Effect of recombinant human erythropoietin on inflammatory status in dialysis patients

Abelardo Aguilera and Rafael Selgas
Servicio de Nefrologı´a, Hospital de la Princesa, Madrid, Spain

Abstract
Under normal conditions, inflammatory status is regulated by a complex equilibrium between plasma and intracellular mediators. This equilibrium is broken in patients receiving dialysis, which can lead to chronic inflammation causing deleterious consequences on their organs and systems. During recent years, substances that can inhibit the effects of inflammation have been sought. The results of these investigations have produced controversial data on the effects of recombinant human erythropoietin (epoetin) and, in this review, the effects of epoetin on the inflammatory status of dialysis patients are discussed. Aspects discussed include biomarkers of inflammation, and the relationships between epoetin, growth factors, endothelial dysfunction, endothelial fibrinolytic capacity, endothelial damage and oxidative stress. Relationships between epoetin and inflammation in non-uraemic patients are also addressed, as are associations between the malnutrition–inflammation–atherosclerosis syndrome and endothelial dysfunction. It is concluded that although epoetin administration in non-uraemic rats has been shown to have an anti-inflammatory and cytoprotective effect, the mechanisms responsible for regulating inflammation in uraemia are very complex and partially contradictory. The changes in pro-thrombotic and pro-atherogenic factors in dialysis patients require further study to evaluate all the factors implicated in the atherogenic process.

Keywords: biomarker; dialysis; epoetin; inflammation; malnutrition–inflammation–atherosclerosis (MIA) syndrome; uraemia

Inflammation in uraemia
Conceptually, inflammation includes the activation of monocytes, T and B lymphocytes, the complement cascade, cytokines and various growth factors [1]. In normal conditions, the inflammatory status is regulated by a complex equilibrium between plasma and intracellular mediators. C-reactive protein (CRP), tumour necrosis factor-α (TNF-α), interleukins (IL-1, -6, -12 and -18), interferon-γ (IFN-γ), nuclear transcription factor-κB (NF-κB), chemokines, granulocyte–macrophage colony-stimulating factor and α-melanocyte-stimulating hormone are pro-inflammatory agents [1,2], whereas transforming growth factor-β (TGF-β), IL-2, IL-4, IL-10, IL-13, IFN-α and adiponectin are anti-inflammatory molecules [1,3–5]. This equilibrium is broken in patients receiving dialysis, which can lead to chronic inflammation causing deleterious consequences on their organs and systems.

In patients with uraemia, the accumulation of inflammatory mediators is higher than in healthy people, due to the lack of renal catabolism. The residual renal function (RRF) is not only more important than the dialysis dose, but specifically protects against the deleterious effects of inflammation [6].

Endogenous factors such as advanced glycation end-products, low-density lipoprotein (LDL)-cholesterol, plasma levels of angiotensin II, cytokines, homocysteine and lipoprotein-a [Lp(a)], and exogenous factors such as endotoxin from dialysate, intravenous iron, vascular access, catheter, dialysis membrane, virus infection, gingivitis and chronic infections (by Helicobacter pylori or Chlamydia pneumoniae) promote the formation and activation of NF-κB from free oxygen radicals, and are exacerbated in uraemia. NF-κB activates several genes in T and B lymphocytes, as well as in other cells, causing a stimulation of growth factor production, oxidative and carbonyl stress, complement activation and IL-6 production. As a result of chromosome 1 activation, IL-6 and finally
CRP are produced, marking the end of the inflammatory cascade [1,7].

During recent years, considerable efforts in the search for substances which can inhibit the effects of inflammation have been made. The results of these investigations have produced controversial data on the effects of recombinant human erythropoietin (epoetin). Here, the effects of epoetin on the inflammatory status of dialysis patients will be reviewed.

Inflammation biomarkers: role in diagnosis and follow-up

The malnutrition–inflammation–atherosclerosis (MIA) syndrome has been proposed as being the negative effect of systemic inflammation in uraemic patients [8]. The MIA syndrome produces inflammatory molecules which tend to exacerbate cardiovascular (CV) and nutritional complications, as well as traditional CV risk factors, such as hypertension, dyslipidaemia, obesity, a sedentary lifestyle and the pathophysiological effects of smoking [9].

Almost all markers used to diagnose the MIA syndrome are inter-related, making it difficult to list the function and effects of each individual biomarker. Many markers of the MIA syndrome are considered to be markers of inflammation, CV risk factors, nutrition and endothelial function (Figure 1). Thus, there is considerable difficulty in monitoring the different components of the MIA syndrome. Albumin and cholesterol are markers of nutrition as well as markers for CV risk factors.

When the endothelium is stimulated under normal conditions, tissue plasminogen activator (tPA), thrombomodulin (TM), nitric oxide (NO), von Willebrand factor (vWF), TGF-β and factor VII are released (Figure 2). tPA is a fibrinolytic glycoprotein, which is released during exercise, the venous occlusion test (VOT) and the desmopressin test [10,11]. TM induces the release of protein-C, a powerful inhibitor of fibrin formation. The level of TM represents the number of dead endothelial cells because TM is released in response to injury and, thus, is a marker of endothelium turnover. NO inhibits platelet adhesion and aggregation, whereas vWF has the opposite effect and stimulates platelet adhesion and aggregation [12–14]. TGF-β stimulates the release of plasminogen activator inhibitor type-1 (PAI), which in turn inhibits tPA. tPA, PAI, Lp(a) and factor VII are considered to be markers of CV risk factors [15,16]. Markers of endothelial dysfunction include high plasma concentrations of PAI, TM, factor VII and Lp(a) and low plasma concentrations of tPA and NO [11,15–17].

We recently conducted a study investigating the effects of epoetin on markers of endothelial function and inflammation, and on endothelial growth factors implicated in CV complications. We studied three groups of patients who were starting epoetin therapy; 12 patients were receiving haemodialysis (HD), 16 were receiving peritoneal dialysis (PD) and seven were pre-dialysis patients. Patients were matched by age, time on dialysis, atherosclerosis score and dose of epoetin [11].
A VOT was performed before and after epoetin administration; right-arm VOT consisted of applying a sphygmomanometer cuff, which was inflated to a pressure midway between systolic and diastolic values (median mean arterial pressure) [10]. Serum samples were taken before and after VOT.

Changes in individual markers of inflammation induced by epoetin

After 2 months of epoetin therapy, all patients showed a progressive increase in plasma TNF-α levels. Significantly, this increment was not associated with the cachectic effects frequently associated with TNF-α. Conversely, plasma levels of leptin, a power anorexigen, decreased [18]. The explanation for this apparently contradictory effect could be that epoetin treatment is associated with an improvement in immune status [19]. It is also known that epoetin treatment is associated with improvements in nutritional status, possibly caused by an increase in appetite secondary to decreased plasma concentrations of leptin [20]. Levels of other markers, such as CRP and IL-6, did not change after 2 months of epoetin therapy.

Epoetin, growth factors and inflammation

Harpel et al. [21] demonstrated that the association between TGF-β and Lp(a) is pro-atherogenic. There is a significant linear correlation between the levels of TGF-β and Lp(a) in non-epoetin-treated patients ($r = 0.4, P < 0.05$). After 2 months of epoetin treatment, the concentration of TGF-β and the strength of the correlation between TGF-β and Lp(a) concentrations increased ($r = 0.61, P < 0.01$). However, in uraemic [22] and non-uraemic [23] patients with severe atherosclerosis, the concentration of TGF-β is severely depressed. In these situations, an inverse relationship with Lp(a) exists, whose levels are elevated [24]. This apparent contradiction in the relationship between TGF-β and Lp(a) may be explained, at least in part, by the elevation of inflammatory molecules (TNF-α) by epoetin, resulting in a chronic decrease in TGF-β plasma levels [25].

TGF-β may be considered as a double-edged sword, because TGF-β has both anti-inflammatory and pro-fibrotic actions. Locally, an increase in TGF-β concentration induces endothelial monocyte migration, proteoglycan accumulation mediated by platelet-derived growth factor (PDGF), and smooth muscle cell proliferation [26,27]. In our series, epoetin treatment caused a decrease in PDGF, which may be considered as a positive effect. At baseline, however, we found a significant linear correlation between PDGF and LDL-cholesterol ($r = 0.45, P < 0.05$), making the relationship pro-atherogenic [28,29], and this correlation increased after 2 months of epoetin treatment. Some authors have demonstrated a synergistic effect.
with this combination in inducing atherosclerosis plaque growth [28–30]. In our study, the levels of vascular endothelial growth factor (VEGF) and other inflammatory molecules, such as monocyte chemoattractant protein-1 (MCP-1) and vascular chemoattractant monocyte, did not change with epoetin therapy, although we previously found that epoetin treatment caused an increase in VEGF levels [31]. An association between epoetin and VEGF is supported by the finding that epoetin causes an increase in VEGF mRNA expression and neovascularization [32].

The results we obtained from this study were similar to those found by other researchers [33], which showed that epoetin treatment was associated with decreased plasma levels of PDGF and increased levels of MCP-1, which is associated with stenosis of the arteriovenous anastomosis. Similarly, Ikegaya et al. [34] found a high expression of erythropoietin receptors and TGF-β1 in samples of stenotic arteriovenous anastomoses.

**Epoetin, endothelial fibrinolytic capacity and inflammation**

Fibrinolysis is regulated by the opposing actions of PAI and tPA, as both agents regulate plasmin concentration. When fibrinolysis is activated in normal conditions, plasminogen is transformed by tPA into plasmin, which is then degraded in the liver by PAI [16]. It has been demonstrated that patients with severe atherosclerosis or unstable angina have a lower fibrinolytic capacity mediated by a baseline increase in PAI and decrease in tPA endothelial release [35]. Uraemia per se is associated with spontaneous increased levels of tPA, although the reason for this is still unknown [11,16].

In our study, epoetin induced a decrease in PAI and tPA pre-VOT. As PAI is a CV risk factor [10,15,16], one would assume that a decrease in PAI would have a beneficial effect. The concomitant fall in the tPA ratio (post- and pre-VOT), however, may result in a powerful inhibition of endothelial fibrinolytic capacity and, consequently, produce a pro-atherogenic environment [13]. This situation worsens further in uraemic patients as the fibrinolytic capacity is reduced [11].

PAI and tPA are also considered to be pro-inflammatory markers [9,16,36]. We believe that the elevation of PAI and tPA induced by epoetin treatment is partially independent of the inflammatory pathway, indicating a direct effect of epoetin on the endothelium. Epoetin receptors have been identified in the endothelium, which supports this hypothesis [37]. At the same time, changes in TNF-α levels associated with epoetin may alter tPA and PAI plasma levels, suggesting a systemic inflammatory action. It has been demonstrated both in vivo and in vitro that cytokines (e.g. IL-1 and TNF-α), mediated by PAI, act on the endothelium and induce a profound alteration in its fibrinolytic capacity [9,16]. In this study, we did not find any significant differences in the levels of PAI when measured pre- and post-VOT, indicating that other mechanisms, such as an alteration in protein hepatic metabolism, could be modulating PAI levels. Liver protein metabolism disorders have been described previously in patients suffering with both malnutrition and inflammation [12]. The stimulation of cultured human umbilical vein endothelial cells with IL-1 and TNF-α reduces tPA synthesis [15], causing a decrease in the endothelial fibrinolytic capacity. As tPA can be considered a pro-inflammatory molecule [14], the elevation of tPA pre- or post-VOT may result in a decrease in the tPA ratio, inducing pro-coagulant phenomena.

**Epoetin, markers of endothelial damage and inflammation**

Nitrate (NO₃) representing NO, TM and vWF are considered to be markers of endothelial damage [11–13], and are affected by inflammatory molecules [10,16,36,38,39]. When the endothelium is damaged, vWF is released, stimulating platelet aggregation. High levels of vWF have been found in different types of endothelial damage, and is considered to be a true CV risk factor because it promotes thrombosis [10,16,17]. Elevated plasma levels of vWF have been described in patients receiving dialysis [11,17]. vWF is not excreted by the kidneys, however, and so increased plasma levels of vWF in patients with chronic kidney disease are not caused by an excretion dysfunction [10,11]. vWF is also an acute-phase reactant and is increased by cytokines [16]. In HD patients, other factors, such as the activation of platelets by the dialysis membrane, contribute to high vWF levels [17,40]. Epoetin did not change vWF levels, however, in any of our study groups.

TM is an integral membrane protein expressed on the surface of endothelial cells, and is released in response to injury and, thus, represents the number of injured endothelial cells [10,13,36]. As TM is excreted renally, the plasma levels of TM in uraemic patients are high [10,13]. TM released in the plasma activates CRP, which inhibits fibrin formation [36]. In our study, we did not find any differences in the plasma concentrations of TM between patients with and without inflammation. It is well known that inflammatory mediators, proteolysis and oxidation induce a downregulation of TM [16,17]. In cultured cells, however, stimulation with near lethal doses of TNF-α induced an increase of TM, indicating endothelial injury [10,16,36]. According to our results, TM levels increase in HD patients (Table 1). We previously have described increases in plasma levels of TM when values are adjusted for RRF in PD patients [13].

NO is a potent vasodilator, synthesized by endothelial cells, and is able to inhibit platelet adhesion, the release of mitogenic factors and vascular smooth muscle cell proliferation [41]. Deficient NO production and early atherosclerosis [33,38,42] have been observed following administration of recombinant TNF-α (r-TNF-α), causing an impairment of
endothelium-dependent relaxation. Moreover, in cultured endothelial cells, TNF-\(\alpha\) administration reduces the half-life of NO synthase mRNA [39]. In our results, although epoetin therapy increases the level of TNF-\(\alpha\), there was a non-significant increase in NO\(_3\), indicating that other factors, such as nutritional status, could participate in NO regulation [41].

The effects of epoetin on the endothelium and markers of inflammation in uraemia are summarized in Figure 3. All of these changes were more evident in patients receiving HD and PD than in patients with chronic kidney disease (Tables 2 and 3), suggesting that RRF is a protective factor.

**Table 1.** Levels of markers of inflammation in HD patients pre- and post-venous occlusion test (VOT)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-epoetin (pg/ml)</th>
<th>Post-epoetin (pg/ml)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>276 ± 124</td>
<td>363 ± 174</td>
<td>0.7</td>
</tr>
<tr>
<td>TGF-(\beta)</td>
<td>27 ± 8</td>
<td>37 ± 7</td>
<td>0.01</td>
</tr>
<tr>
<td>tPA ratio</td>
<td>0.69 ± 0.37</td>
<td>0.58 ± 0.25</td>
<td>0.05</td>
</tr>
<tr>
<td>TM (ng/ml)</td>
<td>265 ± 132</td>
<td>299 ± 123</td>
<td>0.054</td>
</tr>
<tr>
<td>MCP-1 (pg/ml)</td>
<td>529 ± 139</td>
<td>498 ± 122</td>
<td>0.05</td>
</tr>
<tr>
<td>VCAM (ng/ml)</td>
<td>1542 ± 501</td>
<td>1760 ± 553</td>
<td>0.07</td>
</tr>
<tr>
<td>PDGF (pg/ml)</td>
<td>1486 ± 531</td>
<td>1310 ± 434</td>
<td>0.07</td>
</tr>
</tbody>
</table>

**Table 2.** Levels of markers of inflammation in PD patients pre- and post-VOT

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-epoetin (pg/ml)</th>
<th>Post-epoetin (pg/ml)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>256 ± 112</td>
<td>263 ± 163</td>
<td>NS</td>
</tr>
<tr>
<td>TGF-(\beta)</td>
<td>27 ± 6</td>
<td>32 ± 6</td>
<td>0.05</td>
</tr>
<tr>
<td>tPA ratio</td>
<td>0.66 ± 0.26</td>
<td>0.49 ± 0.25</td>
<td>0.05</td>
</tr>
<tr>
<td>TM (ng/ml)</td>
<td>223 ± 141</td>
<td>258 ± 111</td>
<td>NS</td>
</tr>
<tr>
<td>MCP-1 (pg/ml)</td>
<td>401 ± 121</td>
<td>453 ± 132</td>
<td>NS</td>
</tr>
<tr>
<td>VCAM (ng/ml)</td>
<td>1384 ± 401</td>
<td>1286 ± 332</td>
<td>NS</td>
</tr>
<tr>
<td>PDGF (pg/ml)</td>
<td>1560 ± 701</td>
<td>1765 ± 476</td>
<td>0.052</td>
</tr>
</tbody>
</table>

**Table 3.** Levels of markers of inflammation in pre-dialysis patients pre- and post-VOT

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-epoetin (pg/ml)</th>
<th>Post-epoetin (pg/ml)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>236 ± 132</td>
<td>245 ± 131</td>
<td>NS</td>
</tr>
<tr>
<td>TGF-(\beta)</td>
<td>25 ± 4</td>
<td>25 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>tPA ratio</td>
<td>0.70 ± 0.23</td>
<td>0.58 ± 0.32</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TM (ng/ml)</td>
<td>178 ± 65</td>
<td>200 ± 98</td>
<td>NS</td>
</tr>
<tr>
<td>MCP-1 (pg/ml)</td>
<td>488 ± 94</td>
<td>485 ± 141</td>
<td>NS</td>
</tr>
<tr>
<td>VCAM (ng/ml)</td>
<td>1309 ± 465</td>
<td>1164 ± 160</td>
<td>NS</td>
</tr>
<tr>
<td>PDGF (pg/ml)</td>
<td>1638 ± 730</td>
<td>1061 ± 427</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

NS = not significant.

Fig. 3. A summary of the effect of epoetin on the endothelium and markers of inflammation in uraemia. Nitrate (NO\(_3\)), thrombomodulin (TM) and von Willebrand factor (vWF) are considered to be markers of endothelial damage. Epoetin treatment was associated with a decrease in leptin, tissue plasminogen activator (tPA) and transforming growth factor-\(\beta\) (TGF-\(\beta\)), and an increase in tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\)), plasminogen activator inhibitor type-1 (PAI-1), TM and platelet-derived growth factor (PDGF). No change in nitric oxide (NO) and vWF following epoetin treatment was observed in our study groups.
Epoetin, oxidative stress and inflammation

The balance between plasma and intracellular oxidative and antioxidative mechanisms is responsible for maintaining the structural and functional integrity of the epithelium. One of the most important antioxidant factors, the intracellular enzyme systems (catalase, superoxide dismutase and glutathione peroxidase), and malonaldehyde were reduced by epoetin treatment [43,44]. Moreover, epoetin per se enhances superoxide anion formation [43]. In theory, the elevation of TNF-α associated with epoetin treatment might exacerbate OS in uremic patients [18]. Other authors, however, have shown an improvement in OS associated with epoetin treatment [45–47]. This apparent contradiction could imply that there is no unique marker for OS, and so these changes in oxidative and antioxidative mechanisms should be analysed globally.

Epoetin and inflammation in non-uraemic patients

Studies in non-uraemic rats suggest that treatment with epoetin has a systemic anti-inflammatory, anti-apoptotic and neuroregenerative effect. Junk et al. [48] used male Sprague-Dawley rats, anaesthetized for 45–60 min and cannulated in the right eye, and raised the intraocular pressure to 120 mmHg to produce retinal ischaemia. One group of rats received 5000 IU/kg of epoetin before the ischaemia, and the second group (control) received saline solution. The results showed that the epoetin-treated group had a reduced level of intracellular deoxynucleotidyltransferase, an important modulator of apoptosis. The authors concluded that epoetin treatment has a neuroprotective effect in ischaemic injury.

In another study, the effect of epoetin (1000 and 5000 IU/kg via intraperitoneal injection) was investigated in female Wistar rats (180–300 g) subjected to experimental spinal cord compression for 1 min. Partial motor function was recovered by day 12 after injury, and motor function was fully recovered by day 28. The time of recuperation was significantly shorter in the treated rats than in the control group receiving saline, and was epoetin dose dependent. This effect was associated with increased NO synthase [49,50] and circulating VEGF, resulting in an increase in the collateral circulation. The anti-inflammatory effect was mediated by a decrease in the cerebrospinal fluid concentration of IL-6 and TNF-α, and decreased gene expression for NF-κB [48,51,52]. The anti-apoptotic effect was mediated by modulation of Janus kinase-1,2/signal transducer (AKT1 and 2) from the caspase pathway [51,53].

MIA syndrome and endothelial dysfunction

MIA syndrome is a complex syndrome that in its maximal expression (MIA-2) is associated with high morbidity and mortality. The importance of inflammation has been studied since 1996 in renal and non-renal populations. Renal populations have the highest risk of CV complications, ~5-fold higher than in populations with no increased levels of markers of inflammation [54]. The pathogenesis of MIA remains unknown, however, because the accumulation of pro-inflammatory cytokines by renal retention is mirrored by that of anti-inflammatory cytokines [1,5,12,55]. Moreover, the injection of r-TNF-α in vivo induced a non-antibody-mediated tolerance [56].

To assess the effect of the accumulation of these substances with opposite actions in dialysis patients, we analysed a group of patients who had developed MIA syndrome. In 32 PD patients, clinically stable at baseline, we measured endothelial function and markers of inflammation both at baseline and after 2 months. There was an increase in inflammatory markers (CRP and TNF-α) in 17 patients, accompanied by a deterioration in endothelial function (Table 4). We therefore hypothesize that endothelial dysfunction could be the link between the different components of the MIA syndrome (Figure 4).

Finally, the effect of epoetin on the inflammatory status is paradigmatic because epoetin is able to decrease or increase some pro- and anti-inflammatory molecules, depending on the context or the type of tissue. For example, both TNF-α and leptin are pro-inflammatory molecules [18] and epoetin increases the plasma concentration of TNF-α without increasing its typical catabolic effect; this increase may be associated with an improvement in immune response. On the other hand, epoetin reduces plasma levels of leptin which may be associated with improved nutritional status via an increase in appetite [18].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before MIA</th>
<th>MIA</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/l)</td>
<td>9.8±8.3</td>
<td>23.0±7.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>30±12</td>
<td>48±16</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Albumin (mg/dl)</td>
<td>3.7±0.3</td>
<td>3.4±0.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PAI (ng/ml)</td>
<td>7.0±3.1</td>
<td>12.1±2.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>tPA ratio</td>
<td>2.1±0.3</td>
<td>1.1±0.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Nitrate (NO3) (µmol/l)</td>
<td>34±12</td>
<td>51±10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Lp(a) (ng/ml)</td>
<td>37±9</td>
<td>55±12</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>VCAM (ng/ml)</td>
<td>1301±405</td>
<td>1514±281</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Table 4. Levels of markers of endothelial function in patients before and after the onset of MIA syndrome

Conclusions

Although epoetin administration in non-uraemic rats has an anti-inflammatory and cytoprotective effect, the mechanisms responsible for regulating inflammation in uraemia are very complex and partially contradictory. The changes in pro-thrombotic and pro-atherogenic factors in dialysis patients require further study to evaluate all the factors implicated in the atherogenic process.
Conflict of interest statement. None declared.

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