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Review Article

# The c-Kit and Nitric Oxide Synergy During Arteriogenesis: a Systematic Review

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## Abstract

**Background:** Around 30% of critical limb ischemia (CLI) patients are not eligible for the current medical treatments. One of the benefits of vascular c-Kit signaling is to induce therapeutic arteriogenesis. The present review summarizes the effects of c-Kit signaling in endothelial nitric oxide synthase (eNOS)/ nitric oxide (NO) pathway during arteriogenesis in a hindlimb ischemia scenario.

**Methods:** A systematic review was performed following an electronic search of PubMed, Embase, ClinicalTrials.gov and Cochrane databases. Manuscripts published in the English language until January 2023 were identified and selected based on the vascular c-Kit and eNOS/NO signaling in neovascularization on both, animal models and cell culture assays.

**Results:** The importance of c-Kit in the vascular system has been shown under physiology and pathological condition. Known that Ischemia leads to hypoxic environment driving the neovascularization process through endothelial sprouting and new capillaries formation. c-Kit is up-regulated during this process and the majority of the studies demonstrate the angiogenic role of this receptor and its ligand in cancer. However, the role of c-Kit in ischemia diseases, specifically in arteriogenesis during hindlimb ischemia has been observed. Further, the effects of c-Kit in NO pathway that has also been reported, could explain, at least in part, the molecular mechanism involving c-Kit in arteriogenesis.

**Conclusion:** Recent evidence shown the beneficial role of vascular c-Kit in arteriogenesis and the impact of c-Kit deficiency in the dysfunction of NO signaling; however, the available scientific data on this topic is still scarce. Further studies to deeply investigate whether vascular c-Kit/eNOS/NO signaling play a major role in arteriogenesis are still needed to provide important new insight on the treatment of CLI.

**Keywords:** transmembrane receptor tyrosine kinase; niric oxide; arteriogenesis

## Introduction

Peripheral arterial disease (PAD), a common occlusive disease which affects around 8.5 million people in the United States(Allison et al., 2007), is among the leading causes of morbidity worldwide(Norgren et al., 2007). PAD is characterized by a narrowed or occluded artery, which leads to ischemia of the lower limb(Criqui & Aboyans, 2015), and eventual critical limb ischemia (CLI)(Rutherford et al., 1997). CLI is defined by ischemia pain rest, gangrene (tissue loss) and amputation, which indicates the dysfunction of the neovascularization process in maintaining minimal blood flow recovery (Stoyioglou & Jaff, 2004). Treatment options for PAD/CLI patients are usually limited to traditional surgery bypass, endovascular revascularization, or limb amputation(Norgren et al., 2007). However, bypasses and endovascular procedures are not recommended for patients with comorbidities, sepsis/limb gangrene, or inappropriate vascular anatomy, which represents a minimum of 30% of the patients with CLI(Norgren et al., 2007). Consequently, the comprehensive knowledge about neovascularization has led studies to develop new targets as a therapeutic approach to mitigate the deleterious effects of PAD/CLI(Heil et al., 2004).

Recent publications studied the molecular mechanisms of arteriogenesis in an attempt to develop therapeutic approaches for PAD/CLI(Grundmann et al., 2007; Schirmer et al., 2009; Schirmer & van Royen, 2014). c-Kit is a tyrosine kinase receptor, which has been extensively studied in the oncologic scenario as an angiogenic molecule(Stankov et al., 2014), and has been

shown to play a beneficial effect against hindlimb ischemia by improving arteriogenesis and blood flow recovery(Bosch-Marce et al., 2007). The c-Kit receptor is also involved in other pathological conditions such as atherosclerosis(Wang et al., 2007) and inflammation (Dentelli et al., 2007; König et al., 1997). For a long time it was even considered a marker of stem cells(Ashman, 1999) and was utilized in cardiac cell therapy strategies (Vagnozzi et al., 2019). However, recently, c-Kit has been identified as a marker of cardiac endothelial differentiation(Li et al., 2008; Liu et al., 2016) and its expression in vascular cells has also been confirmed(Hollenbeck et al., 2004; Matsui et al., 2004). Moreover, c-Kit deficiency has been shown to impair endothelial nitric oxide synthase (eNOS)/ nitric oxide (NO) function(Hernandez et al., 2019), one of arteriogenesis' essential signaling pathways. In this review we explore experimental advancements of the potential axis associated with vascular c-Kit signaling – eNOS/NO pathway appears to emerge as a potential therapeutic mechanism to improve arteriogenesis during hindlimb ischemia.

## Methods

### Eligible studies

A systematic search of the experimental evidence on the role of vascular c-Kit/eNOS/NO signaling in neovascularization was performed in PubMed, Embase, ClinicalTrials.gov and Cochrane databases were carried out by two independent investigators. References of related reviews were also added manually. The key terms used: "Ischemia" AND "c-Kit" AND "Nitric Oxide" AND "neovascularization" OR "arteriogenesis" OR "angiogenesis". Primary research studies included for comparison involve animal, cell culture, as well as human subjects. The last search update was performed on January 2023.

### Inclusion and exclusion criteria

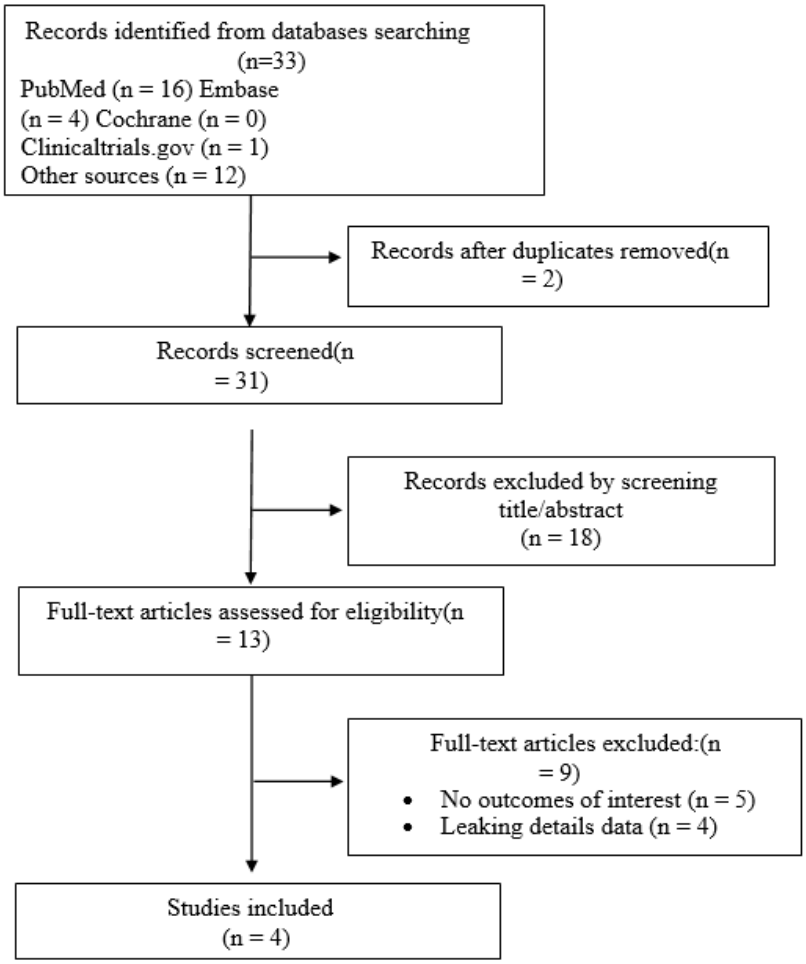
Studies were selected following inclusion criteria 1) evaluation of the c-Kit transmembrane association eNOS signaling in the neovascularization; 2) studies focusing on mechanism of animal model or cell culture or case-control study. Comments and editorial were used such as exclusion criteria. Important reviews involving c-Kit are discussed where applicable in order to keep the review complete, but not compiled in the data tables.

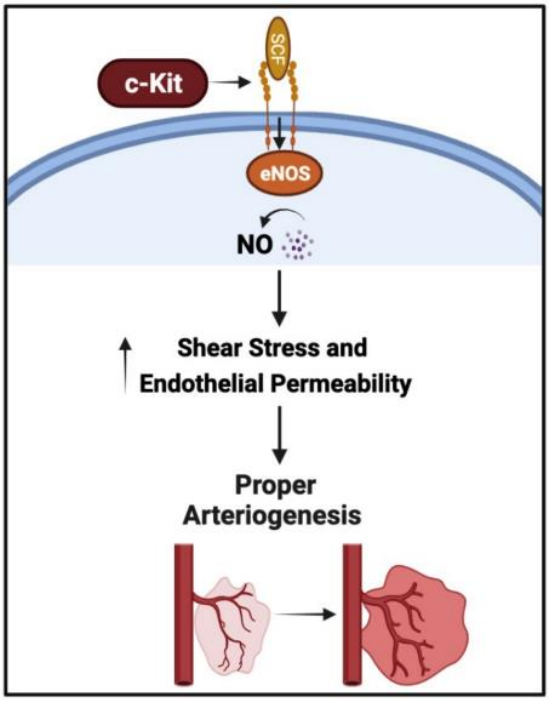
### Data extraction

Two independents investigators performed data extraction. We assessed study methodology, the role of c-Kit and eNOS/NO signaling pathway in arteriogenesis, and the relevance of the ischemic environment. Studies were screened based on 1) title, abstract fitting the applicable inclusion criteria. 2) a full-text analysis using same inclusion criteria. Duplicate articles and publishing not original article were excluded.

### Characteristics of studies

A total of 33 articles were screened from Pubmed [16], Embase [4], ClinicalTrials.gov [1], Cochrane [0], and other sources [12] (Figure 1). The characteristics of each study are shown in Table 1 and 2.






| Publication Information | Research Model | Intervention | Analysis | Regulation of Signaling Pathways | Signaling Pathway(s) Activated | Outcomes |
|---|---|---|---|---|---|---|
| Shao-Chih Chiu; *J Nutr Biochem.* (2014) | hEPCs | 1)Treated with EPA or DHA; at concentrations of 25, 50 and 125μM) in 10% FBS MCDB-131 for 8 h.<br>2) EPA or DHA at concentrations of 50μM in 10% FBS MCDB-131 for different time periods: 0, 0.5, 1, 2, 4 h | 1)Monoclonal antibodies against c-Kit, VE-cadherin, and actin.<br>2) Monoclonal antibodies against p-Akt, t-Akt, p-GSK3β, t-GSK3β,p-eNOS, t-eNOS, p-ERK 1/2 and t-ERK 1/ | ↑c-Kit; ↑PI3-K/Akt/eNOS.<br>↑↑ EPA treatment induced the phosphorylation of the Akt, eNOS, ERK 1/2and GSK-3β.<br>↑↑ EPA treatment phosphorylation of eNOS and GSK3βwas induced at latertime points (between 1 and 4 h) | up-regulation of the c-Kit protein as well as the phosphorylation of the ERK1/2, Akt and endothelial nitric oxide synthase signaling molecules in hEPCs. | Neovasculogenesis |
| Ji Yeon Kim; *Arterioscler Thromb Vasc Biol.* (2014) | HUVEC | Pretreated with phosphoinositide 3-kinase inhibitor (10 μmol/L) or Akt inhibitor (20 μmol/L) before stimulation with SCF (20 ng/mL) | 1)Nitrite production after stimulation with PBS, SCF, or VEGF.<br><br>2) HUVECs were transfected with eNOS-specific small interfering RNA (siRNA; si-eNOS) or control siRNA (si-Cont) | ↑Nitrite<br>↑ SCF-induced production of nitrite in HUVECs transfected with si-Cont, si-cKit → Permeability si-eNOS<br>↑Permeability si-Cont | SCF-induced cKit activation stimulated NO synthesis in ECs via phosphoinositide 3-kinase/Akt-dependent eNOS activation. | Permeability |
| hEPCs: human endothelial progenitor cell ; HUVEC: human umbilical vein endothelial cell ; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; Arrows indicate whether markers regulated by increased (↑) or decreased (↓) in expression and/or activation. | | | | | | |

**Table 1:** Characteristics of studies on regulation of signaling c-Kit pathways.

| Publication Information | Research Model | Regulation of Signaling Pathways | Insights | Outcomes |
|---|---|---|---|---|
| Diana R. Hernandez; *Biochem Biophys Res Commun.* (2019) | Mesenteric arteries and primary aortic SMCs from c-Kit deficient (Kit $^{W/W-v}$) and wild type ($Kit^{+/+}$) mice | ↓↓ sGCβ1<br>↓21.9% in SNP-induced vasorelaxation in $Kit^{W/W-v}$ | Endothelium-independent relaxation showed significant dysfunction of NO signaling in c-Kit deficient SMCs. | Vascular reactivity and remodeling |
| Lei L. Chen, *PLoS One (2017)* | HUVEC and GISTs | ↑ ET3 by SCF (HUVEC)<br>↑ WM793 melanoma cells express KIT | SCF-expressing cells communicate with neighboring KIT-expressing cells directly or indirectly.<br>SCF-KIT signaling induces timely local ET3 synthesis<br>NO diffuses into neighboring cells, thus acts in both SCF- and KIT-expressing cells | Endothelial function |

SMCs: smooth muscle cells; HUVEC: Human umbilical vein endothelial cell; GISTs: Gastrointestinal stromal tumors; sGCβ1: soluble guanylyl cyclase beta 1; ET3: endothelin-3; NO: nitric oxide; SNP: sodium nitroprusside; Arrows indicate whether markers regulated by increased (↑) or decreased (↓) in expression and/or activation.

**Table 2:** Literature on c-Kit signaling and nitric oxide interaction.

## Results

### c-Kit receptor

#### *Structure*

c-Kit is a transmembrane receptor tyrosine kinase which has essential properties as a regulator of growth, differentiation, migration, and proliferation in the hematopoietic cell(Lennartsson et al., 2005). Previous studies show that the major cognate ligand for KIT(145-KD transmembrane glycoprotein) is the stem cell factor (SCF)(Ashman, 1999), responsible for its activation and function, which causes receptor dimerization and autophosphorylation(Lennartsson et al., 2005).

Initially, the viral oncogene vc-Kit was identified, followed by sequencing and its homologue, c-Kit, in which mutations in the identified steel factor demonstrate that stem cell factor is the cognate ligand for KIT(Lennartsson et al., 2005). Structurally, c-Kit is a member of the platelet-derived growth factor (PDGF) family of kinases. It is composed of five immunoglobulin-like motifs in the extracellular portion, and of a 70-100 residue hydrophilic kinase insert domain, which forms its intracellular portion(Li et al., 2008). The intracellular part of c-Kit starts with the juxtamembrane region, which holds great importance for regulation of c-Kit kinase activity. The kinase domain consists of two subdomains, tyrosine kinase domain one and two, which are interrupted by a kinase insert sequence. As mentioned, c-Kit is expressed in cells from hematopoietic, germ cell and melanoblast lineages(M.-S. Kim et al., 2008), suggesting that its signaling is highlighted by the nervous system, placenta, heart, lung and kidney in the median of pregnancy(Lennartsson & Rönnstrand, 2012). c-Kit is also expressed in several other bone marrow populations and in the vascular bed(Hollenbeck et al., 2004). Its activation depends on either dimerization or oligomerization of monomeric receptor molecules(Lemmon & Schlessinger, 2010), which occur quickly, with dimers being detected only minutes after the addition of the ligand(V. C. Broudy et al., 1998). For a review on the structure, regulation, and splicing of the c-Kit protein and/or mRNA expression, see Reference(Lennartsson & Rönnstrand, 2012).

#### *c-Kit regulation, function, and its effect on vasculature*

The inappropriate expression or activation of c-Kit is associated with a variety of human diseases. Markers of progenitor cells, c-Kit positive cells, differentiate into blood and/or vascular endothelial cells, playing an important role in the amplification and mobilization of these specialized cells(Matsui et al., 2004). c-Kit participates in vital functions of the human body, including fertility, homeostasis, and melanogenesis; as expected, the global c-Kit knockout mice are not viable(Lennartsson & Rönnstrand, 2012; Waskow et al., 2002) c-Kit downstream signaling has been studied in several different systems. In one report, the c-PKC mediated the phosphorylation of the endogenous c-Kit receptor on serine 746, resulting in decreased overall tyrosine phosphorylation of c-Kit upon steel factor stimulation, showing that this specific feedback mechanism of c-PKC mediated phosphorylation of the c-Kit receptor has consequences for both proliferation and survival of HSC-like cell lines(Edling et al., 2007). This receptor also appeared to regulate a variety of vital cellular processes such as differentiation, proliferation survival, metabolism, motility, migration, and maturation of the cells(V. Broudy et al., 1994).

Regarding the vasculature, c-Kit and SCF are both present in vascular cells, including smooth muscle cells (SMCs)(Lennartsson & Rönnstrand, 2012) and endothelial cells (ECs)(Matsui et al., 2004). Additionally, it has been shown to play a critical role in hematopoiesis, cancer, atherosclerosis(Song et al., 2019) and angiogenesis. The relationship of c-Kit and vascular system is observed under normal physiology and pathological conditions. In cancer tumors, SCF/c-Kit signaling participates in the maintenance of the vasculature. Hypoxia-inducible factor (HIF)-1α is observed as a mediator in the expression of SCF in response to hypoxia and epidermal growth factor(Han et al., 2008). However, since the stimulation of c-Kit ligand is also known to stabilize and upregulate HIF-1α(Pedersen et al., 2008), a positive feedback loop is preferred in this situation, potentially contributing

to tumor angiogenesis even in the absence of hypoxia. The majority of tumor cells shown aberrant expression of c-Kit and/or Kit ligands(Turner et al., 1992), which when inhibited by c-Kit antibodies attenuates tumor growth by blocking angiogenesis(Fang et al., 2012).

In the heart, c-Kit positive cells expressing the lysine transferase Setd4, induced an increase in capillaries of neonatal and adult mice. The authors showed that Setd4 regulates the quiescence of c-Kit positive cells through the PI3K-Akt-mTOR signaling pathway via H4K20me3; leading to cardiac improvement after myocardium infarction through neovascularization(Xing et al., 2021). Although initial reports had treated c-Kit positive cells essentially as stem cells, especially in the heart where they can become myocardial, endothelial, or smooth muscle cells(Molkentin & Houser, 2013; Sultana et al., 2015), recent studies provide elegant approaches, suggesting that c-Kit positive cells in murine hearts are actually an important population of endothelial cells(Nadal-Ginard et al., 2014; Sultana et al., 2015; Van Berlo et al., 2014). The importance of c-Kit in neovascularization has also been analyzed by using heterozygous c-Kit mutant mice. When the bone marrow of c-Kit mutant mice is replaced with wild type cells it leads to an improvement of the infarcted myocardium due to the angiogenic cytokines environment(Fazel et al., 2006).

In vitro assays also revealed the substantial importance of SCF in human EPCs activity through the c-Kit receptor. Interestingly, the SCF-induced increase in neovascularization activity was substantially greater in EPCs compared to human umbilical vein endothelial cells (HUVECs). This high responsiveness of EPCs to SCF was explained by the finding that the cell-surface expression of c-Kit is greater in EPCs than in HUVECs(K. L. Kim et al., 2011). Altogether, the reported data confirmed the importance of SCF/c-Kit signaling to the neovascularization process, primarily during angiogenesis, but also during arteriogenesis in limb ischemia.

## Signaling pathway leading to eNOS/NO, c-Kit, and arteriogenesis

### *Limb Ischemia and Neovascularization*

Peripheral artery disease is often caused by accumulation of atherosclerotic plaques. Aging(Curb et al., 1996), smoking(Willigendael et al., 2005), hypercholesterolemia(KROON et al., 1995), diabetes(Gerald et al., 1992), hypertension(WINSOR, 1950), and chronic kidney disease(Liew et al., 2008) risk factors contribute to worsening of symptoms. CLI is secondary to severe stenosis/occlusion of arteries due to deficient compensatory angiogenesis and arteriogenesis. Arteriogenesis is the process of growth and enlargement of pre-existing collaterals that can function as natural bypasses. This process along with hypoxia mediated sprouting of new capillary networks (angiogenesis)(Carmeliet, 2000) ensure sufficient blood to the ischemic tissue after stenosis. Similar to the coronary reserve, effective arteriogenesis is essential to those human ischemic limbs that require a large volume of blood through pre-existing collaterals. Moreover, PAD patients who develop arteriogenesis efficiently are less likely to progress to CLI. Therefore, an effective therapy to enhance arteriogenesis would represent a much-needed alternative to protect the limbs of patients at risk of CLI and its devastating consequences.

### *The Role of Nitric Oxide Pathway in Arteriogenesis*

Arteriogenesis is a complex, multifactorial process that involves endothelial activation, inflammation and vascular cells proliferation. During the adaptive response to ischemia there is a decrease in blood pressure distal to the occlusion, leading to augmentation of shear stress inside the collateral arteries, now used as a new route for the blood to reach lower extremities(Pipp et al., 2004). The increased shear stress in the collaterals leads to an upregulation of adhesion molecules in the endothelium (such as I-CAM and V-CAM)(Scholz et al., 2000), which attract and activate leukocyte (specially monocytes(Heil et al., 2002) and lymphocytes(Stabile et al., 2003)), and their trans-endothelial migration. Together, the activated endothelium and the cytokines released by monocytes (such as TNF-α and VEGF) increase the chemotaxis of additional monocytes to the collateral site, resulting in proliferation of ECs and SMCs(Heil et al., 2006; Shizukuda et al., 1999). This process increases endothelial nitric oxide synthase (eNOS) which leads to greater production of NO-induced vasodilation. The vasodilation occurs once NO is diffused into vascular smooth muscle cells and initiates the NO/cGMP pathway. Activation of soluble guanylate cyclase generates cGMP, thereby activating protein kinase G. This process decreases cytoplasmic calcium, leading to smooth muscle cell relaxation(Denninger & Marletta, 1999).

NO is a major contributor to arteriogenesis during two distinct moments, after an arterial occlusion and after an increase in shear stress. Following an arterial occlusion, as an initial response to the ischemic environment, NO-mediated vasodilation occurs temporarily, leading to increased perfusion of collaterals. This perfusion creates an enhanced endothelial wall shear stress, which activates flow-sensitive potassium channels, which promotes the calcium influx that induces NO release(Cooke et al., 1991). In response to high shear stress, endothelial cells cultured under flow conditions show increased eNOS expression and NO release(Fisslthaler et al., 2000). Moreover, research has shown that the diameter of collaterals and blood perfusion in the ischemic limb is significantly reduced in eNOS−/− and L-NAME treated mice, subjected to femoral artery ligation(Park et al., 2010). Xuming Dai et al suggests that eNOS deficiency results in reduced density of native collaterals, remodeling and perfusion after ligation, presenting a greater ischemia area(Dai & Faber, 2010). Yu et al(Yu et al., 2005) and Lloyd et al(Lloyd et al., 2005) demonstrated reduced post-ischemic arteriogenesis as eNOS activity is blocked, concluding that eNOS is required for post-ischemic blood flow recovery and arteriogenesis in a mouse model of hindlimb ischemia. Similarly, inhibition of eNOS has been shown to inhibit various types of vascular remodeling, migration and proliferation of endothelial cells.

In the arteriogenesis process, other vasoactive substances (i.e. bradykinin and prostacyclin) and several growth factors such as PDGF, bFGF, TGF-β and VEGF are similarly stimulated by shear stress. While the VEGF receptor is one of the major tyrosine kinase receptors involved in arteriogenesis(Lloyd et al., 2005) other tyrosine receptors such as c-Kit also seems to be involved in this process.

### *Effects of c-Kit in Arteriogenesis*

Recent studies on hindlimb ischemia animal models have demonstrated the role of c-Kit in arteriogenesis. According to Bosch-Marce et al(Bosch-Marce et al., 2007), c-Kit loss-of-function decreases the expression of angiogenic cytokines, and impairs the blood flow recovery after hindlimb ischemia surgery. It is also reported that global c-Kit mutant mice have impaired blood flow recovery and arteriogenesis after limb ischemia(Hernandez et al., 2018). Moreover, the greater expression c-Kit after arterial injury in SMCs(Hollenbeck et al., 2004) and in the ischemic limb in comparison to non-ischemic limb after a femoral artery occlusion further suggests a major role of c-Kit in vascular injury and arteriogenesis. However, the mechanism by which c-Kit can improve arteriogenesis during hindlimb ischemia is still unknown. Due to involvement of c-Kit positive bone marrow cells in the arteriogenesis process, bone marrow transplantation using wild type cells in c-Kit global mutant mice were performed to test whether the impaired arteriogenesis of the mutant mice was caused by the loss of c-Kit in the bone marrow. Interestingly, the data suggested that the impaired arteriogenesis in the c-Kit mutant mice was actually associated with loss of vascular c-Kit. Evidence for this showed that the bone marrow transplantation did not interfere with the poor outcomes in blood flow recovery and collateral remodeling of c-Kit mutant mice. Furthermore, neither the greater ischemic tissue damage nor foot dysfunction observed in these mutant mice were alleviated by bone marrow transplantation. These data together indicated that vascular c-Kit signaling is a requisite for preventing tissue necrosis and proper arteriogenesis.

### *Effects of c-Kit in eNOS/NO Pathway*

It is known that several different mechanisms can generate nitric oxide, involving enzymatic and non-enzymatic pathways. The enzymatic production of NO by eNOS is one of the most studied and most relevant pathways associated with vasodilatation. However, the various ways to generate NO by other non-classical pathways, reflect the complexity of the activation and interaction process. Regarding vasculature, eNOS activity involves protein-protein interactions, post-translational regulations, and serine/threonine phosphorylation(Dudzinski & Michel, 2007). There are multiple extracellular stimuli that regulate eNOS function and control NO bioactivity in the vessel wall(Dudzinski & Michel, 2007). Although the activation of eNOS/NO pathway and the role of NO in arteriogenesis are already described in the literature, recent evidence suggests that c-Kit might affect this pathway and as well as improve arteriogenesis. These findings suggest that the beneficial effects of c-Kit in arteriogenesis could potentially occur through the eNOS/NO pathway.

A very interesting study from Kim et al.(J. Y. Kim et al., 2014) demonstrated that SCF binding to c-Kit in ECs results in the activation of multiple downstream signaling molecules including NO generation. c-Kit activation stimulated NO synthesis via phosphoinositide 3-kinase/Akt-dependent eNOS activation, inducing vascular permeability. In other words, SCF enhances endothelial permeability by increasing eNOS-mediated NO production. Moreover, the inhibition of eNOS using inhibitors or small interfering RNA decreased the capacity of SCF to increase endothelial permeability. Further, our group(Hernandez et al., 2019) using mesenteric arteries from c-Kit deficient (KitW/W-v) and littermate control (Kit+/+) mice, measured vascular reactivity by a pressure myography and demonstrated the dysfunction of NO-mediated relaxation in c-Kit deficient SMCs. We also observed higher blood pressure in c-Kit deficient mice (compared to control) when fed a high salt diet, which was associated with the c-Kit/sGC pathway that leads to impaired NO signaling in c-Kit deficiency. Another interesting study Chen et al.(Chen et al., 2017) demonstrated that SCF/c-Kit signaling in the endothelium led to local endothelin-3 (ET3) synthesis and secretion in ECs. After bind in its receptor, ET3 increases cellular Ca2+ leading eNOS/NO activation. The authors concluded that the c-Kit-ET3-NO axis leads to vasodilation maintaining vascular homeostasis(Chen et al., 2017). These data together suggest that c-Kit influences eNOS/NO-mediated vasodilation in a positive manner which could interfere with developmental remodeling of pre-existing collaterals.

## Perspectives and conclusion

This review summarizes the importance of eNOS/NO pathway and c-Kit for arteriogenesis during limb ischemia. While NO is a potent vasodilator activated by shear stress and responsible for increased blood flow through collaterals, loss of vascular c-Kit impairs blood flow recovery, collateral remodeling, and consequently induces tissue damage and foot dysfunction. Herein, we also discussed the few studies that reported the link between c-Kit and the eNOS/NO pathway, demonstrating the potential benefits of c-Kit in a functional eNOS/NO pathway.

In conclusion, we believe that, at least in part, the potential mechanism by which c-Kit influences a proper arteriogenesis is through the activation of eNOS/NO pathway, however, further investigations are required to specifically identify the entire mechanism and confirm that the effect of c-Kit in the NO pathway leads to greater arteriogenesis. Lastly, this axis c-Kit – eNOS/NO – arteriogenesis is largely unexplored, and could be a potential therapeutic opportunity to enhance compensation of major arterial occlusion and further prevent the progression to critical limb ischemia.

## Limitations

The present study gives an overview of mechanism available involving c-Kit and nitric oxide in neovascularization specially during limb ischemia. Some limitations should be reported since this article focus in the local effects of c-Kit in the vasculature without discussing the data that shows c-Kit as a marker of stem cells. Further, most of the literature that reports the effects of c-Kit in neovascularization is related to tumor angiogenesis, which was not the focus of this review; wherefore myocardial ischemia was considered here. The major limitation in the systematic review was the crossing of the MeSH terms “c-Kit in arteriogenesis”, and “c-Kit in the eNOS/NO pathway”. Therefore, the axis- c-Kit-eNOS/NO pathway - arteriogenesis is a novel concept in particular for clinical investigation. However, we believe that this is an important topic that needs further investigation.

## Conflict of Interest/Disclosure

The authors declare that they have no conflict of interest

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