REVIEW ARTICLE

Nitric Oxide in β-Thalassemia

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Abstract

Nitric oxide (NO) has multiple physiologic functions and its decrease or increase is associated with pathophysiology of diseases. NO is produced by nitric oxide synthase (NOS)-dependent and NOS-independent pathways. The NO concentrations and rate of production from different pathways are determinants of its biological functions. NO at low levels produced by constitutive NOS maintains adequate blood flow and inhibits platelets in normal situation, while NO at high levels produced by inducible NOS plays role in pathophysiologic process. In NOS-independent pathway, NO is produced from nitrite by the nitrite reductase activity of deoxyhemoglobin. In thalassemia, iron overload and oxidative stress lead to endothelial dysfunction and decreased NO, which are associated with platelet hyperactivity and pulmonary hypertension. Here, the preclinical and clinical studies of NO-related and nitrite therapy in β-thalassemia are reviewed.

Keywords: Nitric oxide, thalassemia, nitrite

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ไนตริกออกไซด์ในโรคเบต้าธาลัสซีเมีย

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บทคัดย่อ

ไนตริกออกไซด์มีหน้าที่หลายอย่างในทางสรีรวิทยา การลดหรือเพิ่มของไนตริกออกไซด์เกี่ยวข้องกับสรีรพยาธิวิทยาของโรคต่าง ๆ ไนตริกออกไซด์ถูกสังเคราะห์จากวิถีที่อาศัยเอนไซม์ nitric oxide synthase (NOS) และวิถีที่ไม่อาศัย NOS ความเข้มข้นและอัตราการสังเคราะห์ไนตริกออกไซด์เป็นปัจจัยที่กำหนดหน้าที่ในทางชีววิทยา ไนตริกออกไซด์ที่ความเข้มข้นต่ำซึ่งถูกสังเคราะห์จาก NOS ที่ทำงานต่อเนื่องทำให้คงการไหลของเลือดและยับยั้งเกล็ดเลือดในภาวะปกติ ในขณะที่ไนตริกไซด์ที่ความเข้มข้นสูงถูกสังเคราะห์จากเอนไซม์ inducible NOS มีบทบาทในกระบวนการพยาธิสรีรวิทยา ในวิถีที่ไม่อาศัย NOS ไนตริกออกไซด์ถูกผลิตจากไนไตรท์โดยการทำงานของ nitrite reductase ของฮีโมโกลบินที่ปราศจากออกซิเจน ในโรคธาลัสซีเมีย ภาวะเหล็กเกินและ oxidative stress ทำให้เกิดความผิดปกติในการทำงานของเซลล์เยื่อบุหลอดเลือดและลดไนตริกออกไซด์ ซึ่งมีความเกี่ยวข้องกับภาวะเกล็ดเลือดไวเกินและความดันของหลอดเลือดสูงในปอด บทความนี้เป็นการทบทวนการศึกษาที่เกี่ยวข้องกับไนตริกออกไซด์และไนไตรท์ในการรักษาโรคธาลัสซีเมีย

คำสำคัญ: ไนตริกออกไซด์, โรคธาลัสซีเมีย, ไนไตรท์

Nitric oxide

Nitric oxide (NO) is a gaseous molecule previously thought to be an air pollutant as a product of fossil fuel combustion. The discovery of endothelial derived relaxing factor in 1980 has discovered the physiologic roles of NO. From many decades of NO study, NO is acknowledged as a key essential molecule in physiology and pathophysiology of many human organ systems. Because of its short half-life, NO reaches and activates targets in paracrine manner. More recently, there are increasing evidences of endocrine activity of NO. NO is converted to more stable forms such as nitrite anion (NO_2^-) and nitrosated proteins which are transported in blood to distant targets. At targets, nitrite can be reduced to NO under acidotic hypoxic conditions through a reaction catalyzed by reductase activity of deoxygenated heme proteins, including deoxyhemoglobin.[1]

Synthesis of nitric oxide

Two major types of NO biosynthesis are NOS-dependent pathway and NOS-independent pathway. NOS enzymes are family of enzymes catalyzing the production of NO from L-arginine. There are 3 isoforms of NOS: neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2) and endothelial NOS (eNOS or NOS3). nNOS and eNOS are constitutive NOS (cNOS) enzymes which produce NO continuously at low physiologic concentrations to maintain vascular homeostasis. In NOS-independent pathway, NO is produced from nitrite[2] and through the entero-salivary nitrate-nitrite-NO pathway.[3] Regarding the bioactivation of nitrite to NO, nitrite is reduced to NO by many heme-containing proteins and molybdenum-containing proteins under hypoxia (Table 1).

For example, deoxyhemoglobin in RBC can reduce nitrite to NO under hypoxia, contributing to hypoxic vasodilation phenomenon and platelet inhibition.[1,4] In addition, xanthine oxidase in hypoxic tissues is also able to convert nitrite to NO. During ischemia, NO inhibits cellular respiration in mitochondria.[5,6] The latter suggests the protective effect of NO and nitrite in ischemic-reperfusion injury. In the enterosalivary nitrate-nitrite-NO pathway, nitrate in blood is secreted into saliva.[7,8] Nitrate in saliva is reduced to nitrite by nitrate reductase of oral commensal bacteria located in deep crypts of the posterior part of tongue. Thereafter, salivary nitrite is swallowed and absorbed at duodenum into blood.

Biological functions of nitric oxide

NO regulates many physiological processes; for example, the cardiovascular homeostasis, neurotransmission, cell proliferation, immune response[9], and apoptosis[10] (Table 2). NO is constitutively produced by eNOS and nNOS. NO from nNOS is involved in learning, memory and neurogenesis. NO synthesized in the central and peripheral nervous system by nNOS acts as a neurotransmitter that stimulates guanylyl cyclase pathway and results in vascular smooth muscle relaxation.[11,12] NO produced by eNOS in endothelial cells maintains adequate tissue perfusion by induction of vasorelaxation, inhibition of platelet aggregation, and inhibition of neutrophil and platelet adhesion to endothelial cells. On the other hand, iNOS is an inducible enzyme found primarily in inflammatory cells. The expression of iNOS is induced by bacterial lipopolysaccharide, cytokines and many agents.

In contrast to low nanomolar concentrations of NO produced by eNOS and nNOS, NO is produced by iNOS at high concentrations and rapid rate, leading to cytotoxicity to microorganism and tumor cells.

Table 1. Nitrite conversion to NO by proteins and enzymes.

Proteins	Cellular or tissue location	Reaction rate constant ($M^{-1}.s^{-1}$)	Optimal condition(s)
Heme proteins			
Deoxyhemoglobin[13-15]	Red blood cells	1.23×10^{4}	Hypoxia
Deoxymyoglobin[16]	Skeletal and cardiac muscles	12.4	Hypoxia
Deoxyneuroglobin[17]	Neurons of both the peripheral and central nervous system	0.12	Hypoxia
Cytochrome c oxidase[18]	Intermembrane space of mitochondria in many tissues	NA	Hypoxia, pH ≤ 6
eNOS[19-21]	Endothelial cells	NA	Hypoxia
Molybdenum-containing proteins			
Xanthine oxidoreductase[22]	Cytosolic enzyme in many tissues	22.9×10^{-3}	Hypoxia, pH ≤ 6
Aldehyde oxidase[23]	Cytosolic enzyme in many tissues	2.7×10^{-3}	Hypoxia, pH = 6.0
Mitochondrial amidoxime-reducing component (mARC)[24]	Mitochondria in many tissues	mARC-1: 0.6 mARC-2: 0.2	Hypoxia, pH = 6.4

NA = No available data

Table 2. Signaling and functions of NO produced from different sources.

Sources	NO or NO_x levels	signaling	Function and effects
NOS-dependent			
nNOS	Low levels, 12 nM/μg protein in supernatant of central catechol-aminergic neuronal cell line[25]	sGC-cGMP-PKG	• Long term regulation of synaptic transmission • Involved in memory function[26] • Central regulation of blood pressure • Regulation of smooth muscles, including blood vessel, gastrointestinal tract[27], and penile corpus cavernosum[24,28]
iNOS	High levels, 40 μM nitrite in tumor cell lysis[29], 1.45 μg/mL nitrate in squamous cell carcinoma tissue[30]	Peroxynitrite production[31]	• Cytostatic and cytotoxic to cells including parasitic microorganism[32] and tumor cells[29] • Cause DNA strand breaks and fragmentation[33] • Inhibition of DNA repair in tumor cells[34]
eNOS	Low levels, 50-300 nM nitrite in blood	sGC-cGMP-PKG	• Vasodilation[35,36] • Inhibition of platelet aggregation and adhesion • Inhibition of leucocyte adhesion and vascular inflammation[37] • Inhibition of smooth vascular muscle proliferation[38,39] • Promotion of angiogenesis[40]
NOS-independent			
Nitrite reduction to NO		sGC-cGMP-PKG	Hemoglobin-derived NO • A major pool of NO, responsible for hypoxic vasodilation[1] • Prevention of hypertension[41] Myoglobin-derived NO • Downregulation of cardiac energy status[42] • Protection against myocardial infarction by inhibition of cellular respiration, limitation of ROS generation and inhibition of mitochondrial enzymes[43]
Dietary nitrate		• Nitrate is reduced to nitrite by oral bacterial nitrate reductase • sGC-cGMP-PKG	• Gastroprotective effect by increasing gastric mucosal blood flow and mucus production[44] • Lowering blood pressure and inhibition of platelet aggregation[45-47]

Abbreviations: cGMP, cyclic guanosine monophosphate; NOS, nitric oxide synthase; PKG, protein kinase G; ROS, reactive oxygen species; sGC, soluble guanylyl cyclase

Fate and metabolism of NO

After produced in endothelial cells, NO diffuses to vascular smooth muscle cells. NO binds and stimulates soluble guanylyl cyclase (sGC), an enzyme recognized as NO receptor (Figure 1). sGC is a heme-containing protein that converts guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP), a second messenger that causes activation of protein kinase G (PKG). Activation of PKG induces downstream cascades leading to inhibition of inositol trisphosphate (IP_3)-mediated release of calcium from sarcoplasmic reticulum (SR). PKG phosphorylates the voltage-gated calcium channels, causing the channel inhibition. Phosphorylation of phospholamban by PKG disinhibits the calcium-ATPase, allowing sequestration of cytosolic calcium into SR. Altogether, the processes result in a decrease in cytosolic calcium and smooth muscle relaxation. In normoxia, NO is oxidized rapidly by oxyhemoglobin, yielding nitrate and methemoglobin with the rate constant close to diffusion limit ($6–8\times10^7\ M^{-1}s^{-1}$). In contrast to normoxic condition, NO reacts with deoxyhemoglobin, yielding iron-nitrosyl hemoglobin (an NO adduct with hemoglobin) under hypoxia. S-nitroso hemoglobin (SNO-Hb) is also generated from the interactions between NO and thiol group of cysteine-93 of β-globin.[48]

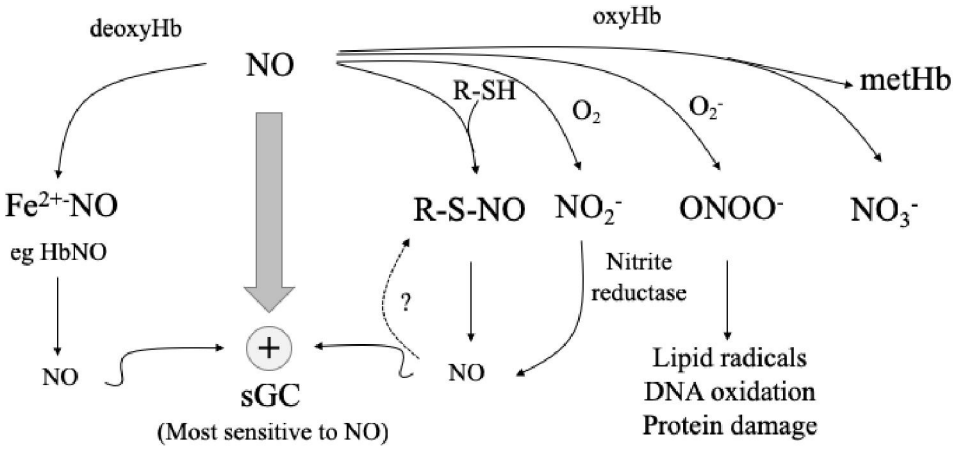

Figure 1. Fate and metabolism of NO. The most sensitive target of NO for biological actions is soluble guanylyl cyclase (sGC). NO at physiologic concentrations (low nM) activates sGC, resulting in vasodilation and platelet inhibition. NO also binds to ferrous ion (Fe^{2+}) in deoxygenated heme proteins such as deoxyhemoglobin (deoxyHb) to form iron-nitrosyl hemoglobin (HbNO). NO interacts with sulhydryl group (R-SH) of protein to form S-nitroso adduct (R-S-NO). Because HbNO and R-S-NO can release NO, they are considered as NO carriers. NO can be oxidized by oxygen (O_2) to be nitrite anion (NO_2^-), by superoxide anion (O_2^-) to form peroxynitrite ($ONOO^-$), and by oxyhemoglobin (oxyHb) to form nitrate (NO_3^-). The reaction of NO with oxyHb yields nitrate and methemoglobin (metHb). Nitrate is biologically inactive because there is no human enzyme that can reduce it to nitrite and NO, while nitrite can be converted to NO by the nitrite reductase of heme proteins.

Thalassemia

Thalassemia is an inherited disorder of hemoglobin synthesis. Hemoglobin is an iron-rich protein in RBC which works as an oxygen carrier from lungs to other tissues. Thalassemia patients carry a defective globin gene associated with inability to produce normal hemoglobin or reduction in synthesis of hemoglobin. RBC of thalassemia patients are destroyed early by the reticuloendothelial system.

Chromosome 11 contains genes for β-globin synthesis. β-thalassemia minor or β-thalassemia traits have one abnormal β-globin gene. Normal hemoglobin or hemoglobin A contains 2 α- and 2 β-globin chains. In β-thalassemia, the insufficient or absent production of β-globin chain leads to the decrease of hemoglobin A levels. From the recently large cohort study[49], the prevalence of β-thalassemia in Thailand is 12.5%. β-Thalassemia is classified into 2 types, including thalassemia major (Cooley's anemia) and thalassemia intermediate.

The clinical severity of β-thalassemia ranges from mild or non-transfusion-dependent thalassemia to severe or transfusion-dependent thalassemia. Hemolysis, ineffective erythropoiesis and reduction in hemoglobin synthesis result in anemia in β-thalassemia. Ineffective erythropoiesis causes bone marrow expansion. Marrow expansion results in deformities of skeletal bones and a variety of growth and metabolic abnormalities.[50] Extramedullary hematopoiesis in β-thalassemia patient results in enlargement of spleen and liver. The major cause of death is from cardiac disorders. From a retrospective study in 447 transfusion-dependent β-thalassemia patients[51], the most common morbidities were endocrinologic (44.7%) and cardio-vascular (41.3%). The 20-year survival rate is 85-88%. The 40- and 60-year survival rates are 63 and 54%, respectively.[52,53]

Pathophysiology

The decrease in β-globin in β-thalassemia results in excessive α-globin accumulation in RBC. The excessive α-globin chain is destroyed by specific erythroid protease.[54] The excessive α-globin chains are unable to form tetramer, but precipitated in the RBC precursors in bone marrow as inclusion bodies.[55] Ineffective erythro-poiesis and shortened RBC lifespan result in anemia in these patients. Iron overload occurs because of blood transfusions and increased intestinal iron absorption. Increased levels of heme, hemichrome and iron are responsible for reactive oxygen species (ROS) generation which causes damages to lipids and proteins through oxidative stress.[54] Oxidation of RBC membrane by ROS causes membrane rigidity and loss of deformability. Defective RBCs are destroyed by reticuloendothelial system more easily than normal RBC.

Cardiovascular complications in β-thalassemia

Heart diseases The most common complications in β-thalassemia are cardio-vascular disorders. Cardiac complications are the primary causes of death and the major causes of morbidity in thalassemia. Cardiomyopathy in thalassemia is categorized into 2 phenotypes: dilated left ventricular cardiomyopathy and restrictive left ventricular filling.[56] β-Thalassemia patients have an increased risk of arrhythmia, including premature atrial contractions, premature ventricular contractions and atrial fibrillation. In 47 β-thalassemia major patients with preserved left ventricular function[57],

abnormal ventricular depolarization and repolarization were observed. From a cross-sectional study in 120 patients with β-thalassemia intermedia and major[58], both atrial and ventricular arrhythmias were detected. Premature atrial contractions were found in 23.3% and 36.6% of β-thalassemia intermedia and major, respectively. The prevalence of atrial fibrillation ranges from 14 to 20% in β-thalassemia major patients, depending on the detection method.[59,60] Acute pericarditis and myocarditis may develop and lead to systolic heart failure in these patients.[61]

Hypercoagulable stage There are case reports of ischemic stroke, deep vein thrombosis and pulmonary embolism in β-thalassemia.[62] There are many possible mechanisms of hypercoagulability in thalassemia.[63] Increased platelet aggregation[64,65] and increased expression P-selectin and CD63[66] (surface markers of platelet activation) were reported. The levels of anticoagulant proteins (protein C, protein S, D-dimer and fibrinogen) and antithrombin III decrease in β-thalassemia.[67,68] Microparticles could induce platelet activation in thalassemia[69]. Microparticles from splenectomized hemoglobin E/β-thalassemia (HbE/β-thal) patients enhances platelet activation by increasing P-selectin expression and induction of platelet-neutrophil aggregation.

Pulmonary hypertension (PH) PH is a serious vascular complication with poor prognosis, which eventually causes right-sided heart failure in β-thalassemia.[70] By using echocardiography as a screening tool, elevated tricuspid regurgitant velocity (TRV) was found in 50% of β-thalassemia patients.[71] Although the echocardiography is used to identify patients at risk of PH, this technique may report a false positive error in patients who have high cardiac output.[72] Thus, the right heart catheterization should be used to diagnose PH. By right heart catheterization, the prevalence of PH was reported to be 1.1% and 4.8% in β-thalassemia major and intermedia, respectively.[73] PH in thalassemia and other hemolytic diseases is classified to Group 5 PH (PH with unclear multifactorial mechanisms).[74] Splenectomy, advanced age, hemolysis, and hypercoagulability are risk factors for PH in β-thalassemia.[75] Time after splenectomy has positive correlation with increased TRV in β-thalassemia patients.[76] Hypercoagulability and thrombosis which are found after splenectomy play roles in the development of PH.[77,78] In the absence of spleen, there is an increase in activated thrombin which activates platelets and coagulation cascade, leading to thrombosis (Figure 2).[79,80] In splenectomized patients, platelet activation correlated with TRV and cell-free hemoglobin.[81,82]

Medications for β-thalassemia

The hemoglobin concentrations in thalassemia are therapeutically maintained at 7 to 10 g/dL. In β-thalassemia intermedia, blood transfusion may be unnecessary while it is required in β-thalassemia major.[83] Repeated transfusion leads to iron overload and organ dysfunction, including diabetes mellitus, cardiomyopathy and liver disease. Iron chelator therapy with parenteral drug (deferoxamine) or oral drugs (deferiprone and deferaxirox) is necessary.[84,85] Additionally, hydroxyurea is used for induction of γ-globin (to increase fetal hemoglobin) in β-thalassemia.[86] Hydroxyurea can cause 1 to 5 g/dL increase in hemoglobin.[87] However, the response to hydroxyurea varies in these patients. The adverse effects of hydroxyurea are cytopenia, opportunistic infection, azoospermia, and hypomagnesemia.[88] For treatment and prevention of thrombosis, aspirin is generally prescribed as anti-platelet drug.[62]

Recently, hematopoietic stem cell transplantation is a curative treatment for thalassemia.[89] The success rate of transplantation is 80-90%; however, the transplantation therapy is successful in young children (≤14 year of age[90]) with HLA-identical sibling donor. The availability of HLA-matched donors, patient's age, risk of graft rejection, and graft-versus host disease are limitations of transplantation in thalassemia.[91]

Nowadays, gene therapy is a strategy to overcome the lack of HLA-match donor using transduced autologous hematopoietic stem cells.[92] The β-globin-expressing lentivirus is transduced into autologous $CD34^+$ cells. Patients undergo myeloablation before infusion of β-globin-expressing cells. Although the long-term efficacy and safety has not been documented, gene therapy has shown promising data. This therapy eliminates the need of blood transfusion in 22 patients with severe β-thalassemia without serious adverse effects.[92]

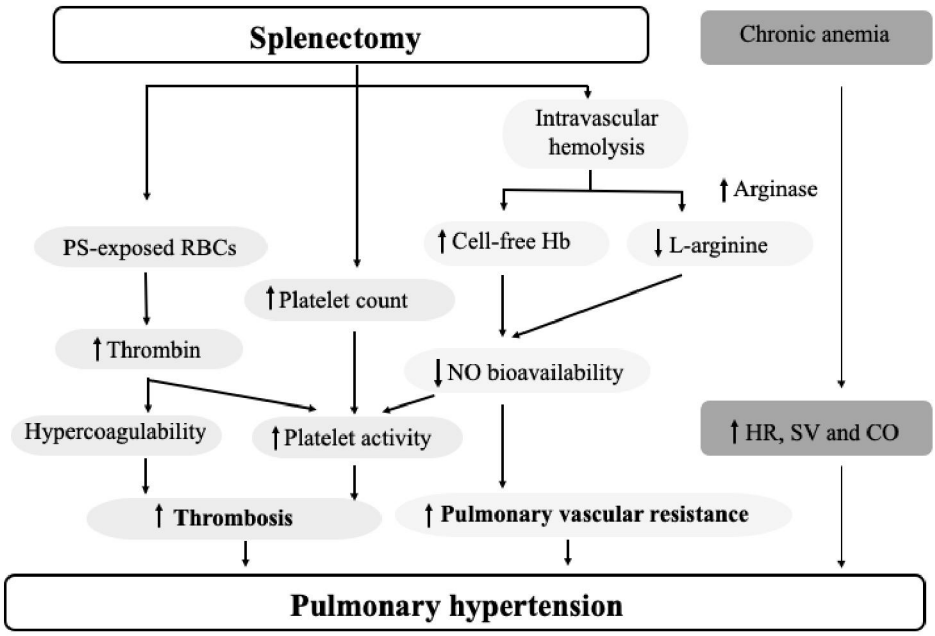

Figure 2. Proposed pathophysiology of pulmonary hypertension in β-thalassemia. After splenectomy, β-thalassemia patients have increased levels of phosphatidylserine (PS)-exposed red blood cells (RBCs), platelets and cell-free hemoglobin (Hb). PS-exposed RBC activates thrombin. Intravascular hemolysis is present after splenectomy, allowing the release of hemoglobin and arginase from RBC. Plasma arginase and cell-free hemoglobin decrease NO availability. Increased thrombin and platelet activity are factors leading to increased pulmonary vascular resistance and pulmonary hypertension. Hemodynamic changes due to anemia such as increases in heart rate (HR), stroke volume (SV) and cardiac output (CO) produce adverse effects on the heart and pulmonary vessels.

NO in thalassemia

Because NO has short half-life (milliseconds) in blood, nitrite as stable NO derivative is measured as a marker of endothelial function. Approximately 70% of plasma nitrite is from NO produced by eNOS in endothelial cells.[93] Even if the endothelial dysfunction has been reported in β-thalassemia[94,95], the NO level is controversial due to different methods of measurement. The blood nitrite levels are 176±17 nM (mean±SD) in healthy volunteers when measured by chemiluminescence NO analyzer.[96] Although most studies reported a decrease in blood nitrite levels in β-thalassemia (Table 3), its levels are different. For example, the nitrite levels measured by Griess assay was in micromolar ranges[97] whereas those measured by chemiluminescence method was in nanomolar ranges. By chemiluminescence method, we found a decrease of nitrite in RBC and correlation of nitrite with severity of pediatric HbE/β-thal.[98] The nitrite levels exhibited negative correlations with hemolytic and oxidative stress markers. In contrast to most studies, increased nitrite was reported in RBC of adult HbE/β-thal[99,100], which could be explained by medications. Deferiprone can increase blood nitrite in healthy volunteers and HbE/β-thal patients. The patients who had chronic deferiprone therapy had increased nitrite levels in RBC. *In vitro* experiment demonstrated that deferiprone increased eNOS activity by inducing phosphorylation of eNOS at Ser1177 in primary human pulmonary artery endothelial cells.[99] In addition, hydroxyurea and deferoxamine also increase eNOS phosphorylation.[101,102] Increased iNOS expression due to chronic inflammation is another possible cause of increased nitrite in blood of adult HbE/β-thal. NO_x levels were reported in many studies.[103-106]

Table 3. Changes in NO_x (nitrite+nitrate), nitrite and nitrate levels in β-thalassemia.

		Healthy	β-thal	Method
NO_x level (µM)				
Kukongviriyapan et al.[95]		31.6±16.9	35.0±23.4	Griess assay with NR
El-Hady et al.[105]		33.0±8.9	12.1±5.1*	Griess assay with NR
Singer et al.[81]		7.2±2.0	9.1±12.0	Chemiluminescence
Satitthummanid et al.[104]		117.2±27.3	135.8±11.3	Griess assay with NR
Uaprasert et al.[106]		178.2±17.6	132.4±32.5*	Griess assay with NR
Nitrite level (nM)				
Suvachananonda et al[98]	RBC:	331.0±21.1	154.3±21.8*	Chemiluminescence
	Plasma:	67.2±8.1	82.9±11.1	
Sriwantana et al. [99]	RBC:	112.2±12.4	179.2±27.4*	Chemiluminescence
	Plasma:	63.9±12.6	98.0±12.5	
Chamchoi et al.[100]	WB:	163.6±40.9	217.8±95.8*	Chemiluminescence
Nitrate level (µM)				
Suvachananonda et al[98]	Plasma:	18.8±1.2	22.2±2.0	Chemiluminescence
Sriwantana et al.[99]	Plasma:	24.2±2.4	28.5±2.8	Chemiluminescence

Values are expressed as mean±SE *Significant differences (p<0.05) compared to healthy groups. NR, nitrate reductase; RBC, red blood cell; WB, whole blood.

Role of NO and development of PH in β-thalassemia NO is an essential molecule that regulates vascular homeostasis. Despite unexpected elevated nitrite found in adult patients, reduced NO bioavailability and endothelial dysfunction is well documented in thalassemia.[94,95] Both extravascular hemolysis and intravascular hemolysis are present after spleen removal.[107] Cell-free hemoglobin can scavenge NO at 1000-fold faster rate than hemoglobin inside RBC.[108] Hemolytic markers such as cell-free hemoglobin and lactate dehydrogenase (LDH) had positive correlations with TRV, suggesting the contribution of hemolysis on development of PH. Moreover, arginase from lysed RBC degrades L-arginine, leading to decreased NO bioavailability.[109] In β-thalassemia patients with elevated TRV, L-arginine in plasma decreased and had negative correlation with TRV. In HbE/β-thal, decreased nitrite reductase (NR) activity of HbE were associated with PH.[100] Due to the difference in redox properties between HbE and hemoglobin A (HbA), the rate of NO generated by NR of HbE decreases about 2.5 folds compared to HbA in vitro.[110] In HbE/β-thal, NO generation from NR of deoxyhemoglobin dialysate decreased in HbE/β-thal.[100] The NR activity showed negative correlation with TRV of HbE/β-thal patients.

Potential therapeutic use of NO in thalassemia Because cyclic guanosine monophosphate (cGMP) is a messenger for vasodilatory effect of NO, inhibition of phosphodiesterase-5 (PDE-5) by sildenafil is used for PH in thalassemia. The efficacy of sildenafil for PH in thalassemia has been demonstrated in several case reports and small open-label trials. Improved PH symptoms and echocardiographic parameters were reported in 2 β-thalassemia patients undergoing 15- and 24-months treatment with sildenafil.[111,112] An open-label trial of sildenafil in 7 β-thalassemia patients showed the improvement of New York Heart Association Functional (NYHA) class, echocardiographic parameters and 6-minute walk distance.[113] In other study, sildenafil caused improvement of NYHA and echocardiographic parameters in 10 β-thalassemia patients without improvement in 6-minute walking distances.[114] Up to date, there is no randomized control trials of sildenafil for PH in thalassemia.

NO can induce pulmonary vasodilation. However, the use of NO gas is limited by difficulty in method of delivery.[115] The local delivery of nitrite by inhalation is a promising approach for PH in β-thalassemia. Inhaled nitrite is converted to NO in lungs with small systemic effect. Inhaled nebulized nitrite was investigated in 5 HbE/β-thal patients with PH. Inhalation of nitrite (15 and 40 mg) immediately decreased pulmonary artery pressure as measured by right heart catheterization and echocardiography.[116] The effect of inhaled nitrite disappeared after end of inhalation. The increase in exhaled NO after nitrite inhalation indicated the local conversion of nitrite to NO in lungs of HbE/β-thal patients. Moreover, inhaled nitrite inhibited platelet activity and increased the phosphorylated vasodilator-stimulated phosphoprotein in platelets.[117] Long-term trial of inhaled nebulize nitrite are under investigation in β-thalassemia patients with PH.

Conclusion

NO is essential to maintain vascular homeostasis and enough perfusion. NO is synthesized constitutively from eNOS to serve these functions. Because the activity of eNOS is decreased during hypoxia, NO is instead produced from nitrite

via the nitrite reduction catalyzed by deoxyhemoglobin. In thalassemia, the presence of endothelial dysfunction and possible impaired eNOS activity are believed to cause a decrease in NO bioavailability, contributing to platelet hyperactivity and pulmonary hypertension. Considering the protective effects of NO on vasculature and decreased NO in thalassemia, NO or nitrite therapy is promising strategy to treat vascular complications in thalassemia.

Statement of conflict of interest

The authors declared no conflict of interest.

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