Triterpenoid inducers of Nrf2 signaling as potential therapeutic agents in sickle cell disease: a review

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Abstract Sickle cell disease (SCD) is an inherited disorder of hemoglobin in which the abnormal hemoglobin S polymerizes when deoxygenated. This polymerization of hemoglobin S not only results in hemolysis and vaso-occlusion but also precipitates inflammation, oxidative stress and chronic organ dysfunction. Oxidative stress is increasingly recognized as an important intermediate in these pathophysiological processes and is therefore an important target for therapeutic intervention. The transcription factor nuclear erythroid derived-2 related factor 2 (Nrf2) controls the expression of anti-oxidant enzymes and is emerging as a protein whose function can be exploited with therapeutic intent. This review article is focused on triterpenoids that activate Nrf2, and their potential for reducing oxidative stress in SCD as an approach to prevent organ dysfunction associated with this disease. A brief overview of oxidative stress in the clinical context of SCD is accompanied by a discussion of several pathophysiological mechanisms contributing to oxidative stress. Finally, these mechanisms are then related to current management strategies in SCD that are either utilized currently or under evaluation. The article concludes with a perspective on the potential of the various therapeutic interventions to reduce oxidative stress and morbidity associated with SCD.

Keywords oxidative stress; Nrf2; triterpenoids; sickle cell disease; vaso-occlusion; CDDO-Me

Introduction

Sickle cell disease (SCD) is a monogenic disorder that is inherited in an autosomal recessive fashion. The cause of SCD is a point mutation at the 6th codon of the β-globin gene causing substitution of valine for glutamic acid (β6-Glu—Val) [1–3]. This substitution results in a mutant hemoglobin molecule, hemoglobin S (Hb S), that when deoxygenated, polymerizes to produce rigid, sickle-shaped erythrocytes that are prone to oxidative stress and have a shortened life span [2].

An individual has SCD when they are either homozygous for Hb S or are compound heterozygous for Hb S and another clinically significant hemoglobin variant or β thalassemia [2,3]. The main pathophysiological mechanisms underlying the clinical manifestations of the disease are chronic hemolysis and intermittent vaso-occlusion associated with ischemia-reperfusion injury [2,4]. At the level of the microvasculature SCD is characterized by endothelial cell activation, abnormal adherence of blood cells to endothelium, inflammation, oxidant damage and nitric oxide depletion [3]. The aforementioned processes all contribute to the progressive organ damage that is associated with SCD [1–4]. An increased level of fetal hemoglobin (Hb F) ameliorates many clinical manifestations of SCD [5–7]. The presence of Hb F inhibits polymerization of sickle hemoglobin by reducing cellular concentration of Hb S and by formation of a mixed tetramer (α2βγγ) that does not participate in polymerization [8]. Consequently, research efforts have focused heavily on approaches that might lead to increased levels of Hb F in patients with SCD [9]. However, there is renewed interest in the role of oxidative stress not only as a key contributor to the pathophysiology of SCD [1,4,10–12], but also as a target for therapeutic intervention. Investigations focused on oxidative stress pathways and the production of reactive oxygen species (ROS) are pointing to potential therapeutic targets that may be exploited to either prevent or reduce the
frequency of complications of SCD [11,12]. Triterpenoids are a class of molecules with potent activities against ROS generation [13,14]. This review will focus on the role of synthetic triterpenoids that activate Nrf2 as potential therapeutic agents that prevent organ dysfunction in SCD.

**The Nrf2 antioxidant pathway**

Nrf2 is a basic leucine zipper transcription factor that has been identified as a regulator of stress response and redox balance [15]. The role of Nrf2 in multi-organ protection has been described [16]. The importance of targeting the Nrf2 pathway for therapeutic intervention in SCD is underscored by results from a study by Sangokoya et al. which showed microRNA miR-144 repressed Nrf2 levels in reticulocytes and that the highest level of miR-144 was in a subgroup of SCD patients who had the most profound anemia [17]. Under basal conditions, Nrf2 is bound to Kelch-like ECH associated protein (Keap1) in the cytoplasm and is quickly ubiquitinated. Under conditions of electrophilic stress, Nrf2 is released from Keap1, protected from degradation in the cytosol and accumulates in the nucleus, where it binds to the antioxidant response element (ARE) present on the promoters of several genes [18]. Fig. 1 provides a graphical representation of the Keap1-Nrf2 pathway. Binding of Nrf2 to the ARE leads to transcription of genes involved in cellular defense in a manner referred to as the coordinated phase II response. These Nrf2 target genes include, but are not limited to, heme oxygenase 1 (HO-1) (the inducible form of heme oxygenase), glutamate cysteine ligase catalytic and modifier subunits (GCLC and GCLM, respectively), glutathione S-transferase (GST), and NADPH quinone oxidoreductase 1 (NQO1). HO-1 catalyzes the rate-limiting step in the breakdown of heme to biliverdin and carbon monoxide both of which cause relaxation of vasculature. GCLC catalyzes the rate-limiting step in formation of glutathione, a powerful antioxidant, and NQO1 catalyzes the breakdown of reactive quinones. The expression of superoxide dismutase (SOD) which catalyzes the dismutation of superoxide (O$_2^-$) and of catalase (CAT) which breaks down hydrogen peroxide is Nrf2-dependent as well [19]. Nrf2 is therefore a key regulator of the redox balance.

**What is oxidative stress?**

During aerobic metabolism, free radicals such as superoxide (O$_2^-$) and nitric oxide (NO) are generated by NADPH oxidases, xanthine oxidase (XO) and nitric oxide synthases [4]. Acting as intermediates, these free radicals form other reactive species such as hydrogen peroxide (H$_2$O$_2$) and hydroxyl ions (OH$^-$). In low