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Review Article

A Review on Erythropoietin

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Abstract

Normally, EPO levels vary inversely with hematocrit if the kidney is not adversely damaged. Hypoxia stimulates EPO release, which, in turn, stimulates bone marrow erythrocyte production. High blood levels of RBC, hemoglobin, hematocrit, or oxygen suppress the release of EPO. Because the major site of production of erythropoietin is in kidney,it should be cared for. The health professionals should do every thing possible to enlighten the public on the proper way of caring for kidney. Those who have urinary tract infection should be properly diagnosed by qualified Medical Laboratory Scientist and Sensitive test done and the urologist take the treatment of the patients seriously. Some drugs that could cause damages to the kidney should be avoided. The Federal government should try in improving the health sector especially the laboratory and erythrpoietin level should be determined for patients with kidney diseases to correct the havoc it may cause if not detected early. When the patient is found anaemic exogenous erythropoietin can be used in the treatment.

Keywords erythrpoietin , RBC, hemoglobin, hematocrit,

Introduction

Erythropoietin also known as **EPO**, is a glycoprotein hormone that controls erythropoiesis. It is a cytokine for erythrocyte precursors in the bone marrow. Human EPO has a molecular weight of 30.4 kDa.

Also called **hematopoietin** , it is produced by interstitial fibroblasts in the kidney in close association with peritubular capillary and tubular epithelial tubule. It is also produced in perisinusoidal cells in the liver. While liver production predominates in the fetal and perinatal period, renal production is predominant during adulthood. In addition to erythropoiesis, erythropoietin also has other known biological functions. For example, it plays an important role in the brain's response to neuronal injury (Siren et al.,2001). EPO is also involved in the wound healing process (Haron et al.,2003).

Exogenous erythropoietin is produced by recombinant DNA technology in cell culture. Several different pharmaceutical agents are available with a variety of glycosylation patterns, and are collectively called erythropoiesis-stimulating agents (ESA). The specific details for labelled use vary between the package inserts, but ESAs have been used in the treatment of anemia in chronic kidney disease, anemia in myelodysplasia, and in anemia from cancer chemotherapy. Boxed warnings include a risk of death, myocardial infarction, stroke, venous thromboembolism, and tumor recurrence (FDA,2011). Exogenous erythropoietin has been used illicitly as a performance-enhancing drug; it can often be detected in blood, due to slight differences from the endogenous protein, for example, in features of posttranslational modification.

The primary role of erythropoietin is an essential hormone for red cell production. Without it, definitive erythropoiesis does not take place. Under hypoxic conditions, the kidney will produce and secrete erythropoietin to increase the production of red blood cells by targeting CFU-E, proerythroblast and basophilic erythroblast subsets in the differentiation. Erythropoietin has its primary effect on red blood cell progenitors and precursors by promoting their survival through protecting these cells from apoptosis.

Erythropoietin has a range of actions including vasoconstriction-dependent hypertension, stimulating angiogenesis, and inducing proliferation of smooth muscle fibers. It can increase iron absorption by suppressing the hormone hepcidin (Ashby et al.,2010).

Multiple studies have suggested that EPO improves memory. This effect is independent of its effect on hematocrit (Miskowiak et al.2007). Rather, it is associated with an increase in hippocampal response and effects on synaptic connectivity, neuronal plasticity, and memory-related neural network (Adamcio et al.,2008; Adamcio et al.,2010). EPO may have effects on mood (Miskowiak et al.,2007).

Erythropoietin has been shown to exert its effects by binding to the erythropoietin receptor (EpoR) (Middleton et al.,1999;Livnah et al.,1998).

EPO is highly glycosylated (40% of total molecular weight), with half-life in blood around five hours. EPO's half-life may vary between endogenous and various recombinant versions. Additional glycosylation or other alterations of EPO via recombinant technology have led to the increase of EPO's stability in blood (thus requiring less frequent injections). EPO binds to the erythropoietin receptor on the red cell progenitor surface and activates a JAK2 signaling cascade. Erythropoietin receptor expression is found in a number of tissues, such as bone marrow and peripheral/central nervous tissue. In the bloodstream, red cells themselves do not express erythropoietin receptor, so cannot respond to EPO. However, indirect dependence of red cell longevity in the blood on plasma erythropoietin levels has been reported, a process termed neocytolysis.

Erythropoietin levels in blood are quite low in the absence of anemia, at around 10 mU/ml. However, in hypoxic stress, EPO production may increase 1000-fold, reaching 10,000 mU/ml of blood. EPO is produced mainly by peritubular capillary lining cells of the renal cortex, which are highly specialized, epithelial-like cells. It is synthesized by renal peritubular cells in adults, with a small amount being produced in the liver (Jacobson et al.,1957; Fisher et al.,1996). Regulation is believed to rely on a feedback mechanism measuring blood oxygenation (Jelkam et al.,2007). Constitutively synthesized transcription factors for EPO, known as hypoxia-inducible factors, are hydroxylated and proteosomally digested in the presence of oxygen.

Erythropoietin (EPO) is a complex molecule, which regulates red blood cell production in the bone marrow. Recombinant human EPO (rHuEPO) is commercially available and is widely used for the treatment of anemia. In recent years, additional nonerythropoietic tissue/organ protective properties of EPO have become apparent, in particular for kidneys

EPO is a 30.4 kD glycoprotein and class I cytokine consisting of 165 amino acids (Mocini et al.,2007). EPO has four acidic oligosaccharide side chains (3 N-linked and 1 O-linked) and contains up to 14 sialic acid residues. Its carbohydrate portion contributes 40% of its molecular weight (Mocini et al.,2007). The N-linked polysaccharide side chains appear to be important for the biosynthesis and secretion of EPO, enhance its stability in blood, and limit hepatic clearance, thus facilitating the systemic transit of EPO from kidney to bone marrow (Boissel et al.,1993).

The variable nature of the sialic acid content gives rise to EPO isoforms with differences in charge. As the number of sialic acid groups on the carbohydrate portion of EPO increase, so does its serum half-life, whereas receptor-binding capacity decreases (Cartlin et al.,2002;Elliot et al.,2004;Rush et al.,1995;Rush et al.,1993;Middleton et al.,1999;Weidemann and Johnson,2009). Clearance, however, appears to have a stronger influence on *in vivo* activity than receptor-binding affinity.

Each EPO molecule has two EPO receptor (EPOR) binding sites. There are two affinities of the EPOR for EPO in solution: one of high and one of low affinity (needs 1,000 times the concentration of EPO for activation) (Weidemann and Johnson,2009).

Erythropoietin (EPO)

Erythropoietin, also known as erythropoetin or erthropoyetin or EPO, is a glycoprotein hormone that controls erythropoiesis, or red blood cell production. It is a cytokine for erythrocyte precursors in the bone marrow. Human EPO has a molecular weight of 30.4 kDa.

Also called hematopoietin or hemopoietin, it is produced by interstitial fibroblasts in the kidney in close association with peritubular capillary and tubular epithelial tubule. It is also produced in perisinusoidal cells in the liver. While liver production predominates in the fetal and perinatal period, renal production is predominant during adulthood. In addition to erythropoiesis, erythropoietin also has other known biological functions. For example, it plays an important role in the brain's response to neuronal injury(Siren et al.,2001). EPO is also involved in the wound healing process(Haron et al.,2003).

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

Red blood cell effects of EPO

The principal physiological function of EPO is red blood cell production, which results from a tightly controlled proliferation and differentiation pathway (Salahudeen et al.,2008). Early hematopoietic progenitor cells differentiate into burst-forming unit-erythroid cells (BFU-Es). Continuous stimulation with EPO triggers the differentiation of CFU-Es into erythroblasts, which lose their nuclei to form reticulocytes. After a few days, reticulocytes lose reticulin and become erythrocytes (red blood cells). Reticulocytes and erythrocytes stop expressing EPOR and cease being responsive to EPO (Silva et al.,1999).

EPO-binding to EPORs on erythroid progenitor cells leads to activation of the JAK2-STAT5 signaling pathway and phosphorylation of PI3K and Akt1 [Chatterjee et al.,2007; Mocini et al.,2007). Akt-mediated phosphorylation of Bad in the Bad-Bcl-xL complex releases the antiapoptotic protein Bcl-xL, which suppresses erythroid progenitor cell apoptosis (Joyeux-Faure et al.,2005). Akt also is involved in several pathways that promote cell survival and antiapoptotic effects through inhibition of FOXO3a, inactivation of GSK3β, induction of XIAP, inactivation of caspases, and prevention of cytochrome C release. These effects not only enhance the erythropoietic properties of EPO but appear to be important in the protection of other cell types and may contribute to the reported neuronal and renal protective effects [Chatterjee et al.,2007).

Nonhematopoietic roles

Erythropoietin has a range of actions including vasoconstriction-dependent hypertension, stimulating angiogenesis, and inducing proliferation of smooth muscle fibers. It can increase iron absorption by suppressing the hormone hepcidin(FDA,2011).

EPO also affects neuronal protection during hypoxic conditions (stroke, etc.)(Siren et al.,2001). Trials on human subjects are not yet reported; if proven to be a viable treatment of heart attack and stroke patients, it could improve the outcome and quality of life. The reasoning behind such a proposal is that EPO levels of 100 times the baseline have been detected in brain tissue as a natural response to (primarily) hypoxic damage.

Multiple studies have suggested that EPO improves memory. This effect is independent of its effect on hematocrit(Miskowiak et al.,2007). Rather, it is associated with an increase in hippocampal response and effects on synaptic connectivity, neuronal plasticity, and memory-related neural networks(Adamci et al.,2008; Adamci et al.,2010). EPO may also be an effective treatment for depression(Miskowiak et al.,2007; Miskowiak et al.,2010).

Mechanism of action

Erythropoietin has been shown to exert its effects by binding to the erythropoietin receptor (EpoR)(Middleton et al.,1998;Livnah et al.,1998).

EPO is highly glycosylated (40% of total molecular weight), with half-life in blood around five hours. EPO's half-life may vary between endogenous and various recombinant versions. Additional

glycosylation or other alterations of EPO via recombinant technology have led to the increase of EPO's stability in blood (thus requiring less frequent injections). EPO binds to the erythropoietin receptor on the red cell progenitor surface and activates a JAK2 signaling cascade. Erythropoietin receptor expression is found in a number of tissues, such as bone marrow and peripheral/central nervous tissue. In the bloodstream, red cells themselves do not express erythropoietin receptor, so cannot respond to EPO. However, indirect dependence of red cell longevity in the blood on plasma erythropoietin levels has been reported, a process termed neoc.

Synthesis and regulation

Erythropoietin levels in blood are quite low in the absence of anemia, at around 10 mU/ml. However, in hypoxic stress, EPO production may increase 1000-fold, reaching 10,000 mU/ml of blood. EPO is produced mainly by peritubular capillary lining cells of the renal cortex, which are highly specialized, epithelial-like cells. It is synthesized by renal peritubular cells in adults, with a small amount being produced in the liver(Jacobson et al.,1957;Fisher et al.,1996). Regulation is believed to rely on a feedback mechanism measuring blood oxygenation(Jelkman,2007). Constitutively synthesized transcription factors for EPO, known as hypoxia-inducible factors, are hydroxylated and proteosomally digested in the presence of oxygen.

Medical uses

Erythropoietins available for use as therapeutic agents are produced by recombinant DNA technology in cell culture, and include Epogen/Procrit (epoetin alfa) and Aranesp (darbepoetin alfa); they are used in treating anemia resulting from chronic kidney disease, inflammatory bowel disease (Crohn's disease and ulcer colitis) and myelodysplasia from the treatment of cancer (chemotherapy and radiation), but include boxed warnings of increased risk of death, myocardial infarction, stroke, venous thromboembolism, tumor recurrence, and other severe off-target effects(FDA,2011).

Blood doping

Erythropoiesis-stimulating agents (ESAs) have a history of use as blood doping agents in endurance sports, such as horseracing, boxing, cycling, rowing, distance running, race walking, snowshoeing, cross country skiing, biathlon, and triathlon. The overall oxygen delivery system (blood oxygen levels, as well as heart stroke volume, vascularization, and lung function) is one of the major limiting factors to muscles' ability to perform endurance exercise. Therefore, the primary reason athletes may use ESAs is to improve oxygen delivery to muscles, which directly improves their endurance capacity. With the advent of recombinant erythropoietin in the 1990s, the practice of autologous and homologous blood transfusion has been partially replaced by injecting erythropoietin such that the body naturally produces its own red cells. ESAs increase hematocrit and total red cell mass in the body, providing a good advantage in sports where such practice is banned. In addition to ethical considerations in sports, providing an increased red cell mass beyond the natural levels reduces blood flow due to increased viscosity, and increases the likelihood of thrombosis and stroke. Due to dangers associated with using ESAs, their use should be limited to the clinic where anemic patients are boosted back to normal hemoglobin levels (as opposed to going above the normal levels for performance advantage, leading to an increased risk of death).

Though EPO was believed to be widely used in the 1990s in certain sports, there was no way at the time to directly test for it, until in 2000, when a test developed by scientists at the French national antidoping laboratory (LNDD) and endorsed by the World Anti-Doping Agency (WADA) was introduced to detect pharmaceutical EPO by distinguishing it from the nearly identical natural hormone normally present in an athlete's urine.

In 2002, at the Winter Olympic Games in Salt Lake City, Dr. Don Catlin, the founder and then-director of the UCLA Olympic Analytical Lab, reported finding darbepoetin alfa, a form of erythropoietin, in a test sample for the first time in sports. At the 2012 Summer Olympics in London, Alex Schwazer, the gold medalist in the 50-kilometer race walk in the 2008 Summer Olympics in Beijing, tested positive for EPO and was disqualified.

Since 2002, EPO tests performed by US sports authorities have consisted of only a urine or "direct" test. From 2000–2006, EPO tests at the Olympics were conducted on both blood and urine. However, several

compounds have been identified that can be taken orally to stimulate endogenous EPO production. Most of the compounds stabilize the hypoxia-inducible transcription factors which activate the EPO gene. The compounds include oxo-glutarate competitors, but also simple ions such as cobalt(II) chloride.

Cycling

Synthetic EPO is believed to have come into use in cycling about 1990. In theory, EPO use can increase VO2max by a significant amount, making it useful for endurance sports like cycling. Italian antidoping advocate Sandro Donati has claimed that the history of doping in cycling can be traced to the Italian Dr Francesco Conconi at the University of Ferrara. Conconi had worked on the idea of giving athletes tranfusions of their own blood in the 1980s. Donati felt this work "opened the road to EPO because blood doping was a trial to understand the role of EPO".

Dr Michele Ferrari, a former student and mentee of Conconi had a controversial interview mentioning the drug in 1994, just after his Gewiss-Ballan team had a remarkable performance in the La Flèche Wallonne race.

History

In 1905, Paul Carnot, a professor of medicine in Paris, and his assistant, Clotilde Deflandre, proposed the idea that hormones regulate the production of red blood cells. After conducting experiments on rabbits subject to bloodletting, Carnot and Deflandre attributed an increase in red blood cells in rabbit subjects to a hemotropic factor called hemopoietin. Eva Bonsdorff and Eeva Jalavisto continued to study red cell production and later called the hemopoietic substance 'erythropoietin'. Further studies investigating the existence of EPO by K.R. Reissman and Allan J. Erslev (Thomas Jefferson Medical College) demonstrated that a certain substance, circulated in the blood, is able to stimulate red blood cell production and increase hematocrit. This substance was finally purified and confirmed as erythropoietin, opening doors to therapeutic uses for EPO in diseases such as anemia (Jelkman,2007).

Haematologist John Adamson and nephrologist Joseph W. Eschbach looked at various forms of renal failure and the role of the natural hormone EPO in the formation of red blood cells. Studying sheep and other animals in the 1970s, the two scientists helped establish that EPO stimulates the production of red cells in bone marrow and could lead to a treatment for anemia in humans. In 1968, Goldwasser and Kung began work to purify human EPO, and managed to purify milligram quantities of over 95% pure material by 1977. Pure EPO allowed the amino acid sequence to be partially identified and the gene to be isolated (Jelkman,2007). Later, an NIH-funded researcher at Columbia University discovered a way to synthesize EPO. Columbia University patented the technique, and licensed it to Amgen. Controversy has ensued over the fairness of the rewards that Amgen reaped from NIH-funded work, and Goldwasser was never financially rewarded for his work.

In the 1980s, Adamson, Joseph W. Eschbach, Joan C. Egrie, Michael R. Downing and Jeffrey K. Browne conducted a clinical trial at the Northwest Kidney Centers for a synthetic form of the hormone, Epogen, produced by Amgen. The trial was successful.

In 1985, Lin et al isolated the human erythropoietin gene from a genomic phage library and were able to characterize it for research and production. Their research demonstrated the gene for erythropoietin encoded the production of EPO in mammalian cells that is biologically active in vitro and in vivo. There is industrial production of recombinant human erythropoietin (RhEpo) for treating anemia patients .
In 1989, the US Food and Drug Administration approved the hormone Epogen, which remains in use today.

The structure of the EPO Molecule

EPO is a 30.4 kD glycoprotein and class I cytokine consisting of 165 amino acids (Mocini et al.,2007). EPO has four acidic oligosaccharide side chains (3 N-linked and 1 O-linked) and contains up to 14 sialic acid residues. Its carbohydrate portion contributes 40% of its molecular weight (Mocini et al.,2007) . The N-linked polysaccharide side chains appear to be important for the biosynthesis and secretion of EPO, enhance its stability in blood, and limit hepatic clearance, thus facilitating the systemic transit of EPO from kidney to bone marrow (Boissel et al.,1993) .

The variable nature of the sialic acid content gives rise to EPO isoforms with differences in charge. As the

number of sialic acid groups on the carbohydrate portion of EPO increase, so does its serum half-life, whereas receptor-binding capacity decreases (Catlin et al.,2002; Elliott et al.,2004; Rush et al.,1995; Rush et al.,1993; Middleton et al.,1999). Clearance, however, appears to have a stronger influence on in vivo activity than receptor-binding affinity.

Each EPO molecule has two EPO receptor (EPOR) binding sites. There are two affinities of the EPOR for EPO in solution: one of high and one of low affinity (needs 1,000 times the concentration of EPO for activation)(Weidemann and Johnson,2007).

Physiological stimuli for EPO Production/release

Approximately 90% of systemic EPO in adults is produced by peritubular interstitial fibroblasts in the renal cortex and outer medulla of the kidney. A feedback mechanism involving oxygen delivery to the tissues appears to regulate EPO production(Jelkmann et al.,2007). Hypoxia-inducible factor (HIF) regulates transcription of the EPO gene in the kidney, which determines EPO synthesis. This process is dependent on local oxygen tension. HIF is quickly destroyed in well-oxygenated cells through ubiquitylation (tagging for degradation in the proteasome) by the von Hippel-Landau tumor suppressor protein (pVHL), but when oxygen delivery decreases, pVHL ceases its proteolysis of HIF, increasing the levels of HIF, which subsequently increases EPO production(Diskin et al.,2008; Bahlmann and Fisher,2009).

Structure of EPO Receptors

The EPO receptor (EPOR) is a 66 kD membrane glycoprotein typically consisting of 484 amino acids and 2 peptide chains; it belongs to a large cytokine and growth factor receptor family (Boissel et al.,1993). Binding studies have demonstrated that the EPOR has different affinities for EPO and that EPOR isoforms with higher affinity for EPO may be responsible for the erythropoietic effects of EPO, whereas isoforms with a lower affinity for EPO binding may have nonerythropoietic effects, such as tissue protection (Johnson et al.,2008).

The cytoplasmic domains of the EPOR contain a number of phosphotyrosines that are phosphorylated by the activation of a member of the Janus-type protein tyrosine kinase family (JAK2), which is bound to the common beta subunit of the EPOR (Percy et al.,2004). In addition to activating the mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and protein kinase B (Akt) pathway, phosphotyrosines also serve as docking sites for signal transducer and activators of transcription (STATs), such as STAT5. Dephosphorylation of JAK can be induced by phosphatase with the consequent internalization and degradation of the EPO/EPOR complex, which marks the end of EPO activity. This prevents overactivation, which may lead to excessive erythrocytosis (Li et al.,2004).

The main pathways of the effects of EPO. The intracellular domain of the EPOR contains phosphotyrosines, which are phosphorylated by activation of a member of the Janus-type protein tyrosine kinase family (JAK2) bound to the EPOR.

Post-receptor (intracellular) effects of EPO

There are a number of common pathways through which EPO exerts its erythropoietic effects that also appear to confer tissue protection. EPO binds to two EPORs, which become joined as a homodimer and change. This activates JAK2, which is bound to the common beta subunit of the EPOR (Percy et al.,2004) and leads to phosphorylation of tyrosine residues of the EPOR, which activates a number of signaling pathways.

EPO classically signals through the "signal transducer and activator of transcription 5" (STAT-5) pathway. The STAT proteins are direct substrates of Janus kinases (JAKs), which results in tyrosine phosphorylation of the STATs as well as phosphorylation of the phosphatidylinositol 3-kinase (PI3K) and subsequent phosphorylation of Akt.

The principal component of pathways that promote anti-apoptotic effects is Akt, which inactivates caspases, the major mediators of apoptosis, mitochondrial dysfunction, and subsequent release of cytochrome C (Rusai et al.,2010). EPO's ability to maintain cellular integrity and prevent inflammatory apoptosis is closely linked to maintenance of mitochondrial membrane potential, modulation of Apaf-1, inhibition of cytochrome C release, and inhibition of caspases. Recent data also indicate that serum and glucocorticoid-regulated kinase-1 (SGK1)

may contribute to the mediation of EPO's renoprotective effects (Myklebust et al.,2009).

Apoptotic pathways influenced by EPO. Activated STAT5 promotes transcription of promitogenic and antiapoptotic genes associated with apoptotic regulation and cytoprotection. Akt promotes cell survival and antiapoptotic effects by (Mocini et al.,2007)) inhibiting forkhead .

The phosphorylation of mitogen-activated protein kinases (MAPKs) appears to contribute to the cell protection EPO confers (Boissel et al.,1993). Protein kinase C (PKC) also is involved in inhibition of apoptosis and cell survival. It regulates the EPO-induced erythroid proliferation and differentiation and interferes with phosphorylation of the EPOR, making it a likely upstream modulator of the EPOR.

EPO may be involved in modulation of cellular calcium homeostasis by increasing calcium influx . Nuclear factor-kappaB (NF-kB), a mediator of inflammatory and cytokine response, is implicated in EPO signaling. The cytoprotection of EPO partly depends on Akt and subsequent NF-kB activation. NF-kB plays a role in the release of EPO during HIF-1 induction; Akt can increase NF-kB and HIF-1 activation with resultant increase in EPO expression (Yang et al.,2003).

Finally, induction of heat shock protein 70 (HSP70) by EPO is related to renal protection in ischemic kidneys (Lui et al.,2007). HSP70 prevents apoptosis by inhibiting movement of apoptosis inducing factor (AIF) to the nucleus (Beere et al.,2002)and by preventing Apaf-1/cytochrome C binding in the cytosol (Elliott et al.,2008).

The pleiotropic effects of EPO

The tissue protective or "pleiotropic" effects of EPO beyond erythropoiesis have been shown in the kidney in many animal and some clinical studies.

Its tissue protective effects may be elicited through the EPOR homodimer via JAK2-STAT5 activation and inhibition of apoptosis or may be mediated by a second EPOR isoform heterodimer composed of an EPOR monomer and the cytokine receptor, common beta subunit (CD-131). For example, carbamylated EPO (CEPO) does not bind to the classical EPOR isoform and is devoid of hematopoietic activity; however, it can provide tissue protection in the kidney, supporting the existence of a heteroreceptor EPO isoform, which mediates tissue protection (Westenfelder et al.,1999). It is clear that the relationship of EPO with its receptor is extremely complex. Therefore, further investigation is required to fully understand the EPOR heterodimer isoform, and the mechanisms and pathways involved in its tissue protective activity.

Animal and in vitro studies

Many animal studies have shown that EPO administration protects kidney tissue from damage and improves renal function in ischemia-reperfusion (IR) and contrast-induced injury models of AKI (Chatterjee,2007; Ates et al.,2005; Esposito et al.,2009; Gong et al.,2004; Imamura et al.,2007; Johnson et al.,2006) in which EPO reduced kidney dysfunction by decreasing apoptosis. In addition, EPO has been shown to reduce the expression of proinflammatory mediators, TNF-alpha and IL-2, in IR renal injury and reverse the effect of endotoxin on the antioxidant, renal superoxide dismutase (SOD). These anti-inflammatory properties of EPO also suggest involvement of the NF-kB pathway in its kidney protection.

Animal Studies Of EPO in nonischaemic models of AKI

CEPO: The administration of carbamylated EPO (CEPO), which does not bind to the classical EPOR, also provides renal tissue-protective effects. In an IR rat model, CEPO markedly reduced apoptosis and increased tubular epithelial cell proliferation. Moreover, CEPO was more protective against IR injury to tubular epithelial cells than EPO in this study. In an in vitro model performed by the same team, CEPO promoted more capillary formation than EPO and also appeared to protect the kidneys from IR injury by promotion of angiogenesis (Vaziri et al.,1994). This protective effect requires mitogenesis and endothelial progenitor cell differentiation, proliferation, and migration.

EPO activates endothelial nitric oxide synthase, and this effect on the endothelium may be critical for the renal tissue protective effects of EPO. EPO is an extremely potent stimulator of endothelial progenitor

cells, whose function is partly dependent on nitric oxide bioavailability. Endothelial progenitor cells appear to be involved in endothelial recovery after injury. EPO limits AKI in part by stimulating vascular repair and by mobilizing endothelial progenitor cells and increasing tubular cell proliferation (Westenfelder et al.,1999). These findings suggest that EPO may exert a protective effect via an interaction with the microvasculature.

Angiogenesis and EPO's renoprotective effects may be influenced by vascular endothelial growth factor (VEGF). Nakano and colleagues found that the vascular EPO/EPOR system promoted postischemic angiogenesis by upregulating the VEGF/VEGF receptor system, both directly by promoting neovascularization and indirectly by mobilising endothelial progenitor cells and bone marrow-derived proangiogenic cells (Nakano et al.,2007). It appears that angiogenesis is impaired and blood vessels are less responsive to VEGF in the absence of EPOR.

EPO in AKI

AKI is common in critically ill patients (Bagshaw et al.,2008; Ostermann and Chang,2007) and is independently associated with increased mortality, and with prolonged length of stay. It escalates both the human and financial costs of care. Therefore, it seems desirable to investigate treatments with potential to ameliorate or prevent AKI.

Some injury pathways for AKI in the critically ill include exposure to endogenous and exogenous toxins, metabolic factors, ischemia and reperfusion insults, neurohormonal activation, inflammation, and oxidative stress. Of these, ischemia-reperfusion may be the most common. EPO can prevent or reduce injury and assist renal repair and recovery through limitation of apoptosis, promotion of neovascularization, anti-inflammatory action, and tissue regeneration.

Investigation of potential treatments for AKI has had limited success to date; however, from the results of animal and some limited preliminary human studies, therapeutic use of EPO seems promising for those "at risk" for AKI.

Clinical trials of EPO in AKI

One randomized, placebo-controlled, clinical trial of preoperative EPO in 71 patients who underwent elective coronary artery bypass graft (CABG) surgery reported renoprotective effects (Song et al.,2009). EPO was given at a dose of 300 U/kg IV immediately preoperatively and was associated with a reduction in the incidence of AKI from 29% to 8% ($p = 0.035$) and improved postoperative renal function as indicated by a smaller increase in SCr (% increase at 24 hours of 1% vs. 15%, $p = 0.04$) and a smaller decline in estimated GFR (% change at 24 hours of +3% vs. -5%, $p = 0.04$) postoperatively.

Potential risks of EPO

Pure red cell aplasia

Despite the numerous benefits of EPO, there are some risks. Pure red cell aplasia is a rare adverse event, which is characterized by anemia, low reticulocyte count, absence of erythroblasts, resistance to EPO, and neutralizing antibodies against EPO. This is an extremely rare complication.

Cancer patients

EPO administration in patients with cancer has been associated with increased mortality and enhanced tumor growth (Bohlius et al.,2008). The underlying mechanisms remain uncertain, but patients with certain malignancies may be in a hypercoagulable state, making EPO administration unadvisable.

Thrombosis

Recent studies and clinical trials have found an increased rate of thrombosis with EPO, which has mainly been observed in patient groups with higher than conventional levels of hemoglobin (> 120 g/L). Exclusion of patients with hemoglobin >120 g/L from clinical trials of EPO minimizes the risk for thrombosis. Nonetheless, systematic assessment for thrombosis should be performed in any EPO trials of critically ill patients because they have an increased risk for thrombosis.

Hypertension

Hypertension occurs in approximately 30% of patients who receive long-term EPO treatment and appears to involve increased endothelin release, upregulation of tissue renin and angiotensin production, changes in the balance of vasoactive substances (prostaglandin/prostacyclin/thromboxane), and an elevation of calcium by EPO (at least in chronic kidney disease) that impairs the vasodilating action of nitric oxide. It is advisable that patients with uncontrolled hypertension do not participate in trials of EPO in AKI.

Carbamylated EPO, a cytoprotective, nonerythropoietic derivative of EPO may not exhibit the same risks as EPO and holds great interest as a future tissue-protective therapy. However, it requires further experimental testing before it can be safely evaluated in clinical trials.

Clinical information

Normally, EPO levels vary inversely with hematocrit if the kidney is not adversely damaged. Hypoxia stimulates EPO release, which, in turn, stimulates bone marrow erythrocyte production. High blood levels of RBC, hemoglobin, hematocrit, or oxygen suppress the release of EPO.

Primary polycythemia (polycythemia vera) is a neoplastic (clonal) blood disorder characterized by autonomous production of hematopoietic cells. Increased erythrocytes result in compensatory suppression of EPO levels. Findings consistent with polycythemia vera include hemoglobin >18.5 gm/dL, persistent leukocytosis, persistent thrombocytosis, unusual thrombosis, splenomegaly, and erythromelalgia (dysesthesia and erythema involving the distal extremities).

Secondary polycythemias may either be due to an appropriate or an inappropriate increase in red cell mass. Appropriate secondary polycythemias (eg, high-altitude living and pulmonary disease) are characterized by hypoxia and a compensatory increase in red cell mass. EPO production is increased in an attempt to increase the delivery of oxygen by increasing the number of oxygen-carrying RBCs. Some tumors secrete EPO or EPO-like proteins; examples include tumors of the kidney, liver, lung, and brain. Such increases result in inappropriate secondary polycythemias.

Abnormal EPO levels also may be seen in renal failure. The majority of EPO production is in the kidneys. Therefore, chronic renal failure may result in decreased renal EPO production and, subsequently, anemia. In addition to the kidneys, the liver also produces a small amount of EPO. Thus, anephric patients have a residual amount of EPO produced by the liver.

Chronic renal failure patients, as well as patients with anemia due to a variety of other causes including chemotherapy, HIV/AIDS, and some hematologic disorders may be candidates for treatment with recombinant human EPO. Recombinant EPO compounds used to treat anemia include epoetin alpha and darbepoetin. Epoetin alpha is a 165 amino acid glycoprotein produced in mammalian cells and has an identical amino acid sequence to natural human EPO. It has 3 oligosaccharide chains and a molecular mass of 30.4 kDa. Darbepoetin alpha is a 165 amino acid glycoprotein that is also produced in mammalian cells. It has 2 additional N-linked oligosaccharide chains and a molecular mass of 37 kDa. There are no specific assays for measuring recombinant EPO compounds. Drug levels can only be roughly estimated from the cross reactivity of the compounds in EPO assays.

Because results obtained with 1 commercial EPO assay may differ significantly from those obtained with any other, it is recommended that any serial testing performed on the same patient over time should be performed with the same commercial EPO test.

Heterophile antibodies may interfere in this assay. Lower EPO levels than expected have been seen with anemias associated with the following conditions: rheumatoid arthritis, AIDS, cancer, ulcerative colitis, sickle cell disease, and in premature neonates.

After allogeneic bone marrow transplant, impaired EPO response may delay EPO recovery.

Patients with hypergammaglobulinemia associated with multiple myeloma or Waldenstrom disease have impaired production of EPO in relation to hemoglobin concentration. This has been linked to increased plasma viscosity.

There is some diurnal variation in EPO levels. For optimal results in serial patient monitoring, all specimens should be collected at the same time of day. The diurnal variation is minimal in normal individuals (<20%), but in hospitalized patients with a variety of illnesses, as well as ambulatory patients with chronic lung disease, serum EPO concentrations can be 20% to 60% higher at night than early in the morning. This phenomenon is most pronounced in patients with EPO levels within approximately 2-times the upper limit of the normal population reference interval(Tefferi,1995;Hoagland,1995;Casadeval,2003; Fisher,2003;Strippoli et al.,2005).

Erythropoietin in health and disease

Under normal steady-state conditions, the concentration of circulating EPO is that amount necessary to maintain the red cell mass and to replace senescent and dying cells. Sensitive radioactive assays of EPO have revealed this level normally to be approximately 10 to 25 mU/mL(Cotes,1982). However, serum titers can vary considerably.For example, at a hematocrit of 30%, EPO levels can range from 50 to 500 mU/mL. It was once believed that the overall inverse relationship between serum EPO and hematocrit in anemic patients was lost oncehematocrit fell below 33%, and it now seems that the inverse correlation between serum EPO level and hematocrit holds in anemic patients even if hematocrit is greater than 33%. Despite the difficulty in assigning a strict value to normal EPO levels, it is generally recognized that patients with diseases associated with varying degrees of anemia manifest EPO levels outside the generally accepted range. For example, endocrine renal function decreases in parallel with excretory renal function resulting in EPO deficiency and anemia once creatinine clearance falls below 40 mL/min/1.73 m2. However, even severely diseased kidneys are capable of producing some EPO; despite the deficiency, the inverse relationship between EPO level and hematocrit remains intact, although it functions at a lower level. Ninety percent of patients with the myelodysplastic syndrome present with anemia. However, their anemia is not secondary to EPO deficiency as their renal function usually is intact. The myelodysplastic syndrome comprises a group of disorders in which a clonal abnormality of hematopoietic stem cells exists that may progress to an acute leukemia state. Ineffective hematopoiesis is an early feature of myelodysplastic syndrome and causes the associated anemia, but may occur in nonanemic patients. In contrast to the situation in chronic renal failure, there is only a weak inverse correlation between hematocrit and EPO level despite the presence in many myelodysplastic syndrome patients of active erythropoiesis and even erythroid hyperplasia. While EPO levels vary greatly in myelodysplastic syndrome patients for the same or similar hemoglobin concentrations often serum EPO levels are elevated, but there is not the expected close relationship of EPO level with the degree of anemia Interestingly, the highest EPO levels are seen in those patients with erythroid hypoplasia. Furthermore, patients with sickle cell anemia have low EPO levels for their degree of anemia.

Human immunodeficiency virus (HIV) infection is associated with defects in hematopoiesis, including decreased proliferation of hematopoietic progenitor cells and increased destruction of mature cells. These events may be secondary to the influence of HIV infection of progenitor cells on bone marrow stromal elements.4' Regulatory cytokines also are disturbed; thus, hematopoietic cytopenias are common. In patients with acquired immunodeficiency syndrome (AIDS) treated with zidovudine, which causes additional significant bone marrow suppression, two distinct types of anemia can be observed42: one associated with macrocytosis and low serum EPO and the other with normocytic red blood cells and high serum EPO. This distinction becomes important when considering treatment of such patients with exogenous EPO. Polycythemia rubra vera is a myeloproliferative disorder marked by autonomous overproduction of erythrocytes and variable overproduction of granulocytes and platelets. Evidence suggests there is an acquired sensitivity of erythroid precursors to EPO, and EPO levels in polycythemia rubra vera are usually low. Because the life span of red blood cells remains unchanged during pregnancy,(Pitchard and Adams,1960) this change in red blood cell mass is most likely secondary to decreased erythropoiesis. Although absolute EPO levels increase over nonpregnant values throughout pregnancy and correlate with hematocrit in the third trimester, delivery, and postpartum, no such correlation is evident earlier in pregnancy. Erythropoietin levels remain relatively low for the degree of anemia at this stage, thus likely accounting for the observed decrease in erythropoiesis and total red blood cell mass seen early in pregnancy.

Conclusion

Normally, EPO levels vary inversely with hematocrit if the kidney is not adversely damaged. Hypoxia stimulates EPO release, which, in turn, stimulates bone marrow erythrocyte production. High blood levels of RBC, hemoglobin, hematocrit, or oxygen suppress the release of EPO. Because the major site of production of erythropoietin is in kidney,it should be cared for.The health professionals should do every thing possible to enlighten the public on the proper way of caring for kidney.Those who have urinary tract infection should be properly diagnosed by qualified Medical Laboratory Scientist and Sensitive test done and the urologist take the treatment of the patients seriously.Some drugs that could cause damages to the kidney should be avoided.The Federal government should try in improving the health sector especially the laboratory and erythrpoietin level should be determined for patients with kidney diseases to correct the havoc it may cause if not detected early.When the patient is found anaemic exogenous erythropoietin can be used in the treatment.

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