



BMH Med. J. 2017;4(4):116-121 **Review Article**

Bench to Bedside: Future Therapies For Treatment Of Anemia In Patients With Chronic Kidney Disease

KM Murali, MD, DM

Wollongong Hospital and University of Wollongong

Address for Correspondence: Dr. KM Murali, MD, DM, Senior Staff Specialist and Clinical Associate Professor, Wollongong Hospital and University of Wollongong. Email: km_murali@hotmail.com

Keywords: anemia, chronic kidney disease

Introduction

The link between chronic kidney disease (CKD) and anemia has been known for over 180 years ever since it was highlighted by the legendary Richard Bright [1]. The key etiological factors responsible for anemia due to renal disease were identified in the 1950s as relative deficiency of erythropoietin and reduced red cell survival as well as abnormalities in iron metabolism [2]. This knowledge was not translated to therapeutics for the next three decades and red cell transfusions were the main-stay of treatment of renal anemia till recombinant human erythropoietin (rHuEPO) was introduced in the 1980s [3]. Recurrent transfusions, in addition to causing HLA sensitization, frequently resulted in iron overload with subsequent parenchymal iron deposition and organ dysfunction - a well-known scenario in the pre-erythropoietin era and use of iron chelation therapy was a feasible therapeutic option in long-term dialysis patients [2]. This situation changed dramatically with the widespread clinical use of erythropoiesis stimulating agents (ESA), and polar opposite to the iron overload problems, most patient on erythropoietin therapy required oral or parenteral iron supplements to replenish iron stores for optimal erythropoiesis [4]. Over the last three decades developments in molecular engineering lead to the modification of the original rHuEPO to longer acting analogues such as darbepoetin (Aranesp[®]) and methoxy polyethylene glycol-epoetin beta (Mircera[®]) and a raft of EPO biosimilars [5]. In India, where the cost of medications constrains the universal access to ESA therapy, biosimilar erythropoietins offer a relative advantage in that they cost less than half the price of the premium brands [6]. Nevertheless, the progress in clinically prescribed ESA therapy since the discovery of rHuEPO has resulted from modest modification of existing knowledge rather than a revolutionary break from convention. Over the last few years newer molecules capable of revolutionizing the therapy of renal anemia have been or are being developed and tested in clinical trials. Some of these have the potential to become realistic treatment options in the near future.

Can there be a better therapy for renal anemia than erythropoietin?

The need for a better erythropoietic agent has always existed. The available therapies - rHuEPO and its analogues are costly and require parenteral administration, which can constrain its use in special patient groups and locations. Despite the consistent relationship between low hemoglobin and

adverse patient outcomes in observational studies, randomized controlled trials have demonstrated an increased risk for death or adverse outcomes with treatment using ESA targeting higher hemoglobin [7]. While this paradoxical finding may be related to the factors such as achieved hemoglobin, the impact of hypertension or the effect of intravenous iron, it has been suggested that the thrombotic effects of EPO may well be due to the drug itself, especially in high doses [8]. The intermittent administration of high dose ESA cannot be expected to biologically mimic the low level continuous physiological secretion of EPO [7].

Iron supplementation is an integral part of ESA therapy in the treatment of renal anemia [4]. End stage renal failure patients have reduced intestinal iron absorption, increased iron loss through haemodialysis and require greater iron turnover to facilitate ESA-driven red cell production [9]. Subjects with even normal iron stores prior to ESA therapy require concomitant administration of iron to achieve optimal erythropoietic response [8]. Intravenous iron is superior to oral iron therapy when used with ESAs in achieving hemoglobin targets [9]. In recent years there is a trend towards increased use of intravenous iron in dialysis patients with unprecedented increase in ferritin concentrations [10]. Intravenous iron therapy has the potential to exacerbate the already high level of oxidative stress prevalent in dialysis patients and has been thought to increase the risk of infections, cardiovascular events and death [9]. In the absence of high quality evidence, the impact of this ubiquitous therapy on mortality and other relevant outcomes in dialysis patients is difficult to quantify [10].

It is therefore obvious that the main-stay therapeutic options for the treatment of renal anemia are fraught with safety concerns, prompting the search for a more efficient and less harmful therapy. Better understanding about the erythropoietic mechanisms has fueled research that has led to the development of several newer molecules to treat renal anemia, some of which have the potential to revolutionize its therapy in the coming years.

Advances in EPO regulation and Iron homeostasis

It has been known since 1950s that anoxia to one or both kidneys stimulate the secretion of erythropoietin [2]. Hypoxic conditions alter gene expression in the cells, inducing a myriad of proteins essential for cell survival [11]. These changes are initiated by a heterodimeric nuclear transcription factor called hypoxia-inducible factor (HIF), that is made up of an oxygen regulated alpha-subunit and a constitutive beta-subunit. When oxygen is available, prolyl hydroxylase domain containing (PHD) enzymes hydroxylates the alpha-subunit of HIF, facilitating its binding to Von Hippel-Lindau (VHL) tumor suppressor protein and subsequent degradation [12], by the E3 ubiquitin ligase complex [11]. PHD enzymes are inhibited by a decrease in cellular oxygen and iron levels, interfering with the hydroxylation of HIF alpha-subunits, thereby inhibiting their VHL binding and degradation. PHD inhibitors lead to stabilization of HIF, promoting transcriptional activation of several genes including that for EPO [12].

Iron homeostasis is critically linked to erythropoiesis. Since our body has no mechanism to excrete excess iron, its main regulation is at the level of intestinal absorption. Hepcidin, secreted by the liver is the master regulator of iron homeostasis in humans and other mammals [11]. It is also known as HAMP (Hepcidin anti-microbial peptide) and was originally identified as a liver expressed anti-microbial peptide (LEAP) with activity against a number of bacterial and fungal species [13]. It is partly regulated by oxygen and iron levels in the cells - the same factors that regulate red cell production through HIF, which may thus be the main link between erythropoiesis and iron homeostasis [11]. Hepcidin regulates iron by altering the cell surface expression of ferroportin, which is the only known iron exporter protein at all sites [14]. Hepcidin binds to ferroportin leading to its internalization and degradation thereby inhibiting iron transport at all sites - in the intestine and at the tissue level [13].

Once dietary iron is absorbed by the enterocyte it can be stored in ferritin or mobilized to circulation

by ferroportin. Once in the blood stream, iron is loaded to the circulating carrier protein transferrin, which binds to transferrin receptor on target cells and gets internalized. Inside the cells, the iron is unloaded from the transferrin, which is then shuttled back to the circulation and made available for another round of iron transport. The iron released inside the cell, can either be utilized for synthetic purposes or stored mainly as ferritin primarily in the liver. When the physiologic need arises the iron stored in ferritin can be recycled through the reticulo-endothelial system, again transported by ferroportin [14]. By downregulating ferroportin at all locations, hepcidin inhibits iron mobilization at the sites of iron absorption (duodenal enterocytes), storage (mainly hepatocytes) and recycling (macrophages of reticuloendothelial system) [13]. The net result of its action is reduced iron availability or utilization.

Patients with chronic kidney disease have high levels of hepcidin thought to be related to diminished renal clearance and chronic inflammation [15]. Excess hepcidin may be an important contributor to the anemia and functional iron deficiency seen in patients with chronic kidney disease.

Iron status and chronic inflammation are the two major stimuli that increase hepcidin production in the liver [13]. Iron mediated hepcidin regulation, works mainly through the signaling involving proteins belonging to the transforming growth factor beta superfamily - BMP/SMAD (bone morphogenetic protein/ sma and mothers against decapentaplegic) pathway, where haemojuvelin (HJV) plays a key role [16]. Increased expression of inflammatory cytokines is common in patients with kidney disease and is thought to be an important contributor to the renal anemia [17]. Inflammation stimulates the synthesis of hepcidin through the IL-6/JAK/STAT3 (Interleukin-6/Janus kinase/ Signal transducer and activator of transcription 3) signaling [16].

The major repressors of hepcidin, thereby improving iron availability or iron utilization, include erythropoiesis, hypoxia and hormones like testosterone. Though EPO therapy leads to reduction of hepcidin, it has been suggested that the effect is not direct, because EPO does not repress hepcidin when co-treated with inhibitors to erythropoiesis [13]. However hepatocytes have EPO receptors and direct inhibition of hepcidin synthesis has also been proposed [11]. Hypoxic conditions lead to inhibition of PHD enzyme resulting in stabilization of HIF, which can repress hepcidin indirectly through its effect on erythropoiesis and directly through hypoxia responsive elements in the hamp gene [13]. Though hypoxia can inhibit hepcidin expression through non-HIF pathways, HIF appears to be the key molecule that links erythropoiesis and iron homeostasis[11].

Future erythropoiesis stimulating agents

Several novel modulators of erythropoiesis targeting newly recognized pathways are in varying stages of development. Some of them may be available for routine clinical use in the near future. These drugs can be broadly categorized as agents targeting hypoxia inducible transcription pathways, modulators of hepcidin expression and agents targeting proteins belonging to TGF-beta superfamily [18].

PHD inhibitors / HIF stabilizers

Small-molecule drugs that inhibit PHD enzymes, resulting in stabilization of HIF and increased production of endogenous erythropoietin have been developed [19]. There are three subclasses of PHD enzymes, PHD 1, 2 and 3. Inhibition of PHD2 is capable of inducing near maximal EPO production in the kidneys, while inhibition of all three PHDs was needed to induce extra -renal, predominantly hepatic EPO production [18]. The stimulation of endogenous erythropoietin production by the HIF stabilizers may offer the advantage of increasing the EPO levels above a certain threshold on a pulsatile daily basis, mimicking natural secretion of erythropoietin which may be safer than administering pharmacological concentrations of erythropoietic proteins [5]. This may

be particularly appealing since higher doses erythropoietin have been consistently associated with poor clinical outcomes [7].

Another potential advantage is their beneficial effect with respect to iron utilization. Conventional EPO requires replete iron stores for optimal erythropoiesis, often necessitating the intravenous administration of iron. HIF stabilizers by inhibiting hepcidin lead to better iron absorption and utilization with the potential to reduce the need for intravenous iron [18]. Another distinct advantage of these small-molecule HIF stabilizers is that they are orally active, which would be expected to be associated with better product stability and lower the cost of production and distribution [19]. Roxadustat (AstraZeneca) Vadadustat (Akebia), Daprodustat (GlaxoSmithKline) and Molidustat (Bayer) are currently undergoing phase 2 or phase 3 trials [20].

It is also possible that PHD inhibitors compared to EPO have a beneficial effect on the high cardiovascular risk observed in patients with chronic kidney disease. Such benefits could be argued to result from the perceived favourable effects in comparison to EPO, on cardiovascular risk factors including blood pressure, glucose tolerance and lipid profile as well as the proposed ability to protect organs from ischaemic injury [19]. The results of clinical trials that are currently underway evaluating whether PHD inhibitors/ HIF stabilizers offer any advantage with respect to cardiovascular endpoints at comparable haemoglobin levels will define the role of these agents as an alternative to ESA and iron therapy in the treatment of CKD patients with anaemia [20].

PHD Inhibitors/HIF stabilizers have the potential to cause several adverse effects. Some of these are extension of their physiologic effect, while others are related to the off-target effects from the inhibition of other 2-oxoglutarate dependent enzymes [19]. Higher levels of hemoglobin has been associated with increased risk of thrombotic events in patients treated with ESA [7] and the effect is unlikely to be different with PHD inhibitors. Persistent HIF activation in patients with genetic mutations involving HIF pathway may be linked to the development of cancers (VHL disease associated with renal cell cancer and pheochromocytoma) and pulmonary hypertension (Chuvash polycythemia and HIF2a mutations are associated with severe pulmonary hypertension) [19]. However the concern that PHD inhibitors/HIF stabilizers may cause these adverse events were not substantiated in phase 2 trials studying these drugs [20].

Various 2-oxoglutarate dependent enzymes, structurally similar to the PHD enzymes are involved in methylation of RNA, DNA and histone as well as hydroxylation of ankyrin repeats and matrix formation [19]. Inhibition of these enzyme could cause a variety of off-target effects, which are not currently known. Phase 2 trials have not demonstrated any serious pattern of side effects and phase 3 studies of these agents are currently underway[18]. It is quite possible that that these agents could become the mainstay therapy of renal anaemia within the next decade if the phase 3 trials turn out to maintain haemoglobin without causing excess risk of cardiovascular or other systemic adverse effects in comparison to EPO therapy.

Hepcidin modulators for treatment of renal anaemia

Drug candidates that directly inhibit hepcidin are in much earlier phases of development in the therapeutics of renal anaemia [18] compared to PHD inhibitors. Indirect, but significant suppression of hepcidin is achieved by PHD inhibitors that stabilize HIF which may offer a dual advantage of erythropoietin stimulation and improved iron utilization [19]. Though heparin, vitamin D and erythropoietin use also lower hepcidin, none of these options will significantly lower the levels in CKD patients at doses used in standard clinical practice [18]. Drugs that sequester hepcidin (antibodies, anticalins), inhibit BMP/SMAD/HJV pathway or IL6/STAT3 pathway or agents that affect hepcidin transduction (siRNA/shRNA) or stabilize ferroportin have the potential to be effective in conditions where hepcidin excess is a cause or contributor to anaemia [16].

Most of the drugs that repress hepcidin are being developed to target anaemia of inflammation and

are not specific to treat anaemia of renal disease [18]. Trials to evaluate these agents in patients with renal disease have a narrow scope and the trial initiated by Ferrumax Pharmaceuticals, Inc. evaluating sHJV.Fc (FMX-8), in patients with renal anaemia interfering with haemojuvelin pathway, was terminated due to an inability to recruit patients that meet the inclusion criteria [21]. Nevertheless, hepcidin will remain an important therapeutic target in the future and hepcidin modulation - increasing or decreasing hepcidin expression, offers promise in the treatment of a number of hematological disorders with link to iron metabolism, like hereditary hemochromatosis, polycythemia vera, anaemia of inflammation and iron-refractory iron deficiency anaemia [14].

Other candidates in the pipeline

Sotatercept is a molecule that trap activins, a family of protein belonging to TGF-beta superfamily and is being evaluated in the therapy of anaemia due to renal disease [22]. The drug was initially used in a trial to improve bone mineral density in post-menopausal women and serendipitously noted to increase haemoglobin [18]. The drug caused only mild adverse effects and its use was not associated with development of anti-drug antibodies [22]. The agent promotes late stage erythroid precursors and has a mechanism of action distinctly different from that of EPO [18]. Clinical trials are underway using Luspatercept (ACE 536) an agent analogous to Sotatercept, acting as a ligand trap for members in the TGF-beta superfamily [22].

Synthetic peptides that mimic EPO, like Peginesatide, Pegolsihematide and EMP antibody fusion protein as well as EPO gene therapy have also raised some expectations as potential novel approaches to treatment of renal anaemia [18]. Of this Peginesatide was recalled only one year after it was rolled out for routine clinical use in the USA, due to serious side effects [22].

Conclusions

Recent advanced in the understanding of pathways in the EPO regulation and iron homeostasis have led to development of novel treatment strategies, that offer great promise in the therapeutics of anaemia of renal disease. PHD inhibitors/HIF stabilizers offer the best hope of revolutionizing the management of renal anaemia within the next decade, but hepcidin inhibitors may capture a niche area in the treatment of renal anaemia patients with certain characteristics. In the meantime, erythropoietin and their longer acting analogues remain the mainstay of therapy more than three decades after they were first rolled out for clinical use. The future however looks bright and the light at the end of the tunnel is fast approaching.

References

1. Bright R: Cases and observations: Illustrative of renal disease accompanied by the secretion of albuminous urine. *Guys Hosp Rep* 1836, 1:338.
2. Adamson JW, Eschbach J, Finch CA: The kidney and erythropoiesis. *Am J Med* 1968, 44(5):725-733.
3. Eschbach JW, Egrie JC, Downing MR, Browne JK, Adamson JW: Correction of the anemia of end-stage renal disease with recombinant human erythropoietin. Results of a combined phase I and II clinical trial. *N Engl J Med* 1987, 316(2):73-78.
4. Humphries JE: Anemia of renal failure. Use of erythropoietin. *The Medical clinics of North America* 1992, 76(3):711-725.
5. Macdougall IC: New anemia therapies: translating novel strategies from bench to bedside. *Am J*

Kidney Dis 2012, 59(3):444-451.

6. Khanna U: The economics of dialysis in India. Indian journal of nephrology 2009, 19(1):1-4.
7. Fishbane S, Besarab A: Mechanism of increased mortality risk with erythropoietin treatment to higher hemoglobin targets. Clin J Am Soc Nephrol 2007, 2(6):1274-1282.
8. Bunn HF: Erythropoietin. Cold Spring Harb Perspect Med 2013, 3(3):a011619.
9. Besarab A, Coyne DW: Iron supplementation to treat anemia in patients with chronic kidney disease. Nat Rev Nephrol 2010, 6(12):699-710.
10. Charytan DM, Pai AB, Chan CT, Coyne DW, Hung AM, Kovesdy CP, Fishbane S, Dialysis Advisory Group of the American Society of N: Considerations and challenges in defining optimal iron utilization in hemodialysis. J Am Soc Nephrol 2015, 26(6):1238-1247.
11. Shah YM, Xie L: Hypoxia-inducible factors link iron homeostasis and erythropoiesis. Gastroenterology 2014, 146(3):630-642.
12. Lee FS, Percy MJ: The HIF pathway and erythrocytosis. Annu Rev Pathol 2011, 6:165-192.
13. Rishi G, Wallace DF, Subramaniam VN: Heparin: regulation of the master iron regulator. Biosci Rep 2015, 35(3):31.
14. Vyoral D, Jiri P: Therapeutic potential of hepcidin - the master regulator of iron metabolism. Pharmacological research 2017, 115:242-254.
15. Babitt JL, Lin HY: Mechanisms of anemia in CKD. J Am Soc Nephrol 2012, 23(10):1631-1634.
16. Poli M, Asperti M, Ruzzenenti P, Regoni M, Arosio P: Heparin antagonists for potential treatments of disorders with hepcidin excess. Frontiers in pharmacology 2014, 5:86.
17. Yilmaz MI, Solak Y, Covic A, Goldsmith D, Kanbay M: Renal anemia of inflammation: the name is self-explanatory. Blood Purif 2011, 32(3):220-225.
18. Schmid H, Jelkmann W: Investigational therapies for renal disease-induced anemia. Expert Opin Investig Drugs 2016, 25(8):901-916.
19. Maxwell PH, Eckardt KU: HIF prolyl hydroxylase inhibitors for the treatment of renal anaemia and beyond. Nat Rev Nephrol 2016, 12(3):157-168.
20. Gupta N, Wish JB: Hypoxia-Inducible Factor Prolyl Hydroxylase Inhibitors: A Potential New Treatment for Anemia in Patients With CKD. Am J Kidney Dis 2017, 69(6):815-826.
21. Blanchette NL, Manz DH, Torti FM, Torti SV: Modulation of hepcidin to treat iron deregulation: potential clinical applications. Expert Rev Hematol 2016, 9(2):169-186.
22. Jelkmann W: The ESA scenario gets complex: from biosimilar epoetins to activin traps. Nephrol Dial Transplant 2015, 30(4):553-559.