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ERYTHROPOITIEN: EFFECTS, APPLICATIONS AND INDUSTRIAL PRODUCTION SYSTEMS

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ABSTRACT

Human erythropoietin is a hormone primarily produced for regulating the process of red blood cell formation. The study was carried out to evaluate effects, functions, applications, future prospects and industrial production methods of this glycoprotein. Roles other than just erythropoiesis which show promising clinical scope are discussed. Pros and cons of different cultural systems for production of recombinant erythropoietin are explained and compared. At present mammalian culture systems using Chinese Hamster Ovary (CHO), Baby Hamster Kidney (BHK), Human HT1080 cell lines are the main industrial source for production of recombinant erythropoietin but other systems such as yeast and plant cell cultures do also have an immense potential which is still to be utilizable on commercial levels. Optimizations and advances in improving quality and productive outcomes are also visualized.

KEYWORDS: Erythropoietin, RHuEPO, Mammalian culture System, Yeast culture system, Plant culture system.

INTRODUCTION

For more than 55 years, it has been known that a plasma factor is responsible for production of red blood cells (Diane M. Ridley, 1994). Erythropoietin was first isolated from an anemic patient. The first ever hematopoietic factor been cloned was human erythropoietin and was cloned on the basis of partial amino acid sequencing (Jacobs K, 1985; Jae Seong Lee, 2012). Erythropoietin gene is situated on chromosome 7 (Marsden, 2006). Kidney and liver are the main organs involved in the production of erythropoietin (Dunn A, 2007). Low level of tissue oxygenation or hypoxia is the stimulatory factor for the production of erythropoietin. Erythropoietin is also produced locally in by testis, spleen, lungs and brain in a very minute amount and is not found to be involved in the process of erythropoiesis. Rather acts locally and has some functions other than erythropoiesis (Jelkmann, Regulation of erythropoietin production, 2011; Jelkmann, Physiology and pharmacology of erythropoietin, 2013). Erythropoietin is a heavily glycosylated, 166 amino acid hydrophobic glycoprotein and has a molecular weight of 39000. 40 % of the molecule comprises of sialic acid. In the erythropoietic process, erythropoietin binds to the EPO receptor located on the erythroid progenitor cells in the bone marrow and induces the production of red blood cells. Soon after the successful isolation and purification of erythropoietin, works started to make artificial laboratory made erythropoietin especially because at that time the only available method of treatment for patients of chronic kidney disease was regular blood transfusions. The discovery of recombinant human erythropoietin (RHuEPO) in 1985 was a revolutionary one and after some successful trials it was granted with a license for human use in 1988 (T Ng, 2003). RHuEPO proved useful for avoiding or reducing repeated transfusions in chronic kidney disease patients, treatment of myelodysplasia, anemia associated with HIV infections (Sowade B, 1998).

RHuEPO can be produced by yeast cell culture systems, plant cell culture systems or mammalian cell culture systems. Presently RHuEPO is being produced commercially in mammalian cultures but benefits of other methods can also be exploited in order to increase yield, reduce cost and enhance product quality. The researchers are making efforts in achieving this goal. Measurement of plasma erythropoietin has also a significant value in diagnosing and differentiating different types of polycythemia and anemia. Measurement of RHuEPO in vitro is also helpful in monitoring therapy (Marsden, 2006). RHuEPO administration increases oxygen transport to muscles as it increases red cell mass. It was found to be used by athletes to enhance their performance. This use is considered to be illegal and is banned. Measurements of

RHuEPO in body fluids of athletes do also provide a fine way to determine whether the drug has been used for this illicit purpose or not (Marsden, 2006). Moreover, in vivo measurement methods in laboratory rats or mice are also available in some research laboratories to determine biological activity of this glycoprotein.

Functions and Effects

Erythropoietin is primarily involved in speeding up the development of erythroid precursors and release of reticulocytes from the bone marrow (Diane M. Ridley, 1994; Carsten Lundby, 2011). Erythropoietin maintains red cell mass in body and after a loss of red blood cells from body rapidly increases red cell recovery. Apart from kidney and liver produced hormone, erythropoietin can also be produced by brain, lungs, spleen and testis locally. Erythropoietin produced locally by these tissues seems to have no effect on the erythropoietic process but is found to be involved in some other functions. Erythropoietin produced by brain cells acts locally as neuroprotective factor. Erythropoietin is found to be involved in cytoprotective role for heart, kidneys and blood vessels as well (Brines M, 2006; Jelkmann, Regulation of erythropoietin production, 2011). Locally produced erythropoietin has also been found to have an anti-inflammatory effect (Jelkmann, Physiology and pharmacology of erythropoietin, 2013). There may be some involvement of erythropoietin in the production of platelets. Studies have shown that there may be some relation between erythropoietin and platelet production. Experiments have shown that there is an increased production of megakaryocytes in-vitro with administration of erythropoietin but the data is not yet utilizable for clinical purposes. It could prove beneficial for increasing platelet production in patients of thrombocytopenia (Jecko Thachil, 2013).

Industrial Production of Rhuepo

RHuEPO as described earlier got license for human use in 1988 for treatment of chronic kidney disease patients of anemia. In 1989, US Food and Drug Administration (FDA) approved its use in this circumstance. Several other approvals including European and Japanese approvals were also been obtained for this purpose (Diane M. Ridley, 1994). It quickly proved to be useful in treating several types of anemia and polycythemia as well. Accordingly, the need for its industrial scale production increased and large scale biopharmaceuticals started producing RHuEPO for treating these human diseases. There are several cultural systems available for production of RHuEPO. The most widely use system for its production is mammalian cell culture system. RHuEPO is produced commercially by using Chinese Hamster Ovary (CHO) cells. Methylotropic yeast such as Pichia Pastoris is also been in use by some small scale industries for RHuEPO production. Plant culture techniques are not yet been exploited for its industrial production but are immensely under consideration. Mammalian culture system is the biggest industrial system for production of recombinant human erythropoietin. Immortalized Chinese Hamster Ovary (CHO) cells are usually used for this purpose (Kim JY, 2012). Baby Hamster Kidney (BHK) and Human HT 1080 cell lines are also in commercial use for production of RHuEPO. Mammalian cells were the first ones to be used commercially for the production of recombinant proteins including erythropoietin. The reason behind is that fully functional glycosylated and properly folded proteins can be produced in them easily (Hossler P, 2009; Son YD, 2011). Because the erythropoietin is to be used by mammals (humans), mammalian post-translational modifications could be done naturally. Also at that time in the earlier 1980's, it was not possible to glycosylated these proteins in other systems. Productivity rates in earlier days was 0.1 to 1 pg/cell/day (Wurm F, 1999) in 1990's but now with some optimizations and advances, productivity rates of 20-60 pg/cell/day is quiet possible to obtain (Arnold L Demain, 2009).

Table 1: Mammalian system in commercial use for RHuEPO production (Jae Seong Lee, 2012)

Cell line	Generation	Example or Product name
CHO	1st	Epoetin alfa, Epoetin beta and Epoetin theta
CHO	2nd	Darbepoetin, methoxy PEG epoetin beta, Epoetin zeta
BHK	1st	Epoetin omega
Human HT1080	1st	Epoetin delta

CHO= Chinese Hamster Ovary, BHK= Baby Hamster Kidney, PEG= Polyethylene glycol

Yeasts have a long history of use for industrial fermentation processes (Hamilton SR, 2003; Arnold L Demain, 2009; Daly R, 2005). Yeasts such as S.cerevisae and Pichia Pastoris are two most commonly used yeasts in industries. Moreover, glycosylation of recombinant proteins in yeasts is quiet possible to achieve nowadays. Furthermore, genetically engineered Pichia Pastoris secretory pathway has been successfully achieved to produce human N-type glycosylated proteins (Choi BK, 2003). Researchers have managed to produce human glycosylated erythropoietin in Pichia Pastoris by exchanging enzymes responsible for yeast glycosylation with those of mammalian glycosylation (TU, 2004; Arnold L Demain, 2009; Hamilton SR D. R., 2006). Mammalian proteins are glycosylated using D-mannose sugars bonded covalently to asparagines linked N-acetyl-D glucosamine molecules. High yield is the main advantage of using yeast system as a tool for production of recombinant erythropoietin, so that the cost is minimized. Yeast strains are well known to produce post-translational modifications. Moreover, proteins can be secreted into the extracellular matrix or broth if proper

sequences are attached to the structural genes. Plant cell cultures are also able to carry out mammalian post-translational modifications because they are higher eukaryotes. Moreover, production is also cost effective. For more than 25 years, plant cells are producing recombinant proteins but their commercial production is not yet made feasible. RHuEPO is reported to have been produced in N. tabacum host expression system (Stephan Hellwig, 2004). Researchers are making efforts to produce RHuEPO in plants on a commercial scale.

Table 2: Comparison of Parameters in Different Cultural Systems for RHuEPO production (Arnold L Demain, 2009).

Parameter	Mammalian system	Yeast system	Plant system
Approximate Yield	10-20 mg/L	20-30 g/L	1 pg/g FW
Cost	High	Least	Low
Affection by human pathogens	High risk	Least risk	Low
Commercial Production	Yes	No	No

FW= Fresh weight

Optimizations

The recombinant erythropoietin with a longer plasma half life, increased biological activity ad decreased receptor binding affinity performs better and hence is more beneficial. Hyperglycosylation and increased sialic acid content results in three fold increase in serum/plasma half life. Moreover, sialated RHuEPO is proven to have 1000 fold potent biological activity (Jae Seong Lee, 2012). Half life of RHuEPO can also be increased by binding it with molecules such as Polyethylene glycol (IC, 2005) or other proteins (Joung CH, 2009) so that release of RHuEPO is slowed down. With methoxy PEG RHuEPO, serum half life can be increased up to 130 hours in serum (Jae Seong Lee, 2012). Furthermore, a totally synthetic erythropoietin synthetic erythropoiesis protein (SEP) was developed which consists of 166 amino acid chain and has a molecular weight of 51 Kilo Dalton. It has two to three folds greater in-vivo half life (Jae Seong Lee, 2012).

Various other strategies at genetic engineering level resulted in increasing production yield. Vectors containing erythropoietin gene and all the regulatory elements of human cytomegalovirus major immediate early gene produced a 2.5 fold increased yield (Maraiti NYK, 2010). Also by increasing nutrient utilization and decreasing or inhibiting cell death, RHuEPO production proved to be increased (Han YK, 2011). Fully robotic systems are also working in market these days for production of epoetin alfa in CHO cell cultures (FM, 2004)

Clinical Applications

RHuEPO is primarily used to maintain hemoglobin concentration slightly above 10g/dl in anemic patients who would otherwise be on mercy of transfusions only. They are usually used for treating anemia associated with chronic kidney disease (CKD), anemia associated with zidovudine treatment, anemia associated with chemotherapy treatment of cancers and anemia in premature infants. The major target of using RHuEPO is to provide a substitute for repeated transfusions. But they are no more a substitute to red blood cell transfusion in severe hemorrhages or traumas, severe blood loss during surgeries and severe anemia which require immediate attention (Jelkmann, Physiology and pharmacology of erythropoietin, 2013). As described earlier, erythropoietin is not merely an erythropoietic substance. It is shown to have roles in angiogenesis, wound healing, brain development, neuroprotection (Maiese, 2016), cardiovascular protection and anti-inflammation (Arcasoy, 141, 14-31). Erythropoietin has been reported for treating different ocular diseases with encouraging results (Kaveh Abri Aghdam, 28, 5-11). Erythropoietin administration is shown to give promising results in acute kidney injury associated with cardiac surgery (Jahan Che Penny-Dimri, 2016). There is an immense ongoing effort to develop RHuEPO variants which could be used to achieve these effects.

Harmful Effects

RHuEPO administration could cause thromboembolism and hypertension. Administration of RHuEPO stimulates tumor growth because cancer cells do express a low level of erythropoietin receptor protein. It should not be especially administrated to patients of uncontrolled hypertension. They are also contra-indicated in patients with hypersensitivity to non human derived substances. Risks for fetus are not well studied so should be used cautiously in pregnancy (Jelkmann, Physiology and pharmacology of erythropoietin, 2013).

CONCLUSION

RHuEPO production can be made cost effective and total yield can be enhanced by utilizing yeast or plant cultural systems. Yeast systems as well as plant systems have the potential to compete with other expression platforms for commercial production of RHuEPO. The overall target for researchers should be to produce in these systems functionally and structurally equivalent RHuEPO to its native counterpart. Applications of RHuEPO which have shown promising clinical outcomes should be evaluated and encouraged.

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