Institution: King's College London

Unit of Assessment: 3B - Pharmacy and Nutritional Sciences

Title of case study: Iron Chelators and Hepcidin Analogues for Therapeutic Use

1. Summary of the impact

Use of the iron chelator drug deferiprone – first developed by researchers at King's College London (KCL) – has extended the lives of thalassaemia patients and is of great utility for those with cardiac problems as it can remove excess iron from the heart. For this reason deferiprone has more recently gained United States approval. KCL researchers have also developed methods for the synthesis and analysis of markers of iron chelation therapy that are being utilised in clinical trials by Novartis Pharmaceuticals and Vifor Pharma and by clinicians. Several neurodegenerative diseases are associated with elevated brain iron levels and the use of deferiprone is also being investigated in clinical trials by ApoPharma and hospitals in the UK and France.

2. Underpinning research

Thalassaemia is the most prevalent inherited single-gene disorder in the world. Over 60,000 a year are born with beta-thalassaemia, the most common type needing treatment. Life-saving and prolonging therapy for thalassaemia includes regular blood transfusions; however, as these can lead to iron build-up in the blood, iron chelation therapy is also necessary as a counteractive measure. The mainstay chelation drug, desferoxamine, is administered via intramuscular infusion over an 8 hour period, five times per week in an unpleasant, time-consuming and expensive procedure. Research carried out at King's College London (KCL) by Prof Robert Hider (1987-2008, Emeritus Professor of Medicinal Chemistry) and Dr Sukhi Bansal (1989-present, Reader in Pharmaceutical Science) have brought about both a new class of therapeutic agent for thalassaemia and the development of analytical methods to monitor patient progress when undergoing chelation treatment.

Introduction of more easily administrated orally active iron chelators has reduced the dependence on desferoxamine. Included in these is deferiprone, designed by researchers at KCL in the 1980's and widely used since 1999. In addition to the advantages of oral chelation with deferiprone in terms of quality of life, KCL researchers specifically designed this drug with the ability to facilitate the movement of iron across membranes and thus remove excess iron from heart and endocrine tissue (Dobbin PS, et al. 1993). This gives deferiprone an advantage over similar chelators as heart failure due to iron overload is the predominant cause of death in those with beta-thalassæmia major.

With heart failure a major concern, monitoring of iron levels is essential for people undergoing repeated blood transfusion and accompanying iron chelation. Monitoring is equally as essential for those undergoing iron-replacement therapy for conditions such as iron-deficiency anaemia. Such monitoring can be carried out through analysis of levels of hepcidin, a peptide hormone involved in regulating plasma iron load. KCL researchers have been instrumental in refining a mass spectrometry (MS) assay for hepcidin to be used in clinical practice. One important aspect of this was the synthesis of hepcidin in the form of [^{15}N,$^{13}C_2$]Gly12,20-hepcidin for use as a reliable internal standard. The assay is based on the quantitative detection of the hepcidin signal by MS relative to the heavy isotope-labelled internal standard that is added at a known concentration. Because the signal intensity is linear for both hepcidin and [^{15}N,$^{13}C_2$]Gly12,20-hepcidin, the concentration can be calculated directly from the ratio of the signal responses (Bansal SS, et al. 2009a; Bansal SS, et al. 2009b; Bansal SS, et al. 2010). Iron overload can also be determined through levels of non transferrin-bound iron and KCL researchers have additionally developed and patented a fluorescence-based flow cytometry method for quantification of this marker that is being utilised in clinical trials (Hider RC, et al. 2010).

Following on from showing its advantages in cardiac tissue, researchers at KCL have also demonstrated that deferiprone is capable of crossing the blood brain barrier (Habgood MD, et al. 1999). Here, it can scavenge excess brain iron, as shown in a KCL study where following 4 weeks of administration of a ferrocene derivative to rats to increase the iron concentration up to 50%; subsequent treatment with deferiprone caused a significant iron content decrease (Ward RJ, et al. 1995). This research is now being translated into potential therapies for neurodegenerative conditions.

3. References to the research (indicative maximum of six references)

Bansal SS, Halket JM, Bomford A, Simpson RJ, Vasavda N, Thein SL, Hider RC. Quantitation of hepcidin in human urine by liquid chromatography-mass spectrometry. Anal Biochem 2009a;384(2):245-53. Doi: 10.1016/j.ab.2008.09.045 (30 Scopus citations)

Bansal SS, Halket JM, Fusova J, Bomford A, Simpson RJ, Vasavda N, Thein SL, Hider RC. Quantification of hepcidin using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 2009b;23(11):1531-542. Doi: 10.1002/rcm.4033 (27 Scopus citations)

Bansal SS, Abbate V, Bomford A, Halket JM, Macdougall IC, Thein SL, Hider RC. Quantitation of hepcidin in serum using ultra-high-pressure liquid chromatography and a linear ion trap mass spectrometer. Rapid Commun Mass Spectrom 2010;24(9):1251-259. Doi: 10.1002/rcm.4512. (19 Scopus citations)

Dobbin PS, Hider RC, Hall AD, Taylor PD, Sarpong P, Porter JB, Xiao G, van der Helm D. Synthesis, physicochemical properties, and biological evaluation of N-substituted 2-alkyl-3-hydroxy-4(1H)-pyridinones: orally active iron chelators with clinical potential. J Med Chem 1993;36(17):2448-458. Doi: 10.1021/jm00069a002 (139 Scopus citations)

Habgood MD, Liu ZD, Dehkordi LS, Khodr HH, Abbott J, Hider RC. Investigation into the correlation between the structure of hydroxypyridinones and blood-brain barrier permeability. Biochem Pharmacol 1999;57(11):1305-310. Doi: http://dx.doi.org/10.1016/S0006-2952(99)00031-3 (46 Scopus citations)

Hider RC, Silva AM, Podinovskaia M, Ma Y. Monitoring the efficiency of iron chelation therapy: the potential of nontransferrin-bound iron. Ann N Y Acad Sci 2010;1202:94-9. Doi: 10.1111/j.1749-6632.2010.05573.x (10 Scopus citations)

Ward RJ, Dexter D, Florence A, Aouad F, Hider R, Jenner P, Crichton RR. Brain iron in the ferrocene-loaded rat: its chelation and influence on dopamine metabolism. Biochem Pharmacol 1995;49:1821-826. Doi: http://dx.doi.org/10.1016/0006-2952(94)00521-M (31 Scopus citations)

Grants

PI(s)	Title	Awarding Body	Amount	Dates
Hider RC	Development of Chelators for Neurodegeneration	British Technology Group	£250,000	2004-2007
Smith N, Bansal S, Bomford A, Raja K, Hider RC, Simpson R	Hepcidin: a new marker for iron stores	Guy's and St Thomas Trustees	£90,000	2005-2007
Hider RC, Bansal S	Development of iron complexes for the treatment of anaemia and hepcidin analogues	Vifor	£470,000	2006-2012
Hider RC, Porter J	Novel approaches to quantification and speciation of plasma non transferrin-bound iron: implication for prevention of iron mediated toxicity	Wellcome Trust	£365,900	2010-2011

4. Details of the impact

FDA approval for KCL's deferiprone

Work at King's College London (KCL) led to the development of the iron-chelator deferiprone, a drug that has had a major worldwide impact on the treatment of systemic iron overload in people with beta-thalassaemia. Independent studies have shown that in combination with desferoxamine is it currently the most efficient method of chelation-based iron removal (1a). Although approved for European use since 1999, until recently it was not available in the United States. Prior to the

introduction of deferiprone, cardiac failure due to iron overload still accounted for 67% of deaths in thalassaemia major. Combination treatment was shown to prevent or reverse cardiac complications (1a). Such findings led to extensive US patient lobbying in support of deferiprone, for instance as guided by the Cooleys Anemia Foundation who encouraged patients with thalassaemia to write to the Food and Drug Administration (FDA) in support of deferiprone's new drug application (1b). Approval was finally gained in 2011 due to an unmet need for a choice of iron chelation therapy in those for which blood transfusion leads to potentially fatal cardiac iron burden (1c,d). Since then, the American Heart Association's consensus statement on 'cardiovascular function and treatment in beta-thalassaemia major' recommends that "the first principle of management of acute heart failure is control of cardiac toxicity related to free iron by urgent commencement of infusion of high-dose intravenous deferoxamine augmented by oral deferiprone" (1e).

KCL research leads to efficient monitoring of iron chelation

With the increasing therapeutic use of iron chelators there is an increasing desire of clinicians to be able to monitor the efficacy of iron chelation in plasma and urine. At the other end of those needing monitoring is the group of patients who require iron replacement therapy, for instance those with certain types of iron-deficiency anaemia. While such therapy for these patients is vital, too much can be toxic. Two suitable parameters closely related to iron overload are the iron regulatory peptide hormone hepcidin and non transferrin-bound iron (NTBI). Clinical analysis of hepcidin is through mass spectrometry and a highly important development in the use of this was the synthesis by KCL researchers of a reliable internal standard in the form of a synthetic hepcidin. KCL houses one of the few labs worldwide capable of the synthesis of such. Important also was the refinement of the method of analysis itself, also carried out at KCL. Prior to this, methods either lacked specificity and/or required extraction or quantitation techniques that gave rise to potentially variable recovery of hepcidin.

Research at KCL led to the development of an assay that has both high rates of recovery from biological matrices and is highly reproducible. This assay has been widely adopted by hospital clinicians and the international pharmaceutical industry and KCL is currently undertaking commercial hepcidin analysis for nine clinical trials being carried out by Novartis Pharmaceuticals (Camberley, Surrey) and Vifor Pharma (St. Gallen, Switzerland), the two major companies involved in the manufacture of iron-based pharmaceuticals. Vifor Pharma writes in a letter of support for KCL that they "recognise the significant impact that the research by Bansal and Hider has had in the area of developing a robust assay for the measurement of hepcidin in clinical samples." They particularly highlight the findings of Bansal 2009a, 2009b and 2010 (2a).

Iron overload may also be determined through the monitoring of NTBI and the fluorescence-based method of detection of NTBI developed at KCL, whose patent was published in June 2010 (2b), is being utilised by Vifor Pharma (2a), Wageningen University in the Netherlands and Heidelberg University in Germany. It is also being used at three major clinical centres in London: Imperial College, University College London and KCL.

Clinical studies of iron chelation for neurodegenerative diseases

KCL has also led the field in the development of iron chelators for the treatment of neurodegeneration. Iron has been shown to accumulate in specific regions in the brain in neurodegenerative diseases such as Parkinson's disease (PD), a disorder that affects 5% of those over 80. Labile iron also accumulates in the mitochondria of patients with Friedreich's ataxia (FA), which can lead to oxidative damage in the brain, heart and endocrine glands and ultimately contribute to an early death. Chelation therapy provides a novel approach for the treatment of these devastating conditions and investigation has now been brought to the clinical trial phase. In a one year pilot trial in Italy involving four patients with pantothenate kinase-associated neurodegeneration (PKAN) and two with parkinsonism and focal dystonia, administration of deferiprone led to decreased iron accumulation in the globus pallidus of two of the patients and a mild-to-moderate motor improvement in three. This study particularly mentions the discoveries of Hapgood 1999 (3a).

ApoPharma, the manufacturers of deferiprone, are now carrying out larger scale trials for patients with PKAN (3b) and have completed a long-term safety, tolerability and efficacy study for patients with FA (3c,d). A letter of support from the President of ApoPharma, notes that publications including Ward 1995, Hapgood 1999 and Hider 2010 “have contributed to the recognition of the potential of deferiprone for its ability to facilitate the removal of iron from a wide range of sensitive tissues, including the heart, endocrine organs and brain.” They also say how “Iron chelation is emerging as a promising therapeutic strategy for the treatment of several forms of neurodegenerative disease and deferiprone is becoming the chelator of choice for such therapy” (3e). Imperial College London (3f) and University Hospital, Lille, France (3g) are also carrying out pilot trials for PD patients, the former of which specifically cites Ward 1995 when discussing this treatment.

5. Sources to corroborate the impact

1. **FDA approval for KCL’s deferiprone**
   a. Farmaki K, et al. Normalisation of total body iron load with very intensive combined chelation reverses cardiac and endocrine complications of thalassaemia major. B J Haematol 2010;148(3):466-75. Doi: 10.1111/j.1365-2141.2009.07970.x
   b. Cooleys Anemia Foundation letter for patients: http://www.thalassemia.org/action-alert-write-to-the-fda-supporting-chelator-application-2/
   c. FDA approval of deferiprone: http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm275836.htm
   d. FDA New Drug Application: http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/OncologicDrugsAdvisoryCommittee/UCM271537.pdf
   e. Pennell DJ, et al. Cardiovascular Function and Treatment in β-Thalassemia Major: A Consensus Statement from the American Heart Association. Circulation 2013;128(3):281-308. Doi: 10.1161/CIR.0b013e31829b2be6

2. **KCL research leads to efficient monitoring of iron chelation**
   a. Letter of professional corroboration on file from Vifor Pharma
   b. Patent: Hider RC, Ma YM, Podinoskaia M, Schiable U. Measurement of nontransferrin bound iron. GB1007209.8, Published 16.6.2010. http://www.ipo.gov.uk/p-pj-ukappfiled.htm?StartYear=2010&StartDay=16th%20-%206317&startMonth=June&EndYear=2010&EndDay=16th%20-%206317&EndMonth=June&searchid=1342416

3. **Clinical studies of iron chelation for neurodegenerative diseases**
   a. Abbruzzese G, et al. A pilot trial of deferiprone for neurodegeneration with brain iron accumulation. Haematologica 2011;96(11):1708-711. Doi: 10.3324/haematol.2011.043018. Clinicaltrials.gov Identifier: NTC00907283
   b. A Two-arm Efficacy and Safety Study of Deferiprone in Patients With Pantothenate Kinase-associated Neurodegeneration (PKAN): http://clinicaltrials.gov/ct2/show/NCT01741532?term=deferiprone&rank=1
   c. A Study Investigating the Safety and Tolerability of Deferiprone in Patients With Friedreich's Ataxia: http://clinicaltrials.gov/ct2/show/NCT00530127?term=deferiprone&rank=10
   d. A Study Investigating the Long-term Safety and Efficacy of Deferiprone in Patients With Friedreich's Ataxia: http://clinicaltrials.gov/ct2/show/NCT00897221?term=deferiprone&rank=2
   e. Letter of professional corroboration on file from Apo Pharma Inc.
   f. Imperial College London Trial: A Pilot Clinical Trial With the Iron Chelator Deferiprone in Parkinson's Disease (DeferipronPD): http://clinicaltrials.gov/ct2/show/NCT01539837?term=deferiprone&rank=4
   g. University Hospital, Lille Trial: Efficacy and Safety of the Iron Chelator Deferiprone in Parkinson's Disease (FAIR-PARK-I): http://clinicaltrials.gov/ct2/show/NCT00943748?term=deferiprone&rank=3