Uncoupling of the VEGF-endothelial nitric oxide axis in diabetic nephropathy: an explanation for the paradoxical effects of VEGF in renal disease

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Nakagawa T. Uncoupling of the VEGF-endothelial nitric oxide axis in diabetic nephropathy: an explanation for the paradoxical effects of VEGF in renal disease. Am J Physiol Renal Physiol 292: F1665–F1672, 2007; doi:10.1152/ajprenal.00495.2006.—In many forms of experimental kidney diseases, renal VEGF is low, and administering VEGF can be shown to be protective. A paradox occurs in diabetes, in which renal VEGF levels are high and administering VEGF can be shown to be deleterious. We have hypothesized that endothelial dysfunction induced by hyperglycemia or other factors may underlie the pathogenic mechanisms of a high VEGF state. VEGF normally stimulates endothelial nitric oxide (NO) release and acts in concert with elevated NO levels as a trophic factor for vascular endothelium. The increased NO derived from the endothelial cell acts as an inhibitory factor that prevents excess endothelial cell proliferation, vascular smooth muscle cell proliferation, and macrophage infiltration. In the setting where NO bioavailability is reduced in diabetes, high levels of VEGF lead to excessive endothelial cell proliferation, vascular smooth muscle cell proliferation, and macrophage infiltration. In the setting where NO bioavailability is reduced in diabetes, high levels of VEGF lead to excessive endothelial cell proliferation, stimulation of macrophage chemotaxis, and vascular smooth muscle cell activation. Consistent with this hypothesis is our recent observation that diabetes induced in endothelial NO-deficient mice results in clinical and histological features identical to human diabetic nephropathy. The discovery of the key role for impaired endothelial NO bioavailability in the stimulation of VEGF and VEGF-dependent disease may provide key insights into not only the pathogenesis of diabetic nephropathy but also the utility and hazard of administering VEGF as a treatment for kidney disease.

NO; angiogenesis

ACUTE AND CHRONIC RENAL DISEASES are frequently associated with impaired angiogenesis with capillary loss (28, 30, 41, 50). In these conditions, stimulating angiogenesis may be an important way to prevent the progression of renal disease. One of the most important angiogenic factors is VEGF, which is constitutively expressed in glomerular podocytes and proximal and distal tubules. In a number of experimental models of kidney disease, a loss of glomerular and peritubular capillaries can be shown, often in association with a reduction in renal VEGF expression (28, 29, 41, 50). The administration of VEGF has been shown to stimulate angiogenesis with improvement in function and a reduction in scarring (29, 30). These studies suggest that VEGF could be an indispensable factor to improve renal function in renal disease. In contrast, in experimental diabetic nephropathy renal and systemic VEGF levels are elevated (6, 68) and VEGF participates in the progression of diabetic nephropathy (7, 12).

Great insights could be provided if we could develop a better understanding of why VEGF is deleterious in diabetic disease as opposed to nondiabetic disease. In other words, understanding how an increase in VEGF might contribute to diabetic nephropathy would be of interest.

Here, we present a hypothesis, which we call “VEGF-endothelial nitric oxide (NO) uncoupling,” to account for these findings.

The “Bright Side” of VEGF in Kidney Disease

VEGF has been found to be protective in numerous experimental renal diseases (28, 30, 41, 50) (Table 1). One of the best examples is the rat remnant kidney model (28). In this model, renal VEGF expression precipitously falls beginning 4 wk after induction of the model and decreased VEGF expression correlates with progressive impairment in renal function (29). Importantly, the fall in VEGF is accompanied by a progressive loss in both glomerular and peritubular capillaries and correlates with the development of glomerulosclerosis and tubulointerstitial fibrosis, respectively (29). Furthermore, the loss of VEGF in tubules coincided with macrophage infiltration, possibly related to the fact that certain macrophage cytokines inhibit VEGF expression in the tubular cells in vitro (29). The protective role of VEGF in renal disease was directly demonstrated by the evidence that systemic administration of VEGF121 stimulated capillary repair, reduced renal fibrosis, and preserved renal function. Furthermore, the renoprotective...
Table 1. **Diverse effects of VEGF on different types of renal diseases**

<table>
<thead>
<tr>
<th>Protective Effects of VEGF</th>
<th>Deleterious Effects of VEGF</th>
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<tbody>
<tr>
<td>Remnant kidney</td>
<td>Diabetic nephropathy</td>
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<tr>
<td>Female remnant kidney</td>
<td>Angiotensin II infusion model</td>
</tr>
<tr>
<td>Cyclosporine nephropathy (acute phase)</td>
<td>Cyclosporine nephropathy (chronic phase)</td>
</tr>
<tr>
<td>Anti-Thy-1 glomerulonephritis</td>
<td>l-NAME-treated animal</td>
</tr>
<tr>
<td>Anti-GBM glomerulonephritis</td>
<td>Remnant kidney with l-NAME</td>
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GBM, glomerular basement membrane; l-NAME, Nω-nitro-l-arginine methyl ester.

**Effect of estrogen, which preserves the microvascular endothelium, was also partly mediated by VEGF (31).**

The beneficial effect of VEGF might not be only due to endothelial protection. Cyclosporine (CsA)-induced arteriolopathy is also reversed by VEGF treatment (30). Since NO is thought to inhibit vascular smooth muscle cell (VSMC) proliferation and migration (75), VEGF-induced endothelial nitric oxide release could prevent the activation of VSMC, resulting in amelioration of arteriolar disease. This notion was supported by a clinical trial, in which patients with peripheral vascular disease undergoing angioplasty for femoral-popliteal disease were treated with antirestenosis gene therapy involving percutaneous arterial gene transfer of VEGF165 (24). In 14 patients treated with VEGF, no stenosis or only minimal neointimal hyperplasia occurred (75%); revascularization was required for restenosis in only 4 patients (22%) after 9 mo of follow up. These data suggest that VEGF may not only improve endothelial cell function but maintain vascular integrity.

Recently, Karumanchi’s group (42) has identified soluble VEGF receptor-1 (sFlt-1), which is an endogenous VEGF inhibitor, as the likely cause of preeclampsia. Preeclamptic placentas were found to overexpress sFlt-1, and blood levels were high and correlated with the severity of the disease. Most importantly, overexpression of sFlt-1 in normal or pregnant rats resulted in features of preeclampsia, including glomerular endotheliosis. Consistent with the hypothesis that a lack of VEGF may predispose to glomerular injury is the finding that selective knockout of VEGF in the podocyte results in a lesion that also has characteristics of glomerular endotheliosis (9). Furthermore, we have found that the overexpression of sFlt-1 in rats resulted in hypertension and renal disease, both of which were reversed by administration of VEGF121 (Ohashi R, Nakagawa T, and Karumanchi SA, unpublished observations).

VEGF is also considered to have protective effects on several renal diseases, such as acute rejection, acute glomerulonephritis, the early phase of remnant kidney, the uninephrectomized kidney, and acute ureteral obstruction (29, 41, 50, 64). Thus VEGF has great promise in the treatment of a wide variety of renal conditions.

**The “Dark Side” of VEGF in Kidney Disease**

In contrast to the reported benefit of VEGF, several animal models reported deleterious effects of VEGF in renal disease (Table 1). This is most evident in diabetes, in which VEGF is thought to play a role in the pathogenesis of diabetic retinopathy, nephropathy, and vascular disease (7, 12). Unlike models of chronic kidney disease in which VEGF levels are low, VEGF and VEGFR-2 levels are increased in the kidneys of patients with type 1 (6) and type 2 diabetes (68). The increase in VEGF was found to mediate glomerular and tubular hypertrophy, increase urinary albumin excretion and stimulate glomerular hyperfiltration in animal models of both type 1 and type 2 diabetes (7, 12). VEGF has also been found to be a critical mediator of diabetic retinopathy (23).

A second example is the angiotensin II (ANG II) infusion model. ANG II induces systemic hypertension with afferent arteriolar disease and tubulointerstitial injury (38). Interestingly, ANG II infusion increased plasma VEGF levels as well as local expression of VEGF and the VEGF receptor (Flt-1 and KDR) along with vascular inflammation in the aortic wall (77). The pathogenic role of VEGF was suggested by the finding that blockade of VEGF by sFlt-1 gene transfer could attenuate ANG II-induced inflammation and remodeling in the aortic wall even though systemic hypertension and cardiac hypertrophy were not inhibited. We have also found that the administration of VEGF121 did not provide significant benefit to rats which were pretreated with ANG II. Instead, the administration of VEGF tended to increase the afferent arteriolar medial hypertrophy (39).

A third example is the rat model treated with Nω-nitro-l-arginine methyl ester (l-NAME), which is an inhibitor of NO synthase (NOS). l-NAME induces vascular disease and systemic hypertension (76), which is associated with de novo vascular expression of VEGF. Indeed, we validated prior studies by others (15) that blocking of NOS with l-NAME worsened renal function in the remnant kidney model. In particular, renal arteriolar injury was severe in this model with marked increases in VSMC proliferation, excessive endothelial cell proliferation, and severe perivascular fibrosis (27). Interestingly, these injuries were associated with de novo expression of VEGF in VSMC. Another group also reported that coronary arterial disease induced by l-NAME was associated with increased VEGF expression (76). In this study, blocking of VEGF with sFlt-1 was able to inhibit the vascular injury along with reduction of MCP-1 and TGF-β expression. These data suggest that VEGF could have a deleterious effect on the vascular system when NO levels are severely reduced with l-NAME.

**Uncoupling of VEGF with Endothelial NO is a Common Pathway in These Models**

Normally, an elevation in VEGF expression should result in elevated endothelial NO levels, since VEGF increases both endothelial NOS (eNOS) expression and NO release from endothelial cells (78). However, in diabetes, despite high levels of VEGF, endothelial NO levels are low. There are several mechanisms to account for the low endothelial NO bioavailability. First, glucose can scavenge NO (2). Second, there is an impairment of eNOS activation (8). A third mechanism could be oxidative stress, which quenches NO to form peroxynitrite (5). Fourth, the formation of advanced glycation products in diabetes may also result in the consumption of endothelial NO (3). Fifth, both asymmetric dimethyl arginine and uric acid are commonly elevated in diabetes and can reduce endothelial NO bioavailability (33, 71). Finally, NO may bind to glycosylated deoxyhemoglobin (43).
We have also reported that high doses of ANG II in rats result in a dramatic reduction in eNOS expression with marked reduction in urinary nitrite excretion in the kidney (14, 38). Given that ANG II infusion upregulates VEGF expression in VSMC (11, 58, 77), renal microvascular injury could potentially relate to the low endothelial NO levels and high VEGF levels. A similar situation occurs with L-NAME treatment, in which increased vascular VEGF expression and low NO levels correlate with the development of vascular disease (27, 76). Thus all three conditions have a similar profile when it relates to VEGF and endothelial NO (Fig. 1). In other words, under conditions in which the endothelial NO system is intact, VEGF could engage the NO-dependent pathway to maintain vascular integrity. However, an impairment in endothelial NO bioavailability would result in the engagement of VEGF to an NO-independent pathway, with potentially deleterious consequences (Fig. 2).

In CsA nephropathy, eNOS activation has shown to be activated in the acute phase (1). This evidence could account for the acute protective effects of VEGF (28–30, 41, 50), which can theoretically engage eNOS. However, it has been shown that eNOS activation is reduced in the late phase (37, 40, 57). VEGF might induce more vascular disease in the late phase when eNOS activation is reduced.

Diabetic eNOS Knockout Mouse: A Model on Uncoupling of VEGF-Endothelial NO

Recently, we tested the hypothesis that VEGF-NO uncoupling might contribute to diabetic renal disease in endothelial NO gene knockout (eNOS KO) mice. We found that renal VEGF expression was increased in diabetic eNOS KO mice induced by streptozotocin, indicating that uncoupling of VEGF with eNOS occurs in this model. Interestingly, diabetic eNOS KO mice developed hypertension, proteinuria, renal insufficiency, and excessive mortality. More importantly, this mouse develops advanced diabetic nephropathy, including mesangiolysis (Fig. 3B), mesangial nodular lesions, and arteriolar hyalinosis, consistent with human diabetes (60, 67). The diabetic lesions were also associated with an increase in glomerular capillaries with endothelial cell proliferation as well as marked macrophage infiltration (Fig. 3F). Interestingly, extra small vessels in periglomerular areas were present in the diabetic eNOS KO mouse (Fig. 3D), similar to that reported in human diabetic nephropathy (44).

The uncoupling hypothesis requires a state of high VEGF and low endothelial NO for abnormal angiogenesis to occur. While blockade of NO synthesis both in vitro and in vivo causes some rebound elevation of VEGF (69), in the eNOS KO mouse the basal VEGF levels were not high within the kidney (47), and this likely explains the impaired angiogenesis that has been consistently observed in this mouse strain (35). However, hyperglycemia is a known stimulus of VEGF, and in the diabetic eNOS KO mouse the renal levels of VEGF were elevated (47). Consistent with our hypothesis, the lowering of blood glucose with insulin resulted in normalization of VEGF levels and a remarkable improvement in all features of diabetic nephropathy, including the excessive angiogenesis, the renal lesions (mesangial expansion, mesangiolysis and nodule formation), proteinuria, renal function, and blood pressure (47). These data confirm that disturbance of the VEGF-NO axis can markedly accelerate diabetic renal disease but also document an important relationship between this alteration and excessive endothelial cell proliferation and other aspects of diabetic renal disease. The observation of a second risk factor (VEGF-NO uncoupling) may help explain why only a minority of diabetic subjects develop renal and retinal disease.

Diabetic Nephropathy: Evidence of Excessive Angiogenesis Due to VEGF-NO Uncoupling

Abnormal angiogenesis plays a pathophysiological role in the development of diabetic retinopathy. In contrast, less is known about the contribution of enhanced angiogenesis in diabetic nephropathy. Given the uncoupling hypothesis, we hypothesized that abnormal angiogenesis in the diabetic kidney could occur.

There are a couple of reports demonstrating the presence of abnormal angiogenesis in diabetic nephropathy (Table 2). For example, new vessels are formed in the glomerulus, glomerular vascular pole region, and Bowman’s capsule in diabetic patients (44, 54). In the glomerulus of the diabetic rat, it has been topologically demonstrated that abnormal angiogenesis is associated with new capillary formation with dilatation, but not by capillary elongation (49). Min and Yamanaka (44) have elucidated the renal morphology from 94 patients with computer image analysis of the three-dimensional reconstructions
and found that most of these capillaries connect at a point after two or three branchings from the afferent arteriole, the opposite end of the vessels outside the glomerulus anastomoses to the peritubular capillary.

Interestingly, these abnormal vessels correlate with glomerular VEGF expression (26). The mechanisms by which VEGF may cause pathophysiological changes in these conditions are not absolutely clear. However, several possibilities exist. First, neovascularization at the glomerular vascular pole could provide a mechanism for bypassing renal autoregulation, leading to inappropriate transmission of systemic pressures into glomeruli. Inappropriate transmission may also occur with the development of preglomerular arteriolar disease and hyalinosis (25). This increased transmission of systemic pressure may predispose to glomerular hypertension with pressure-induced endothelial injury and mesangiolysis. Second, the high VEGF levels coupled with local endothelial NO deficiency can augment the prochemotactic effects of VEGF, leading to local macrophage infiltration (63) that might stimulate mesangiolysis (72) or mesangial matrix accumulation. Finally, the increase in total glomerular capillaries will acutely increase the total area for filtration and hence may facilitate the hyperfiltration observed with early diabetic disease (51). Interestingly, studies in animals with experimental diabetic nephropathy have demonstrated that over time glomerular capillary density falls as do VEGF levels (16), leading to the characteristic low VEGF state with reduced capillary numbers observed in other chronic renal diseases (29). Thus the abnormal VEGF-NO axis is likely to be more important in the development of diabetic nephropathy, but as renal disease...
progresses the same processes as observed in all chronic renal diseases may take over.

Additionally, acute mesangial injury in glomerulonephritis leads to mesangiolysis, mesangial proliferation, and matrix expansion, all of which may resolve spontaneously (20, 73). On the other hand, although these mesangial injuries are also characteristic of diabetic nephropathy, chronic glomerular injury is the consequence (45, 60, 67, 74). It is possible that this might be accounted for by reduced eNOS activity in diabetes. In fact, it has been shown that eNOS deficiency accelerates progression of anti-glomerular basement membrane antibody glomerulonephritis (19). Hence, mesangial function could be partially dependent on endothelial NO.

**Mechanism for Dysregulated Endothelial Cell Proliferation**

Many studies suggest that endothelial cell proliferation in response to VEGF is intricately associated with endothelial NO release. However, it is likely that this proliferative response and NO production are differently regulated by two types of VEGF receptors. Recently, we and other groups have reported that the binding of VEGF to the KDR receptor is responsible largely for the endothelial proliferative response (4, 48). On the other hand, NO production is regulated by the Flt-1 receptor (4, 48) while over time KDR stimulation could be involved in the increased expression of eNOS mRNA and protein (66). These receptors are functionally balanced to maintain endothelial integrity. In fact, Flt-1 deficiency increases endothelial cell proliferation.

**Table 2. Evidence of abnormal angiogenesis in diabetic nephropathy**

<table>
<thead>
<tr>
<th>Features</th>
<th>Location</th>
<th>Model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thin wall of capillary</td>
<td>Glomerulus</td>
<td>Human</td>
<td>54</td>
</tr>
<tr>
<td>Basement membrane in Bowman’s capsule</td>
<td>Basement membrane in Bowman’s capsule</td>
<td>Human</td>
<td>54</td>
</tr>
<tr>
<td>New vessel formation</td>
<td>Glomerular vascular pole</td>
<td>Human</td>
<td>26, 44, 51, 52, 53</td>
</tr>
<tr>
<td>Anastomosis to intraglomerular capillary and to peritubular capillary</td>
<td>Basement membrane in Bowman’s capsule</td>
<td>Human</td>
<td>44</td>
</tr>
<tr>
<td>Increase in capillary number with dilation</td>
<td>Glomerulus</td>
<td>STZ rat</td>
<td>49</td>
</tr>
<tr>
<td>Increase in endothelial number</td>
<td>Glomerulus</td>
<td>db/db mouse</td>
<td>18</td>
</tr>
<tr>
<td>Increase in endothelial number</td>
<td>Peritubular capillary</td>
<td>STZ rat</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C57BL6 mouse</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>eNOS KO mouse</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mouse</td>
<td>47</td>
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</table>

STZ, streptozotocin; eNOS KO, endothelial nitric oxide synthase knockout.

**Fig. 4. Western blotting for endothelial ICAM-1 expression in response to VEGF.** Blocking of NO with 1 mM N^\text{G}-nitro-L-arginine methyl ester (l-NAME) enhances ICAM-1 expression in response to 10 ng/ml VEGF in human aortic endothelial cell. 1, Control; 2 and 3, VEGF (10 ng/ml); 4 and 5, VEGF (10 ng/ml)+l-NAME (1 mM).

**Fig. 5. Overview of the VEGF-NO uncoupling hypothesis.** A: NO-dependent pathway (coupling of VEGF with endothelial NO). In endothelium, Flt-1 contributes to NO production, whereas endothelial cell proliferation is regulated by KDR. On the other hand, NO production is regulated by the Flt-1 receptor. Flt-1 deficiency increases endothelial cell proliferation while over time KDR stimulation could be involved in the increased expression of eNOS mRNA and protein. These receptors are functionally balanced to maintain endothelial integrity. In fact, Flt-1 deficiency increases endothelial cell proliferation.

B: NO-independent pathway (uncoupling of VEGF with endothelial NO). Endothelial NO bioavailability is reduced in certain physiological conditions, such as diabetes. In endothelium, once NO bioavailability is reduced, a compensatory increase in VEGF expression as well as a disruption of negative regulation in the vascular system in response to VEGF occur. As a consequence, VEGF engages KDR to enhance endothelial cell proliferation while Flt-1 on macrophages and VSMC can be activated to induce vascular injury.
number, resulting in an overcrowded and disorganized vasculature (13) while major vessels fail to develop in embryos lacking KDR (65).

Recently, we have presented in vitro evidence that uncoupling of VEGF with NO could induce excessive endothelial cell proliferation, in which NO inhibition (using L-NAME or high glucose) enhanced VEGF-mediated endothelial cell proliferation. On the other hand, exogenous NO inhibits endothelial cell proliferation in response to VEGF. Interestingly, this enhanced endothelial proliferation is accompanied by an increase in KDR mRNA expression and subsequent ERK1/2 activation. Compatibly, NO inhibition could further enhance VEGF-mediated ERK1/2 activation. Blocking either the KDR receptor or ERK1/2 was able to prevent the endothelial proliferative response. These data suggest that the negative regulatory effects of NO on endothelial cell proliferation may be mediated by activation of the KDR pathway. In contrast, NO administration inhibited this enhanced response. Furthermore, the NO release was regulated by Flt-1, rather than KDR in these cultured endothelial cells (4, 48). Taken together, the Flt-1-NO pathway is likely negatively regulating the KDR-ERK1/2 pathway to maintain endothelial integrity (48).

NO-Independent Pathway Activates VSMC and Macrophages in Renal and Vascular Injury

While VEGF is vasculoprotective, VEGF may induce inflammation by stimulating both macrophage migration and VSMC activation. In this process, NO could also play a critical role as a negative regulator of VEGF action (10, 32, 62). Besides endothelial cell proliferation, an inhibition or absence of endothelial NO could also contribute to activation of VSMC and immune cell infiltration in vascular injury. Indeed, eNOS deficiency results in enhanced leukocyte adhesion to endothelial cells along with P-selectin expression (36) and also exacerbates neointimal atherosclerosis with VSMC proliferation in a mouse model of carotid artery ligation (75). In the kidney, pharmacological or genetic blocking of endothelial NO was found to accelerate leukocyte and platelet infiltration into the glomerulus in experimental glomerulonephritis (19). In vitro studies using endothelial cells also demonstrate that NO inhibition induced MCP-1 expression (61). Interestingly, uncoupling of VEGF with NO could enhance this inflammatory response. Indeed, we also found that intercellular adhesion molecule-1 expression in response to VEGF was enhanced by blocking NOS in endothelial cells (Fig. 4). Thus endothelial NO may have a critical role in protecting the host from vascular injury.

Compatibly, we found that uncoupling of VEGF with eNOS in diabetic eNOS KO mice was associated with severe macrophage infiltration in the kidney (Fig. 3F). We hypothesized that the presence of endothelial NO may also retard the activation of macrophages by VEGF. Recently, we have demonstrated that exogenous NO inhibited macrophage migration in response to VEGF (63). Interestingly, VEGF upregulates Fli-1 expression, which is also blocked by exogenous NO in macrophages. The other important issue for cell migration might be stress fiber formation (59). In our experiment, we also found that VEGF induced F-actin reorganization in macrophages. This F-actin formation was mediated by Fli-1; therefore, exogenous NO also blocked F-actin formation (63). Thus NO could negatively regulate Fli1 expression, which could be a key mechanism for VEGF-induced macrophage migration.

Counterarguments

There are several counterarguments to consider. First, one could wonder why an infusion of VEGF should have beneficial effects in models of renal disease (28, 30, 34, 41) as many of these models are associated with some degree of endothelial dysfunction. However, there is supportive evidence that the degree of endothelial dysfunction is likely not severe in these models (28, 34, 56, 70) and that the VEGF-NO axis is largely intact. Second, the role of NO is complicated in VEGF-induced endothelial cell proliferation since NO positively or negatively regulates endothelial cell proliferation in response to VEGF (4, 55, 78). Gooch et al. (17) demonstrated that low concentrations of NO stimulated, whereas higher NO levels inhibited, endothelial cell proliferation. Therefore, the response may be dependent on the degree of eNOS activity. Third, there is also evidence that eNOS gene deficiency may result in impaired angiogenesis in response to tissue ischemia (46). However, it was also found that local expression of VEGF was not markedly enhanced even in ischemic tissue in this model. Therefore, complete uncoupling was unlikely to be present. Finally, since VEGF has a beneficial effect on tubulointerstitial fibrosis in the remnant kidney model, one might also wonder whether VEGF treatment is an option for treatment of tubulointerstitial fibrosis in a progressed diabetic nephropathy. However, we may need to be cautious to perform VEGF therapies in diabetic subjects, because eNOS activation may still be reduced in the late phase due to the effect of high glucose, oxidative stress, and advanced glycation end products. VEGF treatment might therefore be associated with uncoupling of VEGF with eNOS, which could further exacerbate monocyte infiltration and VSMC activation and cause abnormal endothelial proliferation.

Conclusions

In conclusion, endothelial NO likely plays a critical role in the maintenance of vascular integrity (Fig. 5A). However, once this negative regulation is lost due to reduced NO availability, then VEGF action may become excessive. In this situation, endothelial cell proliferation and induction of adhesion molecules occur through KDR, whereas VSMC activation and immune cell infiltration are mediated via Flt-1, leading to vascular injury (Fig. 5B). Therefore, endothelial NO could play a critical role under VEGF stimulation. The cooperation between these two VEGF receptors might be well balanced by endothelial NO to maintain vascular integrity.

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