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Novel Approaches in Erythropoietin

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Abstracts

Erythropoietin, or EPO, is a glycoprotein hormone that controls erythropoiesis, or red blood cell production. It is a cytokine for erythrocyte (red blood cell) precursors in the bone marrow. Also called hematopoietin or hemopoietin, it is produced by the peritubular capillary endothelial cells in the kidney, and is the hormone that regulates red blood cell production. The existence of a hormone that controls RBC production was first suggested by the experiments of Paul Carnot in 1906, who created anemic rabbits and then transfused their serum into recipient rabbits. EPO is produced by peritubular cells in the adult kidney, and in hepatocytes in the fetus. In adults, a small amount is also produced by the liver. The rate of Epo synthesis and secretion depends on local oxygen concentrations; hypoxia is the main stimulus for Epo production. Although the use of erythropoietin has been studied in critically ill patients, erythropoietin has not been shown to be effective in this setting. In a randomized controlled trial, erythropoietin insignificantly reduced mortality among critically ill patients. In 1983, the gene coding for EPO was identified, leading to its synthesis as epoetin-alfa by American genetic research corporation, Amgen, who patented the drug under the name Epogen. In 1989, another company, Ortho Biotech, a subsidiary of Johnson and Johnson, began marketing the drug under license as Procrit in the US, and Eprex in the rest of the world.

Key words: Erythropoietin, Erythropoesis Stimulating Agents.

Introduction

1. Erythropoietin:

1.1 Definition:

Erythropoietin, or EPO, is a glycoprotein hormone that controls erythropoiesis, or red blood cell production. It is a cytokine for erythrocyte (red blood cell) precursors in the bone marrow. Also called hematopoietin or hemopoietin, it is produced by the peritubular capillary endothelial cells in the kidney, and is the hormone that regulates red blood cell production. It also has other known biological functions. For example, erythropoietin plays an important role in the brain's response to neuronal injury. EPO is also involved in the wound healing process.

When exogenous EPO is used as a preformance-enhancing drug, it is classified as an erythropoiesis-stimulating agent (ESA). Exogenous EPO can often be detected in blood, due to slight difference from the endogenous protein, for example in features of posttranslational modification[1, 2].

1.2 History:

The existence of a hormone that controls RBC production was first suggested by the experiments of Paul Carnot in 1906, who created anemic rabbits and then transfused their serum into recipient rabbits. The observation that recipient rabbits increased their RBC production above normal supported his idea, but isolation and purification of the active component took several decades. Extensive studies were carried out in patients with aplastic anemia, leading to a major breakthrough in 1977 when small amounts of erythropoietin were purified from the urine of these patients. Amino acid sequence data from this protein were used in subsequent efforts to clone the gene for erythropoietin in 1983.The gene was then inserted into a suitable mammalian cell line, Chinese hamster ovary cells, allowing large-scale manufacture of the protein as a commercial product. It was approved for use in 1991. About $10B was spent worldwide in 2006 for treatment of patients with rHuEpo, with about $2B for the cost of treating Medicare patients on dialysis[3-5].

1.3 Structure:

EPO is a glycoprotein with a molecular mass of 30.4 kD. Its structure includes a 165-amino acid backbone with three N-linked carbohydrates attached to asparagines at amino acid positions 24, 38, and 83 and one O-linked carbohydrate attached to Ser126 .The carbohydrate residues allow for many possible isoforms and contribute to the stability of the hormone in vivo. Darbepoetin was created through site directed mutation of two amino acid residues, allowing for two additional N-linked carbohydrate chains[6].

1.4 Production:

EPO is produced by peritubular cells in the adult kidney, and in hepatocytes in the fetus. In adults, a small amount is also produced by the liver. The rate of Epo synthesis and secretion depends on local oxygen concentrations; hypoxia is the main stimulus for Epo production. The serum concentration of Epo in adults is normally 4-27 mU/mL. In adults with non-renal anemias, the serum concentration tends to increase with the severity of the anemia.

1.5 Actions:
EPO's activities depend on successful interaction with its receptor, which is prominent on the surface of developing RBC in the bone marrow. Epo signaling acts to prevent or retard apoptosis, i.e., it acts as a survival factor for developing cells. The increase in RBC mass brought about by Epo stimulation of the bone marrow completes a self-regulating feedback loop, since (other things being equal), the increased RBC mass would lessen the hypoxia experienced by the kidney and thus, lessen Epo production[6].

1.6 Uses:
Anemia in chronic kidney disease
Erythropoietin may be used in patients with chronic kidney disease. In this setting, the goal hemoglobin should be 11.3 g per deciliter. In patients who require renal dialysis, iron should be given with erythropoietin. Use of erythropoiesis-stimulating agents for anemia related to cancer may increase mortality[7-9].

1.7 Route of administration:
For injection dose : For severe anemia in Adults: The usual dose is 80 to 120 Units per kilogram (kg) of body weight three times a week, injected IV or subcutaneously. The dose is gradually decreased by 25 Units per kg of body weight every four weeks or more until the lowest effective dose is reached. Most patients who have low iron stores require concurrent iron therapy for optimal response[10].

1.8 Adverse effect: Erythropoietin is associated with an increased risk of adverse cardiovascular complications in patients with kidney disease if it is used to increase hemoglobin levels above 13.0 g/dl. The FDA released an advisory on March 9, 2007, and a clinical alert on February 16, 2007, about the use of erythropoeisis-stimulating agents. The advisory noted these drugs had a "higher chance of serious and life-threatening side effects and/or death...and had a higher rate of deep venous thrombosis". Erythropoietin may increase blood pressure. Pure red cell aplasia: Caused by formation of antibodies to EPO. Thought to be due to change in the immunogenicity of the EPO molecule[14, 15].

1.9 Causes of inadequate response to EPO:[16]
- Iron deficiency.
- Chronic blood loss.
- Folate or vitamin B 12 deficiency.
- Infection/inflamation.
- Malnutrition.
- Hemolysis.
- Osteitis fibrosa.
- Aluminium toxicity.
- Hemoglobinopathies.(eg.alfa & beta thalassemias, sickle cell anemia).
- Multiple myeloma & malignancy.
- Use of ACE-I agents.

1.10 Benefits:

Resolution of anemia can provide many benefits, including improved exercise tolerance and increased ability to carry out the activities of daily life. Avoidance of transfusions is another important benefit[11].

1.11 Risks and Complications:

- Adverse cardiovascular events such as heart attacks and strokes have been reported with Epo in the context of chemotherapy. These safety concerns were discussed in May 2004 by the FDA and a report is available.
- Absolute or functional iron deficiency may develop during erythropoietin therapy. Almost all patients will require supplemental iron during Epo therapy in order to support the increased rate of red blood cell production.
- Hypertension is a common effect and occurs in 20% to 30% of patients.

1.12 Precautions:

- Blood counts will be monitored before receiving erythropoietin and regularly while on the drug erythropoietin. This allows the doctor to determine if patients are candidates for this treatment and if the dose the patient is receiving needs to be increased or decreased.
- Blood pressure should also be monitored regularly while on erythropoietin. Patients who have high blood pressure that is not under control should not use erythropoietin.
- Patients may be instructed to take oral iron tablets while on erythropoietin to increase the drug's effectiveness.
- It is not recommended to give erythropoietin to patients who have cancer, such as leukemias, arising from their bone marrow.
- Patients with a known previous allergic reaction to erythropoietin or the drug albumin should tell their doctor.
- Patients who may be pregnant or trying to become pregnant should tell their doctor before receiving erythropoietin[17].

1.13 Interactions:

- In clinical studies erythropoietin did not have any drug interactions.
- In addition to taking oral iron replacement, patients should increase their intake of iron in their diet. This would include eating foods such as red meats, green vegetables, and eggs.
- Patients should tell their doctors if they have a known allergic reaction to erythropoietin or any other medications or substances, such as foods and preservatives. Before taking any new medications, including non-prescription medications, vitamins, and herbal medications, the patients should notify their doctors.

1.14 Signs of Toxicity/Overdose:

Acute overdosing is unlikely to cause a problem.
Chronic overdosing may cause too high of a red blood cell count and other related problems.

1.15 EPO receptor:

By epo binding to its receptors,the following events take place:

- Increased ca uptake.
- Increased phosphorylation of receptor as well as of several intracellular proteins.

- Increased glucose uptake within 1 hour.
- Increase in transferrin receptors after 6 hours.
- HB synthesis beings after 12 months[12-13].

2. Types of Erythropoietin:

2.1 Epoetin alfa

In 1983, the gene coding for EPO was identified, leading to its synthesis as epoetin-alfa by American genetic research corporation, Amgen, who patented the drug under the name Epogen. In 1989, another company, Ortho Biotech, a subsidiary of Johnson and Johnson, began marketing the drug under license as Procrit in the US, and Eprex in the rest of the world.

Epoetin-alfa is formulated as a colorless liquid in a solution of sodium chloride buffered with sodium citrate or sodium phosphate, and is packaged, for injection, in 1mL vials containing; either 2000, 3000, 4000, or 10,000 International Units (IU) of epoetin-alfa, 5.8 mg sodium citrate, 5.8 mg sodium chloride, and 0.06 mg citric acid in water.

2.2 Epoetin beta

In 1988 the German pharmaceutical company, produced its own recombinant erythropoietin; epoetin-beta, marketed as Neo Recormon.

Epoetin-beta (Recormon) comes in 1000 IU/0.3mL, 2000 IU/0.3mL, 3000IU/0.3mL, 4000 IU/0.3mL, 5000 IU/0.3mL, 6000 IU/0.3mL, 10,000 IU/0.6mL, and 30,000 IU/0.6mL solutions; and contains urea, sodium chloride, sodium phosphate, and water, in pre-filled syringes for injection. Not available in the USA.
The clinical efficacy of both epoetin-alfa and epoetin-beta is similar.

2.3 Darbepoetin alfa

In 2005, Amgen patented a new erythropoietic, darbepoetin alfa, under the brand name Aranesp® Although very similar to EPO, Aranesp®, when administered, has a longer active life than EPO and is approved for use in patients with chronic renal disease, whether or not they are on dialysis.

2.4 Epoetin delta

This is one of the newest agents currently available. Called DYNEPO®, this agent is also produced by recombinant technology, from human cell lines. DYNEPO® acts like other epoetins and is also indicated for anemia related to chronic kidney disease. It has received considerable attention in the sports world because DYNEPO® resembles human EPO and may not be detected by standard urine tests. comparing epoetin-alfa to delta, demonstrated the latter's efficacy for the correction of anemia; however, further studies are needed.

2.5 Recombinant Human Erythropoietin:

Recombinant erythropoietin means EPO derived using recombinant DNA technology. It is abbreviated as rHuEPO. Endogeneous erythropoietin and recombinant human erythropoietin (rHu-EPO) are similar with respect to their biological and chemical properties except for some differences in their carbohydrate chains[18].

3 Erythropoietin Test:
The erythropoietin test measures the amount of a hormone called erythropoietin ((EPO) in blood. The hormone acts on stem cells in the bone marrow to increase the production of red blood cells. It is made by cells in the kidney, which release the hormone when oxygen levels are low.

Blood is drawn from a vein, usually from the inside of the elbow or the back of the hand. The site is cleaned with germ-killing medicine (antiseptic). The health care provider wraps an elastic band around the upper arm to apply pressure to the area and make the vein swell with blood.

Next, the health care provider gently inserts a needle into the vein. The blood collects into an airtight vial or tube attached to the needle. The elastic band is removed from your arm. Once the blood has been collected, the needle is removed, and the puncture site is covered to stop any bleeding. In infants or young children, a sharp tool called a lancet may be used to puncture the skin and make it bleed. The blood collects into a small glass tube called a pipette, or onto a slide or test strip. A bandage may be placed over the area if there is any bleeding.

When the needle is inserted to draw blood, some people feel moderate pain, while others feel only a prick or stinging sensation. Afterward, there may be some throbbing. This test may be used to help determine the cause of anemia, polycythemia (high red blood cells) or other bone marrow disorders. A change in red blood cells will affect the release of EPO. For example, persons with anemia have too few red blood cells, so more EPO is produced[19].

The normal range is 0-19 milliunits per milliliter (mU/mL).

4 Erythropoietin therapy:
4.1 Treating Anemia in Patients with Cancer:
4.2 Guidelines:
Two guidelines are available to provide support for the use of erythropoietin for cancer patients. The 2006 update of the National Comprehensive Cancer Network (NCCN) guidelines advise considering erythropoietin therapy in patients with a Hgb value of 11 g/dL or less, while the American Society of Oncology and the American Society of Hematology (ASCO, ASH 2002) note a Hgb value of less than 10 g/dL as the starting point. Both NCCN and ASCO/ASH state that doses should be titrated to maintain a Hgb level of 12 g/dL.

According to the NCCN guidelines, responders are defined as patients with an increase in Hgb of at least 1 g/dL within four weeks of initiating treatment with epoetin alpha and six weeks of initiating darbepoetin alfa. Patients should continue to receive erythropoietin until they reach a Hgb level of 12 g/dL. For those who do not respond, the dose should be titrated. If patients continue to be nonresponders after an additional four weeks at a higher dose of epoetin alfa or six weeks at a higher dose of darbepoetin alfa, erythropoetic agents should be stopped. For patients with rapidly escalating Hgb (an increase of >1 g/dL in two weeks), the dosage should be reduced by 25%.

4.3 Iron Supplementation:
Iron supplementation is frequently required for patients receiving erythropoietic agents. In ambulatory patients, oral products are used, with each formulation containing different amounts

of elemental iron (Table 3). Ferrous fumarate, ferrous gluconate, and ferrous sulfate contain 33%, 12%, and 20% elemental iron, respectively. The percentage of absorbed iron decreases as the dose increases, and patients can experience constipation, dark stools, or nausea. A typical dosage is 50 to 100 mg elemental iron three times daily for six months. Caution should be employed in patients with hepatic disease, since the liver stores iron[20].

Conclusion

Consideration and treatment of all possible causes of anemia are key to providing effective treatment and achieving the goal of improved QOL. Following evaluation and treatment of other etiologies of anemia in cancer patients with nonmyeloid malignancies who are receiving chemotherapy, erythropoietic agents such as epoetin alfa or darbepoetin alfa should be considered depending on the patient's symptomatology when Hgb falls below 11 g/dL. If patients are responsive to erythropoetic agents, therapy should be continued at the same dose until achieving a Hgb of 12 g/dL. When Hgb reaches 12 g/dL or greater, the erythropoetic agent should be discontinued. Following discontinuation, if Hgb falls to 10 g/dL, therapy should be resumed. For nonresponders, the dosage should be titrated. If no response is achieved after eight weeks for epoetin alfa therapy or 12 weeks of darbepoetin alfa therapy, other causes of anemia should be considered. Iron levels should be monitored monthly, and patients should receive iron supplements as indicated.

5 Treatment of anemia in CRF:

5.1 K/DOQI guideline:

- Hb is the measurement of choice.
- Anemia work up(hct/hb, rbc indices, reticulocytes, serum Fe, TIBC, transferrin saturation, serum ferritin, faecal occult blood) should be done in patients with hb<11g/dl in premenopausal women, and hb<12g/dl in men and post menopausal women.
- Target hb for EPO therapy should be (hb 11-12g/dl(hct 33-36%)). medical justification is needed to maintain hb>12g/dl.
- Iron stores:target tranferrin saturation>20% and serum ferritin>100ng/ml. intravenous iron (50-100 mg/week for 10 weeks)should be given to patients with resistant anemia or receiving large EPO doses even if these targets are met. can be repeated. no value in maintaining saturation >50% or ferritin >800 ng/ml.
- Serum ferritin and transferrin saturation should be monitored monthly in patients not receiving IV Fe, and 3monthly in those receiving IV Fe, and then 3monthly once a target hb is reached. iron stores should be monitored 3-6monthly in patients with CRF not on EPO.
- Supplemental Fe is necessary. orally at least 200mg elemental Fe per day. intravenously iron dextran or sodium ferric gluconate complex(iron gluconate), repeated necessary(after initial test dose).oral Fe is unlikely to maintain Fe stores in HD patients,and most should receive IV Fe regularly.
- EPO should be administered subcutaneously as preferred route.
- Initial EPO dose 80-120 units/kg/week in 2 or 3 doses SC(120-180 units/kg/week if given IV).strategies for titrating dose of EPO are given.
- Hb should be monitored every 1-2weeks after initiation or changes to EPO therapy.once target achieved,monitor every 2-4 weeks.

▪ Inadequate response to EPO most commonly due to Fe deficiency.other causes include:infection,inflamation chronic blood loss,osteitis fibrosa,aluminium toxicity,hemoglobinopathies,folateorB12deficiency,myeloma,malnutrition, haemolysis,and ACEI.

▪ Transfusion are indicated in severely anaemic patients with signs or symptoms and EPO resistance with chronic blood loss.

▪ EPO possible adverse effects:BP should be monitored in all patients receiving EPO.antihypertensive therapy may need to be increased.previous seizures are not a contraindication to EPO use.no need to increase surveillance of access thrombosis in HD patients treated with EPO.patients treated with EPO do not need more intensive potassium monitoring,not more heparin.

5.2 Newer Erythropoesis Stimulating Agents {ESA's}:

1. Protein based ESA therapy.

EPO.
Darbapoietin.
Continuous erythropoietin receptor activity.
Synthetic erythropoiesis protein.

2. Small molecule ESA.

Peptide based.

3. Novel strategies.

HIF stabilizers.
HCP inhibitors.
EPO gene therapy.

1. Protein based ESA therapy:

1.1 EPO:

Route & frequency of administration:

The preferred route of administration is subcutaneous route (sc), because it can achive a 25-50% dose reduction of EPO & consequently a reduction in cost. Patients however should be explained about the rare risk of developing PRCA (Pure red cell aplasia). In patients on hemodialysis the intra venous route may be used. Once a week, subcutaneously. Patients on hemodialysis twice weekly administration may be appropriate. Dose of EPO can be increased as required.

Dose of EPO:

EPO should be started at a dose of 80-120 IU/kg/week
following initiation of EPO therapy,HB monitoring should be performed once in 2 weeks till the target HB is achived.
Once the target HB is achived, HB monitoring should be performed once every month.

▪ A 1 gm% rise in HB is necessary with EPO therapy at the end of 2 weeks. EPO dosage can be increased by 50% till the target HB is achived.

▪ If the rise in HB is > 2gms % at the end of 2 weeks, the dose should be reduced by 25%.

▪ If the target HB is reached or exceeded, reduce the dose of EPO by 25%.

▪ When a patient is unable to tolerate subcutaneous route of administration, he/she can be administered EPO by the IV route once or twice weekly.In such a situation the dose of EPO has to be increased by 25%.

▪ Further dose titration may be perfomed as per the previous recommendation.

▪ During inter-current infection, hematocrit response to EPO may be reduced.however, the previous dose of EPO should be continued.

1.2 Darbepoetin alfa

▪ Higher isoforms (↑ sialic acid residues) of recombinant human EPO more potent biologically due to a longer circulating half-life than the lower isomers.

▪ 22 sialic acid residues, compared with recombinant or endogenous EPO, which support a maximum of 14 sialic acid residues.

▪ Amino acid sequence at sites not directly involved in binding to the EPO receptor.

▪ greater metabolic stability – t1/2 in human of darbepoetin alfa increases three-fold (25.3 h) compared with epoetin alfa (8.5 h).

▪ The half-life after subcutaneous administration is doubled from approximately 24h to approximately 48 h.

▪ allows less frequent dosing, with most patients receiving injections once weekly or once every other week.

▪ once-monthly dosing with darbepoetin alfa possible in selected patients- {clinically stable and who do not yet require dialysis}.

Table 1: Dosing and monitoring of EPO-α and darbepoetin-α

	EPO-α	**Darbepoetin-α**
Dose (s.c.)	80-120 U/kg/week	0.45 µg/kg/week
Interval (Recommended)	Divided 2x/week	Weekly[23]
Interval (Range)	Up to once every 2-3 weeks	Up to Monthly
Monitoring	Every 2 weeks until stable; then monthly	Every 2 weeks until stable; then monthly
Dose Adjustment (25%-50%)	Every 2 weeks	Monthly

1.3 Continuous Erythropoietin Receptor Activity:

methoxy-polyethyleneglycol polymer chain + EPO molecule

molecular weight of CERA approximately 60 kD, compared with EPO (30.4 kD).

the half-life of circulating CERA is considerably prolonged compared with that of epoetin: at approximately 130 h.

Less frequent dosing regimens of once every 2 wk and once every month have been tested in Phase II and Phase III clinical trials.

CERA vs EPO at receptor level:
Lower receptor binding affinity
Slower receptor association rate and slightly faster dissociation rate
Higher concentration maintained at the EPO-R expressing target cells due to slower consumption (internalisation)
Greater potency in vivo
Continuous activation of receptor,closely mimicking the body's natural control of RBC formation.
Starting doses of C.E.R.A.

Previous epoetin dosage (IU/wk)	C.E.R.A. starting dosage (µg/2wk)	C.E.R.A. starting dosage (µg/4wk)
<8000	60	120
8000-16000	100	200
>16000	180	360

C.E.R.A.,Continuous erythropoietin receptor activator.

1.4 Synthetic erythropoiesis protein (SEP) :
A 51-kD protein-polymer construct which contains two covalently attached polymer moieties. Like darbepoetin alfa and CERA, this polymer stimulates erythropoiesis through activation of the EPO receptor, and with a longer circulating half-life than for EPO alone.
Large EPO fusion proteins, of molecular weight 76 kD, have been designed from cDNA encoding two human EPO molecules linked by small flexible polypeptides.
Genetic fusion of EPO with the Fc region of human IgG (Fc-EPO) promotes recycling out of the cell upon endocytosis via the Fc recycling receptor and provides an alternative mechanism for enhancing circulating half-life.

The same effect may be achieved by fusing EPO with albumin.
CTNO 528, which is an EPO-mimetic antibody fusion protein

- Enhanced serum half-life.
- No structural similarity to EPO.
- Single subcutaneous dose of CTNO 528 in rats showed a more prolonged reticulocytosis and hemoglobin rise compared with treatment with epoetin or darbepoetin alfa.
- A peak reticulocyte count occurring after 8 d, the maximum hemoglobin concentration being seen after 22 d.
- None of the 24 participants in this study developed antibodies against the molecule.

An Fc-EPO fusion protein has been successfully administered in a Phase I trial to human volunteers as an aerosol, with a demonstrable increase in EPO levels associated with an increase in reticulocyte counts.

Other delivery systems for EPO have been investigated

- Ultrasound-mediated transdermal uptake.
- Orally via liposomes{rats}.

▪ Mucoadhesive tablets containing EPO and an absorption enhancer for oral administration have been studied in rats and dogs.

2. Small-Molecule ESAs:

2.1 Peptide-Based ESAs

20 amino acids , unrelated in sequence to EPO but still bound to the EPO receptor
identified by random phage display technology.
Induce the same conformational change in the EPO receptor that leads to JAK2 kinase/STAT-5 intracellular signaling as well as other intracellular signaling mechanisms, resulting in stimulation of erythropoiesis both in vitro and in vivo.

2.1.1 Hematide (Affymax, Palo Alto, CA)

▪ A pegylated synthetic dimeric peptidic ESA - found to stimulate erythropoiesis in experimental animals .

▪ The half-life of Hematide in monkeys ranges from 14 to 60 h. depending on the dosage administered.

▪ The primary route of elimination for the peptide is the kidney .

▪ A Phase I study in healthy volunteers showed that single injections of Hematide caused a dosage-dependent increase in reticulocyte counts and hemoglobin concentrations .

▪ Phase II studies have demonstrated that Hematide can correct the anemia associated with CKD.

▪ Hematide may be administered either intravenously or subcutaneously, and dosing once a month is effective.

Potential Advantages :

Greater stability at room temperature.
Lower immunogenicity compared with conventional ESAs.
Much simpler (and cheaper) manufacturing process, avoiding the need for cell lines and genetic engineering techniques.

Antibodies against Hematide do not cross-react with EPO, and similarly anti-EPO antibodies do not cross-react with Hematide.

▪ First, even if a patient does develop anti-Hematide antibodies, these should not neutralize the patient's own endogenous EPO, and the patient should not develop pure red cell aplasia.

▪ Second, patients with antibody-mediated pure red cell aplasia should be able to respond to Hematide therapy by an increase in their hemoglobin concentration, because Hematide is not neutralized by anti-EPO antibodies.
This latter hypothesis has already been confirmed in animals.

3. Novel Strategies:

3.1 Hypoxia Inducible Factor Stabilizers:

The first oral therapy for the treatment of anemia in CKD is also in phase – 2 of clinical trials. This oral agent is hypoxia–inducible factor (HIF) stabilizers. HIF is now recognized to be a key regulator of erythropoietic gene expression. Besides erythropoiesis HIF also regulates iron absorption, energy metabolism, pH, and angiogenesis. HIF is negatively, regulated by a prolyl

hydroxylase enzyme in presence of oxygen. The HIF stabilizer inhibits the prolyl hydroxylase enzyme which reduces the susceptibility of HIF to degradation and increase erythropoietin production in pseudo-hypoxia conditions. Erythropoietic Ph inhibitor induce complete erythropoiesis by coordinately regulating induction of EPO and improving bioavailability and utilization of iron. FG-2216 is a first generation pH inhibitor that elevates endogenous EPO and hemoglobin in healthy subjects and patients with CKD. FG-4592 is a second generation oral pH inhibitor being evaluated to treat anemia of chronic disease . Phase I studies have shown it to be safe with no serious adverse events with FG-2216. The hemoglobin increase observed was consistent with the level obtain with rHuEPO and Darbepoietin alfa.

Advantage

Orally active.

Upregulate other genes involved in the process of erythropoiesis, notably those that improve iron utilization.

Disadvantage

At least 100 other genes are upregulated by inhibition of the prolyl hydroxylases including hypoxia-sensitive genes, such as vascular endothelial growth factor with the risk for potentiation of tumor growth .

In mid-2007, during one of the Phase II clinical trials of FG-2216, a female patient developed fatal hepatic necrosis that was temporally related to the introduction of this compound .The Food and Drug Administration has for now suspended any further clinical trials with HIF stabilizers.

3.2 Hemopoietic Cell Phosphatase Inhibition:

- The potential importance of this molecule in mediating responsiveness to EPO therapy in population of hemodialysis patients responding poorly to EPO.
- The gene for SHP-1 has been cloned, and SHP-1 inhibitors have been identified.
- In vitro inhibition of SHP-1 resulted in a dosage-dependent erythroid proliferation although the HCP inhibitors have not yet been tested in humans
- These orally active agents, however, could potentially be used as adjuvant therapy to enhance the responseto other ESAs or even to enhance the patient's own endogenous EPO.

3.3 EPO gene therapy:

It is possible that gene therapy may be able to supplant the need for exogenous EPO administration in patients with CKD. As an example, the potential efficacy of this approach was demonstrated in a study of uremic mice, in which myoblast transfer of human EPO gene led to persistent secretion of human EPO and correction of the anemia. Osada and Ebihara reported on the results of gene therapy with human erythropoietin gene as a method of treating anemia of renal origin. They studied mice with polycystic kidney disease, transfected cells with an adenovirus vector and human EPO gene, and inserted these cells intraperitoneally.

6 Non hematopoetic effects of ESA:[22]

6.1 Effects of ESA therapy at a cellular level:

- Increases vascular resistance and resistance to the vasodilatory action of nitric oxide via impact on calcium influx in vascular smooth muscle cells.

▪ It increases the number of circulating erythrocytes primarily by preventing apoptosis of erythroid progenitors.

▪ ESA prevents neuronal apoptosis with results of recent studies show that systemically administered ESA is neuroprotective in vivo.

6.2 ESA stimulates angiogenesis:

▪ With increased number of circulating stem cells (CD34þ cells) low doses of ESA treatment in haemodialysis patients.

▪ Endothelial progenitor cell proliferation and differentiation is also regulated by ESA.

6.3 Pleiotropic renoprotective actions of ESA:

▪ ESA reduce the renal dysfunction and injury caused by oxidative stress, hypoxia and haemorrhagic shock, by reducing caspase activation and apoptotic cell death. {The study suggests that epoetin can ameliorate chronic as well as acute renal failure.}

▪ Potential areas for renoprotection are donor kidneys before transplantation or clamping of the renal arteries during surgery for aortic aneurysms.

▪ Non-haemopoietic roles for ESA in the kidney, such as mitogenesis.

6.4 Asialoerythropoietin:

▪ Neuroprotective properties.

▪ Reduces tissue injury in models of cerebral ischaemia, spinal-cord compression, and sciatic nerve crush-injury.

6.5 Carbamylated ESA analogues:

▪ Do not bind to the classical ESA receptor yet protect against stroke, spinal-cord injury, and diabetic neuropathy as well as ESA but without haemopoietic activity.

▪ The ability of such ESA analogues to reduce ischaemic injury of other organs and to ameliorate renal failure awaits investigation.

▪ So far, the renoprotection induced by ESA and its analogues remains experimental.

7 Effect of immunosupressants:[23]

▪ Mycophenolate mofetil and tacrolimus are associated with a lower haematocrit .

▪ The highest prevalence of post-transplant anaemia (57%) has recently been described in sirolimus treated patients.

▪ Gene expression of proteins related to erythropoiesis is reduced in transplant recipients during rejection episodes.

▪ ESRD patients with a failed kidney transplant exhibit worse anaemia and ESA resistance index.

References

[1] Siren AL et al. *Proc Natl Acad Sci USA* **(2001**); 98: 4044–4049.
[2] Haroon ZA, Amin K, Jiang X, Arcasoy MO. Am. *J.* Pathol**. 2003** ;163(3): 993–1000.
[3] Miyake T, Kung CK, Goldwasser E. *J Biol Chem* **1977**;252:5558-5564.
[4] Lin FK, Suggs S, Lin CH, et al., *Am. J.* **Pathol 1985**;82: 7580-7584.

[5] Browne JK *et al*. *Harb Symp Quant Biol*.**(1986)**; 51:693-702.
[6] Eschbach JW, Kelly MR, Haley NR, Abels RI, Adamson JW. *N.* Engl. *J.* Med. **1989**; 321 (3): 158–63.
[7] Singh AK, Szczech L, Tang KL, *et al*. *N.* Engl. *J.* Med**. 2006**;355 (20): 2085–98.
[8] Macdougall IC, Tucker B, Thompson J, Tomson CR, Baker LR, Raine AE. *Kidney Int.* **1996;** 50 (5): 1694-9.
[9] Tonelli M, Hemmelgarn B, Reiman T, *et al*. *CMAJ*, **2009**;180 (11): E62–71.
[10] Corwin HL, Gettinger A, Fabian TC, *et al*. *N.* Engl. *J.* Med**. 2007**; 357 (10): 965–76.
[11] Zarychanski R, Turgeon AF, McIntyre L, Fergusson DA. CMAJ *:* Canadian Medical Association journal **2007;**177 (7): 725–34.
[12] Parisotto R, Wu M, Ashenden MJ, et al. *Haematologica* **2001**;86 (2): 128–37.
[13] Drüeke TB, Locatelli F, Clyne N, Eckardt KU, Macdougall IC, Tsakiris D, Burger HU, Scherhag A. *Engl. J. Med.* **2006**; 355 (20): 2071-84.
[14] FDA Public Health Advisory; Erythropoiesis Stimulating Agents (ESAs): Epoetin_alfa (marketed as Procrit, Epogen), Darbepoetin alfa (marketed as Aranesp).
[15] Dr. bharat shah. *Indian best practicies guidelines* ;**2003**;3:125-127.
[16] FDA Briefing Document: Safety Concerns Associated with Aranesp (darbepoetin alfa) Amgen, Inc. and Procrit (epoetin alfa) Ortho Biotech, L.P., for the Treatment of Anemia Associated with Cancer Chemotherapy. May 4, **2004**;130-145.
[17] Jelkmann W. *European journal of haematology* **2007**;78 (3): 183–205.
[18] Spinowitz et al. *Curr Med Res Opin* **2006**; 22:2507-13.
[19] Bahlmann FH . *Kidney Int* **2003**; 64: 1648–1652
[20] Skibeli V, Nissen-Lie G, Torjesen P. *Blood.* **2001** Dec;98(13):3626-34.
[21] Jermy levy,julie morgan;Oxford handbook of dialysis, **2004**;2:620-650.
[22] De Santo NG, Cirillo M, Kirsch KA, et al. *Semin Nephrol.* **2005** ;25(6):379-87.
[23] Percy MJ, Furlow PW, Lucas GS, et al. *N Engl J Med.* **2008** ;358(2):162-8.