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Endothelin receptor antagonists in sickle cell disease: a promising new therapeutic approach

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Abstract

Sickle cell disease (SCD) is a genetic hematologic disorder that is characterized by a variety of potentially life threatening acute and chronic complications. Currently, hydroxyurea is the only clinically approved pharmacological therapy for the treatment of SCD, and the continued prevalence of severe disease complications underscores the desperate need for the development of new therapeutic agents. Central features of the sickle cell disease milieu, including hypoxia, oxidative stress, and thrombosis, are established enhancers of endothelin-1 (ET-1) synthesis. This conceptual connection between ET-1 and SCD was confirmed by multiple studies that demonstrated markedly elevated plasma and urinary levels of ET-1 in SCD patients. Direct evidence for the involvement of ET-1 signaling in the development of SCD pathologies has come from studies using endothelin receptor antagonists in SCD mice. This review summarizes recent studies that have implicated ET-1 signaling as a mechanistic contributor to renal, vascular, pulmonary, and nociceptive complications of sickle cell disease and discusses the potential for the use of ET receptor antagonists in the treatment of SCD.

Keywords

Endothelin-1; sickle cell disease; nephropathy; pain; pulmonary hypertension

Introduction

Sickle cell disease (SCD) is a common genetic hematologic disorder that affects millions of people worldwide[1]. The medical management of SCD has led to a steady improvement in the lifespan of patients throughout the past fifty years, and as a result, a variety of chronic complications have emerged as major medical issues in SCD[2,3]. The only clinically approved disease-specific drug for the treatment of SCD is hydroxyurea, which is thought to primarily function to induce expression of fetal hemoglobin, ultimately leading to a

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reduction in red blood cell sickling[3,4]. This treatment strategy has been demonstrated to produce a significant reduction in disease severity[5,6]. Despite this, patients with SCD continue to suffer from chronic end-organ damage as well as painful, and potentially lethal, acute exacerbations of the disease, known as vaso-occlusive crises (VOC). These acute and chronic disease manifestations continue to affect SCD patients due to the lack of pharmacological therapies targeted to mechanisms underlying their etiology. Thus, there is clearly a large unmet therapeutic need in SCD.

Endothelin-1 (ET-1) is a 21 amino acid peptide produced by a variety of cell types throughout the body and acts by binding to two distinct receptor subtypes, ET_A and ET_B[7]. ET-1 was originally identified as a sub-nanomolar potent vasoconstrictor[8], and it is now additionally known to be a pro-inflammatory[9], mitogenic[10], natriuretic[11], and nociceptive mediator[12]. These often detrimental actions of ET-1 are mediated primarily by activation of the ET_A receptor, and in some tissues can be counter-balanced by activation of the ET_B receptor[13]. The cellular synthesis of ET-1 has been demonstrated to be increased in the setting of hypoxia[14], oxidative stress[15], decreased nitric oxide bioavailability[16], and thrombosis[17], all of which are well-established features of the SCD milieu[18-21]. The first evidence supporting a connection between ET-1 and SCD came from a study demonstrating that exposure of endothelial cells to sickle erythrocytes increases their synthesis of ET-1[22]. This discovery led to multiple published reports that documented markedly increased plasma and urinary levels of ET-1 in SCD patients[23-28]. Notably, plasma ET-1 levels were shown to be elevated to a significantly greater extent in SCD patients experiencing VOC. Together, these data clearly indicate that elevated ET-1 levels are a biomarker of the SCD process but do not directly implicate ET-1 signaling as a contributing factor to the pathophysiology of SCD.

Further evidence to support a connection between ET-1 and SCD comes from two gene polymorphism studies in SCD patients. The first study demonstrated that an ET-1 gene polymorphism is associated with risk of VOC in SCD patients[29], and the second demonstrated an association of another ET-1 gene polymorphism with SCD when compared to controls in an African cohort[30]. Together, these studies implicate ET-1 as an important disease-modifying gene in SCD, and suggest that differential ET-1 signaling can modulate SCD severity.

The first direct evidence to suggest that ET-1 plays a mechanistic role in SCD pathology came from a published report in which SCD mice were treated with the dual ET_A/ET_B receptor antagonist, bosentan[31]. This study demonstrated that bosentan had protective effects in both kidneys and lungs by preventing both vascular congestion and inflammation following VOC, and additionally, demonstrated a complete protection from VOC-mediated mortality in SCD mice. Of note, the magnitude of the effects observed by Sabaa et al. suggested that ET-1 signaling is a major contributory mechanism to central features of SCD pathophysiology.

Collectively, these studies established the basis for investigation of mechanisms by which ET-1 contributes to SCD pathophysiology, and a growing number of labs are working in this area[32-34]. Importantly, ET-1 is an established mechanistic mediator of chronic kidney

disease, vascular dysfunction, pulmonary hypertension, and chronic pain, all of which occur in SCD. Based on these associations, the National Institutes of Health's National Heart Lung and Blood Institute has established a center to investigate the therapeutic potential for endothelin receptor antagonists in SCD by funding an Excellence in Hemaglobinopathies Research Award (NIH Grant U01HL117684). The current review focuses on evidence implicating ET-1 as a major player in the pathogenesis, treatment, and prevention of sickle nephropathy, as well as in the pain hypersensitivity and pulmonary complication of SCD (Figure 1).

Therapeutic potential of endothelin antagonists in sickle cell nephropathy

Sickle cell nephropathy (SN) represents one of the most serious complications of SCD, accounting for up to 18% of mortality in patients[35,36]. SN is characterized by structural and functional abnormalities in the kidney, and these abnormalities vary in incidence and onset. An early-onset urine concentration defect occurs in nearly all SCD patients[37]. This disease manifestation is reversible with blood transfusion therapy in the first decade of life but irreversible later, due to rarefication of the capillary network and loss of tubule segments that establish the hyperosmotic environment within the renal medulla[38-40]. Hematuria and progressive glomerulopathy occurs in a large subset of patients[41]. Glomerulopathy in SN leads to subsequent proteinuria and reduced GFR, which are risk factors for increased mortality among patients with SCD[42]. Although the pathophysiology of SN remains to be fully elucidated, chronic hemolysis-related endothelial dysfunction is currently considered as one of the key mechanistic factors contributing to the disease process[43,44].

In the renal medulla, ET-1 is an established mediator of natriuresis and diuresis by signaling to ET_B receptors in the collecting duct, with subsequent action on epithelial sodium channels and aquaporins[45-47]. Additionally, ET-1 can regulate medullary vascular tone by acting on microvascular ET_A receptors[48]. The microenvironment of the renal medulla is characterized by an oxygen tension that is among the lowest of any tissue in the body, and this oxygen tension is known to be decreased further in a variety of disease states[49]. Hypoxia increases ET-1 expression within the renal medulla, and in SCD, hypoxia contributes to red blood cell sickling and vascular congestion in the vasa recta[50]. Thus, in SCD, hypoxia-induced ET-1 production in the renal medulla may exacerbate vascular congestion, leading to a further reduction in interstitial osmolality and a resultant impairment in urinary concentrating ability. Results from our laboratory support this hypothesis. We generated chimeric endothelial-specific ET-1 knockout mice that express human hemoglobin S via bone marrow transplantation with marrow derived from humanized SCD mice. Humanized SCD mice have mouse globin genes knocked out and targeted insertion of human globin genes at the same loci, resulting in mice expressing human hemoglobin S[51]. Using our chimeric endothelial-specific ET-1 KO SCD mice, we demonstrated preserved urine-concentrating ability, indicating that endothelial-derived ET-1 is a major mediator of renal medullary dysfunction SCD mice[52]. The mechanism of the effect in this genetic system may be through preservation of medullary blood flow, which ultimately could prevent damage in the renal medulla. ET receptor antagonists are known to exert pro-angiogenic effects, which could limit capillary rarefication in the renal medulla, and prevent the urine concentrating

defect via another mechanism[53]. Thus, future studies are needed to determine if ET-1 receptor blockade may display a similar therapeutic effect in SCD.

Urinary ET-1 excretion is an index of renal ET-1 production, and in SCD patients, elevated urinary ET-1 excretion correlates with microalbuminuria[28]. This observation suggests a pathophysiological link between increased renal ET-1 production and impairment of glomerular function in SCD. In addition, abundant evidence for endothelial dysfunction in SCD[43,44] suggests that dysfunction in the glomerular endothelium may promote ET-1 release from the glomerular endothelial cells, thereby causing glomerular damage and albuminuria. Previous studies in our laboratory showed that hypoxia specifically increases glomerular ET-1 expression in C57BL6J mice, but not in endothelial-specific ET-1 knockout mice[54], suggesting a potential mechanism for ET-1-induced nephropathy in SCD. However, despite many studies implicating ET-1 in numerous glomerulopathies, the mechanism by which elevated ET-1 production contributes to glomerular dysfunction in SN remains unknown[55-57]. Sabaa et al.[31] first demonstrated the potential importance of ET receptor antagonism as a preventive or therapeutic approach in SN. Using doppler ultrasound to study the renal hemodynamics of bosentan treated SCD mice, investigators demonstrated a substantial decrease in vascular resistance during hypoxia/reoxygenation-induced VOC, renal microvascular congestion, and systemic inflammation[31]. More recently, chronic bosentan therapy was shown to attenuate both glomerulomegaly and glomerulosclerosis, as well as prevent glomerular lesions in SCD mice[58]. Results from our laboratory support these observations. We have recently demonstrated a beneficial effect of the ET_A receptor antagonism, using ambrisentan, on glomerular injury in humanized SCD mice. Interestingly, our results showed that long-term treatment with ambrisentan fully prevented both elevated glomerular permeability to albumin as well as albuminuria and proteinuria in humanized SCD mice[59]. Moreover, as reactive oxygen species are implicated in a long-term pathology of SCD[60], we have shown that chronic administration of ET-1 increases glomerular reactive oxygen species production through the ET_A receptor[54]. This effect was prevented by ET_A receptor blockade in SCD mice[61], providing a confirmation of ET_A-dependent reactive oxygen species mediated glomerular injury in SCD. These results suggest that ET-1, via ET_A receptor activation, leads to glomerular injury, and suggest that chronic ET_A antagonism should be considered as a prospective therapy to prevent the onset of SN in SCD patients.

Together, these studies suggest a critical role of ET-1 in initiating and/or mediating renal injury in SCD and highlight the potential for chronic ET receptor antagonism as a pharmacological intervention to prevent the development and delay progression of renal involvement in SCD.

Therapeutic potential of endothelin antagonists in chronic pain management in SCD

Pain is a chronic complication of SCD that is acutely exacerbated during VOC, and associated with inflammation and tissue injury[62,63]. Analogously, ET-1 is chronically elevated in the plasma of SCD patients at baseline, further elevated during the course of painful VOC, and significantly reduced at asymptomatic follow-up[25]. In children with SCD, both plasma ET-1 level and the ratio of plasma ET-1[64] to the peptide vasodilator apelin[65]

have been demonstrated to be positively correlated with baseline pain rating. In a mouse model of SCD, dorsal root ganglia, where cell bodies of sensory neurons are located, were found to have elevated ET-1 expression at the mRNA level[66]. The involvement of ET-1 in the elicitation of pain in peripheral locations has been well documented by administration of exogenous ET-1 in both humans and animal models of nociception[67-71]. Moreover, several studies have demonstrated the ability of ET receptor antagonists to alleviate clinical pain in patients with systemic sclerosis and Raynaud's phenomenon, two conditions in which disease manifestations share some similarity with SCD[72-74]. Nevertheless, two other studies have demonstrated negative results in these conditions[75,76]. Painful skin ulcers, which are seen in systemic sclerosis and have been shown to respond to ET antagonism, also occur in SCD[73]. A case report documented completed remission of ulcers in a SCD patient treated with bosentan, an effect that may be related to the pro-angiogenic effect of ET receptor antagonism[77]. Taken together the current body of evidence establish a strong foundation for the hypothesis that ET-1 signaling contributes to hyperalgesia in SCD.

The mechanisms underlying a potential role of ET-1 signaling in the pathogenesis of pain in SCD remain unknown. This understanding will be particularly important when considering translating preclinical findings to humans, as both ET_A and dual ET_A/ET_B receptor antagonists are available for clinical use. In animal models of nociception, peripheral ET_A receptors can be generally considered to mediate hyperalgesia, suggesting that ET_A antagonism may be the appropriate approach in SCD[78]. However, the role of peripheral ET_B receptors is less clear, as ET_B receptors have been demonstrated to have pro-algesic[79], neutral[80], and analgesic effects[81] depending on experimental conditions. Therefore, the use of dual ET_A/ET_B receptor antagonists in the treatment of pain in SCD could produce additional benefit over ET_A antagonism alone or, alternatively, may decrease or eliminate the analgesic effect of ET_A antagonism. A recent comprehensive review has discussed mechanisms by which ET-1 signaling induces nociception[78]. Thus, this review will focus on new findings that may be mechanistically related to a role of ET-1 signaling in hyperalgesia in SCD patients.

Recently it has been shown that transient receptor potential vanilloid 1 (TRPV1) channels mediate mechanical sensitization of nociceptor terminals, resulting in mechanical allodynia in SCD mice[82] Inflammatory mediators, including ET-1, released following VOC events can maintain the persistent sensitization of TRPV1 channels in SCD[83,84] Moreover, other studies showed that subcutaneous administration of ET-1, via direct sensitization of TRPV1, results in prolonged mechanical allodynia[85] Interestingly, ET_A receptors are co-localized with TRPV1 channels in HEK293 cells, and ET-1 leads to potentiation of TRPV1 activity via ET_A receptors[84] Furthermore, studies performed by Yamamoto et al. also highlight the importance of ET-1 in peripheral nociceptive signaling associated with pain. Investigators demonstrated that in sensory neurons, ET-1 induces Ca^{2+}-dependent activation of protein kinase Cε, which is well known to phosphorylate TRPV1 channels[86,87]. These studies establish the basis for the hypothesis that in SCD, elevated ET-1 production results in excessive ET_A receptor activation on sensory neurons, resulting in potentiation of TRPV1 activity, and ultimately pain hypersensitivity. Future studies of this hypothesis may provide evidence for the use of ET receptor antagonists as an analgesic therapy in SCD. Importantly,

ET receptor antagonists may provide a non-habit-forming alternative to opioid analgesics that are currently widely used in the treatment of pain in SCD.

Therapeutic potential of endothelin antagonists in pulmonary complications in SCD

Pulmonary hypertension is a serious SCD complication that occurs in a subset of SCD patients and is a risk factor for mortality in SCD[88]. ET-1 is a well-established mediator of pulmonary hypertension, and ET receptor antagonists represent an approved therapy for this disease[33,89]. Based on the success of ET receptor antagonism in treating pulmonary hypertension arising from other etiologies, a clinical trial was initiated to investigate the therapeutic efficacy of bosentan to treat pulmonary hypertension in SCD[90]. Unfortunately, this clinical trial was prematurely discontinued because of the small sample size. However, bosentan was well tolerated in this small SCD cohort. Moreover, a study in SCD mice demonstrated that bosentan decreased lung injury scores following hypoxia/normoxia[31]. These studies suggest potential therapeutic benefit of ET receptor antagonists in pulmonary complications in SCD.

The current knowledge about the ET receptor subtypes and their functions raises the question: Should patients with SCD be treated with a selective or combined ET receptor antagonist? There have been many studies investigating the efficacy of ET receptor antagonists in pulmonary hypertension, but there have been only limited studies to investigate the advantage of targeting specific ET receptors to treat chronic lung disease in patients with SCD[33]. Pulmonary ET_B receptors clear approximately 40% of circulating ET-1 in patients with pulmonary hypertension[91], as well as inhibit ET converting enzyme-1[92]. Therefore, ET_B receptor antagonism could have detrimental effects. Knowledge about general ET_A and ET_B receptor functions provides a basis for the idea that selective ET_A receptor antagonism could represent a superior strategy the treatment of pulmonary complications in SCD by allowing ET_B mediated anti-inflammatory and nitric oxide-producing effects. Furthermore, a recent report supports this argument by demonstrating that ET_A receptor blockade protects against pneumolysin-induced barrier dysfunction in SCD[93], thus linking ET-1 with lung injury pathways in SCD through the activation of ET_A receptors. However, it also has been reported that in the setting of prolonged elevated ET-1 expression in conditions of reduced nitric oxide bioavailability, ET_B receptor signaling may contribute to additional reactive oxygen and nitrogen species, potentially adding to the effect of nitric oxide dysregulation in SCD[94,95]. Moreover, changes in the expression and signaling role of ET receptors in condition such as pulmonary hypertension in SCD may deviate significantly from physiological conditions. ET_B receptor expression is known to be upregulated in pulmonary hypertension[96]. Further, the recent concept of ET receptors heterodimerisation[97] suggests that the ET_B receptors may adopt the function of the ET_A receptors[98] and may provide justification for use of dual ET receptor antagonist in treating pulmonary hypertension in SCD. Additionally, ET_B receptor mediated activation of Gardos channels on red blood cells stimulates dehydration, which can contribute to sickling of hemoglobin S[99,100] and potentially lead to VOC events and progression of the disease. Further investigations are warranted to resolve the question of the optimal ET receptor antagonism strategy to prevent pulmonary injury in SCD.

Conclusion

The studies herein reviewed have provided direct evidence that ET-1 is a mechanistic mediator of diverse complications that occur in distinct organ systems in SCD. Together, they suggest that elevated ET-1 signaling may be a central mechanism linking many salient features of SCD pathophysiology. This concept provides great potential for conducting more detailed studies on the clinical benefit of ET receptor antagonism in both the management of acute exacerbations and chronic complication in SCD patients. This is particularly critical. Additionally, as elevated ET-1 expression has been demonstrated across organ systems in SCD, endothelin converting enzyme inhibition could also be explored in the treatment of SCD. New therapies are desperately needed for the treatment of SCD, where an ideal therapeutic agent would target an upstream mechanism of organ dysfunction in order to provide maximal benefit. Currently, a pilot trial of the ET_A receptor antagonist, ambrisentan, is underway in a cohort of SCD patients with evidence of renal complications (NIH Grant U01HL117684). This study will determine the safety and tolerability of ET_A antagonism is SCD patients, and may provide evidence on the efficacy of this intervention to reduce SCD-mediated renal dysfunction. Future studies are needed to investigate the mechanism(s) underlying the involvement of ET-1 signaling in the herein described SCD complications and to determine if ET-1 plays a role in other SCD complications, such as stroke and skin ulceration.

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Figure 1.

Factors known to cause elevated ET-1 expression are salient features of the SCD milieu, and ET-1 is an established mediator of multiple organ-specific pathologies that occur in SCD.