NEW CONCEPTS IN SICKLE CELL ANEMIA

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SUMMARY:

Current knowledge on the pathophysiology of sickle cell anemia is reviewed and potential therapeutic options are reviewed and discussed. It is hoped that better understanding of the pathophysiology will improve the care given to these patients as well as their quality of life.
Key words : Sickle cell anemia –Pathophysiology – Treatment

RESUME :

Il s'agit d'une revue de la littérature sur les connaissances récentes de la physiopathologie de la drépanocytose ainsi que les options potentielles du traitement.
Le souhait est que la meilleure connaissance de la physiopathologie, permette d'améliorer la prise en charge et la qualité de vie de ces patients.
Mot clés : Drépanocytose –Physiopathologie - Traitement

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I - INTRODUCTION

Sickle cell anemia (SCA), is an inherited condition resulting from an abnormality in the β-chain of adult hemoglobin (HbA). The amino acid valine replaces glutamic acid which is normally present at the sixth position at the amino terminal. On deoxygenation HbS polymerizes and forms long crystals that distort the erythrocyte membrane [1-2].

The normal post natal hemoglobins include HbA ($\alpha_2\beta_2$), HbF ($\alpha_2\gamma_2$) and HbA_2 ($\alpha_2\delta_2$). The minor adult hemoglobin component HbA_2 is seen only when significant amounts of HbA are present. At birth, less than 1% of HbA_2 is seen, by 12 months 2-3.4% and throughout life the normal ratio of HbA to HbA_2 is about 30:1. By 6-12 months of age, the normal adult hemoglobin pattern appears, and HbF is present only in traces. High levels of β-chain and thus HbS are not produced until 3-6 months post natal life so symptoms of the disease are rare before 3 months [3].

Although little is known about the mortality from inherited hemoglobin disorders in developing countries, it is clear that in sub-Saharan African, many children die from them early in life. Both in the USA and Jamaica, the peak incidence of death among those affected with these disorders is at 1-3 years of age, usually due to infection. Recent data from the USA, suggests that among affected adults, the median age of death is 42 years for males and 48 years for males[4].

In recent years, there has been impressive progress in understanding the molecular pathogenesis and development of new therapeutic agents, for SCA.

1I - PATHOPHYSIOLOGY [1,2,5]

When sickle hemoglobin (hemoglobin S) is deoxygenated, the replacement of β6 glutamic acid with valine results in a hydrophobic interaction with another hemoglobin molecule, triggering an aggregation into large polymers. Sickle cell trait is benign, because the cellular concentration of hemoglobin S is too low for polymerzation to occur and it is hemoglobin S polymers that cause the cellular injury responsible for the clinical manifestations.

When deoxygenated, cells containing hemoglobin assume a banana or sickle shape and transmission electron microscopy reveals the presence of bundles of fibres oriented along the long axis of the cell.

The kinetics of polymer formation are critical determinants of the shape and morphology of the cells. When deoxygenation is rapid, multiple independent polymerization events result in a granular texture that does not alter the cell's disk-like shape. Contrarily, when deoxygenation is slow, a single nucleus of aggregated molecules of deoxygenated HbS is formed. This nucleation is followed by the growth and alignment of fibres, transforming the cell into a classic sickle cell shape. The distortion of the shape of the cell by projections of aligned hemoglobin, has a critical role in perturbing the structure and function of the membrane in SS red cells. The rate and extent of polymer formation in a circulating SS red cell, depend on 3 independent variables :

- the cells' degree of deoxygenation
- the intracellular hemoglobin concentration
- the presence or absence of HbF

Although the mean intracellular hemoglobin concentration and mean density of the overall population of SS red cells are close to those of normal cells, the density distribution of SS red cells is unusually broad. The increase in the least dense SS cells is due primarily to a high number of reticulocytes with a relatively low intracellular hemoglobin concentration. The presence of a substantial population of very dense cells is the result of polymerisation-induced membrane damage leading to enhanced dehydration. The end result of this process is the irreversibly sickled cell, with an elongated shape, even after fully oxygenated and lacks polymers. Since the rate of polymerisation of deoxygenated hemoglobin S is dependent on the hemoglobin concentration, dense SS cells is much likely to become distorted and rigid and thus contribute to the vaso-occulsive and hemolytic aspects of the disease.

The most important contributors to the dehydration of SS red cells are potassium-chloride cotransport and Ca++ - activated K+ efflux. In normal AA red cells, the former transport mechanism is active only in reticulocytes. Potassium – chloride cotransport is induced by cell swelling and acidification.

SS red cells have increased amounts of calcium, compartmentalized within intracellular vesicles, with normal steady-state concentrations of Ca++ in the cytosol. When the cell membrane is distorted by sickling, there is a transient increase in cytosolic Ca++.

The increase is sufficient to trigger the Ca++ - dependant (Gardos) K+ channel, thus providing a second pathway for sickling-induced loss of K+ and water and leading to cell dehydration.

The Gardos channel is activated by both increased intracellular calcium levels and agonists such as prostaglandin E_2. Since the potential for a sickled cell to initiate a vaso-occlusive event depend mostly on whether the rate of polymer formation is within the range of the capillary transit time, anything that retards transit of SS red cells in the microcirculation can have a critical effect on the pathogenesis of vaso-occulsion in SCA. Studies under static and dynamic conditions have demonstrated that SS red cells have a sticky surface and attach more readily than normal cells to monolayers of cultured endothelial cells. Recent studies have delineated the molecular interactions responsible for the adhesion of SS red cells to endothelium:

- reticulocytes especially those from SS individuals have on their surface the integrin complex, $\alpha_4\beta_1$, which binds to both fibronectin and vascular-cell adhesion molecule 1. This latter molecule is expressed on the surface of endothelial cell, especially after activation by inflammatory cytokines as tumour necrosis α, interferon-γ IL-1β, vascular endothelial growth factor (VEGF), thrombin, histamine and the effects of hypoxia and reperfusion.
- reticulocytes that are prematurely realeased form, the bone marrow ("stress"reticulocytes) in hemolytic disease display additional adhesive ligands that facilitate interactions between sickle cells and endothelial cells.
- microvascular endothelial cells and a subpopulation of sickle reticulocytes have CD36, which binds to thrombospondin secreted by activated platlets.
- several other plasma proteins including very – high – molecular weight forms of von Willebrand factor, may make an important contribution to adhesion.
- granulocytes interact with sickle cells and endothelial cells, and are stimulated to release injurious cytokines.

From extensive in vitro studies, in recent years, it has been concluded that sickle red cells indeed exhibit an increased adhesive phenotype and many cell adhesion receptors, plasma protons and sub endothelial matrix components are involved in mediating adhesive interactions. As the phenomenon of cell adhesion is better understood, it is likely that anti-adhesion therapies may become viable therapeutic alternatives in the management of vaso-occulsive crisis in the next decades. Although the major effects of SCA, result from the non-delivery of oxygen by abnormal blood, it is also likely that oxygen delivery may also be impaired by increased blood viscosity without primary adhesion and occlusion as described above. The viscosity of a fluid is defined as the resistance to flow of that fluid and is determined by some factors as temperature and the intrinsic properties of the fluid.

Several studies done to define the intrinsic characteristics that are altered in SCA, all indicate that membrane viscosity and the internal viscosity and deformability in SCA is markedly altered even when the cell is fully oxygenated. These are markedly increased when the hemoglobin is deoxygenated. This is because the hemoglobin molecules are no longer randomly arranged when deoxygenated but form structured, elongated arrays. The increase in viscosity is greater when deoxygenation is sudden and rapid than when it is gradual. The increased erythrocytic intracellular and membrane viscosity translates directly into whole blood viscosity.

Another important determinant of blood viscosity is the hematocrit. For normal blood, viscosity rises linearly as the hematocrit is increased. At very high hematocrits, viscosity may rise at a greater rate than the rate of rise of hematocrit, and oxygen delivery can start to decrease at hematocrits of 45-50% and the decrease becomes dramatic at a hematocrit of 60%. This decrease reflects the diminished circulation in small vessels as whole blood viscosity rises. The effect of hematocrit is more striking when HbS is examined. At full oxygen saturation, the curve relating viscosity to hematocrit is much steeper than for normal hemoglobin and deoxygenation makes it steeper still.

The curve relating oxygen delivery to hematocrit is strikingly shifted (even with full oxygenation) and indicates that the diminution of oxygen delivery begins at hematocrits of 30-35% and is striking at hematocrits of 45%. Diminished oxygen delivery results in tissue hypoxia and further desaturation of the hemoglobin.

The effects of increased viscosity on the circulation are seen in the larger vessels than the effects of adherence and occlusion. If the consequent hypoxia is sufficiently prolonged in a sufficiently large volume of tissue, necrosis results. Another important effect of increased whole blood viscosity is the increased tendency to thrombosis, probably due to the slowed rate of circulation. Thrombosis can be seen on either the arterial or venous side of the circulation.

Since the main effect of an increased hematocrit in SCA is a striking increase in blood viscosity, its measure can serve as a surrogate for the effect of increased viscosity in the pathogenesis of the disease.

III - THERAPEUTIC APPROACHES

Treatment for SCA is rapidly evolving as better understanding of the pathophysiology improves.

The following section reviews newer and emerging treatments.

1) Chemical inhibition of hemoglobin S [2,6]
The substitution of valine for glutamic acid at position 6, creates a hydrophobic pocket in the hemoglobin tetramer that readily polymerises on deoxygenation. The development of drugs that specifically bind sickle hemoglobin and inhibit polymerisation was based on biochemical principles for disrupting the hydrophobic interactions between the deoxyhemoglobin S tetramers, and has been further refined using the three dimensional structure of hemoglobin S and site mutagenesis. The development of a safe and effective inhibitor agent is a great challenge. The ideal agent would be readily absorbed through the gastrointestinal tract, circulate in the plasma without binding strongly to plasma proteins, readily penetrate the erythrocyte membrane and bind strongly and specifically to hemoglobin S to inhibit polymerisation. It should also not affect physiologic oxygen transport or bind to other biologically important molecules. A large amount of drug would be necessary to bind to the approximately 400 g of hemoglobin in patients with SS disease.
Unfortunately no antisickling drug so far tested, has a ratio of efficacy to toxicity that is high enough to merit clinical use.

2) Reduction of the intracellular hemoglobin concentration [2,6]

Since the rate of polymerisation of sickle hemoglobin is dependent on Hb S concentration, any treatment that lowers the mean corpuscular hemoglobin concentration is rationale. Clotrimazole (an antifungal drug) specifically inhibits the Gardo's channel, inhibiting K+ and water loss from SS red cells and thus reducing the intracellular hemoglobin concentration. Magnesium supplementation also effectively retards K+ and water loss from SS red cells in vitro. Two trials have shown the effectiveness of zinc sulphate and piracetam in preventing sickle cell related crisis in a total of 246 patients, by preventing red blood cell dehydration [7]. A reduction in pain crisis was shown in the piracetam study over a one year period and in the zinc trial a significant reduction in the total number of pain, hemolytic, aplastic and sequestration crisis was observed over a period of one and a half years. While these results are encouraging, larger and / or longterm multicenter studies are needed to further evaluate efficacy.

3) Role of vasoactive modulators in sickle cell anemia [6]

The sickle erythrocyte has been shown to produce adverse morphologic changes and modulations of the vasoregulatory molecules in exposed endothelial cells.
Nitric oxide is an important regulator of normal vascular tone and also binds directly to hemoglobin at both the heme iron and thiol groups, and is delivered to the tissues as oxygen is delivered. Experimental studies in the rat show that nitric oxide maintains organ blood flow in the presence of sickle red cells. In man, inhaled nitric oxide increases oxygen affinity in sickle red cells, and is well tolerated. Thus, nitric oxide may be a useful therapy for the treatment of vasoocculsion in SCA both as a vasomodulator and as an antisickling agent.

4) Treatment of sickle cell painful episodes [8]

Current treatment of acute painful episodes of SCA is primarily supportive and includes hydration, heat packs, oxygen, antibiotics, analgesics and blood transfusions. Painful episodes and the associated end organ damage are caused by microvascular occlusion, tissue ischemia resulting from complex interactions between the sickle erythrocytes, endothelium, platlets, plasma clotting factors and certain inflammation mediators.
Poloxamer 188 (Rheoth RxÒ), a nonionic block copolymer composed of blocks of hydrophobic polyoxpropylene and hydrophilic polyoxyethylene has hemorrheologic properties and has been shown to improve microvascular blood flow by lowering viscosity and adhesive frictional forces. Rheoth RxÒ provides a hydrated, poorly compressible barrier that blocks hydrophobic adhesive interactions (cell-cell, cell-protein, and protein-protein) in the blood. As a result, there is a reduction in blood viscosity, erythrocyte aggregation, adhesion to vascular endothelium, and an improvement in microvascular blood flow, thus reducing painful episodes in SCA.

5) Induction of fetal hemoglobin synthesis

Nearly 56 years of clinical and basic research have established that high hemoglobin F concentrations reduce the severity of SCA by preventing formation of hemoglobin S polymers [1,5].

The first drug to be tested was 5 – azacytidine, an antineoplastic drug, and was shown to increase HbF concentration, in phlebotomized baboons. But was abandoned because HbF production was via cytoxicity rather than gene hypomethylation [5].

Analogues of butyrate as well as acetate and other short-chain fatty-acid derivatives also appear to induce HbF production [2,6]. Presently the use of these drugs remains experimental [5].

Hydroxyurea is currently the only drug in widespread use, known to stimulate HbF production. It is relatively non-toxic, its myelosuppressive effects reversible and not known to induce tumours [2]. It has no role in the treatment of crisis in progress [9].

Hydroxyurea blocks DNA synthesis by inhibiting ribonucleotide reductase from ribonucleoside precursors [5,9]. It acts on bone marrow by its cytotoxic effects, selects a population of erythroblasts that can synthetize increased amounts of HbF. There are no direct effects on gene expression, and bone marrow cellularity may be diminished. Higher concentrations of HbF, reduce the level of HbS polymers and the numbers of deformed, dense and damaged erythrocytes. Cells with a high HbF content survive longer, attenuating hemolysis and leading to a reduction in reticulocytes, circulating granulocytes, monocytes and platelets. Fewer dense, poorly adhesive erythrocytes are less apt to adhere to and perturb the endothelium reducing the likelihood of vasoocculsion. Two other erythrocyte adhesive receptors a_4b_1 integrin and CD34 also drop during hydroxyurea treatment [5,6]. Recent reports suggest novel methods of action which include generation of nitric oxide (NO) by peroxidation, increased erythropoitin and induction of methemoglobin formation [5].

Therapy should be started with 500mg of hydroxyurea or 10 to 15mg/kg of body weight,and after six to eight weeks of treatment, the dose may be increased to 1000mg per day if blood counts are stable [1,10]. Hydroxyurea should be given for a trial period of at least 60 days before a patient is determined not to be responding to the drug [10]. Some authors used higher doses in children (14 - 27mg/kg body weight daily) and had good results and tolerance [11]. In about 10 to 25% of adult patients, hydroxyurea treatment does not cause an increase in HbF, perhaps because of abnormal bone marrow, genetic factors and variations in drug metabolism [1]. Doses of hydroxyurea that achieve fetal hemoglobin responses are at or near threshold of marrow suppresion [10].Studies of hydroxyurea in infants, children and adolescents lag behind adult studies in the appraisal of clinical efficacy.

All trials show an increase of HbF from about 5% before treatment to about 16% after 6 months to 1 year of treatment. A trial in 84 children with a mean age of 10 years gave similar results to those in adults [5]. Longterm effects are not yet defined. Few cases of leukaemia have been reported. Adverse effects on growth and development have not been reported [5]. The safety of hydroxyurea therapy in pregnancy is unclear [9]. Contraception should be practiced by both women and men receiving hydroxyurea and the uncertain outcome of an unplanned pregnancy discussed frankly [5].

6) Bone marrow transplantation

Bone marrow transplantation is the only currently available treatment that can cure SCA. Unfortunately, it is associated with significant mortality, morbidity and failure rates [6]. It was first used in Europe for patients from Africa on the assumption that the risks of disease in their countries outweighed the hazards of transplantation [1]. Children and adolescents younger than 16 years of age who have severe complications (stroke, recurrent acute chest syndrome or refractory pain) and an HLA-matched donor available, are the best candidates for transplantation [1,12]. In a group of 22 children with sickle cell disease who received marrow from HLA matched siblings, 15 (68%) were cured and have remained symptom-free of SCA, 4 (18%) rejected the marrow allograft and SCA recurred, 1 had mixed chimerism with 30% circulating sickle cells and 2 died after transplantation [11]. The patients cured are likely to be infertile and have an undefined risk of chemotherapy-induced malignant condition or other late complications of transplantation [13]. To the small group of patients who survived but were not cured by transplantation, will add the long-term effects of bisulfan and cyclophosphamide to their medical problems, including the possibility that the vascular insult of the conditioning regimen used for transplantation may ultimately exacerbate the underlying SCA[12]. Almost all transplantation cases to date
have utilized bone marrow from HLA-identical siblings. Studies to use unrelated and HLA-mismatched related donor transplantation to expand transplantation for SCA is limited and are still at the preliminary trial phases.

7) Umbilical cord blood transplantation [14]

Umbilical cord blood (UCB) is another source of hematopoietic stem cells undergoing investigation to support transplantation for SCA. UCB has unique properties that makes it potentially useful in this setting.

Studies of $CD34^+$ cells isolated from UCB show enhanced generation of committed hematopocetic progenitor cells, compared to same cells from the bone marrows. UCB is also immunologically naïve compared to adult peripheral blood. Preliminary results indicate that outcomes after UCB transplantation from sibling donors for hemoglobinopathies are similar to those after bone marrow transplantation, but with an additional advantage of a lower rate of graft rejection.

Based on these observations there is a growing interest in facilitating UCB collection and storage from families who currently have a child with sickle cell anemia or hematological disorder and who are expecting another child.

8) Gene therapy

Despite 20 years of research, progress towards implementing gene therapy for sickle cell anemia has been slow and is still at the experimental phase [14].

There has been recent success in developing two transgenic sickle cell mouse lines that express exclusively human hemoglobin and manifest the clinical features of SCA [6].

The principle of gene therapy is based on the fact that a gene can be efficiently inserted into repopulating hematopoietic cells to achieve regulated expression in specific hematopoietic lineages [14].

Relatively low efficiencies of gene transfer with conventional, murine oncoretroviral vectors coupled with the requirement for a very high level of globin gene expression in differentiating erythroblasts, have been major barriers to rapid progress. Recent studies have demonstrated that sickling phenotype can be corrected by retroviral vector mediated gene transfer, into repopulating stem cells in murine models of sickle cell anemia.

Another breakthrough has been the adoption of lentiviral vectors based on human immunodeficiency virus for globin transfer [14]. Great consideration is being given to biosafety related to lentiviral vector production,and strategies to ensure absence of replication of competent retrovirus are mandatory. The risk of mobilization of the vector genome in the context of subsequent HIV infection still remains to be assessed [14].

Early clinical protocols to evaluate gene therapy in patients with SCA are still in Phase I studies, and are designed to evaluate toxicity. Patients likely to participate are severely affected adults with severe disease and low endogenous levels of fetal hemoglobin[14].

Despite major advances in our understanding of the molecular pathology, pathophysiology, and control and management of inherited hemoglobin disorders, thousands of infants and children with these diseases are dying through lack of appropriate medical care. It takes time to establish expertise in developing countries for the control and management of these conditions, and the lessons learnt from developed countries will need to be transmitted to those countries in which they occur at a high frequency[4].

IV- CONCLUSION

This review demonstrates that remarkable progress has been made in recent years to understand the pathophysiology and development of new therapeutic approaches in sickle cell anemia. Although most of this work is still experimental, the future is promising. It is hoped that this research will be translated practically into a form that can be applied in the less developed and poorer countries especially in Africa where up to a third of the population carry the hemoglobin S gene.

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