without the adverse effects associated with the current aluminium and calcium based phosphate binders. This review will describe recent advances and successes in the therapeutic application of the lanthanides. These applications will be discussed in the context of the biological chemistry of the lanthanides.

Director, Biology, AnorMED Inc., #200 20353 64th Avenue, Langley, BC, V2Z 1A6, Canada. E-mail: sfricker@anormed.com; Fax: 604 530 0976; Tel: 604 532 4653



Simon Fricker

Simon Fricker obtained his PhD from the Department of Chemistry and Molecular Sciences, University of Warwick, UK. This was followed by postdoctoral research at the University of Cambridge, and the University of Southampton. He is currently Director, Biology at AnorMED, a Canadian biopharmaceutical company. Prior to joining AnorMED he was a Principal Scientist with the Biomedical Technology group of Johnson Matthey. He has worked on a variety of research projects on metal-based drugs including the development of Fosrenol (lanthanum carbonate) for treatment of hyperphosphatemia.

Biochemistry of the lanthanides

The lanthanides are generally thought of as the group of elements from lanthanum (Z = 57) to lutetium (Z = 71), though strictly speaking they are confined to the 14 ''4f'' elements following lanthanum in the periodic table.[2] However, lanthanum is generally included in this group and will be considered as one of the lanthanides for the purpose of this review. The lanthanides have an electronic configuration of $[Xe]4f^0$ to $[Xe]4f^{14}$. The ''f'' electrons are relatively uninvolved in bonding, with ion formation involving loss of the ''d'' electron. In physiological solution most of the lanthanides are only stable in the trivalent form (Ln^{3+}), with the exception of Ce and Eu which can exist as Ce^{4+} and Eu^{2+}. A particular feature of these elements is the decrease in atomic size and

radius with increasing atomic number, a property known as the lanthanide contraction.

The lanthanides have similar ionic radii to calcium, but by virtue of possessing a higher charge, they have a high affinity for Ca^{2+} sites on biological molecules, and a stronger binding to water molecules. Their coordination number is variable across the series ranging from 6–12, but with a preferred coordination number of 8–9, compared with calcium which has a preferred coordination number of 6. In addition, their spectroscopic properties, resulting from their unusual electronic configuration, make them a useful probe for calcium in biological systems using techniques such as NMR, luminescence or fluorescence spectroscopy.

Many of the biological properties of the lanthanides are a function of their similarity to calcium.[1,2] One of the major physiological effects of the Ln^{3+} is to block both voltage operated and receptor operated calcium channels.[5] The Ln^{3+} can block the Na^{+}/Ca^{2+} synaptic plasma membrane exchange, and inhibit skeletal, smooth, and cardiac muscle contraction by blocking the the Ca^{2+}-ATPase in the sarcoplasmic reticulum of muscle. The Ln^{3+} ions themselves are unable to cross cell membranes but act by blocking the exterior face of the calcium channel. Though the Ln^{3+} cannot gain access to intracellular organelles, they have been used as biochemical probes to study calcium transport in mitochondria and other organelles. The potency of calcium channel blockade increases with the ionic radius of the Ln^{3+} ion. Blockade of the Type-T voltage gated calcium channel by Ln^{3+} has been ranked in the order $Ho^{3+} \approx Y^{3+} \approx Yb^{3+} \geqslant Er^{3+} > Gd^{3+} > Nd^{3+} > Ce^{3+} > La^{3+}$. Blockade of the vasopressin stimulated Ca^{2+} and Mn^{2+} influx across the hepatocyte membrane, and Ca^{2+}-dependent neurotransmitter release, *e.g.* epinephrine, serotonin and dopamine, are examples of receptor operated calcium channel inhibition. Ln^{3+} can block stretch-activated channels in muscle and this may have therapeutic applications for Duchenne muscular dystrophy; a degenerative muscle disease resulting in death due to respiratory muscle failure, in which stretch-induced muscle damage after exercise contributes to long-term muscle degeneration.[6]

The lanthanides can substitute for calcium in proteins,[2] though it should be noted that the Ln^{3+} can also substitute for other metal ions such as Mg^{2+}, Fe^{3+} and Mn^{2+}. Calcium dependent enzymes can either be inhibited by lanthanides, or in some cases be activated to a similar or greater extent than by calcium. It has been proposed that the stimulatory or inhibitory effect of the lanthanides may be a function of the role of calcium in the native enzyme. Where calcium plays a catalytic role the Ln^{3+} substitution leads to inactivation of the enzyme. In cases of inhibition the degree of inhibition is generally dependent upon the ionic radius of the lanthanide. If the calcium plays a structural role then Ln^{3+} substitution should lead to, at least, retention of activity. Examples of enzymes inhibited by Ln^{3+} include Staphylococcal nuclease, the family of cytochrome P450 xenobiotic metabolizing enzymes, and enzymes involved in blood clotting such as prothrombin activation, and Factor X activation. The latter may be the mechanism whereby Ln^{3+} inhibits coagulation. Enzymes stimulated by Ln^{3+} include trypsin and acetylcholinesterase. The Ln^{3+} will interact with other calcium binding proteins and will bind to the calcium binding protein calmodulin, and cause polymerization of collagen, and G actin. Ln^{3+} can also inhibit calcium-mediated processes associated with immune cell function.[1,2] These properties will be discussed in more detail in the context of the potential therapeutic applications of the lanthanides.

Generally the lanthanides are non-toxic, primarily because they cannot cross cell membranes and are therefore not absorbed if ingested orally. However, they are toxic if administered by the intravenous route whereupon they can gain access to cells expressing calcium channels. Acute toxicity *via* this route can manifest itself by a drop in blood pressure followed by cardiovascular collapse and pulmonary paralysis. Chronic toxicity is generally associated with hepatotoxicity, and edema. After intravenous administration the lanthanides are rapidly cleared from the blood and redistributed to tissues, primarily the liver and bone. The lighter lanthanides initially go to the liver where they are associated with a histopathological phenomenon described as fatty liver. They then rapidly redistribute to the bone with a half-life of approximately 10–20 days. The heavier lanthanides accumulate in the bone where they can reside for considerable period of time with a half-life of several years.[7] It should be noted that these phenomena are seen with intravenously injected lanthanide salts. Chelates such as Gd(DTPA), which is used as an NMR contrast imaging agent, is 50 times less toxic than $GdCl_3$ on a molar basis.[5] It is rapidly cleared with a plasma half-life of 20 minutes, and within 3 hours over 80% is excreted in the urine. This is in contrast to $GdCl_3$ where only 2% is excreted after 7 days. This emphasizes two important points when considering metal toxicity and pharmacology. The first is that the biochemical and physiological effects are dependent upon the chemical form and speciation of the metal, *e.g.* oxidation state, salt or complex. The second, more general point is that toxicity is dependent upon the route of exposure.

Immune function and disease

One of the challenges in attempting to elucidate the effects of Ln on cellular function is their inhibitory action on numerous diverse biochemical processes involving calcium. An excellent example is the reported contradictory effects of Ln on immune function. The lanthanides have been reported to inhibit lymphocyte activation, neutrophil chemotaxis and aggregation, Kuppfer cell activity, histamine secretion from mast cells, reduction of reactive oxygen species (ROS), reduction of histamine and serotonin induced vascular permeability, and reduce carrageenin-induced inflammation.[1,2] Conversely, at low doses they appear to enhance some aspects of the immune response such as antibody formation and lymphocyte activation. The lanthanides have been investigated for the treatment of liver toxicity, artherosclerosis and rheumatoid arthritis. A common theme linking these apparently disparate diseases is the interaction of the lanthanides with components of the immune system.

Rheumatoid arthritis is an inflammatory disease characterized by a progressive erosion of the joints resulting in deformities, immobility and a great deal of pain. Chemical mediators such as prostaglandins, leukotrienes and cytokines

drive this progressive inflammatory response. Tissue erosion is mediated by the release of degradative enzymes such as collagenase, and ROS $OH^{\bullet}$ and O_2^{-}, released by the synoviocytes and infiltrating cells. $PrCl_3$, $GdCl_3$, and $YbCl_3$ were able to reduce carrageenin-induced inflammation, and $GdCl_3$ and $Ce(NO_3)_3$ were able to modulate the levels of the inflammatory cytokines IL-2 and TNF-α associated with toxicant-induced liver damage suggesting that Ln^{3+} may be able to modulate the inflammatory process in rheumatoid arthritis. Additionally it was found that Ln^{3+} could inhibit the activity of neutral metalloproteinases such as collagenase. Sm^{3+} proved the best inhibitor of gelatinase and caseinase, while La^{3+} inhibited collagenase the most strongly. Furthermore Ln^{3+} can reduce ROS produced under inflammatory conditions. These observations led to the proposal that Ln^{3+} may have anti-arthritic properties but this application appears not to have advanced beyond these biochemical studies.[2]

Oral administration of $LaCl_3$ in rabbits fed an atherogenic diet resulted in inhibition of the development of atherosclerosis.[8] Oxidation of low-density lipoprotein by ROS is a key step in atherosclerotic plaque formation so reduction of ROS levels is one possible explanation for this observation. An alternative explanation is interference in the involvement of calcium in plaque deposition. Ln^{3+} can also inhibit platelet aggregation which is calcium dependent. As plaque deposition involves both free radicals for LDL oxidation, calcium for the calcification and platelet aggregation all of these process are likely explanations.[1] However, the adverse effects of Ln^{3+} on the cardiovascular system precluded their development as agents for the treatment of atherosclerosis.

An interesting potential application for one Ln^{3+} salt is the hepatoprotective effect of $GdCl_3$ towards toxicant-induced liver damage. It has been suggested that the inflammation cascade plays a critical role in the pathology of liver injury. $GdCl_3$ has been shown to protect against liver damage caused by a variety of toxicants including ethanol, CCl_4, and cadmium.[9–11] The Kupffer cells, which are resident macrophages in the liver, release cytokines and free radicals upon activation by chemicals such as alcohol that contribute to liver injury. The hepatoprotective effect of $GdCl_3$ is primarily due to the inactivation and destruction of the Kuppfer cells.[1] This results in a reduction in cytokine and ROS production. Interestingly, in a model of cadmium-induced liver toxicity Kuppfer cells were depleted by low, mid and high doses of $GdCl_3$, but hepatoprotection was only seen at the higher doses suggesting that Kuppfer cells are not the only contributors to the liver damage.[11] In addition cytokines such as Transforming Growth Factor β (TGF-β) may act on stellate cells to stimulate collagen production thus contributing to liver fibrosis (Fig. 1).[10] There are two possible sources of ROS during liver fibrosis, NADPH oxidase from the Kuppfer cells or hepatocyte cytochrome P450 enzymes. The cytochrome P450 CYP2E1 is induced after challenge by alcohol and CCl_4. However, there was no decrease in CYP2E1 activity in $GdCl_3$ treated rats supporting the hypothesis that the Kuppfer cells are the main target for $GdCl_3$, not the CYP2E1.[9] Similarly $GdCl_3$ can also protect lungs from post-ischemic injury by lowering ROS, the proposed source of ROS being myeloperoxidase from mononuclear phagocytic cells.[12] These

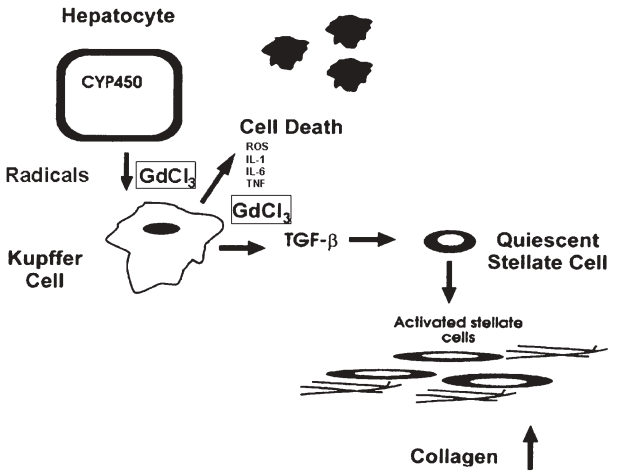


Fig. 1 The hepatoprotective effect of gadolinium chloride in toxicant-induced liver injury.

data suggest that there may be a therapeutic application for $GdCl_3$ in the treatment of liver fibrosis.

Antimicrobial and wound healing properties, and the treatment of burn wounds

There is a high risk of mortality associated with major, full thickness burns. Early mortality is associated with circulatory shock or the acute respiratory effects of inhalation injury. Late deaths are generally attributed to sepsis. In a full thickness burn all three layers of skin, epidermis, dermis and the subcutaneous layer, are destroyed, including all the regenerative elements. The standard treatment for full thickness burns is to surgically excise the wound, and then to cover with an autologous skin graft. However, in cases where the burn is extensive this is not possible, so alternatives such as stored allograft skin, xenografts such as pig skin, and tissue engineered or biosynthetic products are used.[13] Unfortunately, there are instances when these sophisticated skin substitutes are not available such as in a combat setting or in parts of the world where these expensive products are unavailable. Cerium nitrate has been shown to have beneficial effects on burn wounds, and represents a convenient and less expensive alternative. In particular there has been renewed interest in its use since the 2001 September 11th terrorist attacks in New York and Washington.[3]

The initial rationale for using cerium for the treatment of burn wounds was based upon the observed antibacterial effects of Ln^{3+} at the end of the 19th Century.[3] Several cerium(III) salts were reported to have antibacterial activity including acetate, stearate, chloride, and nitrate, and the oxidizing properties of Ce(IV) led to the use of Ce(IV) sulfate as an antiseptic powder. Systematic studies later confirmed that cerium nitrate had broad-spectrum antibacterial activity against a range of bacteria including *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Combination studies were subsequently performed with $Ce(NO_3)_3$ and silver sulfadiazene, another metal-based agent with demonstrated efficacy in the treatment of burn wounds. Initial clinical studies demonstrated a 50% reduction in mortality compared to predicted death

rates.[14] It was assumed that this was due to a synergistic antimicrobial interaction between the two agents. However, subsequent studies were unable to confirm this, and recent literature has shown that cerium nitrate has little anti-bacterial activity against common burn pathogens suggesting that there is an alternative mechanism whereby outcome is improved in burn patients.

It is now known that one of the major contributors to morbidity and mortality from major burns is disruption of the immune system. This is manifested in part by immunosuppression, which makes the victim more susceptible to infection. In addition, dysregulation of the immune response is a contributory factor to the systemic inflammatory immune response (SIRS) and subsequent multi-organ failure syndrome (MOFS) associated with mortality.[14,15] Early excision of the burn wound decreases mortality indicating that a component of the wound is responsible for the immune failure. The burn toxin responsible for the observed immune suppression was identified as a high molecular weight lipid protein complex (LPC) formed by heat-induced polymerization of six skin polypeptides. LPC induces a number of immune responses. It has an effect on cell-mediated immunity particularly on T cell function. The LPC is a potent inhibitor of T-cell growth suggesting that, at least in part, the mechanism of immunosuppression in burn patients is due to LPC inhibition of T-cell activation.

Studies in animal models of burn injury have indicated that cerium nitrate is a modulator of the burn-associated immune response. Both anti-inflammatory agents, and topical treatment with cerium nitrate restored the reduction in the T-cell helper/suppressor ratio observed in an animal model of burn injury. Furthermore, in a model of cell-mediated immunity in which mice with burn injury previously sensitized with 2,4-dinitrofluorobenzene were re-challenged, the usual nadir in the cell-mediated immune response was prevented by treatment with cerium nitrate. Though cerium nitrate can bind to and inactivate LPC, the immunomodulatory effect of cerium nitrate appears to be mediated by its effect on the burn eschar, the dry scab formed on the skin following a burn wound.

Topical application of cerium nitrate leads to a firm, impermeable eschar, which is leather-like in appearance with a greenish discoloration, and is firmly attached to the wound. This is in contrast to the eschar formed with silver sulfadiazene which is typically soft, moist, uneven and macerated.[14] The eschar formed by cerium nitrate contains deposits of insoluble pyrophosphate and carbonate salts, and calcium. It has been proposed that cerium may bind pyrophosphate, thus removing inhibition to local calcium deposition. The resultant eschar acts as a biological dressing, forming an impermeable crust over the wound. This covering may prevent both ingress and egress of bacteria from the wound thus preventing bacterial colonization, and egress of LPC into the systemic circulation thus inhibiting activation of the sepsis cascade and MOFS. This leaves the wound in a clean, healthy state, ready to accept a skin graft.

Cerium nitrate is usually administered in the clinic in combination with silver sulfadiazene. The combination is manufactured commercially as Flammacerium in Europe, and as Dermacerium in South America. The clinical benefit of cerium nitrate is unclear. There are several reports that claim a reduction in mortality with Flammacerium treatment compared with predicted death rates. Conversely there is data that concludes there is no improvement in overall, or sepsis-related, deaths between silver sulfadiazene and silver sulfadiazene/cerium nitrate treated patients. The problem with the clinical data is that there are no reported prospective randomized trials examining the effect of cerium nitrate treatment. In a recent trial comparing silver sulfadiazene and Flammacerium the investigators reported a more rapid re-epithelialization of partial thickness wounds, and a more rapid improvement in the healing of full thickness wounds. As a result the burn wounds were ready to accept skin grafts in a much shorter time frame than the silver sulfadiazene treated wounds. The net benefit of these findings was reduction in the length of stay in hospital and a reduced total cost of treatment, together with an observed reduction in morbidity in the Flammacerium treated patients.[15]

There is a resurge of interest in Flammacerium. It is available in British hospitals where it is approved for use on a named patient basis. In a recent survey it was found that many British burn surgeons choose to use Flammacerium on the basis that it reduces bacterial colonization, suppresses the immune response, and provides a more manageable eschar. Many of the respondents in the survey indicated that they would welcome a wider approval of the drug.[16]

In situations where immediate excision and grafting, either with skin grafts or alternative dressings, is not available, Flammacerium appears to provide a viable alternative with the biological dressing effect of the eschar buying valuable time. This is particularly pertinent in countries where the high cost and low availability of synthetic, biosynthetic and biological dressings limits there utility, or in a situation with mass burn casualties, or a severe emergency setting such as a terrorist attack where immediate treatment is required on a large scale. In spite of the lack of data demonstrating unequivocal improvement on mortality, the benefits of a firm biological dressing that can be safely left for several weeks may provide a valuable role for Flammacerium in burns treatment.

Lanthanides for the treatment of cancer

Lanthanide complexes have found a role in cancer treatment as contrast imaging agents such as Gd(III) DTPA which is commonly used for MRI imaging of tumors. Radioisotopes of lanthanides have also been explored both for imaging and therapy.[2,17,18] These applications have been reviewed elsewhere. Ln^{3+} compounds have also been investigated for their anti-cancer potential. Early clinical reports suggested that cerium(III) iodide had activity against solid tumors. More recently work has focused on lanthanide complexes. Cerium (III), lanthanum(III) and neodymium(III) coumarin complexes were synthesized with ligands such as hymecromone, umbelliferone, mendiaxon, warfarin, coumachlor and niffcoumar. Preclinical studies with these compounds have demonstrated cytotoxicity against the HL-60 myeloid cell line. Also a series of cerium(III) bipyridyl, phenanthroline and related complexes have been reported with *in vitro* activity against cell lines.

Terbium has been shown to enhance the cytotoxicity of cisplatin, possibly by increasing accumulation of drug in cisplatin-resistant cells.[1]

One group of lanthanide complexes that have progressed into clinical trials is the texaphyrins.[19,20] Recently a redox active gadolinium texaphyrin complex has entered phase III clinical trials for the treatment of brain metastases of non-small cell lung cancer. The lutetium texaphyrin has also been investigated as photo-activated agent for the treatment of atherosclerotic plaque in coronary heart disease, and for treatment of age-related macular degeneration (AMD). The structures of these complexes are shown in Fig. 2. These compounds are being developed by Pharmacyclics, Sunnyvale, California.

The texaphyrins can be thought of as extended porphyrins.[19] They are pentaaza, Schiff base macrocycles that resemble porphyrins in being fully aromatic, and form colored complexes. However, there are significant differences that make them attractive from a pharmacological perspective. The texaphyrins are monoanionic ligands containing five, rather than four, coordinating nitrogen atoms in the central core. The central core of the texaphyrin ligand is approximately 20% larger than that of the porphyrins, which enables texaphyrins to form stable 1 : 1 complexes with a range of metal cations, including Ln^{3+}. Metal complexation is also accompanied by ligand oxidation. As a result the macrocyclic skeleton tightens around the metal cation forming a highly stable complex with a high barrier to dissociation. The texaphyrins also differ from their porphyrin counterparts in terms of their redox potential. Whilst both texaphyrins and porphyrins are relatively resistant to oxidation, the redox potentials of the Gd-texaphyrin and Lu-texaphyrin are $E_{1/2}$ of -0.041 V and -0.044 V with respect to normal hydrogen electrode in dimethylformamide respectively, compared with -1.41 V for Zn(II) octaethylporphyrin, meaning that the texaphyrin complexes are more susceptible to reduction. This makes the texaphyrins more redox active in a biological environment with significant consequences for their mechanism of action. In addition, the texaphyrins are dark green in color compared with the purplish-red of porphyrins. This means that they absorb and are activated by light with wavelengths greater than 700 nM. This allows for greater tissue penetration of the activating light, which facilitates their use as agents for photodynamic therapy.

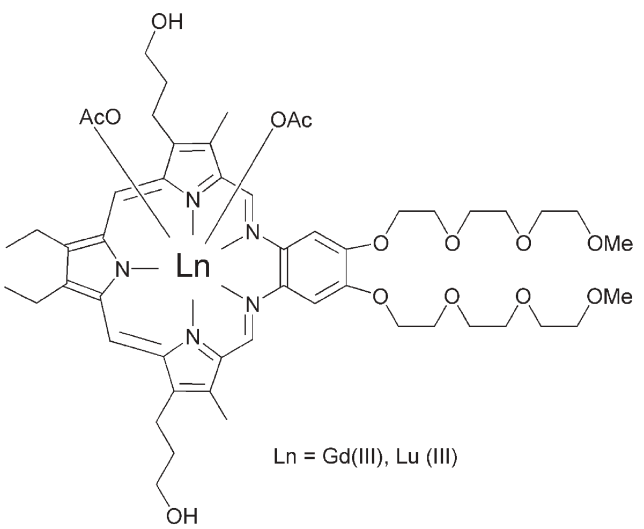


Fig. 2 The structure of gadolinium and lutetium texaphyrin complexes.

The Gd-texaphyrin complex, motexafin gadolinium (MGd, Xcytrin) has been investigated and exploited as a radio- and chemo-sensitizer for cancer treatment based on its unique redox properties.[21] Using pulse radiolysis it was shown that MGd can accept electrons from radicals including O_2^-, and also aquated electrons in the medium, resulting in reduction of the MGd complex. In the absence of oxygen, MGd can act as a sponge to remove electrons formed from the interaction of the radiation with water, resulting in an augmented concentration of hydroxyl radicals. Alternatively, in the presence of oxygen the same initial electron capture event will result in a metastable reduced Gd-texaphyrin complex which can act as an electron donor, reacting with molecular oxygen to form the superoxide anion and regenerate MGd. The combination of these two electron transfer reactions has been described as futile redox cycling.[19] Based on these studies it has been hypothesized that MGd reacts with a variety of intracellular antioxidants to produce ROS.

Reactive oxygen species such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\bullet}$) can interact with biological macromolecules such as protein and nucleic acids. ROS-induced oxidative damage to DNA and protein can lead to apoptosis and cell death. The cell contains a number of defences against ROS-induced injury including the glutathione redox system and thioredoxin reductase, which is involved in maintaining the redox balance of the cell. Pharmacological manipulation of the tumor cell redox balance in favor of increased ROS and increased oxidative stress has the potential to enhance tumor cell death. In *in vitro* studies MGd was found to catalyze the oxidation of ascorbate and NADPH under aerobic conditions, forming hydrogen peroxide. Incubation of the tumor cell lines MES-SA and A549 with MGd in the presence of ascorbate or NADPH resulted in cell death, this was associated with an increase in intracellular ROS. MGd can also catalyze the oxidation of glutathione, dihydrolipoate, and protein thiols. Similarly MGd, in the presence of ascorbate, was cytotoxic towards multiple myeloma cells. Dexamethasone is the first line treatment for multiple myeloma and interestingly MGd was active against both dexamethasone sensitive and resistant cells. The cytotoxicity was linked to ROS production and subsequent apopotosis.[22] Further evidence for ROS-mediated cell death was the attenuation of the cytotoxic effect of MGd by catalase.

The high paramagnetism of Gd allows MGd to be tracked by MRI. Using this technique it has been shown that MGd selectively localizes in tumor cells. In addition pharmacokinetic studies with radiolabelled drug have shown delayed clearance from tumors compared with rapid clearance from blood and normal tissues. These properties of redox activation and tumor cell accumulation have led to the investigation of MGd as a radiosensitizer.

Radiotherapy is one of the most commonly used first line treatments for a number of solid tumors. However, one of the major challenges with radiotherapy is that the hypoxic centre of

a tumor is resistant to radiation. This can be overcome by delivery of hyperbaric oxygen. An alternative strategy is to use a redox active compound to counteract the hypoxia-related radioresistance. To this end halogenated pyridines and nitroimidazoles have been studied, but neither class of compound has proven to result in an improved clinical outcome. Promising indications were seen with MGd in pre-clinical models. MGd in the presence of ascorbate increased the aerobic radiation response of E89 cells, and synergistic effects with L-buthionine-(*S*,*R*)-sulfoximine (BSO), an inhibitor of glutathione synthesis causing glutathione depletion, were seen in MES-SA and A549 cells. A correlation between increased radiation responsiveness with MGd treatment and depletion of cellular high-energy phosphates has been found *in vivo* in a mouse tumor model indicating a disruption in energy metabolism. The resultant depletion of intracellular reducing metabolites and subsequent bioenergetic disruption due to the futile cycling described above would result in enhanced sensitivity to radiation in the hypoxic tumor environment. MGd in combination with radiation, produced significant tumor growth delay compared to irradiated control groups in both single and multifraction radiation studies against EMT6, SMT-F and MCa murine tumors. Interestingly, several other metallotexaphyrins, identical except for the metal ion, were studied in the EMT6 tumor model including lutetium (Lu), europium (Eu), yttrium (Y), and cadmium (Cd) texaphyrin complexes, but only the gadolinium complex, MGd, produced therapeutic enhancement in combination with radiation.

As a result of these promising pre-clinical observations MGd was entered into clinical trials. The safety profile was defined in a series of Phase I and Phase II trials. In a Phase I trial the maximum tolerated dose for a single administration was determined as 22.3 mg kg^{-1}. Importantly, MGd was cleared rapidly with a half-life of 7.4 hours, and MRI demonstrated selective tumor accumulation in both primary and metastatic tumors. This was followed by a Phase Ib/II study in which the drug was given daily by infusion for 10 days in combination with whole brain irradiation to patients with brain metastases.[21] Under these conditions the maximum tolerated dose was 6.3 mg kg^{-1} with hepatotoxicity being the dose-limiting toxicity. Interestingly, adverse events included dose-dependent transient greenish discoloration of skin, urine and sclera, which disappeared 3–4 days after administration of the last dose. This discoloration was attributed to the dark-green color of MGd. Plasma pharmacokinetics after repeat dosing confirmed the rapid clearance of the drug. Based on an evaluation at 2 months, the radiologic response rate in 18 assessable patients in phase II was 72%, with only 12% of patients dying as a result of CNS tumor progression.

Following the favorable results from the Phase I and II trials a Phase III trial was initiated in patients with brain metastases.[23,24] Brain metastases are a frequent complication of many cancers including lung cancer, breast cancer, colon cancer, and melanoma, occurring in as many as 24% of all cancer patients. In lung cancer, the most common cause of brain metastases, up to 50% of patients develop central nervous system (CNS) involvement. Brain metastases occur early in lung cancer, sometimes producing neurologic symptoms at disease presentation, compared with other cancers where CNS spread is usually a later complication. Brain metastases are not amenable to surgical resection and are therefore commonly treated with whole brain irradiation. The median survival of these patients with whole-brain radiation therapy is approximately 4 months, therefore there is a need for new and effective therapeutic interventions.

Over four hundred patients (451) were enrolled into a randomized Phase III trial comparing MGd in combination with whole brain irradiation, with whole brain irradiation alone. The patient population consisted of 251 patients with lung cancer, 75 with breast cancer and 75 with other cancers, with 193 patients in the MGd combination arm, and 208 patients in the whole brain irradiation arm. The primary endpoints were survival and time to neurological progression, with time to neurocognitive progression as one of the secondary endpoints. Emphasis was placed on the neurologic progression because it was considered that a survival endpoint would be of little value as patients with brain metastases frequently die as a result of systemic disease progression. In comparing the two arms, there was no overall difference in survival with median survival times of 5.2 months for the MGD with whole brain irradiation arm compared with 4.9 months for the control arm. However, there were significant differences in time to neurologic progression and neurocognitve function. Combination treatment with MGd resulted in a significantly improved time to neurologic progression of 4.3 months with MGd compared with 3.8 months with whole brain irradiation alone. Interestingly this difference was primarily seen in the lung cancer patients. There was no difference overall in neurocognitve function, but again when analyzed in terms of tumor type there was a marked improvement in time to neurocognitive progression in lung cancer patients. There are a number of reasons why this improvement was restricted to lung cancer patients. The lung cancer patients presented with brain metastases at early initial diagnosis, had only brain as a site of metastasis, had a smaller lesion volume, and had less prior therapy. A further conclusion from this trial was that neurocognitive function correlated with tumor growth and could be predictive of survival.

MGd is now in a follow up international Phase III trial (SMART, Study of Neurologic Progression with Motexafin Gadolinium And Radiation Therapy) in combination with whole brain irradiation for brain metastases in non-small cell lung cancer conducted at centers in North America, Europe and Australia. The company announced preliminary results from this trial in December 2005. As in the earlier trial the time to neurologic progression was longer in the MGd plus whole brain radiation arm compared with the control arm with only whole brain irradiation, though the difference between the two arms did not reach statistical significance overall. However, the difference was statistically significant in the North American patients. This was probably due to the characteristic of that particular patient population which contained more females, shorter time from primary cancer diagnosis to development of brain metastases, and less use of post-randomization chemotherapy compared to the other regions. In North America, the median time to neurologic progression was 24.2 months in the MGd arm compared to 8.8 months for whole brain radiation alone. As in the previous trial no

improvement in survival was observed. Other benefits of the MGd treatment were reduced steroid usage and less need for salvage radiation therapy to the brain.

Preclinical studies have suggested that MGd may also have utility as a chemosensitiser. MGd enhanced the *in vitro* cytotoxic effect of both bleomycin and doxoxrubicin against MES-SA and Rif-1 tumor cells.[25] Both bleomycin and doxorubicin can generate ROS in cells. Bleomycin can chelate iron and *via* reaction with oxygen can generate ROS. Doxorubicin can also form complexes with iron and copper and produce ROS *via* reduction to a semiquinone radical. Combination of MGd with either bleomycin or doxorubicin enhanced the *in vivo* anti-tumor effect of both drugs against the EMT-6 murine tumor. MGd is in clinical trials as combination therapy with several drugs and/or radiotherapy including cisplatin and 5-fluoruracil for advanced head and neck cancer, docetaxel and cisplatin for non-small cell lung cancer, and temozolomide for malignant glioma. MGd is also in clinical trials as a single agent for the treatment of the hematologic malignancies non-Hodgkin's lymphoma and chronic lymphocytic leukemia. It is also being investigated in a combination study with ^{90}Y-ibritumomab tiuxetan (Zevalin). Zevalin is composed of a murine IgG1 anti-CD20 antibody, ibritumomab, covalently linked to the beta-emitter ^{90}Y by a chelator, tiuxetan. This is a novel way of combining radiotherapy with a redox active radiosensitiser.[21]

Other experimental approaches with MGd include the synthesis of conjugates of methotrexate with MGd. Both ester- and amide-linked conjugates were synthesized. The ester conjugate showed greater *in vitro* anti-proliferative activity against the A549 lung carcinoma cell line at short incubation times than did methotrexate alone, whilst the amide conjugate and MGd were inactive alone.

As mentioned earlier the texaphyrins are dark green in color and absorb and are activated by light with wavelengths greater than 700 nM. This activation at wavelengths in the far-red region allows for greater tissue penetration and less absorption by hemoglobin. The lutetium–texaphyrin complex, Motexafin lutetium (MLu), which is activated at a wavelength of 732 nM, has been extensively studied as a photoactivated drug for photodynamic therapy (PDT) based on its high singlet oxygen quantum yield.[19,20] PDT is a treatment modality that uses light to activate a photosensitizer, which, upon activation, produces cytotoxic singlet oxygen. PDT is potentially an attractive treatment for cancer where the tumor is localized. Light is delivered *via* optical fibers placed directly into a tumor; a process called interstitial light delivery. Like MGd, MLu selectively localizes in tumor tissue, and MLu has been investigated as a photosensitiser for cancer treatment. Activity has been demonstrated in murine tumor models EMT-6 and SMT-F, and has been evaluated in dogs for treatment of prostate cancer. MLu has entered clinical trials for metastatic tumors to the skin and recurrent breast cancer to the chest wall.

The most successful application of PDT has been in the treatment of age related macular degeneration, a disease of the retina related to neovascularization. The benzoporphyrin derivative photosensitizer, Visudyne, has shown great utility for treatment of AMD. MLu has also been investigated as a potential treatment for AMD and has shown activity in both *in vitro* and *in vivo* test systems. However, despite these early promising results MLu is not being actively pursued as a PDT for either cancer or AMD, presumably because the viability of PDT for cancer is still a topic of debate, and because of the market success of Visudyne for AMD.

Another feature of MLu is that it selectively targets and accumulates in metabolically active inflammatory cells such as macrophages in atheromatous plaque, probably *via* a LDL-receptor-mediated mechanism, and upon photoactivation causes apoptosis of macrophages. The formation of the atherosclerotic plaque is now seen as being an inflammatory event. Vascular macrophages play a significant role in plaque formation, and contribute to the instability of vulnerable plaque. Disruption of vulnerable plaque is a major causative event in acute coronary attack. MLu has completed a Phase I clinical trial in patients with coronary artery disease.[26] This Phase I safety trial investigated the safety and tolerability of PDT with MLu in patients undergoing percutaneous coronary intervention and stent deployment. MLu was administered to 79 patients by intravenous infusion 18 to 24 hours before surgical procedure, and photoactivation was performed after balloon predilatation and before stent deployment. Clinical outcome was evaluated after 6 months. MLu PDT was well tolerated without serious dose-limiting toxicities, and without serious side effects. It was concluded from this study that coronary MLu PDT was safe. This clinical trial also indicated that MLu PDT could be incorporated into current medical practice; *i.e.*, placement of the light delivery fiber can be accomplished with customary interventional techniques, such as PCTA (percutaneous coronary transluminal angioplasty), and stenting. Current and future work on motexafin lutetium development is focused on the potential use of light-activated motexafin lutetium in the treatment of vulnerable plaque.

Lanthanum carbonate for the management of hyperphosphatemia

Hyperphosphatemia, increased serum phosphate levels, is one of the clinical consequences that accompany end stage renal disease (ESRD).[27] Normal adult serum phosphate levels range from 2.17–4.34 mg dl^{-1} compared with the elevated levels of 6.2–9.3 mg dl^{-1} seen in ESRD patients. The average dietary phosphate intake is around 1000–1500 mg per day. Under normal, healthy conditions phosphate is absorbed in the intestine and excreted *via* the kidney resulting in a net phosphate balance of zero. Phosphate metabolism is intimately linked with calcium metabolism, and is regulated by parathyroid hormone (PTH) and Vitamin D. PTH controls phosphate balance in the body by lowering tubular reabsorption of phosphate by the kidney. Consequently, during renal impairment PTH secretion increases in an attempt to further decrease phosphate reabsorption, and correct the hyperphosphatemia. Furthermore vitamin D metabolism in the kidney is impaired in ESRD resulting in reduced calcium absorption and hypocalcemia. This decrease in calcium can in turn stimulate PTH secretion. In this way the body attempts to correct for the high phosphate levels, but at the expense of

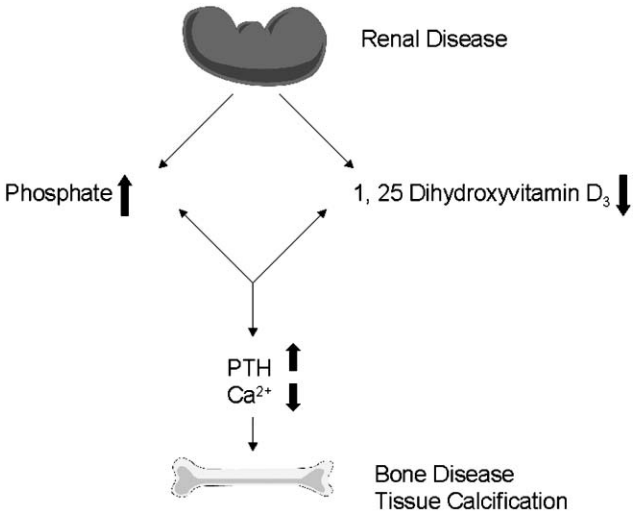


Fig. 3 The interrelationship between the pathological consequences of hyperphosphatemia, PTH, and vitamin D3.

increased parathyroid activity, a state described as secondary hyperparathyroidism. These events are illustrated in Fig. 3.

The pathological consequences of hyperphosphatemia are severe. These interacting events lead to several bone malformations in the joint, a disease known as renal osteodystrophy. Nearly half the deaths of dialysis patients are due to cardiovascular complications prevalent in patients with ESRD. Cardiac and vascular tissue calcification is a major contributor to cardiovascular disease. Factors that may promote tissue calcification are elevated levels of PTH, tissue alkalinity, hyperphosphatemia and the elevated calcium-phosphate product (Ca x P). There is an increased risk of mortality in those patients with a Ca x P of > 72 mg^2 dl^{-2}. Damage caused by calcification of cardiac tissue can lead to arrythmias, left ventricular dysfunction, damage to heart valves, and ultimately complete heart block. In addition hyperphosphatemia and hypocalcemia are associated with atherosclerosis and calciphylaxis (a complication of extra-skeletal calcification). Calcification of soft tissues is found in organs such as the lung, kidney, gastric mucosa, cornea and conjunctiva, and cutaneous and subcutaneous tissues.

Unfortunately hyperphosphatemia cannot be controlled by normal dialysis. Long, slow, nocturnal dialysis may be effective but presents difficulties for the patient.[4,27] Similarly patients on continuous ambulatory peritoneal dialysis (CAPD) fare slightly better because this is a continuous daily treatment. It is difficult to control dietary intake of phosphate as phosphate is associated with protein intake, thus decreasing dietary phosphate is difficult without significant reduction in protein intake. This puts patients with impaired renal function at risk of malnutrition. The associated hypocalcemia and secondary parathyroidism can be treated with calcium supplements and the vitamin D derivative calcitriol, but hyperphosphatemia can interfere with calcitriol therapy. This means that alternative treatment options are needed.

The binding of dietary phosphate in the gut has been the favored option. The ideal phosphate binder should have a high affinity for phosphate and should be able to bind phosphate rapidly. It should have low solubility and little or no systemic absorption. It should be non-toxic, available as a palatable oral dosage form, with a low pill burden.[27] Aluminium-based binders such as aluminium hydroxide were used in the 1970s and early 1980s because aluminium readily forms insoluble and nonabsorbable aluminium phosphate precipitates. However, aluminium hydroxide, which is readily soluble, is absorbed from the gut and was found to be toxic. Aluminium causes CNS toxicity and encephalopathy. There are associated increases in hypercalcemia and cardiovascular calcification, and osteomalacia and adynamic (low turnover) bone disease, and associated bone and muscle pain. As a result, calcium phosphate binders replaced aluminium-based phosphate binders.[28] There are several available calcium-based phosphate binders including calcium carbonate, acetate, alginate and ketoglutarate with calcium carbonate and acetate being the most commonly used. Both calcium carbonate and calcium acetate are effective phosphate binders in dialysis patients and until recently have been the agents of choice. However, the problem with calcium-based agents is that the calcium can be absorbed resulting in hypercalcemia and increased risk of cardiovascular calcification. This can be further complicated if calcitriol is being used to control secondary hyperparathyroidism.

These concerns and challenges with aluminium- and calcium-based phosphate binders have stimulated research into aluminium- and calcium-free binders.[27,28] Renagel (sevalamer hydrochloride) from Genzyme Therapeutics is a hydrogel-cross-linked polyallylamine hydrochloride. The high amine content on the polymer provides a high density of positive charges, which can interact with the phosphate anions resulting in strong phosphate binding. Renagel was shown in clinical trials to be at least as effective as calcium acetate at lowering phosphate levels, with an associated decrease in PTH and Ca x P product, and reduction in hypercalcemia and aortic and coronary calcification. In addition to binding phosphate, Renagel also binds LDL and fat-soluble vitamins such as 1,25 dihydroxyvitamin D3 and vitamin K resulting in beneficial changes in blood lipids. Renagel was approved by the FDA in 1998 and gained approval in Europe in 2000. Uptake in Europe was initially slow because of its high cost, However, a cost-effectiveness study indicated that Renagel reduces the incidence of cardiovascular events thus reducing the risk of hospitalization and overall medical costs while improving patient health. However, the pill burden is high with Renagel, patients need to take 8–16 large pills per day, so patient compliance can be a problem. Recently, a new phosphate-binding drug based on lanthanum carbonate, Fosrenol, has been approved in both the USA and Europe.

The concept for Fosrenol was initially proposed and investigated by the Biomedical Technology Department of Johnson Matthey. It came out of an exploratory project investigating the biological interactions of lanthanides with a view to identifying potential therapeutic applications. Work sponsored by Johnson Matthey at the University of Surrey, UK, highlighted the ease with which the lanthanides formed precipitates with phosphate, frequently a challenge for the investigator.[29,30] In collaboration with Shire Pharmaceuticals a project was initiated to examine lanthanides as phosphate

binders for hyperphosphatemia. *In vitro* phosphate binding studies comparing a number of lanthanide salts and complexes identified lanthanum carbonate as having good phosphate binding properties. Further studies demonstrated that improved phosphate binding was obtained with the tetrahydrate $La_2(CO_3)_3.4H_2O$, with optimal binding at pH 3–5 whilst retaining binding activity across the full pH range of 1–7.[31] This means that lanthanum carbonate can bind phosphate at the low pH of the stomach, as well as at the higher pH values found in the small intestine, duodenum and jejunum, unlike calcium carbonate. In addition, comparative *in vivo* distribution studies showed that lanthanum carbonate had the best biodistribution profile compared with other Ln^{3+} salts, with little to no oral absorption and tissue accumulation, and effectively complete elimination in the feces. A chemical process was developed for manufacture of the tetrahydrate, and with these proof-of principle studies, Shire Pharmaceuticals took on the clinical development of lanthanum carbonate. In 1996 the biopharmaceutical company AnorMED was formed as a spin-off from Johnson Matthey. Patent rights to lanthanum carbonate to treat hyperphosphatemia in renal patients were assigned to AnorMED.

Detailed pre-clinical studies confirmed the phosphate-binding ability of lanthanum carbonate.[4,32] The relative efficacy of lanthanum carbonate was compared with other phosphate binders in a rat model of renal failure. Lanthanum carbonate was equally effective as aluminium hydroxide, and more effective than either Renagel or calcium carbonate, at reducing urine phosphate levels in this model. Lanthanum carbonate is very insoluble and lanthanum, like all Ln^{3+} does not cross biological membranes. Pharmacokinetic studies in animals confirmed that lanthanum carbonate is poorly absorbed when given by the oral route with >90% excreted in the feces, and <0.001% absorbed. No toxicity was observed in animal studies, in particular there were neither cardiovascular nor CNS effects, nor any direct effects on calcium, vitamin D or PTH metabolism. No effect on bone has been observed in normal animals, however at high doses (1000–2000 mg kg^{-1}) an impairment of bone mineralization was seen in rats with chronic renal failure.[33] No such effects have been seen with patients in the clinic, on the contrary improvements are seen in those patients with low turnover bone disease when treated with lanthanum carbonate. This favorably compares with calcium carbonate where there is a tendency to an increase in adynamic bone disease. Further studies in rats have shown that the observed impairment in bone mineralization was due to phosphate depletion at the high doses given, rather than to a direct effect on bone.

The favorable pre-clinical pharmacokinetic and toxicology profile has been reproduced in several Phase 1 and Phase II clinical studies. Phase III clinical studies have been performed in both Europe and North America.[34–37] In general these studies have followed a similar protocol. Patients are screened and go through a 1–3 week ''washout'' period in which they are taken off phosphate binders. Patients with serum phosphate levels > 5.58 mg dl^{-1} are then given Fosrenol to bring the phosphate levels down, and then continued on a maintenance dose for the remaining period of the trial. The control arms consisted of patients either given a placebo or another phosphate binder. The primary endpoint was reduction in serum phosphate. Secondary endpoints included maintenance of serum phosphate levels to a set level, Ca x P product, calcium and PTH levels, and safety and tolerability. When compared with placebo, lanthanum carbonate reduced and maintained phosphate levels, and when compared with another phosphate binder, calcium carbonate, lanthanum carbonate was equally as effective. In long term trials lanthanum carbonate was able to maintain phosphate levels for up to 2 years at levels of <5.9 or <5.6 mg dl^{-1} depending upon the endpoint set by the trial. Dose levels of lanthanum carbonate ranged from 225–3000 mg La per day in the trials. Doses used at initiation of treatment were usually 375 or 750 mg per day, and did not exceed 3000 mg per day, with doses of 1350–2250 mg per day being effective in most patients. In one study the pill burden of lanthanum carbonate compared with calcium carbonate was 12 tablets compared to 18 tablets.[37] To achieve the best effect the tablets have to be taken with or immediately after food.

As would be expected from a non-calcium phosphate binder, lanthanum carbonate treatment resulted in a decrease in the Ca x P product compared with calcium carbonate or placebo. The higher Ca x P product seen with calcium carbonate can be attributed to higher calcium levels. No changes in serum calcium levels have been reported in patients treated with lanthanum carbonate. PTH levels are difficult to assess but generally PTH levels remained stable or showed an increase in lanthanum carbonate treated patients. Lanthanum carbonate is safe and well tolerated over the long term with some patients now having taken the drug for over 4 years. Most reported adverse events are mild-moderate, with gastrointestinal events being the most common, occurring with a frequency similar to that seen with calcium carbonate. The frequency of other adverse events is also similar to that seen in patients on other phosphate binders, and is primarily associated with the problems of treating a very sick patient population rather than drug related.[4,32]

Controlling serum phosphate in patients with ESRD on renal dialysis has been a major problem. None of the available calcium- or aluminium-based phosphate binders match the requirements for an ideal agent, each having its own limitations. The approval of Renagel in part obviates these problems, but its cost and high pill burden present their own challenges. Lanthanum carbonate, Fosrenol, is an alternative non-aluminium, non-calcium phosphate binder. Lanthanum carbonate is well tolerated, poorly absorbed and therefore not accumulated in tissues, binds phosphate effectively across the physiological pH range of the upper gastrointestinal tract, and has no detrimental effect on calcium, vitamin D or PTH metabolism. From clinical data so far it seems that its effectiveness as a phosphate binder will translate into a lower pill burden for patients. Fosrenol therefore represents a significant improvement in treatment options for patients with end-stage renal disease. Fosrenol was approved for use in Sweden in March 2004. Sweden has become the Reference Member State in the European Union Mutual Recognition Procedure for Fosrenol, and represents the first step in securing marketing approval throughout Europe. The FDA granted approval for Fosrenol in the US in

October 2004, it was launched on the US market in January 2005 and had 8% of the US phosphate binding market by December 2005. Fosrenol looks set to be a successful lanthanide-based drug.

Conclusion

The biological properties of the lanthanides, primarily based on their similarity to calcium, have been the basis for research into potential therapeutic applications of lanthanides since the early part of the twentieth century. Many of these early applications have been unsuccessful, with the notable exception of the use of cerium nitrate as a topical cream with silver sulfadiazene for the treatment of burn wounds. The modern approach to burn wound treatment in hospitals with well-established and well funded health care systems is to use sophisticated surgical procedures. However, in countries without such advantages, or in situations where these facilities are not available Flammacerium has a role to play. This role is being evaluated such that Flammacerium may find a wider use in modern burn centers.

A more recent investigational drug is the redox active lanthanide texaphyrin complex, motexafin gadolinium. This is in Phase III clinical trials in combination with whole body irradiation for the treatment of brain metastases in non-small cell lung cancer. This treatment may improve patient quality of life by increasing the time to neurologic progression. The photoactivatable lutetium analogue motexafin lutetium is being investigated as phototherapy for vulnerable atherosclerotic plaque.

The recent success story for lanthanides as therapeutic metals has been the approval for lanthanum carbonate, Fosrenol, as a phosphate binder for the treatment of hyperphosphatemia in renal dialysis patients in both the USA and Europe. Lanthanum carbonate dissociates at the physiological pH of the upper gastrointestinal tract to allow formation of lanthanum phosphate. This insoluble phosphate is eliminated in the feces without significant absorption of lanthanum. Efficacy and safety have been demonstrated in several Phase III clinical trials in both Europe and North America. The approval of Fosrenol is particularly significant as it provides an alternative approach to the control of the intake of dietary phosphate for patients with ESRD without the adverse effects of the current aluminium and calcium based phosphate binders. The success for inorganic chemists is that with Fosrenol there is now a new, clinically approved, metal-based drug.

Acknowledgements

The author would like to acknowledge his colleagues at both Johnson Matthey and AnorMED who together contributed to the discovery of Fosrenol; Richard King, Nigel Powell, Graham Henderson, Christopher Barnard, Barry Murrer, and Michael Abrams.

References

1 K. Wang, Y. Cheng, X. Yang and R. Li, *Met. Ions Biol. Syst.*, 2003, **40**, 707.
2 C. H. Evans, *Triends Biochem. Sci.*, 1983, 445.
3 M. A. Jakupec, P. Unfried and B. K. Keppler, *Rev. Physiol. Biochem. Pharmacol.*, 2005, **153**, 101.
4 F. Albaaj and A. J. Hutchison, *Expert Opin. Pharmacother.*, 2005, **6**, 319.
5 C. H. Evans, *Calcium in Drug Actions. Handbook of Experimental Pharmacology*, Springer-Verlag,Berlin, 1988, p. 1.
6 E. W. Yeung, S. I. Head and D. G. Allen, *J. Physiol.*, 2003, **552**, 449.
7 T. J. Haley, *J. Pharm. Sci.*, 1965, **54**, 663.
8 D. M. Kramsch, A. J. Aspen and C. S. Apstein, *J. Clin. Invest.*, 1980, **65**, 967.
9 D. R. Koop, B. Klopfenstein, Y. Iimuro and R. G. Thurman, *Mol. Pharmacol.*, 1997, **51**, 944.
10 C. A. Rivera, B. U. Bradford, K. J. Hunt, Y. Adachi, L. W. Schrum, D. R. Koop, E. R. Burchardt, R. A. Rippe and R. G. Thurman, *Am. J. Physiol. Gastrointest. Liver Physiol.*, 2001, **281**, G200.
11 E. B. Harstad and C. D. Klaassen, *Toxicol. Appl. Pharmacol.*, 2002, **180**, 178.
12 H. Okutan, C. Savas, I. F. Ozguner, Z. Yonden, V. C. Eren and N. Delibas, *Tohoku J. Exp. Med.*, 2004, **203**, 267.
13 R. Papini, *Br. Med. J.*, 2004, **329**, 158.
14 J. P. Garner and P. S. Heppell, *Burns*, 2005, **31**, 539.
15 C. G. de Gracia, *Burns*, 2001, **27**, 67.
16 J. P. Garner and P. S. Heppell, *Burns*, 2005, **31**, 379.
17 For recent reviews on Lanthanides in medicine/MRI imaging see, *Chem. Soc. Rev.*, 2006, **35**, 6.
18 F. Rosch and E. Forssell-Aronsson, *Met. Ions Biol. Syst.*, 2004, **42**, 77.
19 J. L. Sessler and R. A. Miller, *Biochem. Pharmacol.*, 2000, **59**, 733.
20 T. D. Mody, L. Fu and J. L. Sessler, 'Texaphyrins: Synthesis and Development of a Novel Class of Therapeutic Agents', in *Progress in Inorganic Chemistry*, ed. K. D. Karlin, John Wiley & Sons, Inc., New York, vol. **49**, 2001.
21 A. M. Evens, *Curr. Opin. Oncol.*, 2004, **16**, 576.
22 A. M. Evens, P. Lecane, D. Magda, S. Prachand, S. Singhal, J. Nelson, R. A. Miller, R. B. Gartenhaus and L. I. Gordon, *Blood*, 2005, **105**, 1265.
23 M. P. Mehta, P. Rodrigus, C. H. Terhaard, A. Rao, J. Suh, W. Roa, L. Souhami, A. Bezjak, M. Leibenhaut, R. Komaki, C. Schultz, R. Timmerman, W. Curran, J. Smith, S. C. Phan, R. A. Miller and M. F. Renschler, *J. Clin. Oncol.*, 2003, **21**, 2529.
24 C. A. Meyers, J. A. Smith, A. Bezjak, M. P. Mehta, J. Liebmann, T. Illidge, I. Kunkler, J. M. Caudrelier, P. D. Eisenberg, J. Meerwaldt, R. Siemers, C. Carrie, L. E. Gaspar, W. Curran, S. C. Phan, R. A. Miller and M. F. Renschler, *J. Clin. Oncol.*, 2004, **22**, 157.
25 R. A. Miller, K. W. Woodburn, Q. Fan, I. Lee, D. Miles, G. Duran, B. Sikic and D. Magda, *Clin. Cancer Res.*, 2001, **7**, 3215.
26 D. J. Kereiakes, A. M. Szyniszewski, D. Wahr, H. C. Herrmann, D. I. Simon, C. Rogers, P. Kramer, W. Shear, A. C. Yeung, K. A. Shunk, T. M. Chou, J. Popma, P. Fitzgerald, T. E. Carroll, D. Forer and D. C. Adelman, *Circulation*, 2003, **108**, 1310.
27 F. Albaaj and A. Hutchison, *Drugs*, 2003, **63**, 577.
28 H. H. Malluche and H. Mawad, *Nephrol. Dial. Transplant.*, 2002, **17**, 1170.
29 D. Bingham and M. Dobrota, *Biometals*, 1994, **7**, 142.
30 D. Bingham and M. Dobrota, *J. Inorg. Biochem.*, 1995, **59**, 39.
31 B. A. P. Murrer and A. Nigel, *US Patent Number 5968976*, October 19, 1999.
32 T. Swainston Harrison and L. J. Scott, *Drugs*, 2004, **64**, 985.
33 G. J. Behets, S. C. Verberckmoes, P. C. D'Haese and M. E. De Broe, *Curr. Opin. Nephrol. Hypertens.*, 2004, **13**, 403.
34 W. F. Finn, M. S. Joy and G. Hladik, *Clin. Nephrol.*, 2004, **62**, 193.
35 M. S. Joy and W. F. Finn, *Am. J. Kidney Dis.*, 2003, **42**, 96.
36 W. F. Finn and M. S. Joy, *Curr. Med. Res. Opin.*, 2005, **21**, 657.
37 A. J. Hutchison, B. Maes, J. Vanwalleghem, G. Asmus, E. Mohamed, R. Schmieder, W. Backs, R. Jamar and A. Vosskuhler, *Nephron Clin. Pract.*, 2005, **100**, c8.