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Chapter VII

Therapy of Sickle Cell Disease

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Abstract

Patients suffering from Sickle Cell Disease (SCD) are constantly exposed to a variety of health hazards, particularly infections and acute crises: a regular surveillance of their clinical and haematological situation is therefore necessary.

As for preventive measures, penicillin protection is usually effective to reduce the frequency of infection, together with vaccination against common pathogens. Blood transfusions are given to prevent cerebral vascular accidents.

Among pharmacological agents so far tested, a prominent role is played by compounds which have been proven capable to reactivate the production of Foetal Haemoglobin (HbF), as the presence of significant amounts of this haemoglobin can reduce the incidence and severity of

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complications; to this aim the best compound available at present is hydroxycarbamide (HC), or hydroxyurea, which has demonstrated a beneficial effect on both clinical and blood parameters of SCD patients: this has been very recently linked with a direct action on erythroid genes, like BCL11A, which are involved in the regulation of HbF levels.

Histone deacetylase inhibitors, like butyrate, are also under investigation; vaso-dilators like nitric oxide, failed to give consistent results in recent trials. Pain management includes opiate analgesics, corticosteroids and oxygen administration.

Transfusion therapy is mainly indicated for acute events, but regular transfusions are given to high-risk children for stroke prevention. Red cell exchange has the edge over simple blood transfusions when a fast reduction of HbS amount is required, namely in the presence of neurological symptoms. In patients receiving chronic transfusion regimens iron chelation treatment is necessary to avoid iron overload.

Haemopoietic stem cell transplantation (HST) is the only curative treatment, but is possible only in a limited number of cases. The most promising option is gene therapy, particularly after the introduction of induced pluripotent stem cells (iPS), which appear an appropriate target for permanent integration of a therapeutic transgene: experiments are under way to obtain genetic correction of SCD haemopoiesis as well as β-thalassemia. The insertion of an ankirin insulator into a lentiviral vector is part of the new approaches for the treatment of these disorders.

Introduction

A regular monitoring of SCD patients, performed by qualified medical centres and including both clinical and haematological assessment, is highly recommended: this should be the rule particularly in infants, who have a high incidence of vaso-occlusive and infectious events. To this regard penicillin prophilaxis has been shown to reduce infection frequency in African children a well as well as in developed countries [1; 2]; folate has also been given since long time, in order to stimulate erythrocyte production [3].Vaccination against Streptococcus pneumoniae has been shown to be successful in reducing the infection rate from this pathogen [4].

Prevention of red cell dehydration, due to ion and water loss via the potassium selective pathways, has been attempted with Gardos channel blockers, like senicapot, with some encouraging results [5].

The role of chronic blood transfusion for prevention of stroke has been the object of numerous investigations, with most studies confirming the usefulness

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Pharmacotherapy

It is well known that the main factor in the variable severity of SCD is the level of Foetal Haemoglobin (HbF) produced by patients: for instance, populations showing genetically determined presence of HbF have a mild form of the disease and the incidence of severe clinical complications is also less frequent in the presence of significant levels of such haemoglobin [9]. It is therefore understandable that major attempts have been done to induce in SCD patients by various means a higher level of HbF: among the compounds showing this activity are *Azacytidine* with its analogs, and, to a special extent, *Hydroxycarbamide* (HC).

It was indeed in the early eighties that 5-aza-cytidine was shown able to re-induce production of HbF in adults, as well as in experimental animals [10]. Years later a derivative of azacytidine, namely deoxy-azacytidine or *Decitabine* (DAC), was found effective in reactivating production of HbF and was therefore started in regular trials on SCD patients [11]. It has also been observed that adult SCD patients with multiple complications who did not respond adequately to HC treatment had less vaso-occlusive events and increased haemoglobin levels following treatment with DAC [12].

It is of interest that oral preparations have also been used, particularly in combination with *Tetrahydrouridine* (THU), a competitive inhibitor of *Cytidine Deaminase*, which metabolizes DAC into inactive uridine compounds: oral administration of THU before oral DAC causes DNA hypomethylation in the γ-globin gene promoter, producing large cumulative increase in Foetal Haemoglobin [13].

Hydroxycarbamide (HC) has been known for long time as an oral, well tolerated treatment for some myeloproliferative disorders. Its action on HbF production, first observed in baboons, was then tested and demonstrated in clinical trials on SCD patients [14; 15]; predictably, bone marrow suppression was observed after long treatment, but such effect is reversible.

It has to be noted however that not all patients receiving HC show a good response to treatment: in particular, little clinical improvement is observed in SCD patients with co-inherited α-thalassemia (αThal-SCD). Very recent data point to an elevated haematocrit and a subsequent increase in vaso-occlusive

events as a crucial mechanism, which determines a reduced response in such patients: this is confirmed by a rise in cell-free DNA, a marker of tissue damage [16].

As for the mechanism of action of HC, it was early shown to be mediated through the activation of soluble guanylil-cyclase [17], while latest research has focused on its effects on erythroid gene expression [18]: a study of reticulocyte RNA expression analysis from children with SCD and treated with HC has indeed shown that this compound affects a great number of erythroid genes, including BCL11A and SOX6, which are negative regulators of HbF levels [19]. It is therefore obvious that BCL11 "silencing" can be a reasonable goal and that screening should now be in progress for small molecules (or peptides) that inhibit BCL11A function, either directly or through disruption of protein-protein interactions, thereby producing a significant rise in HbF [20].

Butyrate was the earliest of a number of compounds used in this context because of their action as *inhibitors of histone deacetylase (HDAC inhibitors):* by promoting histone acetylation, butyrate increases the transcription rate of γ-globin gene as well as the translation of γ-globin m-RNA [21]. Other HDAC inhibitors and butyrate itself have also shown a different mechanism of action, namely through the activation of P38MAP kinase pathway: the effector molecules involved in γ-globin activation have been identified as CREB1 and ATF2, acting via the before mentioned pathway, which therefore appears as an important mediator of γ-globin gene regulation [22].

A number of other disparate compounds have been investigated: among them two thalidomide analogs, *pomalidomide and lenalinamide* were tested on early erytroid progenitors *in vitro*, causing increased proliferation of immature erythroid cells and induction of HbF without cytotoxicity; a synergistic effect was also observed in association with HC [23]. Many more compounds, endowed with some activity on the production of γ-globin and HbF, have been recently reviewed [24].

As vaso-occlusive events are a common dramatic feature in SCD, producing acute crisis, *vaso-dilators* have received special attention for prevention and treatment of these situations; in particular *nitric oxide (NO)*, a potent natural vaso-dilator, has been used by inhalation in many trials with some favourable results: however a recent multi-center, double-blind randomized trial showed no significant differences in secondary outcome measures, including length of hospitalization, cumulative opioid usage and rate of acute chest syndrome, between the NO treated patients and a placebo group [25].

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Another pharmacological trial has been carried out in order to correct the increased tricuspid regurgitation velocity (TRV),associated in SCD patients with increased morbidity and mortality, using *sildenafil*, a phospho-diesterase inhibitor, which had been shown to improve exercise capacity in patients with left ventricular systolic dysfunction and pulmonary venous hypertension: a randomized trial however did not show any improvement of exercise capacity or hospitalization length; moreover adverse effects from the drug were observed [26].

Pain Management

Although acute vaso-occlusive pain can be self-limiting, this is the most important complication from the patient's perspective and the most common reason for admission to hospital for both adults and children [27].

In the management of sickle cell crisis the patient should be firstly evaluated for potential infectious, traumatic or surgical causes of pain. Adequate hydration should be provided along with analgesia with narcotics and non-steroidal anti-inflammatory agents.

Opiate analgesia is the mainstay in the management of severe pain. Corticosteroids can also shorten episodes of acute pain, although use of these drugs has mostly stopped because of a high frequency of rebound pain and hospital re-admission.

Another important supportive treatment is oxygen therapy, useful for management of hypoxic states as acute chest syndrome and heart failure.

Providing psychosocial support and reassurance, and allaying anxiety are also important goals.

Blood Transfusion

Simple Transfusion

The aim of blood transfusion therapy in patients with SCA is to replace rigid, sickle-shaped red blood cells with normal cells, restore blood flow and increase oxygen carrying capacity [6; 28].

Transfusion therapy is indicated mainly for treatment of acute events, but in some cases even a long term transfusion therapy can be established [29] (Table 1).

Simple transfusion can be used to improve heart failure associated with an oxygen-carrying deficit, dyspnoea and severe fatigue. Top-up transfusions are consequently usually indicated for episodes of anaemization, acute splenic sequestration, acute chest syndrome, severe sepsis and acute hepatic sequestration.

Table 1. Indication to transfusion therapy in SCD

Top-up transfusion	Regular transfusion	Transfusion may be useful	Transfusion not indicated
Acute anemization	Primary and secondary stroke prevention	Complicated obstetrical problems	Uncomplicated painful episodes
Acute chest	Recurrent acute chest	Refractory and protracted painful episodes	Uncomplicated pregnancy
Syndrome with hypoxia	Syndrome not responsive to hydroxycarbamide	Acute severe priapism when given early in episode	Minor surgery with local anesthesia
Stroke or acute neurological deficit	Progressive organ failure	Refractory leg ulcers	Infections
Multiorgan failure	Recurrent splenic sequestration	Chronic pain	
Preoperatory management			

Regular red blood cells transfusions are indicated for stroke prevention, as a randomized multicentre stroke prevention trial (STOP I) showed that the

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is typically subject to an extended crossmatch for ABO, full Rhesus (Cc/D/Ee), and Kell blood groups [34]. In countries where most of the blood donors have European origin, this procedure reduces alloimmunization by 50% and is recommended practice.

Chronic blood transfusion is associated with iron overload, for this reason iron chelation therapy is essential in chronically transfused patients. *Desferrioxamine* is a parenteral chelator; has short half-life (0.4 hours) and poor oral bioavailability (<2%), urinary and faecal excretion and needs 8 to 12 hours subcutaneous infusion 5–7 days per week; the dosage is 20 mg/Kg/daily to 40 mg/Kg. The compliance can be an issue. Side effects include skin reactions, ototoxicity, retinal toxicity, bone and growth abnormalities, and also Yersinia Enterocolitica infection resulting from the iron mobilization. The oral iron chelator *deferasirox* is increasingly used with evidence of benefit [35]: it has long half-life (8–16 hours) and high oral bioavailability (70%) allows once-daily oral dosing, and results in higher iron loss, due to faecal excretion [36]. One more oral chelator, *deferiprone*, is licensed for thalassaemia only, not yet for SCD.

Another transfusional complication may be viruses' transmission.

Bone Marrow Transplantation and Gene Therapy

Children and adolescents younger than 16 years of age who have severe complications (stroke, recurrent acute chest syndrome, or refractory pain) and have an HLA-matched donor available are the best candidates for transplantation, the only curative treatment. However, 5–10% of patients die for complications related to BMT and another 10% experience graft rejection with the return of SCD [37]. It is anyway of interest that some improvement was noted even if removal of the abnormal cell population is incomplete ("mixed chimerism"): this is due to better growth of healthy red cells, as compared to sickle erythrocytes [38].

Gene therapy is a promising option, as autologous transplantation of genetically corrected haematopoietic stem cells (HSCs) can constitute a chance to treat patients lacking a compatible bone marrow (BM) donor. Moreover, the availability of induced pluripotent stem cells (iPSC), obtained from somatic cells treated with cocktails of specific transcription factors,

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Lentiviral vectors (LV), expressing a transcriptionally regulated human β-globin gene can actually correct haematological defects and organ damage in mice with sickle-cell disease and also can provide a degree of correction of murine β-thalassaemia by gene transfer in murine HSCs as well as in Thalassaemia Major human cells [40].

In a recent development, attention has been directed to the possibility of using a novel lentiviral vector, carrying both the human β-globin gene and an erythroid-specific ankyrin insulator: this vector is able to improve erythropoiesis through a novel mechanism which stimulates the rate of transcription of the transgenic β-globin mRNA during erythroid differentiation so that an increase in adult hemoglobin (Hb A) is obtained in erythroid precursors and in CD34(+) cells isolated from β-thalassaemia and SCD patients [41].

All these investigations show a keen interest for designing new approaches to the treatment of SCD and related diseases and, at the same time, provide a solid rationale for future clinical studies.

Conclusion

SCD is the world's most widespread monogenic disorder, namely due to a single gene alteration, but its clinical features can be modulated by co-existing genetic modifiers and environmental effects (like malaria), as well as ethnic, social, doctrinal factors, exerting a significant influence on its course: the impact of these situations is particularly evident in areas of recent immigration. All this should be taken into account when a therapeutic strategy must be considered, although, in the first place, some general measures can be adopted, like population screening and marital advice, in order to reduce the number of affected babies. Countries with extensive incidence of SCD have also started to practice neonatal and early life screening to detect individuals at risk. Specific treatment in subjects showing signs of SCD symptoms should include traditional preventive measures, like antibiotic protection and vaccinations; blood transfusions should be given to prevent cerebral vascular accidents.

As far as pharmacotherapy is concerned, it is well known that the main influence on variability of SCD is the level of HbF in patient's red cells.

Therefore, attempts have been made by various means to reactivate the HbF production, which is normally switched off perinatally. Such approach has been attempted since the 1980s, ranging from drugs like 5-azacytidine and its derivative, decitabine, to a series of compounds like hydroxycarbamide and a number of histone deacetylase inhibitors like butyrate, which seem to act as epigenetic modifiers. Many other disparate agents have been tried with mixed results, but hydroxicarbamide remains the most effective compound so far available and it has been very recently possible to demonstrate a clear effect of this drug on erythroid genes affecting the haemoglobin switch.

At any rate treatments like bone marrow or cord blood transplantation are so far the only real cure for a limited number of patients with severe haemoglobinopathies. Improved chemotherapy regimens of milder toxicity than those employed in the past have made it possible to obtain a stable, mixed donor-recipient chimerism, with reversal of the SCD phenotype.

A great potential is anticipated for gene therapy, with the possibility of making use of "induced" human stem cells from somatic de-differentiated cells: recent studies are now aiming at targeted insertion of a therapeutic gene into haemopoietic cells. The possibility of undesired effects, like the emergence of potentially oncogenic cell populations, should however be considered.

In the end, studies on gene therapy require great efforts and substantial resources, which can only be provided by cooperation between academic and industrial Institutions.

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