

The Role of Lipids in Inflammation: Review of the Evolving Pathogenesis of Sickle Cell Disease

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Abstract

The pathologic features of sickle cell disease had been known in the past to be as a result of red cell abnormality leading to vascular occlusion, haemolysis and consequent anaemia. Recent knowledge has revealed numerous pathogenetic pathways involving leukocytes, platelets and the vascular endothelium. Complex interactions between the inflammatory cytokines and the membrane lipids in sickle cell present several pathogenetic processes affecting disease severity. The mechanisms of membrane fluidity, aggregation, adhesion and inflammation are strongly associated with membrane lipid constitution. The omega -3 fatty acids via incorporation into the lipid membrane have been found to play a central role in suppressing inflammation in several disease processes. Variations in disease severity have been shown to correspond with levels of fatty acid desaturases involved in the synthesis of these fatty acids. The genes coding for these substances can also be manipulated to achieve a favorable outcome and may provide several possible therapeutic and prophylactic access points. This review aims at exploring these delicate interactions and proffering possible targets to ameliorate disease features. The information and referenced publications quoted in this review were obtained from the PubMed Central database, using the search keywords; inflammation, sickle cell, fatty acids and cytokines.

Keywords: Membrane lipids; Sickle cell disease; Inflammation; Omega-3 fatty acids; Cytokines; Fatty acid desaturases

Introduction

Sickle cell disease is an inherited disorder in which the mutant globin gene produces a less efficient haemoglobin molecule, causing depreciation in the resilience and flexibility of the red cells. Recent advances have unearthed several other effects of this supposed mutant globin gene. These effects are now known to include increased expression and binding of the cellular adhesion molecules to ligands on the vascular endothelium [1] a process similar to what is observed during inflammation [2]. This process involves the neutrophils, monocytes, lymphocytes and platelets as well as the reticulocytes and red cells. The background inflammatory process tends to be exacerbated during episodes of vaso-occlusion and has been proposed to actually initiate them. Inflammation is regarded as the response of living tissue to any form of assault. This response has both vascular and cellular components, with a myriad of chemical interactions mediating the process.

All cells are bound by a membrane considered to be the 'barrier of life', as it is a structure that isolates living cells from their surroundings [3]. One of the main components of all cellular membranes in all living cells, are phospholipids, which make up over 40% of the cell membrane bi-layer. This phospholipid bi-layer, with its interspersed transmembrane proteins acts as receptors of ligands. The asymmetrical distribution of membrane lipids in this phospholipid layer is important in the integrity and survival of most cells. In sickle cell anaemia, there exists an alteration in the distribution of membrane lipids, with exposure of phosphatidyl serine (PS) in some red cells in

circulation [4]. This distorts the membrane and consequently exposes and activates cell adhesion receptors as well as the binding sites of certain enzymes like promthrombinase [5]. It also initiates apoptosis of red cells and its removal by the splenic macrophages. The concentration of the membrane lipids differs across various locations on the membrane, with some regions showing increased concentration of certain fatty acids. These areas are known as "rafts" or "micro-domains".

The Omega-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA), have been observed to have anti-inflammatory effects. Dietary omega-3 fatty acids are easily incorporated to a large extent into the cell membrane lipids and thus influence the composition of the rafts. The mechanisms by which these poly-unsaturated fatty acids carry out these functions are not completely understood. However, recent studies have shown that intake of ω -3 fatty acids decreases levels of C reactive proteins (CRP), IL-6 and tumor necrosis factors (TNF- α) [6]. These actions are hypothesized to be effected mainly via regulations of the transcription factors; nuclear factor κ and peroxisome proliferator activated receptor. Other inflammatory markers which are reduced such as; monocytes chemo-attractant protein 1 (MCP-1), intercellular adhesion molecule 1 (ICAM-1) and lipoprotein-associated phospholipase A2 (LpPLA-2), also play important roles in increasing oxidative stress [7].

The rate limiting enzymes in the production of these fatty acids are the Δ -3 and Δ -6 fatty acid desaturases. The depreciation in activity of these enzymes may be responsible for the depletion of the PUFAs in sickle cell disease (SCD) patients, which occurs to a greater extent in those with severe disease. Protein synthesis is consequent upon DNA transcription and any mutation in genes coding for these enzymes will

invariably affect these function. This review aims to explore the inflammatory pathways involved in SCD as well as the impact of the PUFAs, desaturases and their complex interactions with regards to the myriad of phenotypic presentation observed. The review also focuses on the background genetic variations with regards to FADs activity that may be responsible for these observed phenotypic differences as well as suggest possible therapeutic implications.

Inflammatory proteins and the role of membrane lipids

The red cell membrane is made of a phospholipid bi-layer and the underlying structural protein scaffolding lattice arranged in the format regarded as the vertical and horizontal associations. The membrane phospholipids are made up of a polar head and a non-polar fatty acid tail. There are proteins embedded in this bi-layer that tether them to the underlying protein lattice. The lipids move rapidly within this bi-layer and the areas where a high concentration of these lipids and proteins occur are called 'micro-domains' or rafts. [8]. The lipid contents of these micro-domains to some extent affect the function of the membrane proteins/ receptors embedded in them [9,10]. These rafts which take part in signal transduction are rich in sphingomyelin, cholesterol and saturated glycerol phospholipids [10]. Certain dietary lipids are easily incorporated into the membrane and reduction in membrane cholesterol content tends to cause dissolution of these rafts [11,12].

There also exists an active exchange of lipids between the inner and outer leaves of the bi-layer, this is not random and is controlled by certain proteins in the membrane. Sphingomyelin (SM) and phosphatidyl choline (PC) – the choline containing phospholipids, are more abundant on the outer layer, while phosphatidyl ethanolamine (PE) and phosphatidyl serine (PS) predominate on the inner layer. This balance is maintained by the ATP- dependent membrane transporter called 'flippase', which is a Mg^{2+} -ATPase bound to the membrane [13]. Recently the enzyme amino-phospholipase translocase has also been discovered to be important in the maintenance of the lipid distribution [14]. This dynamic balance is necessary for the optimal functioning and survival of most eukaryotic cells.

The phospholipids in the cellular membrane contain fatty acids (FA), and the type of diet an individual consume will directly determine the type of FA (saturated or un-saturated) that will be incorporated into the phospholipid bi-layer, and consequently will define the 'structural integrity' and physical characteristics of the cellular membrane [3,15, 16]. The lipid composition of the cellular membrane will therefore, govern the effectiveness of its fluidity and hence its membrane fluidity index (MFI) [15-17]. The PUFA's (EPA and DHA) have been exhibited in many studies to increase the MFI and thus create a peculiar environment for the membrane proteins-transporters, ion channels and receptors and by doing this influence their activity. The physical state of the membrane needs to be optimally balanced in terms of fluidity [16] in order for cells to carry out their regular functions and activities maximally. In addition, ideal MF will decrease the cells susceptibility to damage and death significantly[3,15]. These areas of the lipid membrane bi-layer, rich in cholesterol, sphingomyelin and saturated glycerol phospholipids are called rafts or micro-domains. Apart from their membrane function these rafts are also known to influence intra-cellular signaling pathways, [8,18]thus affecting gene expression via their action on transcription factors [19,20]. These fatty acids are known to effect these actions by acting on transcription factors; nuclear factor κ ,

binding protein for the sterol regulatory element [21] and peroxisome proliferator activator receptor (PPAR) α and γ [22,23]. This ability has been ascribed to DHA and EPA to varying degrees depending on the nature of the cell. Any reduction in the incorporation of cholesterol into the membrane is known to cause dissolution of these rafts.

Nuclear factor- κ B upon activation is a potent transcription factor for several inflammatory cytokines; cyclooxygenase (COX)-2, adhesion molecules and nitric oxide synthase. Upon activation by bacterial endotoxins, oxidative stress, UV light or inflammatory cytokines, it moves from the cytosol to the nucleus to enhance production of pro-inflammatory cytokines. Several studies have shown that EPA and DHA inhibit this inflammatory action in monocytes [24], dendritic cells [19], macrophages [25] and endothelial cells [26]. They inhibit monocyte NF- κ B and consequently modulate transcription of TNF- α , [22] (which plays a central role in inflammation) this influences the other factors to indirectly control fatty and triacyl glycerol metabolism. The PPARs are transcription factors which regulate gene expression by binding to retinoic-X-receptors, which are bound by the ligand; 6 cis-retinoic acid [27]. The genes coding for several enzymes involved in β -oxidation are regulated by the PPAR- α isoform, predominantly found in the liver. PPAR- γ is found in inflammatory cells and adipocytes and is involved in increasing insulin sensitivity and regulating production of inflammatory cytokines [28]. PPARs are activated by PUFAs, especially DHA to cause decreased production of IL-6 and TNF- α as well as effects its metabolic role on adipocytes [29]. This is proposed to be the mechanism underlying the effect of omega-3 PUFAs in reducing inflammation and plasma glycerol and improving insulin sensitivity in diabetics.

Other effects of the variations in the lipid membrane are mediated via the availability of arachidonic acid, an important substrate in the production of very important bio-active lipid end products - prostaglandins, thromboxanes and leukotrienes. These eicosanoids are known mediators of inflammation causing, platelet aggregation, smooth muscle contraction and vaso-dilatation. Intake of long chain fatty acids (DHA and EPA) have been observed to cause decrease in the production of these pro-inflammatory eicosanoids from arachidonic acid as well as production of weaker or less inflammatory analogues – prostaglandin E3 and thromboxanes A3 [30]. Recent studies have shown that EPA and DHA are also substrates in complex biosynthetic pathways that give rise to anti-inflammatory mediators known as resolvins, protectins/neuroprotectins and maresins [31-33]. These mediators also take part in immuno-modulation and are currently being hypothesized to be the major pathway through which polyunsaturated fatty acids carry out their anti-inflammatory actions.

The omega 3 fatty acids bind to G-protein coupled receptors; GPR40 and GPR120 are found on Adipocytes and inflammatory cells [34]. The GPR 120 acts via its agonist, GW9508 to inhibit response of macrophages to endotoxins [35]. This is achieved by activating the inhibitory subunit (I κ B) of NF- κ B, and thereby inhibiting production of TNF- α and IL-6. DHA and EPA are known to activate the genes for GPR120, and their anti-inflammatory effects are to some extent dependent on this pathway. Previous studies have shown that the anti-inflammatory effects of DHA, was absent in GPR120 knockdown cells, [27] further substantiating this assertion. This implies that the inhibitory effects of DHA on NF- κ B, most likely occurred via the GPR120 pathway. However, both pathways may be involved in varying degrees in different types of cells.

Alteration in Membrane Lipids in Sickle Cell Disease

In mammalian cells this peculiar distribution of the membrane lipids is of utmost importance in the maintenance of its integrity. The red cells in their journey through areas of high oxygen tension are particularly susceptible to the development of reactive oxygen species [36,37]. Membrane damage due to increased oxidant stress as a result of formation of oxygen free radicals and outstripping of the anti-oxidant mechanisms is the main mechanisms of membrane damage in SC. Intravascular haemolysis of sickle red cells also release haeme protein and the enzyme arginase which mops up nitrous oxide and arginine, respectively. This produces further oxidative stress, resulting in production of more oxygen free radicals. These reactive oxygen species disrupt the apolar acyl chains of the membrane phospholipids and thereby making close-packing of the lipids impossible [29]. These oxidized portions are usually removed by the phospholipases via a deacylation process and replaced by selective uptake from plasma lipids in an ATP-dependent process called re-acylation. Inability to initiate this repair process leads to excessive exposure of PS on the outer leaf of the membrane. This exposure in platelets has been noted to be the initial step in activation of the coagulation cascade [38] and its occurrence in red cells lead to removal by macrophages as well as interactions with other cells and vascular endothelium [5]. In haemoglobinopathies the altered haemoglobin produces added oxidant stress on the anti-oxidant system, as well as the repair process of the membrane phospholipid layer [39,40].

Homozygous S individuals have been shown to have a reduced capacity to handle oxidative stress. This is coupled with their enhanced propensity of the red cell environment to generate oxygen free radical due to its iron content and passage through regions of high oxygen tension, as it traverses the vasculature. There is also an observed reduction in the activity of plasma as well as membrane bound anti-oxidants notably, plasma retinol, α -tocopherol and β -carotene and the red cell Cu/Zn-superoxide dismutase and Se-glutathione peroxidase [41]. The changes in these anti-oxidants have also been observed to correlate with the concentration of the fatty acids DHA and EPA [36], it has not yet become clear which is the cause or effect of the other. However, it has been proposed that the increased generation of oxygen free radicals leads to peroxidation of the more susceptible lipids- n-5 and n-6 fatty acids, and their subsequent depletion. It will however need to be demonstrated that an increase in the antioxidant levels will lead to a subsequent increase in omega 3 fatty acid levels.

Background Inflammation in Sickle Cell

Sickle cell patients with a high leukocyte counts have higher predisposition to develop vaso-occlusive crises as well as several other chronic complications of sickle cell disease. High levels of pro-inflammatory cytokines have been observed in these patients even while in steady state [42-44]. There's increasing evidence of the over-expression of the platelet selectin and its ligand (PSGL-1 or CD162), leukocyte selectin (CD62), β 2 integrin and platelet endothelial cell adhesion molecule-1 [6]. The vascular endothelium is also activated with increased expression of inter-cellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). This background state of smoldering inflammation persists, though on a lower scale in sickle cell patients in steady state. The initial contact and rolling of the leukocytes on the endothelium is mediated via CD62L, while the firmer attachment and diapedesis are mediated by β integrins and CD31 (PECAM-1 platelet endothelial cell adhesion molecule). These leukocytes then bind to each other and to the

platelets, red cells and reticulocytes to form larger aggregates, capable of occluding vascular lumen. This is similar to the initial steps observed in acute inflammatory processes and also involves endothelial activation via TNF- α and interleukin-1 β , released by monocytes. Periodic exacerbation of these events occur as vaso-occlusive crisis when external stimuli, like infections or physiologic stress, cause greater amounts inflammatory cytokines to be released by activated leukocytes.

These cellular adhesion molecules and their ligands occur as trans-membrane proteins and receptors, and their concentrations as well as activity are most likely affected by the lipid composition of the membrane. A large community-based study has shown an inverse relationship between the level of inflammatory biomarkers (notably IL-6, TNF receptor, ICAM-1 and P-selectin) and the blood EPA and DHA levels [45,46]. Drugs like hydroxyurea (HU) have been found to cause improvement even in patients in whom there was no significant change in their haemoglobin F levels. Apart from reducing the neutrophil count (and thereby depleting the overall number of inflammatory cells and ligands available), it has been found to also reduce expression of adhesion molecules by reticulocytes [47] and inhibit the translocation of PS to the outer membrane leaf of the red cell [48].

Possible genetic variations underlying observed changes in lipid metabolism and their possible influence on disease phenotype.

There are peculiar differences which exist in the genome of minority of individuals; these variations may lead to alterations in quantity or activity of certain enzymes. These small scale genetic variations include; single, double or multi-nucleotide polymorphisms (SNP), as well as microindels. Microindels are additions or deletions of extra nucleotides as well as occurrence of repetitions of 1 to 50 nucleotides, or combinations of both within the sequence. SNPs are single base changes in the DNA sequence, seen in less than 1% of individuals, which occur at an approximate frequency of 1 in 1000kb [49] and may or may not be of clinical significance. These mutations may occur in the coding region of genes encoding the fatty acid desaturases or inflammatory cytokines and thereby confer some phenotypic differences in terms of disease severity. Previous studies have observed that SNPs and microindels have been implicated in drug metabolism via their encoding gene- CYP2D6 with loss of enzyme activity and slow metabolism [50,51]. Alterations in the FADS enzyme genes are associated with the variations in the activity of these enzymes [52,53] as well as the K-ras gene in some other patient groups. Polymorphism in the genes coding for the FADS enzymes have been discovered in genome wide association studies by Guan et al. [54].

The FADs enzymes as well as phospholipase-A 2, play a pivotal role in the production of the anti-inflammatory omega-3 fatty acids and pro-inflammatory omega-6 fatty acids. Previous studies have shown low activity of the FADS enzymes as well as omega-3 fatty acids in sickle cell patients with severe disease. Meta-analysis of genes coding for the Omega-6 fatty acids have also revealed that gene loci in other chromosomes as well as distant SNPs may also affect the level and activity of this enzyme [54]. Modifications and changes in the genes coding for these enzymes will lead to loss of FADS enzyme activity or quantity and invariably lead to increased production of IL-6 and TNF- α , with subsequent worsening of the clinical features of the disease. We hypothesize this as a possible explanation for the variations in the phenotypic manifestations observed in sickle cell disease.

Future Therapeutic Implications

Therapeutic targets have so far concentrated on the red cell-induced vaso-occlusion and the haemolysis associated with the disease. This has included attempts at improving hydration, ameliorating anti-oxidant effects, increasing synthesis of HbF, replenishing plasma arginine and improving red cell rheology. This section will however focus on the possible prospective anti-inflammatory and fatty acid metabolic options.

Omega-3 fatty acids

The omega-3 fatty acids play a pivotal role in the synthesis of less inflammatory end products of the cyclooxygenase pathway, but more importantly suppress transcriptions of the genes of potent cytokines, which mediate inflammation. This has been shown by several studies in past in different patient groups, including SC [6] and diabetes, [55] where they have been noted to induce amelioration of the symptoms and improve well-being [56]. Dietary DHA and EPA, after incorporation into the cell membrane, have also been shown in previous studies to increase membrane fluidity and thus affect the activity of the transmembrane proteins [12]. These are natural products with little or no side effects, which given at certain doses, positively influence metabolic pathways to favor reduced inflammation, and consequently reduce disease severity. It must always be remembered that inflammation is an innate defensive mechanism and that its prolonged and sustained suppression, may likely lead to some untoward effects. However the PUFAs as opposed to the saturated FAs competitively bind to the Toll-like receptor 4, through which it influences the transcription of NF- κ , and consequently the COX-2 pathway, while the COX-1 pathway remains relatively untouched [25]. In this way the most beneficial inflammatory proteins and cytokines are to a large extent preserved. The therapeutic threshold for each disease entity, as well as the 'ceiling dose', beyond which severe adverse effects occur, has not yet been adequately established. The ratio of omega-3 to omega-6 fatty acids has also been observed to be of importance, since the excess of omega-6 FAs favors the increased inflammation, insulin resistance and macrophage infiltration [57]. Future research will most likely focus on the synthesizing omega-3 fatty acid analogues and mixtures of various PUFAs in order to achieve an effective dosing and metabolic equilibrium.

Fatty Acid Desaturases and their encoding genes

These enzymes occur as the rate-limiting enzymes in the production of the omega-3 fatty acids [58]. Increase in the production of these fatty acids can also be achieved by increasing the FADS enzyme activity or alternatively targeting their coding genes or transcription factors [59]. The FADS1 and 2 enzymes are encoded by exon 13 on chromosome 11q12.2-13.1 for FADS1 and exon 14 on chromosome 11q12.2, for FADS 2. Alterations in the form of polymorphisms affecting these genes will affect their transcription [52,53] and may be utilized to obtain an increase in production of PUFAs. Also these areas may be targeted by plasmids containing specific desired sequences which are capable of converting severe to mild disease phenotypes.

Hydroxyurea and its analogues

Majority of the effects of HU on sickle cell disease is carried out via its ability to reduce neutrophil count, increase HbF levels [60] and

reduce expression of cellular adhesion molecules [48,61]. Hydroxycarbamide also reduces the exposure of phosphatidyl serine on the surface of platelets and red cells [48]. It has been observed that HU does not cause an increase in HbF in all SCA patients, [62] and does not completely protect against end organ damage [63]. Previous research has also shown that HU mobilizes arachidonic acid from the inner leaflet of the red cell membrane via its action on COX-2 and cytoplasmic PLA-2 [64]. The eicosanoids DHA which is generated as a result of this process is thought to play a part in the therapeutic improvement seen in patients on HU. However the observation of a reduction in fertility and alterations in sex hormones after 6 months of therapy raises some concerns [65]. There arises the need to increase efforts to produce a safer analogue of this very important molecule, which may also be used in combination with drugs acting via other pathways at less toxic doses.

Inhibition of adhesion molecules

Several adhesion molecules are being targeted in on-going trials. The selectin inhibitors, Rivipansel and low molecular weight heparin both act by inhibiting platelet, leukocyte and endothelial adhesion [46]. HU to a large extent also diminishes the cellular and endothelial adhesion molecules by reducing the total number of white cells available for adhesion [66]. Omega 3 fatty acids on the other hand suppress the expression of the adhesion molecules β 2-integrin (CD-11b) and L-selectin (CD62L) in monocytes and granulocytes [6]. Intravenous gamma globulins as well as β 3-integrin inhibitors are also being tested [63]. These molecules are quite appropriate for both prevention and treatment of end organ damage, in some cases. However the search for a treatment of the most prevalent form of crisis- the acute vaso-occlusive (bone pain) still remains elusive. Emergency therapeutic drugs are still required which should be capable of adequately aborting acute vaso-occlusion.

Resolvins/Protectins pathway activators

The resolvins, protectins and maresins are end product of the metabolism of DHA and to a lesser extent EPA, via complex biosynthetic pathways. They seem to play an important role in suppressing inflammation and have been hypothesized to be the major pathway through which n-3 fatty acids achieve these ends [67]. They also play a role in modulating immune functions via their direct effect on gene expression, targeting genes coding for cytokines, cyclooxygenase, nitric oxide synthetase and metalloproteinases [20,68]. Therefore these mediators or their analogues can be given directly, as a form of more specific therapeutic targeting, to achieve these desirable ends.

Targeting genes for transcription factors in the inflammatory pathway

Down-regulation of the transcription factor NF- κ B has been noted repeatedly to be responsible for majority of the actions of most important prospective molecules in trial. Synthetic products targeting this transcription factor can be developed and tried in the clinical setting. Gene therapy has been on the lime-light for some years now, but newer target genes should be explored. Ex-vivo manipulation of autologous haemopoietic stem cells have also been tried, especially for patients who do not have a matched donor [69]. Newer and safer viral vectors have also been developed to overcome the occurrence of genotoxicity. The beta globin gene chain in erythropoietic stem cells had been used in the past, but current and future researches are

focusing on the pluripotent stem cells [70]. It may also be helpful target the inflammatory genes if these makes transfection and uptake more tolerable, as this may serve to ameliorate disease severity.

Declaration of Interests

The authors report no declaration of interests.

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