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# Cis-vaccenic acid induces differentiation and up-regulates gamma globin synthesis in K562, JK1 and transgenic mice erythroid progenitor stem cells

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ABSTRACT

Gamma globin induction remains a promising pharmacological therapeutic treatment mode for sickle cell anemia and beta thalassemia, however Hydroxyurea remains the only FDA approved drug which works via this mechanism. In this regard, we assayed the γ-globin inducing capacity of Cis-vaccenic acid (CVA). CVA induced differentiation of K562, JK1 and transgenic mice primary bone marrow hematopoietic progenitor stem cells. CVA also significantly up-regulated γ-globin gene expression in JK-1 and transgenic mice bone marrow erythroid progenitor stem cells (TMbmEPSCs) but not K562 cells without altering cell viability. Increased γ-globin expression was accompanied by *KLF1* suppression in CVA induced JK-1 cells. Erythropoietin induced differentiation of JK-1 cells 24 h before CVA induction did not significantly alter CVA induced differentiation and γ-globin expression in JK-1 cells. Inhibition of JK-1 and Transgenic mice bone marrow erythroid progenitor stem cells Fatty acid elongase 5 (Elovl5) and $\Delta^9$ desaturase suppressed the γ-globin inductive effects of CVA. CVA treatment failed to rescue γ-globin expression in Elovl5 and $\Delta^9$-desaturase inhibited cells 48 h post inhibition in JK-1 cells. The data suggests that CVA directly modulates differentiation of JK-1 and TMbmEPSCs, and indirectly modulates γ-globin gene expression in these cells. Our findings provide important clues for further evaluations of CVA as a potential fetal hemoglobin therapeutic inducer



## 1. Introduction

The hallmark of current molecular strategies in the therapy of sickle cell anemia (SCA) and beta thalassemia is centered on the pharmacological reactivation of fetal hemoglobin in these patients (Musallam et al., 2013). The central feature of the human β-globin gene expression is the existence of a two stage-specific switch that regulates the expression of hemoglobin from embryonic hemoglobin to fetal hemoglobin to adult hemoglobin (Bieker, 2010; Perrine, 2011; Sankaran et al., 2010). Several studies have documented the clinical benefits of increased fetal hemoglobin synthesis in the hemoglobinopathies (Fibach et al., 1993; Platt et al., 1984). However, Hydroxyurea remains the only FDA approved sickle cell treatment drug that functions by inducing HbF. But its widespread use is limited due to concerns over its long term side effects, and moreover, a significant number of patients do not respond to hydroxyurea therapy (Kinney et al., 1999; Platt et al., 1984; Steinberg et al., 2003). Studies by Perrine and colleagues revealed that butyrate, a short chain fatty acid, induced significant γ-globin gene expression and fetal hemoglobin synthesis in SCA and β-thalassemia patients (Perrine et al., 1987). Butyrate was shown to increase γ-globin gene expression through mechanisms dependent on histone deacetylase inhibition (Perrine et al., 1993).

Bone marrow stem cell transplant (which is unaffordable to a significant population of people living with SCA) remains the only cure for SCA (Walters, et al., 2000), it involves the complete replacement of the individuals' bone marrow using stem cells from a normal compatible donor to replace that of the affected individual. The heightened risks associated with the procedure also contribute to limiting its applications. Gene therapy is also another SCA therapeutic procedure that attempts to overcome the limitations of bone marrow transplant using stem cells from patient's own blood transduced with a lentiviral vector containing an

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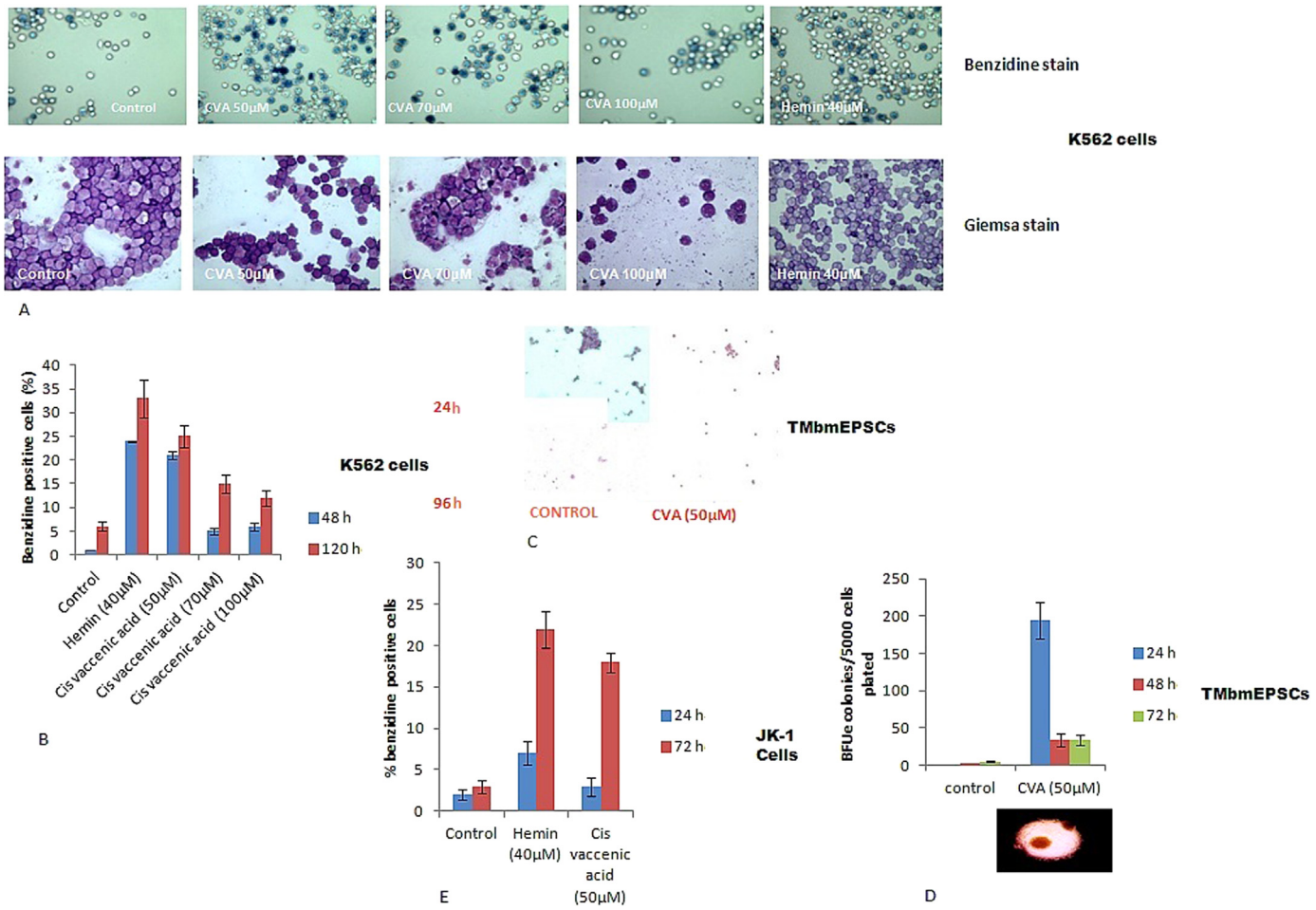


**Fig. 1.** CVA induces differentiation to the erythroid lineage. Differentiation was assayed as a measure of the emergence of hemoglobinized cells positive for benzidine. Analysis was carried out with K562, transgenic mice bone marrow progenitor stem cells and JK-1 cells. A. K562 cells induced with CVA for 72 h and stained with Benzidine and Giemsa stains. B. K562 cells were induced with varying concentrations of CVA and monitored for percentage of benzidine positive cells at 48 and 120 h post induction. C. Transgenic mice bone marrow progenitor stem cells depleted of plastic adherent cells in IMDM supplemented with 20% FBS, 100 U/ml penicillin, 200 μg/ml streptomycin and 2 U/ml EPO. Cells were induced with CVA for 96 h, and cytospin preparations of the cells were stained using benzidine-giemsa stain. D. Percentage of BFUe colony formation from TMbmEPSCs induced by CVA. TMbmEPSCs depleted of plastic adherent cells were seeded in plates containing semisolid IMDM supplemented with 20% FBS, 100 U/ml penicillin and 200 μg/ml streptomycin. E. JK-1 cells induced with 50 μM CVA and monitored for percentage of benzidine positive cells at 24 and 72 h post induction.

anti-sickling gene leading to improved production of healthy RBCs (Pawliuk et al., 2001). However the clinical success of gene therapy has been greatly limited due to the low titers observed as a result of regulatory elements of the β-globin gene locus used for the improvement of the transgene's expression and the eventual silencing of the transgene (Papanikolaou and Anagnou, 2010). More recently, some progress have also been made using zinc finger nucleases or transcription factor activator-like nucleases targeted editing of the abnormal β-globin gene leading to mutations such as frame-shift or deletions or to stimulate homologous recombination thus activating fetal hemoglobin production in these cells (Bauer et al., 2012). This approach however is not advanced enough for human therapeutic purposes and would require precise specificity to prevent off target mutagenic effects which could be deleterious. This underscores the requirement for a pharmacologic intervention which can up-regulate fetal hemoglobin with minimal toxicity.

Primary erythroid progenitor stem cell cultures from bone marrow or peripheral blood remain the best in vitro models for determining potential pharmacologically active agents, although cell lines have been used widely as good in vitro models for drug screening and have been widely used to screen and identify novel gamma globin inducers (Bianchi et al., 2001; Cioe et al., 1981; Gambari, 2003; Zein et al., 2010). Human K562 cells established by Lozzio and Lozzio (1975), co-express ε and γ but not β-globin genes; however, transgenic mouse bone marrow erythroid progenitor stem cells (TMbmEPSCs) and human JK-1 cells express β in addition to γ-globin, similar to the in vivo scenario of humans (Blau et al., 2005; Okunno et al., 1990).

Globin synthesis is developmentally regulated by a host of factors, key amongst which is the Kruppel like factor 1 (*KLF1*) (Zhou et al., 2010). Knockdown of *KLF 1* an erythroid specific transcription factor (Bieker, 2010), in human and mouse adult erythroid progenitors leads to reduced expression of B cell lymphoma 11a (*BCL11a*) and consequently induced γ-globin levels. Studies have also demonstrated that haploinsufficiency of *KLF 1* leads to hereditary persistence of fetal hemoglobin (Zhou et al., 2010) thus illuminating *KLF1* as a molecular target for the reactivation of fetal hemoglobin synthesis in humans.

*In vivo* inhibition of the mechanistic target of Rapamycin (mTOR) synthesis has been shown to remarkably improve erythroid cell maturation and anemia in a model of β-thalassemia (Zhang et al., 2014). (Z) 11 octadecenoic acid also called Cis-vaccenic acid (CVA) an 18 carbon *n*-7 mono-unsaturated fatty acid is biosynthesized in humans by hepatic fatty acid elongase 5 (Elovl5). CVA has also been shown to be the fatty acid precursor of 9-*cis* 11-*trans* octadecenoic acid an isomer of conjugated linolenic acid (CLA), a reaction also catalyzed by Elovl5 (Tripathy and Jump,