Case Study: Immunogenicity of rhEPO
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6.1. Abstract

Erythropoietin is an endogenous growth factor that is required for erythropoiesis. The hormone consists of a single acidic polypeptide chain of 165 amino acids and three N-linked and one O-linked carbohydrate side chains. Autoantibodies against endogenous EPO are extremely rare in humans. Since its introduction as a drug for the treatment of renal and non-renal anemia, antibodies against recombinant human erythropoietin were observed in low frequencies in single cases only. Due to a steep increase in the number of patients developing anti-erythropoietin antibodies during the course of therapy from 1999 to 2004 there is an increased interest in the observation of patients treated with erythropoietin. Moreover, new products as well as modified versions of the natural hormone have entered the market. In addition, modified versions with optimized features as well as peptides mimicking the erythropoietin action are under development and will enter clinical trials soon. These aspects make it essential to screen patients for the presence of anti-EPO antibodies. Despite the lack of a common calibrator, screening assays for the detection of anti-erythropoietin antibodies are available.

6.2. Description of Erythropoietin

6.2.1. Structure and Function

Human erythropoietin (EPO) is an acidic polypeptide consisting of 165 amino acids with a molecular mass of 30.4 kD (Jelkmann 2007). The carbohydrate moiety consists of three tetra-antennary N-linked (Asn 24, Asn 38 and Asn 83) and one O-linked chains (Ser 126) and constitutes up to 40% of the molecular mass (Sasaki et al. 1988). The glycosylation pattern is diverse and leads to a certain degree of heterogeneity. The structure and length of the sialic acids directly affect the biological activity and plasma half-life time of the hormone. It is noteworthy that some N-glycosidic chains are targets of sulfation, of which the biological function is still unknown.

EPO is a member of an extensive cytokine family that includes growth hormone, somatropin, prolactin, interleukins 2 through 7 as well as “colony stimulating factors” (G-CSF, M-CSF and GM-CSF). The hormone is mainly synthesized in the endothelial kidney cells (85–90%). Only minor amounts
are produced in other cell types including hepatocytes and other tissues like brain, uterus, testis and even hair follicles (10–15%). The serum concentration (2–24 mIU/ml) is maintained by a feedback mechanism based on the tissue O$_2$ pressure (pO$_2$), which depends on the hemoglobin concentration, the arterial pO$_2$, the O$_2$ affinity of the hemoglobin and the rate of the blood flow. High serum concentrations of EPO are found in various tumors including renal tumors, hepatic tumors, cerebellar hemangioblastoma and adrenal tumors as a consequence of a reduced oxygen availability, i.e., the synthesis of EPO is stimulated by hypoxia. Decreased serum concentration of EPO in the serum is found in different forms of terminal and pre-terminal kidney insufficiencies as well as anemia of unknown origin like chronic infections, autoimmune diseases, AIDS, hypothyroidism, etc.

During erythropoiesis in the bone marrow EPO binds to its erythropoietin receptor (EPOR) on erythroid progenitor cells, which leads to their final differentiation to erythrocytes via the JAK-STAT-signal pathway. In this way each day more than 200 billion erythrocytes are produced. In addition to the mere erythropoiesis, EPO is also involved in apoptotic processes and stimulates the generation of megakaryocytes. It was shown that EPOR is expressed in numerous cell types including neurons, astrocytes, myocytes and hair follicles (D’Andrea and Zon 1990). The EPO/EPOR interaction was observed in various non-erythroid tissues in the context of cell differentiation, chemotaxis, angiogenesis, activation of intracellular calcium and inhibition of apoptosis. Some studies have demonstrated the neuroprotecting and neurotrophic effects of EPO (Marti et al. 2000, Ehrenreich et al. 2002).

6.2.2. Manufacturers of Recombinant Human EPO and EPO Variants

Since the first launch of recombinant human EPO in 1989 by Amgen, many different recombinant EPO preparations have reached the market. Besides the well-established recombinant EPO preparations, there are also numerous novel preparations under development. In 1989 Amgen launched the first recombinant human EPO preparation (Epogen, epoetin a). Johnson & Johnson has developed, under a license from Amgen, a variant named epoetin β which is known under the names Procrit (in USA) and Eprex (outside USA) or Erypo (in Europe). In 1990 Boehringer Mannheim (now Roche) brought an EPO preparation (epoetin β) under the name of NeoRecormon on the market. Although both preparations are produced in CHO cells, epoetin a and epoetin β show a slightly different molecular mass due to some differences in the glycosylation pattern. Additional variants were developed, like epoietin ω (Elanex Pharmaceuticals) which shows differences in glycosylation due to its production in BHK kidney cells.

Genetically modified EPO versions were developed to achieve optimized pharmacokinetic characteristics compared to natural endogenous human EPO or other recombinant versions (Bunn 2007). Realizing that glycosylation is an important factor for the circulation time of EPO in human plasma, Amgen produced in 2001 a modified EPO preparation (Aranesp, darbepoetin a) with a threefold prolonged serum half-life. This was achieved by the substitution of five amino acids generating additional glycosylation sites. Furthermore, in 2004 Amgen has introduced an Aranesp analog under the name of AMG114 which was shown to have a half-life time in human serum of up to 131 hours.