Erythropoietin and microvascular diabetic complications*

Kai-Uwe Eckardt

Department of Nephrology and Hypertension, University of Erlangen-Nürnberg, Germany

Keywords: anaemia; diabetic nephropathy; diabetic retinopathy; erythropoietin

When recombinant human erythropoietin (EPO) became available for clinical use, the hormone was considered as a specific erythropoietic growth factor and as a more or less unique molecule, since its endogenous production increases in response to reduced oxygen availability. Subsequently, we have witnessed a revolutionary increase in knowledge about the highly conserved ability of virtually all cells to sense oxygen and to translate reductions in oxygen availability into specific patterns of gene expression. This hypoxia response is to a large extent mediated by hypoxia-inducible transcription factors (HIF). EPO is the prime example for a hypoxia-inducible HIF target gene, but more than 100 additional genes have meanwhile also been identified as HIF targets, including genes involved in new vessel formation and anaerobic metabolism. In general, the activation of the hypoxia pathway stimulates responses that increase either oxygen availability or hypoxia tolerance [1].

Consistent with this broader perspective of oxygen sensing and tissue responsiveness to changes in oxygenation, EPO has meanwhile also been found to have actions beyond stimulation of red cell precursors, including pro-angiogenic and cell protective functions. High doses of EPO convey tissue protection in experimental models of ischaemic brain and kidney injury, and trials testing the clinical utility of such effects are underway. Moreover, low levels of EPO gene expression have been found in various tissues other than liver and kidneys, the two organs that are responsible for the synthesis of circulating EPO. In combination, these findings suggest that EPO—in addition to its function as an erythropoietic hormone—can also act as a paracrine growth factor. However, the pathophysiological relevance of EPO effects outside the bone marrow remains poorly understood.

Probably all systems that usually preserve the tissue structure and function can under specific circumstances turn into adverse mechanisms and promote disease processes. Circumstantial evidence suggests that such a mal-adaptive function of the hypoxia response occurs during the course of proliferative diabetic retinopathy (PDR). This devastating complication of diabetes is thought to occur after progressive retinal ischaemia induces expression of angiogenic factors, which lead to retinal vascular proliferation and permeability [2]. Neovascularization and fibrous proliferation can ultimately result in tractional retinal detachment. Local EPO production appears to play an important role in this context. In the vitreous humour of patients suffering from diabetic retinopathy, increased concentrations of EPO have been measured [3,4]; enhanced levels of EPO gene expression were determined in the retina of humans and experimental animals [3,4] and EPO receptor immunoreactivity has been reported in epiretinal membranes [5]. Moreover, the growth stimulating effect of vitreous fluid from patients with PDR on microvascular retinal endothelial cells is blunted when EPO is blocked experimentally with soluble receptors [3]. Although other angiogenic factors such as vascular endothelial growth factor (VEGF) are also increased in eyes affected by PDR, the effect of EPO is independent and is not well correlated with that of VEGF.

In this context, Tong et al. have recently provided very interesting data, suggesting that a promoter polymorphism of the EPO gene is associated with severe diabetic eye and kidney complications [6]. The provocative conclusion of the study is that a genetically determined ability of increased EPO synthesis predisposes diabetic patients to the development of PDR and end-stage renal disease (ESRD).

Promoter polymorphism of the EPO gene in diabetic patients with retinopathy and ESRD

The rational for the study by Tong et al. is based on the well-known facts that only subsets of patients with diabetes develop severe retinopathy and advanced kidney disease, that both complications have a high concordance rate and that they also show familial aggregation. The authors,
In order to determine whether increased EPO production is a critical factor in the progression of diabetic complications, the investigators genotyped single nucleotide polymorphisms from 11 genes possibly involved in angiogenesis. These genetic variations were associated with the manifestation of severe eye and kidney disease. This finding in patients with type 2 diabetes was confirmed in the two other cohorts of patients with type 1 diabetes. The polymorphism was found more frequently in the patients with PDR plus ESRD than in the controls. Although these data are preliminary, it is possible, therefore, that the observed exchange of a single nucleotide leads to a higher activity of the EPO gene. Interestingly, several previous studies have shown that chromosome 7q21, where the EPO gene is located, harbours a locus for increased susceptibility to diabetic nephropathy [7,8], which is consistent with a possible role of EPO in the pathogenesis of diabetic nephropathy.

According to this concept one might postulate that common genetic factors exist that predispose (or protect) patients with diabetes to develop both complications. Therefore, they selected three cohorts of around 375 patients suffering from both PDR and ESRD from three different and much larger cohorts of diabetic patients. Age- and ethnicity-matched diabetic patients without these complications from the same cohorts served as controls. Since PDR is characterized by increased angiogenesis, the investigators genotyped single nucleotide polymorphisms from 11 genes possibly involved in angiogenesis to test for allelic associations. In one of the three cohorts they found a single nucleotide polymorphism located at \(~\sim\)1100 bp upstream of the EPO promoter that was significantly associated with the manifestation of severe eye and kidney disease. This finding in patients with type 2 diabetes was confirmed in the two other cohorts of patients with type 1 diabetes. The polymorphism is located at a putative enhancer binding site and the variant that was found more frequently in the patients with PDR plus ESRD revealed enhanced EPO promoter activity in reporter assays. Although these data are preliminary, it is possible, therefore, that the observed exchange of a single nucleotide leads to a higher activity of the EPO gene. Interestingly, several previous studies have shown that chromosome 7q21, where the EPO gene is located, harbours a locus for increased susceptibility to diabetic nephropathy [7,8], which is consistent with a possible role of EPO in the pathogenesis of diabetic nephropathy.

According to this concept one might postulate that an increased sensitivity of the mechanisms that link EPO gene activity to oxygen availability accelerates eye and kidney disease (Figure 1). Since local oxygen availability depends on (micro) vascular blood supply and blood oxygen content, the progressive anaemia associated with kidney disease together with alterations in the microvasculature of both organs would in fact create a positive feedback loop that might further enhance EPO synthesis and thus disease progression.

Inconsistencies and open questions

Is an increased ability to synthesize EPO thus a critically important factor that predisposes to two of the most serious complications of one of the most relevant global health problems? Although this is an intriguing hypothesis, there are a number of findings that are not consistent with this concept and many questions remain to be addressed to prove its validity. For example, although there is evidence that EPO plays a role in the pathogenesis of PDR (A in Figure 1), the interaction appears to be complex and not uniform. Another recent study by Chen et al. showed in a mouse model of hypoxia-induced retinopathy that the administration of exogenous EPO during the early phase prevents vessel dropout and protects against hypoxia-induced retinal neuron apoptosis, whereas late EPO treatment enhances pathological neovascularization [9]. Previous studies also found protective effects of EPO against photochemical and ischaemia/reperfusion injury of retinal cells [10,11].

In the kidney, hypoxia is also considered to play an important role in the progression of chronic injury and fibrosis, and renal HIF expression has been described in a rat model of diabetic nephropathy [12]. However, this is in contrast to the pathogenesis of PDR, reduced microvascular density, which rather than increased neovessel formation is a hallmark of renal pathology in chronic kidney disease [13], which argues against a common pathomechanism. Moreover, there is no evidence for a direct effect of EPO on the progression of renal disease (B in Figure 1). In fact, a recent study in db/db mice reported that pegylated EPO can inhibit pathological features of diabetic nephropathy [14]. Another study found that cobalt, which stimulates HIF-dependent gene expression, including EPO synthesis, ameliorates renal injury in an obese hypertensive rat model [15]. Since EPO synthesis declines with progressive destruction of renal architecture and loss of renal function, any effect of endogenous EPO in the kidney should also be offset during the progression of the disease (C in Figure 1). Finally it is important to note that several studies showed an inverse rather than positive correlation between the degree of anaemia and the progression of both kidney disease [16,17] and diabetic retinopathy [18,19]. The main cause of renal anaemia is inappropriately low EPO production. Therefore, although serum EPO levels were not usually measured in these studies, this finding obviously contradicts the hypothesis that patients with higher EPO production are at increased risk for retinopathy and ESRD.

Clinical consequences?

Tong et al. suggest that on the basis of their genotype findings, caution may be warranted when maintaining higher haemoglobin (Hb) concentrations by using exogenous EPO treatment in diabetic patients, because this might accelerate progression to PDR or ESRD [6]. Although this conclusion appears plausible at first glance, it may be too simplistic. Apart from the unresolved questions outlined above, the issue is complicated by the fact that expression of the endogenous EPO gene is sensitive to local tissue oxygenation.
In the kidney this has been demonstrated beyond doubt and in the eye it is at least highly likely [3,6]. Therefore, treatment of renal anaemia with EPO will probably suppress endogenous EPO formation. In quantitative terms, this suppression of endogenous EPO synthesis could be far more relevant for local effects in the vicinity of EPO producing cells than a treatment-related increase in circulating hormone levels.

Given the complexity of the underlying pathophysiological mechanisms, only RCTs can determine the net relationship between risks and benefits. In fact, trials of anaemia correction in chronic kidney disease found either no benefit on cardiovascular outcomes or increased mortality risk in patients randomized to higher targets. Theoretically this increased risk observed in some studies could be due to higher Hb levels or extraerythropoietic effects of higher doses of EPO. A recent secondary analysis of the CHOIR study supports the latter possibility, indicating that non-erythropoietic effects of EPO, such as those postulated by Tong et al. may indeed play an important role [20]. Unfortunately, all relevant RCTs in the field of renal anaemia that were conducted so far have not been placebo controlled, which has limited their ability to determine the effects of therapy on various outcomes. The ongoing TREAT trial is the first large multicentre study, testing the effect of anaemia therapy with darbepoietin in comparison to placebo in patients with type 2 diabetes and kidney disease [21]. It will provide urgently needed insight into the effects of EPO therapy in this important patient group. Until the results of TREAT are available it seems prudent to follow the most recent KDOQI guidelines [22].

Acknowledgement. I thank Professor André Reis for helpful discussions of the genetic aspects.

Conflict of interest statement. The author has received consultancy fees and lecture honoraria from companies producing erythropoiesis stimulating agents.

References