Review Article

Erythropoietin use and abuse

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ABSTRACT

Recombinant human erythropoietin (rhEPO) is arguably the most successful therapeutic application of recombinant DNA technology till date. It was isolated in 1977 and the gene decoded in 1985. Since then, it has found varied applications, especially in stimulating erythropoiesis in anemia due to chronic conditions like renal failure, myelodysplasia, infections like HIV, in prematurity, and in reducing peri-operative blood transfusions. The discovery of erythropoietin receptor (EPO-R) and its presence in non-erythroid cells has led to several areas of research. Various types of rhEPO are commercially available today with different dosage schedules and modes of delivery. Their efficacy in stimulating erythropoiesis is dose dependent and differs according to the patient's disease and nutritional status. EPO should be used carefully according to guidelines as unsolicited use can result in serious adverse effects. Because of its capacity to improve oxygenation, it has been abused by athletes participating in endurance sports and detecting this has proved to be a challenge.

Key words: Abuse, continuous erythropoietin receptor activator, darbepoetin, erythropoietin

Introduction

A century of arduous research has stood behind the success of recombinant human erythropoietin (rhEPO) and its analogues in the management of anemia in chronic renal failure and in certain hematological diseases like myelodysplastic syndrome (MDS) and anemia of chronic disease. With the characterization of the erythropoietin receptor (EPO-R), it has been realized that EPO is a pleiotropic hormone with actions on non-erythroid tissues as well. This is an area of current interest and research.[1] However, rhEPO is also abused as an agent in endurance sports because of its ability to increase oxygen carrying capacity of blood by stimulating supra-physiological erythrocytosis. Detection of rhEPO use among athletes is difficult and time consuming because the agent is not easily differentiated from the naturally occurring EPO.

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Historical background

Humoral regulation of hematopoiesis was first identified in 1906 and endogenous EPO was isolated in 1977, with its gene cloned in 1985. A series of initial clinical trials were performed to assess its effectiveness in correcting anemia of chronic kidney disease (CKD). After it proved to abrogate the transfusion requirements and improve the well-being of patients, it was granted license in 1988 as a therapeutic agent for CKD patients.[2-4]

Biochemistry of endogenous erythropoietin and erythropoietin receptor

EPO, a member of the type I cytokine superfamily, was first identified as the hormone that stimulates erythroid progenitors within the bone marrow to mature into erythrocytes. The main site of production of EPO is from the kidney and to a much lesser extent from the liver. In the kidney, certain interstitial fibroblasts appear to be a major source of EPO; however, other studies suggest an important role of proximal tubular cells as well.[5,6]

The human erythropoietin gene is located at chromosome 7q11-22, and consists of five exons and four introns, which produce a post-transcriptional single polypeptide backbone containing 193 amino acids.[7] This undergoes post-translational modification with the addition of three

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N-glycosylation and one O-glycosylation sites and removal of 28 amino acids, resulting in a 165 amino acid polypeptide chain which is the primary structure of the mature EPO. The molecular mass of the polypeptide backbone and the glycosylated form of erythropoietin is estimated to be 18 and 30 kDa, respectively.[8]

EPO acts synergistically with other cytokines to promote the proliferation, differentiation, and survival of progenitor cells in the erythroid lineage and boosts the production of erythrocytes. It does not influence the fate of the pluripotent stem cell, but acts on the colony forming unit-erythroid (CFU-E) cells to prevent their apoptosis and induce expression of erythroid specific proteins. The EPO-R polypeptide is a 66-kDa membrane protein belonging to the cytokine receptor superfamily.[9]

The EPO binding to its receptor results in homodimerization of the receptor, followed by activation of several signal transduction pathways: JAK2/STAT5 system, G-protein (RAS), calcium channel, and kinases [Figure 1]. A gain of function mutation in JAK2 has been reported in patients with polycythemia vera and other myeloproliferative diseases.[10] EPO also acts on angiogenesis, vasculogenesis, regulation of vascular resistance, and neuroprotection.[11]

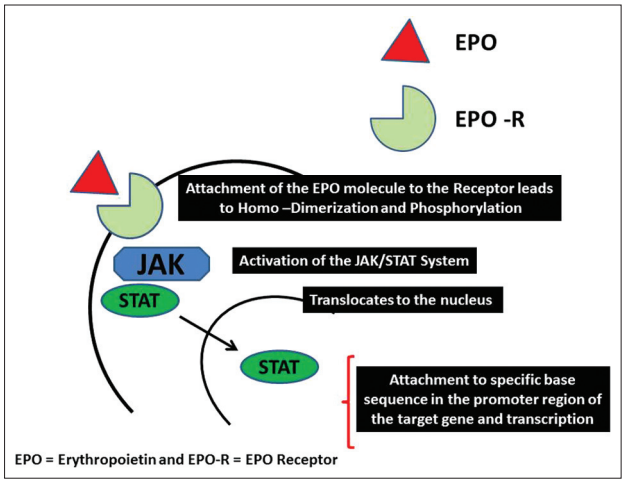

Figure 1: The mechanism of action of erythropoietin

Pharmacology of the Current Preparations of Recombinant Human Erythropoietin Available for Use[12]

a. Erythropoietin alpha: Epoetin alpha is an isoform of recombinant DNA-derived erythropoietin (rEPO), synthesized in Chinese hamster ovary (CHO) cells. It differs from the beta isoform in its migration on isoelectric focusing (IEF) and in a range of lectin-binding assays.[13]
b. Erythropoietin beta: Epoetin beta is also synthesized by CHO cell lines and differs from epoetin alpha in containing:
   i. a greater proportion of more basic isoforms,
   ii. a greater proportion of EPO binding to Erythrina cristagalli agglutinin, and
   iii. isoforms with higher *in vivo*: *In vitro* bioactivity.[13]

Routes of administration of erythropoietin alpha and beta

Apart from the generally recommended subcutaneous (SC) route of administration, intravenous (IV) and intraperitoneal routes have been used to administer EPO. With the increasing reports of pure red cell aplasia (PRCA) with SC route, the Department of Health in UK recommends a change EPO-alpha administration from SC to IV route.[3]

However, SC route has several advantages over IV route like ease of administration, non-requirement of venous access, and up to 30% reduction in weekly rhEPO dose on hemodialysis patients.[14] Although patients on peritoneal dialysis may benefit from intraperitoneal route, a larger dose may be required to maintain the same hemoglobin level. Outside the uremic setting, both IV and SC rhEPO have been employed, but the SC route was used in the majority of the studies. However, there have been no studies to compare the efficacy of these routes [Table 1].[3]

Frequency of administration of erythropoietin alpha and beta

rhEPO can be given once, twice, or thrice weekly depending on the clinical status of the patient as per the level of hemoglobin maintained.

Table 1: Comparison of various types of erythropoietin

Parameter	EPO-alpha	EPO-beta	Darbepoetin alpha	CERA
MW (Daltons)	30,000	30,000	37,000	60,000
Polyethylene glycol conjugation	Absent	Absent	Absent	Present
Glycosylation sites	3	3	5	-
Routes of administration	SC, IV, IP	SC, IV, IP	SC, IV	SC, IV
Half-life (SC admn; hours)	19	20	73	139
Bioavailability after SC administration (%)	20	23–42	37	62
Dose	50–150 IU/kg	20–80 IU/kg	0.45 mcg/kg	0.6–1.2 mcg/kg
Dosing schedule	1–3 times/week	1–3 times/week	Once a week or once in 2 weeks	Once in 2 weeks to once a month

MW: Molecular weight, SC: Subcutaneous, IV: Intravenous, IP: Intraperitoneal , CERA: Continuous erythropoietin receptor activator

c. Darbepoetin alpha (a hyperglycosylated rhEPO): It has five N-glycosylation sites as compared to three in the rhEPO. This is created by a process called "site mutagenesis" and confers higher negative charge and threefold longer half-life. This is based on the principle that increase in number of glycosylation sites may enhance its activity. This helps in giving once a week dosing strategy.

Dosing of darbepoetin

The approved dosage in anemia of CKD is 0.45 mcg/kg IV or SC. Weekly monitoring of hemoglobin is suggested upon initiation of therapy and then to maintain hemoglobin levels <12 g/dl and to avoid increases of hemoglobin >1 g/dl over a 2-week period.[15]

d. Continuous erythropoietin receptor activator (CERA): Methoxy polyethylene glycol-epoetin beta is a third-generation molecule, incorporating a large polymer chain and has an elimination half-life in humans that is up to 6 times longer than darbepoetin alpha and up to 20 times longer than epoetin, making it possible for once in 2 weeks to once a month dosing strategy.[16,17] The successful conversion of patients on epoetin or darbepoetin to CERA has been successfully demonstrated.[18]

Dosing of Continuous erythropoietin receptor activator

The starting dose of CERA would be 125 μg if the patient had previously received <8000 U epoetin weekly or <40 μg darbepoetin weekly, or a dose of 200 μg if the patient had previously received 8000–16,000 units epoetin weekly or 40–80 μg darbepoetin weekly. It is administered either IV or SC. The IV route is recommended for patients receiving hemodialysis because it may be less immunogenic.[19]

CERA can be administered once in every 2 weeks or once monthly to patients whose hemoglobin has been stabilized by treatment with an EPO [Table 2].[20]

Clinical Applications of Recombinant Human Erythropoietin

Anemia associated with chronic kidney disease on dialysis

Rationale: Patients with CKD on dialysis have subnormal endogenous EPO production. Studies have shown that rhEPO treatment corrects anemia and improves quality of life (QOL) in patients with CKD.[21] It also optimizes the patient's hemodynamic status and minimizes the risk of left ventricular hypertrophy, along with improvement in physical performance and cognitive function.[22,23]

Role of rhEPO in patients with CKD in pre-dialysis stage: A review in 1995 showed no improvement of anemia in such patients but they had acceleration to end-stage CKD.[24] However, other studies between 1980 and 2000 have shown that early treatment with rhEPO corrects anemia, avoids blood transfusion, and improves the QOL and exercise capacity.[25]

Anemia of chronic disease

Role of rhEPO in rheumatoid arthritis (RA) patients along with intravenous iron for improvement of anemia has been established. It is also used to increase the volume of autologous blood donation in patients with RA undergoing hip or knee replacement.[26-28]

Anemia in HIV-infected patients

Almost 60% of patients suffering from HIV have anemia, more so if they are on Ziduvudine treatment. If baseline EPO levels are <500 mU/ml, weekly or thrice weekly dose of 100–200 U/kg corrects anemia and improves patient's QOL and survival.[29,30]

Patients on hepatitis C treatment

Hemolysis and resultant anemia is a problem during the treatment of hepatitis C with Ribavirin and Interferon. Treatment with rhEPO and darbepoetin increases the hemoglobin levels and optimal therapy can be continued.[31,32]

Cancer/chemotherapy related anemia

Anemia of chronic disease, a condition characterized by disordered iron metabolism, shortened RBC half-life, and inefficient erythropoiesis, is the major contributor to cancer related anemia.[33]

Rationale: Cancer patients have low serum levels of EPO.[34] Anemia contributes to the poor QOL in cancer patients and reduced response to radiotherapy. Although blood transfusion is the mainstay of treatment in such situations, rhEPO could overcome the problems associated with stored blood such as low 2,3-diphosphoglycerate (2,3-DPG) levels, preservation injury to RBCs, and risk of blood-borne infections.[35]

The European Medicines Agency (EMEA) labels the use of EPO as follows;[36]

Table 2: The dose equivalence of continuous erythropoietin receptor activator

Previous weekly EPO dose (units/week)	Previous weekly darbepoetin alpha dose (mcg/week)	CERA dose	
		Once monthly (mcg/month)	Once every two weeks (mcg/every 2 weeks)
<8000	<40	120	60
8000–16,000	40–80	200	100
>16,000	>80	360	180

EPO: Erythropoietin, CERA: Continuous erythropoietin receptor activator

i. In patients treated with chemotherapy and with an Hb level of ≤10 g/dl, treatment with EPO might be considered to increase Hb by <2 g/dl or to prevent further decline in Hb.
ii. In patients not treated with chemotherapy, there is no indication for the use of EPO and there might be an increased risk of death when EPO is administered to a target Hb of 12–14 g/dl.
iii. In patients treated with curative intent, EPO should be used with caution.
iv. If the Hb increase is at least 1 g/dl above baseline after 4 weeks of treatment, the dose may remain the same or may be decreased by 25–50%.
v. If the Hb increase is <1 g/dl above baseline, the dose of selected EPO should be increased. If after an additional 4 weeks of therapy, the Hb has increased by ≥1 g/dl, the dose may remain the same or may be decreased by 25–50%.
vi. In the case of response, treatment with EPO should be discontinued 4 weeks after the end of chemotherapy.
vii. If the Hb increase is <1 g/dl above baseline after 8–9 weeks of therapy, response to EPO therapy is unlikely and treatment should be discontinued.
viii. If the Hb rises by >2 g/dl per 4 weeks or if the Hb exceeds 12 g/dl, the dose should be reduced by 25–50%.
ix. If the Hb exceeds 13 g/dl, therapy should be discontinued until Hb falls below 12 g/dl and then reinstituted at a dose 25% below the previous dose.

Use of erythropoietin in myelodysplastic syndrome

Although Food and Drug Administration (FDA) announced alert and safety warnings of EPO in non-MDS patients targeting an Hb of >12 g/dl, EPO has been shown to be safe in adult MDS patients. It has become important for symptomatic improvement in patients with anemia and it also reduces the red cell transfusion requirements.[37]

Anemia of prematurity

Neonates born prematurely (before 32 weeks of gestation), weighing less than 1300 g, usually receive multiple blood transfusions to compensate for regular blood sampling required for intensive monitoring.[38]

Rationale: Preterm babies have physiologically low serum levels of EPO and supplementation of rhEPO reduces the requirements of blood transfusions.[39]

It has also been shown that preterm infants receiving rhEPO may have a lower incidence of necrotizing enterocolitis and a reduction in the number of days requiring oxygen support.[40] The reduced oxygen support could be related to the increase in 2,3-DPG levels in RBCs, causing a right shift in the oxygen dissociation curve.[41]

To minimize allogeneic blood transfusions after surgical procedures

rhEPO can minimize blood transfusions in patients undergoing surgical procedures. For instance, cardiothoracic surgery[42] or orthopedic surgery[43] may cause up to a 20% loss of total blood volume. A recent study showed that in patients who were ineligible for autologous donation, a low dose of rhEPO (150 IU/kg/week) given 3–4 weeks before surgery reduced the blood transfusion requirement by nearly 50%.[44]

Critically ill patients

Anemia and the need for blood transfusions are common among patients admitted to intensive care unit. EPO has been used to decrease the need for transfusions with variable results. A recent meta-analysis has shown the reduction in blood transfusions per patient to be very small, with insufficient evidence to determine whether rhEPO results in clinical benefit with acceptable risk.[45]

Avoidance of blood transfusions

This could be because of personal preferences, religious beliefs, rare blood groups, or allosensitization of rare RBCs.[3] Among Jehovah's Witnesses, rhEPO has been successfully employed to avoid blood transfusion for various surgical procedures.[46,47]

Use of erythropoietin in research settings

Heart failure: Recombinant EPO therapy has been found to be useful in patients with heart failure, especially with the cardio-renal anemia syndrome.[48] Some recent studies show reduction in cardiac remodeling, Brain Natriuretic eptide levels, and hospitalization rate, resulting in improvement in left and right ventricular systolic function.[49]

Stroke: There is a lot of interest in the role of EPO as a neuroprotective agent in ischemic stroke based on preclinical studies and one pilot study; however, a recent study failed to show any benefit and raised some doubts regarding the safety of EPO in such patients.[50]

Acute kidney injury: The role of EPO in acute kidney injury (AKI) is undergoing active research and animal studies have revealed a physiological basis for the use of erythropoietin in AKI; however, a recent study failed to show any benefit.[51,52]

Evaluating response to recombinant human erythropoietin

Failure to respond to rhEPO therapy is defined as hemoglobin increase to <10 g/dl after a 4-week standard dosage treatment.[3]

In CKD setting, the resistance is defined as inadequate response to >300 IU/kg/week or a continued need for such a dosage to maintain the target hemoglobin. In myelodysplasia setting, it is >900 IU/kg/week. However,

one has to rule out underlying iron or Vitamin B-12/ folate deficiency, inflammation, blood loss, bone marrow involvement, or PRCA. For optimal rhEPO therapy, iron status of the patient needs to be continually assessed and intermittent intravenous iron therapy may be warranted.

Adverse effects of recombinant human erythropoietin[53]

a. Flu-like symptoms: Commonest side effect which subsides within 24 hours
b. Allergic and anaphylactic reactions
c. Seizures and hyperkalemia: Rare
d. Hyperviscosity
e. Thrombosis: A meta-analysis involving nearly 10,000 cancer patients indicates that treatment with rhEPO increases the risk of thrombosis[54]
f. Hypertension
g. Possibility of cancer progression: There is somewhat less convincing evidence that rhEPO enhances tumor progression[55]
h. Pure red cell aplasia (mainly reported in patients with CKD): Autoantibodies in the serum can neutralize both rhEPO and endogenous EPO. This was mainly observed in CKD patients, especially after SC injection. Its incidence after 2000 has reduced, especially with the IV formulations

Recent Areas of Research in Erythropoietin

With the discovery of EPO-R in non-erythroid tissue, pleiotropic effects of EPO were understood. Some areas of research with EPO as a novel therapeutic agent are mentioned below:

Spinal cord injury (SCI): Recently, research has focused on rhEPO and its effects on SCI treatment as well as the mechanisms such as anti-apoptotic, anti-inflammatory, and edema reduction, leading to neuronal and oligodendrocytes' survival and restoration of vascular integrity.[56]

EPO in depression: A current study is underway to evaluate the potential for EPO to alleviate depression and neurocognitive deficits in affective disorders among treatment-resistant cases.[57]

EPO in diabetes: EPO has been found to affect all phases of wound healing and shows encouraging results for chronic wound healing in experimental animal and human studies, especially in the management of patients with chronic diabetic wounds.[58]

EPO as an immunomodulating agent: A recent article shows that macrophages act as direct targets of EPO which enhances the pro-inflammatory activity and function of these cells.[59]

Abuse of Erythropoietin in Competitive Sports

To improve physical fitness and endurance exercise in sports: Administration of rhEPO increases the body's maximum oxygen consumption capacity, thus increasing endurance and physical fitness. This has led to the misuse of rhEPO in sports.[60] In 1990, the International Olympic Committee (IOC) prohibited the use of EPO in sports. With different types of EPO available in the market, it is even more challenging to detect them from the law enforcement point of view.

The most important recombinant EPOs and analogues misused in sports are:

a. rhEPO
b. Darbepoetin alpha
c. CERA

The detection of EPO abuse has been challenging for the following reasons:[61]
Timing of sampling and availability of specialized dedicated laboratories with immense infrastructure requirements are the major limiting factors in detecting EPO misuse. The other factors playing a role in the detection are follows:

1. It is difficult to discriminate between the endogenous EPO and recombinant exogenous hormone.
2. EPO has a relatively short half-life in serum (the half-life of rhEPO-a is 8.5 ± 2.4 hours when administered IV and 19.4 ± 10.7 hours when administered SC).[62]
3. EPO is undetectable in urine after 3–4 days of injection.
4. Screening in large numbers may be difficult as it requires highly trained technicians and standardization between laboratories.

Methods of detecting misuse of erythropoietin

Direct method of erythropoietin detection

This approach relies on the physicochemical properties of the hormone. It is based on different carbohydrate components of recombinant and endogenous hormones, conferring different net electrical charges and thus distinguishable isoelectric points. This is the underlying principle of the only direct method of rhEPO detection that has been approved by the court of arbitration for sport.[63,64] It uses electrophoretic techniques to separate the isoform profiles of recombinant and endogenous EPO in the urine according to their isoelectric points.[65] Darbepoetin alpha needs to be administered only once a week and as it has five glycosylation sites. Its detection is easier by the Lasne's method due to its different electrical charge.[64]

Indirect methods of detecting misuse of erythropoietin[66]

i. The hematocrit
ii. The reticulocyte hematocrit (fractional volume of the reticulocyte pool in the bloodstream, which equals the product of the number of reticulocytes and their mean corpuscular volume: Ret × MCV Ret)
iii. Macrocyte percentage
iv. EPO concentration
v. Serum soluble transferrin receptor (sTfR)[67]

To use these indirect methods, two statistical models were used: "ON" model and "OFF" model. ON model detects current users and is indicative of accelerated erythropoiesis that occurs during rhEPO use (HE model uses hemoglobin and serum EPO and HES model uses hemoglobin, serum EPO concentration, and sTfR).

OFF model is designed to differentiate between recent rhEPO users and non-users. This is consistent with down-regulation of erythropoiesis, which occurs following discontinuation of rhEPO (HR model uses hemoglobin and reticulocyte count and HRE model uses hemoglobin, reticulocytes, and serum EPO concentration).

Many common hematological conditions and inter-individual genetic variations associated with extreme hematological profiles can also obscure the specificity of these indirect detection methods.[68]

Novel erythropoietin doping strategies

Erythropoietin analogues

a. Synthetic erythropoiesis protein (SEP): SEP consists of a polypeptide chain similar to that of EPO and two covalently attached, branched polymer moieties that bear a total of eight negative charges. These polymers enhance the molecule's stability by protecting it from proteolytic cleavage. It is also less immunogenic as it is synthesized chemically and has less contaminating antigens. However, SEP can be detected by Lasne's method.[69]
b. EPO mimetics: They are small molecules capable of activating EPO-R in a way similar to EPO. On binding to the EPO-R, they cause the receptor to dimerize and activate multiple cellular signaling pathways. Subsequently, multiple genes are transcriptionally induced and mediate proliferative, antiapoptotic, and erythropoietic effects of EPO (e.g. Hematide).[70-72]
c Inhibition of hematopoietic cell phosphatase (HCP): An indigenous negative regulator of the EPO–EPO-R signaling cascade. Combination of EPO mimetics with HCP inhibitors could provide an oral substitute of endogenous EPO with equivalent potency.
d. EPO delivery by cell encapsulation: In this method, the modified cells are enclosed inside semi-permeable membrane polymers that isolate the encapsulated cells and thus prevent antigen recognition and immune rejection. Cell encapsulation as a form of immunoprotection has been shown to enhance erythropoiesis. There are no human studies with this molecule.[73]
e. EPO gene doping: Gene doping is defined as the transfer of genetic material to improve athletic performance.[74] In 2003, the IOC and the World Anti-Doping Authority incorporated gene doping into their list of prohibited practices. Two approaches are used here.
    i. *In vivo* gene transfer through intramuscular injection of a virus containing gene encoding EPO.[75] Other gene delivery methods include plasmid DNA, liposomes, and protein–DNA conjugates, or direct injection of EPO gene into muscles.
    ii. *Ex vivo* gene transfer into cells that are subsequently transplanted into the recipient organism.

At present, human applications with gene doping have not yet materialized. However, there is a potential of misuse of this technology in the future.

Potential strategies for detection of gene doping

Direct methods

1. Since glycosylation of EPO differs in the skeletal muscle and endogenous EPO, Lasne's method would be able to detect the same.
2. Detection of the gene delivery system in the body in case of gene doping: Plasmids, viral vectors, liposomes, and protein–DNA conjugates.
3. Labeling all EPO gene transfer products with genetic "barcodes."[76]

Indirect methods

1. "Hematological passport": In this concept, hematological parameters are monitored sequentially in all the athletes and subject-specific references are generated.[72] This would be useful in finding the genetic polymorphisms/mutations leading to increased endurance in a particular person (e.g. Finnish cross-country skier Eero Mantyranta who won two gold medals in the 1964 Winter Olympics was later identified to have a mutation in the EPO-R gene that caused sustained activation of EPO signaling. Mantyranta's oxygen carrying capacity was increased by 25–50%).[77]
2. "Molecular passport": Sequential determinations of the expression levels of certain EPO target genes by DNA microarray analysis could define athlete-specific reference ranges for the level of expression of these genes. Athletes with gene expression levels above or below their personal range would be considered suspicious for doping.[78]

With novel EPO molecules around the block, misuse of them in sports would be increasing and the challenge would be to provide easy and reliable detection strategies which can be used for mass screening.

Conclusion

There has been significant progress in the last three decades on the development and improvement of EPO and widening of its potential use in humans. With the growing understanding of EPO-R being present in non-erythroid tissue as well, several novel areas of research are currently underway. It is evident that we have not yet realized the full potential of rhEPO. However, it remains a boon and a bane with its potential for abuse.

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