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Hematology and Oncology: Current Research

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Sickle Cell Disease and Transglutaminase 2

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Received: Jun 16, 2022

Accepted: Jul 11, 2022

Published Online: Jul 13, 2022

Journal: Hematology and Oncology: Current Research

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Editorial

Despite the great progress in the therapy of "Sickle Cell Disease" (SCD) there are still unknown mechanisms in the pathophysiology of the disease. The recognition of these hidden pathways is worth discovering in order to apply new treatments. In this letter, I am going to draw attention to my earlier not published observations on the increased activity of Transglutaminase 2 (TG2) in the erythrocytes of patients with SCD. This approach is still new. In addition, data are collected from literature to explain that molecules of urea and amine types which are inhibitors of TG2 activity, can be potent agents in the drug therapy of SCD. Such potential examples can be "Hydroxyurea" (HU) and "L-Glutamine" (Endari) which drugs are widely used in the present therapeutic practice of this disease. Although the experimental data presented here derive from my earlier unpublished and not completely finished results, I would like to stimulate further works to investigate the role of TG2 in SCD.

"Sickle Cell Disease (SCD) is an inherited blood disorder. Currently, an estimated 300,000 affected babies are born each year, more than 80% of whom are in Africa. The rest derives from other regions where malaria was historically endemic, including sub-Saharan Africa, India, the Middle East, and the Mediterranean. The recent migration pattern increases the presence of SCD in the non-malarial regions, too. It is estimated that 100,000 Americans are affected with SCD, the majority of whom are of African descent. SCD is caused by an abnormal form of hemoglobin called hemoglobin S, HbS), in which glutamic acid at position 6 of the beta-globin chain of Hb is changed to valine caused by a single base change (A>T) at codon 6. The genetic causes of SCD include homozygosity (HbSS) and heterozygosity. Another structural variant of beta globulin is HbC causing of genotype HbSC. The fundamental event that underlies the complex pathophysiology and multi-systemic consequences of SCD is the polymerization of HbS that occurs under low oxygen tension leading to the altered structure and function of the Red Blood Cells (RBCs). The damaged (typically sickled shaped) RBCs are not only less flexible compared to normal RBCs, but also highly adhesive. Repeated cycles of sickling and unsickling shortens the lifespan of the damaged erythrocytes to about 1/6th that of normal cells. The outcome is the occlusion of blood vessels in almost every organ of the body and chronic hemolytic anemia, the two hallmarks of the disease, that result in recurrent episodic acute clinical events, of which acute pain is the most common and accumulative organ damage. Acute sickle pain is so severe that it is often referred to as "vaso-occlusive sickle crisis (VOC)". These events trigger a cascade of pro-inflammatory ac-

Cite this article: Sipka S. Sickle Cell Disease and Transglutaminase 2. Hematol Oncol Curr Res. 2022; 5(2): 1017.

tivity setting off multiple pathophysiological factors that also involve neutrophils, platelets, and vascular endothelium." These ideas were reviewed by Salinas Cisneros and Tein in 2020 and 2021 [1,2]. These authors summarized the current advances on therapy for SCD in these works as follows: (1) modifying the patient's genotype, (2) targeting hemoglobin S polymerization, (3) targeting vaso-occlusion, (4) targeting inflammation. From the molecules of drug therapy two agents, "hydroxyurea" (HU) and "L-glutamine" (Endari), do have a special interest and attention from the point of view of this work. According to these authors hydroxyurea (a derivative of urea) is supposed to work via induction of fetal Hemoglobin (HbF), furthermore, it can decrease VOC, acute chest syndrome, number of transfusions, death and inflammation. However, its mechanism of action has not been fully understood. "L-Glutamine" (Endari), a molecule of amine type, is supposed to improve VOC via increasing NADH and NAD redox potential [1]. These observations, however, are not able to explain the disease specific biochemistry of SCD and the common points in the actions of "HU" and "L-Glutamine".

In the current work experimental results are presented to prove that (1) in the erythrocytes of SCD patients the activity of transglutaminase 2 (TG2) is increased considerably compared to healthy controls, (2) two molecules of "urea" origin, BCNU and CCNU, used for cancers can inhibit the activity of TG2 *in vitro*, (3) further data are collected from the literature to show that there are several other molecules of "urea origin" and those of "amine type" which can inhibit the activity of TG2, (4) new proposals are suggested to pay attention to the potential role of transglutaminases in the complex pathophysiology of SCD where the use of TG2 inhibitors can be a form of rational drug therapy.

TG2 is a multifunctional enzyme catalyzing the crosslinking between glutamine and lysine residues and involved in various pathophysiological events [3]. Beside crosslinking activity, TG2 functions as deaminase, GTPase, isopeptidase, adapter/scaffold protein, disulfide isomerase and kinase. TG2 is involved in cell growth, differentiation, cell death, inflammation, tissue repair and fibrosis. Depending on the cell type and stimulus, TG2 changes its subcellular localization and biological activity leading to cell death or survival. In normal unstressed cells intracellular TG2 exhibits GTP-bound closed configuration exerting pro-survival functions. However, upon cell stimulation with Ca^{2+} or other factors TG2 adopts a $Ca2^{+}$-bound open conformation demonstrating a transamidase activity involved in cell death or survival [4].

I was working at Kálmán Laki's Laboratory in the Department of National Institute of Arthritis Metabolism and Digestive Diseases in the National Institutes of Health (NIH, Bethesda, U.S.A.) during 1977/78. Here I found significantly elevated activity of transglutaminase 2 (TG2) in the erythrocytes of patients with SCD compared to healthy controls. That time I was not able to finish the whole work and publish the data.

However, now it can be worth mentioning these results to explain the therapeutic effects of "hydroxyurea" and "L-glutamine" from the aspect of TG2. The conclusions of those results were as follows:

1. The uptake of ^{14}C labelled putrescine by washed erythrocytes was increased about 44 percent on average in 6 black patients with SCD (3 males and 3 women) compared to 6 black healthy controls (3 men and 3 women) measured at 37°C for 6 hours. In healthy white donors of both sexes the uptake of putrescine did not differ from that which was found in the healthy black controls. Putrescine was a substrate for TG2.
2. The similar increased ratios of ^{14}C labelled putrescine were found in the membranes of lysed erythrocytes in SCD patients compared to healthy controls.
3. The SDS polyacrylamide (5%) disc electrophoretic (PAGE) protein measurements were carried out on the lysates of erythrocyte membranes in two parts: a.) the gels were stained by Coomassie brilliant blue, b.) the gels were not stained but cut by blade in pieces of 2 mm thickness and tested for isotope (C^{14} putrescine) contents. Large aggregates of isotope-containing proteins not entering the gels were found on the top of gels in SCD patients, but they were lacking in the controls. According to the protein patterns of stained gels it seemed that the aggregates could mostly derive from crosslinked glycoforin, band 3 and spectrin which molecules were characteristic components of erythrocyte membranes. These findings were direct signs of increased activity of TG2 in the erythrocytes of SCD patients. Supposedly, this enzyme could play a role in the formation of "sickled shaped" red blood cells which were the characteristic elements of disease.
4. The highest TG2 activities were found in the erythrocytes of SCD patients cultured in deoxygenated milieu. The highest ratios of RBCs with "sickled" shape were found also in this group.

The Ca^{2+} mediated crosslinking of erythrocyte proteins by "intrinsic" transglutaminase (TG2) and its inhibition by histamine were observed earlier [5]. We could confirm these results. However, we could still add the demonstration of increased ratios of altered and rearranged protein structures in the cell membranes of SCD patients in consequence of crosslinked proteins by TG2. In this state the cell membranes of erythrocytes could become more rigid losing the original membrane plasticity leading to the formation of "sickle cells" with shortened cell lifespan [6]. The demonstration of increased TG2 activities in the erythrocytes of SCD patients can be a new element to explain the pathology of disease.

Supposedly, the following linkages may exist among HU, L-glutamine, SCD, and TG2: a.) There is an increased activity of TG2 in SCD. b.) Both drugs can inhibit the activity of TG2; c.) The blocking of TG2 activity can contribute to their good therapeutic effects on SCD. There were also earlier proofs that some other derivatives of urea as BCNU (Bis-chloroethylnitrosourea) [7], and CCNU (Chloroethyl-Cyclohexil Nitrosourea) [8] or "phenylthiourea" [9] could inhibit the activity of TG2 *in vitro.* Thus, it can be possible that "HU" also can block TG2. In addition, some other amine molecules out of histamine [6] e.g. cystamine and cysteamine [10] also can inhibit TG2, beside other amine molecules as pentylamine and dansylcadaverine [11]. Therefore, it is also plausible to suppose that "L-glutamine" (Endari) can be an inhibitor of TG2. All these data suggest that the inhibition of TG2 activity in the erythrocytes of SCD patients can contribute to the complex beneficial therapeutic effects of "hidroxyurea" and "L-glutamine". Besides, these drugs can moderate even the clearance of dying red cells induced by Ca^{2+} activated TG2 [12]. Furthermore, the blocking of TG activity can be advantageous not only for the damaged erythrocytes but also for the impaired platelets in SCD [13] as platelets contain factor XIII which is another type of transglutaminases [14]. Thus, both "HU" and "L-

glutamine" can act on factor XIII in the platelets, too. In addition, factor XIII has a role in SCD associated priapism [15], and the inhibition of this enzyme by "HU" and "Endari" can be beneficial to treat the embarrassing symptom.

It is true that "gene therapy" appeared as a new promising way for treatment of SCD. However, the need for drug therapy not only remains in the future [1] but it stimulates further the research to combine "hydroxyl urea" or "L-glutamine" with other agents, too [16].

Summing up, the increased activity of TG2 in the erythrocytes can be a newly recognized significant pathological process in SCD. The inhibition of TG2 can be a new approach in the explanation of the beneficial therapeutic effects of "hydroxyurea" and "L-glutamine" in this disease. The introduction and testing of other new effective inhibitors of TG2 and their combinations can be considered for the complex drug therapy of SCD beside "hydroxyurea" and "L-glutamine".

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