Research Article

Effect of Fe Chelators on Fe Oxidative Cellular Metabolism: An Update on the Role in Human Health

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ABSTRACT

Fe, a micronutrient, is a key element in human nutrition, but its excess could lead to oxidative stress by actively catalyzing the generation of free radicals. The purpose of this mini-review is to briefly summarize the Fe chelators' central role on human health, in terms of oxidative effects. It is shown the impact of the nature of the Fe complexes used in the treatment of Fe deficiencies, and on research studies on the effects of exogenous Fe supplementation in experimental models. The current advances in the search for Fe chelators that could be useful in the treatment of Fe-overload condition generated, either as a consequence of pathological clinical situations or of the treatment of other conditions, are also shown. Even though chelators have been studied for a long time, Fe metabolism is very complex and many advances are still required in order to fully know the nature and function of catalytically active Fe in human health and therapies. Therefore, the research on the specific characteristics of the wide spectra of Fe complexes and Fe chelators employed in therapy, is a field constantly growing.

THE RELEVANCE OF CHELATION IN THE CELLULAR Fe UPTAKE AND METABOLISM

Fe is an essential micronutrient and it is required for the adequate function of all the organisms, and plays a central role in essential cellular functions, such as oxygen (O_2) transport, energy metabolism of mitochondria and electron transport [1]. Besides the significant amount of Fe present in the diet, its bioavailability is low [2]. Complexing agents naturally present in foods, such as sugars, amino acids, ascorbic acid, and glycoproteins, can form complexes with Fe by maintaining it in a soluble form and favoring its absorption, while phytates, phosphates and carbonates, oxalates, bicarbonates, and dietary fiber bind Fe forming relatively insoluble complexes [3]. In animal organisms, Fe enters the body through food intake, and both, Fe^{2+} and Fe^{3+}, can be absorbed by the organisms by different mechanisms and distributed in the cells and the subcellular structures [4,5]. Normal adult individuals have a Fe content in the body of 3-4 g and the concentration is regulated by ingestion and excretion. Fe is transported in the blood bound to specific proteins such as Transferrin (Tf) and Lactoferrin (Lf), and Fe is stored in Ferritin (Ft) and hemosiderin in mammalian cells [2]. Cells can control Fe availability through different proteins, such us intracellular Ft and extracellular Tf [4] and Lf, and the way Fe is chelated is very critical for these processes. The labile Fe pool (LIP) present in all living cells, is defined as the Fe found

in the cytosol bound to low molecular weight compounds, such as ATP, GTP, ADP, citrate, oxalate, etc. [6]. Specifically, mammalian cells can accumulate abnormally high amounts of cytoplasmic Fe when they lack siderophore, which binds Fe through association with a Fe-trafficking protein, the lipocalin 24p3 [7,8]. Although it is still under study, some aspects of intracellular Fe homeostasis are conserved from bacteria through humans. Since Fe excess could lead to cellular damage by oxidative reactions [1], the use of Fe chelators in the designing of strategies to maintain its level within a very tight range of steady state concentrations is critical. This brief review presents the relevant features of Fe chelators and the formed Fe complexes usually employed in many aspects of the studies in this field. The main analyzed points are: Fe chelators and oxidative stress, Fe complexes and Fe chelators used in Fe overload scientific studies, Fe complexes used in the treatment of Fe deficiencies, Fe chelators used in the treatment of Fe overload conditions; that are shortly commented under the Discussion and conclusions section.

Fe CHELATORS AND OXIDATIVE STRESS

Fe contains missing electrons and can be considered as a radical. When Fe is not strongly bound to proteins, its one electron transfer capacity turns it into a critical catalyst for the formation of reactive species of O_2 (ROS) and N_2 (RNS). The generation of these substances (or chemical species) is recognized as a key factor that contributes to the damaging of lipids and proteins in biological membranes and DNA. Recently, Dixon et al. [9] proposed the concept of ferroptosis, a Fe-dependent, non-apoptotic mode of cell death characterized by the accumulation of lipid-ROS. Ferroptosis-inducing factors can directly or indirectly affect glutathione peroxidase activity through different pathways, resulting in a decrease in antioxidant capacity and accumulation of lipid ROS in cells, ultimately leading to oxidative cell death [10]. This Fe-dependent pathway was proposed to play an important regulatory role in the occurrence and development of many diseases and is considered as a promising factor on the treatment and prognosis improvement of related diseases [10]. Thus, it is critical for cells to maintain Fe homeostasis to be able to ensure its supplementation but preventing the excess [11]. Fe catalyzed the conversion of normal by-products of cell respiration, like superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), into highly damaging hydroxyl radical (•OH) through the Fenton reaction or by the Fe^{2+} catalyzed Haber-Weiss reaction, or into equally aggressive Fe^{3+} ions or O_2^-bridged Fe^{2+}/Fe^{3+} complexes. Fe^{3+} can be reduced either by O_2^- or by ascorbate leading to ascorbyl radical production. The ability of a chelator to generate a Fe-complex active or inactive, in terms of free radical production, is reflected by the reduction potential values ($E^{\circ\prime}$ in biological systems). This parameter is used to predict the spontaneity of an electron transfer process by calculating the differences between the $E^{\circ\prime}$ values of the oxidation and reduction half-reactions. A positive $\Delta E^{\circ\prime}$ indicates an energetically favored reaction, and then there is a range of $E^{\circ\prime}$ that potentially maintain the Fe redox cycling capacity. For instance, taking into account $\Delta E^{\circ\prime}$, when Fe is complexed with Ethylenediaminetetraacetic Acid (EDTA) as a chelator, it should be potentially able to react with H_2O_2 to produce •OH, meanwhile when Desferrioxamine (DFO) is the chelator, this property is lost because Fe has more affinity to DFO than to EDTA (4). Inside the cells, the majority of cellular Fe is found within different proteins (Ft, heme proteins, myoglobin and cytochromes or proteins with Fe-S motifs) and different compartments (such as mitochondria and nucleus), while another small Fe fraction exists as LIP, understand as the pool of weakly bound Fe that is not restricted in its catalytic activity towards the generation of ROS. The LIP can be enhanced anytime Fe exceeds the metabolic needs of the cell [12]. Chemical aspects of the Fe chelators, such as Fe-affinity, Fe-selectivity, molecular weight, and lipophilicity, in addition to stability and redox properties of the resultant Fe-complex drastically change the ability of the Fe-complex to catalyze radical generation. Thus, considering all these properties, Fe chelators are important factors to understand the ability of specific forms of Fe complexes to be efficient in terms of, either to fulfill Fe functions or be useful in Fe therapies.

Fe COMPLEXES AND Fe CHELATORS USED IN Fe OVERLOAD SCIENTIFIC STUDIES

Fe overload treatment for scientific studies may lead to different tissue or cellular Fe accumulation. The kinetics of Fe incorporation and its amount depends on the type of Fe complex administered, the dose, the way of administration and the organ under study. For example, the acute administration

of Fe-dextran leads to the deposit of Fe in the endothelial reticulum system [13], meanwhile the administration of Fe in a chronic way as carbonyl-Fe favors the deposition in hepatocytes [14]. In the scientific studies of Fe-dependent effects, the Fe compounds could be given either as parenteral or oral administration and the modality in either an acute, sub-chronic or chronic fashion. The obtained results through intraperitoneal administration of Fe-dextran resemble in either parenteral Fe or transfusion effects. The administration to rats of dietary carbonyl-Fe produces clinical situations, such as occur by Fe accumulation in Hemochromatosis (HH), and in the Fe genetic disorder in which the body simply loads too much Fe [2]. In both models, alterations in the oxidative metabolism and cellular antioxidant defenses were reported in the liver and blood of rats overloaded with Fe [15,2]. As it was mentioned previously, Fe not strongly bound to proteins can participate in the formation of reactive species, so it is important not only the evaluation of the total Fe accumulation but also the LIP content. Also, different treatments applied with the same Fe complex may result in different oxidative stress processes. In this regard, Piloni et al. [16,17] reported that acute and sub-chronic administration of Fe-dextran lead to a maximum Fe deposit in rat brain after 6 and 2 h of treatment, respectively reaching the same total Fe, but with different LIP content.

The total Fe content can be measured through different techniques employing appropriate Fe chelators. After reduction with thioglycolic acid, the absorbance of bathophenanthroline in tissue can be measured by spectrophotometry [16,17]. Fe reduction rate by biological systems could be assessed by employing the chelator 2,2'-bipyridyl, since during reduction under air, the re-oxidation of Fe^{2+} was inhibited by the 2,2'-bipyridyl and the formation rate of the stable Fe^{2+}-(bipyridyl)3 complex is measured at λ =520 nm [18]. The most applied techniques to measure LIP used fluorescent dyes as Fe chelators, such as Calcein (CA) or Phen Green SK [19]. Using the CA-dependent fluorescence technique LIP detection was feasible when Fe^{3+} was chelated to citrate (Fe-citrate), ATP (Fe-ATP), EDTA (Fe-EDTA), or histidine (Fe-histidine), and also when a synthetic Dinitrosyl Fe Complex (DNIC) was employed. However, LIP detection by this dye was not possible when a synthetic Mononitrosyl Fe Complexes (MNIC) was tested, suggesting that this complex was elusive to this method for LIP measurement, and Electron Paramagnetic Electronic (EPR) assays should be employed [12].

Camiolo et al. [20] reported the generation of Fe overload by Ferric Ammonium Citrate (FAC) in human mesenchymal stem cells and liver and intestine of zebrafish, while treatment with α-Lipoic Acid (ALA) resulted in a significant reduction of Fe storage and oxidative stress due to its antioxidant properties. Fe complexes, such as Fe-EDTA, were also used to increase the bactericidal effects of H_2O_2, which is widely used as a disinfectant by generating oxidative stress. Du and Chen [21] reported that *Escherichia coli (E. coli)* added to a beef extract culture medium and treated with H_2O_2, and the Fe-EDTA complex resulted in a large increase in the mortality of E. coli. The death of the cell of *E. coli* was significantly inhibited by the presence of catalase, but not by vitamins C and E, suggesting that •OH were not generated during the Fe-EDTA and H_2O_2 reaction.

Fe COMPLEXES USED IN THE TREATMENT OF Fe DEFICIENCIES

Despite Fe's plentifulness on earth (5% of the earth's crust), Fe deficiency is extremely common in humans, and is the most prevalent cause of anemia worldwide [22]. Erythropoiesis-related demands for Fe are created by three variables: tissue oxygenation, erythrocyte turnover, and erythrocyte loss from hemorrhage. Tissue oxygenation requirements and erythrocyte production generally remain stable during adulthood in the absence of hemorrhage, disease, or altered physical activity, thus Fe homeostasis also remains stable. Fe deficiency has maintained itself as the most common anemia and nutritional disorder despite effective treatment represents a major challenge to public health efforts. Multiple obstacles involving economics, cultural barriers, and infectious diseases converge and make the eradication of this disease more difficult. About 30-50% of anemia in children and other groups is caused by Fe deficiency, over 1.6 billion people are anemic [23], and several hundred million manifest Fe deficiency anemia. How much Fe is needed depends on age and gender. Men need at least 8 mg/day and women need more than 18 mg. Since Fe is absorbed from the food in the small intestine, conditions like celiac disease, ulcerative colitis, or Crohn´s disease could make it difficult the intestinal absorption. Also, surgery such as gastric

bypass and medicines used to lower stomach acid can affect the ability of the body to absorb Fe [24]. It is predicted that increased research and understanding of fundamental Fe biology will assist in devising new strategies aimed toward the global elimination of this disease. Several treatments have been used to counteract Fe deficiencies, such as oral and intravenous (IV) Fe ones. Recently, oral Fe treatments include sucrosomial-Fe, a new generation drug with high absorption and bioavailability and a low incidence of side effects [25]; Fe-sulfate and -fumarate, and ferrous sulfate heptahydrate, a new oral solution with high levels of tolerability in young children with mild or moderate Fe deficiency anemia [26,27]. Oral Fe replacement is the primary treatment strategy for Fe deficiency anemia but may be inadequate for some patients due to intolerance, impaired absorption, significant ongoing bleeding, or nonadherence [28]. For these patients, IV Fe treatments, such as Fe-dextran complex, and non-dextran products, such as Fe-gluconate, Fe-sucrose, ferumoxytol, ferric-carboxymaltose, Fe-polymaltose and Fe-isomaltoside [28,29] may be indicated. IV Fe formulations may be used in those patients intolerant to oral Fe or for conditions in which oral Fe is ineffective or potentially harmful. Some of these possible cases could be some anemias, chronic kidney disease, hereditary hemorrhagic telangiectasia and inflammatory bowel disease [30]. Although IV Fe has been shown to raise hemoglobin levels more effectively than oral Fe, sometimes IV Fe administration can produce hypersensitivity reactions, which in the past were largely due to high molecular weight Fe-dextrans, which are no longer commercially available [30].

However, there are some anemias, like an inherited chronic haemolytic anaemia, the Sickle Cell Disease (SCD), that requires blood transfusion that causes significant Fe overload [31]. In patients with chronic kidney disease, who are receiving long-term dialysis and require Fe treatment, IV Fe therapy was superior to oral Fe. This response was irrespective of the type of IV Fe formulation used and significantly more patients reached an increase in Hemoglobin (Hb) and Ft levels [32]. To evaluate the risk of anaphylaxis among different IV Fe products, Wang et al. [28] compared anaphylaxis risk between Fe-dextran and non-dextran products (Fe-gluconate, Fe-sucrose, and ferumoxytol) in patients which were not receiving dialysis. They have shown that Fe-dextran was associated with increased anaphylaxis risk, as compared with non-dextran formulations at first administration. Recently, it has been accepted that maternal Fe supplementation should be limited to actual insufficiencies, since excess Fe consumption can cause Fe overload and is linked to gestational diabetes, hypertension, and metabolic syndrome [33,34].

Fe CHELATORS USED IN THE TREATMENT OF Fe OVERLOAD CONDITIONS

On the other hand, patients with many pathologies require Fe overload treatment. Severe Fe increases were described in patients due to inappropriately Fe absorption (HH and adult alcoholics) or excessive dietary Fe (siderosis); or excess Fe as a consequence of the treatment of other pathologies (e.g. those mentioned before, atransferrinemia, thalassemia) where after a pathological decrease in Fe content a supplementation is indicated [4,34]. Moreover, EDTA chelation therapy (initially described by the Noble Prize Linus Pauling in 1968) is used as an antioxidant strategy, and in circulatory (e.g. angina) and inflammatory (e.g. rheumatism) disorders [35]. Even though the therapy was recommended by alternative medicine in the treatment of several pathologies, such as cardiovascular disease [36], atherosclerosis [37], and essential hypertension [38], it is not supported by the highest quality of evidence.

Despite the availability of specific Fe administration protocols in deficiency conditions, in some cases, it is necessary the use of Fe chelators, to avoid Fe-overload adverse effects. To be effective for the treatment of Fe overload disorders, ligands should be able to form safe Fe complexes, with no capacity to catalyze redox cycling [4]. So far, the most widely used Fe-chelators are DFO (Desferal®), deferiprone (DFP) or L1 (Ferriprox®), and deferasirox (DFS) or ICL670 (Exjade®). They are the three chelating agents currently approved by the US Food and Drug Administration [20]. Although Fe chelators and their effects are still under study, Anderson et al. [39] have confirmed that cardiomyopathy can be reversed with the IV administration of DFO. Furthermore, Fe chelation therapy can attenuate the progression of liver fibrosis and glucose intolerance in transfusion-dependent patients [20].

Many patients with Myelodysplastic Syndrome (MDS) require red blood cell transfusions for the management of symptomatic anemia. Such transfusions generate high risk of developing Fe overload. It was assessed the safety and efficacy of DFS in low

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or intermediate-1-risk MDS. Patients receiving previous treatment with DFO (Desferal; Novartis, East Hanover, NJ) or DFS (Exjade; Novartis), reduced Fe burden measured by reduction in serum Ft content and plasmatic LIP content [40]. Also, some patients suffering SCD can suffer Fe cardiomyopathy, and Coates and Woods [31] pointed out that it is not clear if organ damage is a consequence of Fe uptake from transfusions used as a treatment, or due to the complications caused by the pathology. However, Meloni et al. [41] and Coates and Wood [31] suggested that it is a Fe related pathology, separable from SCD damage, that was detected in chronically transfused patients. In those cases where SCD patients require chronic transfusion, chelation therapy to reduce plasma and cytosolic levels of labile Fe is necessary. Chelators used in SCD are DFO, DFP (Ferriprox) and DFX (ExJade, JadeNu). Due to difficulties with administration and associated side effects with these three molecules (DFO, DFP and DFX), the search continues for an efficient nontoxic orally active Fe-chelator. In this regard, Pangjit et al. [42] described the properties of 1-(N-acetyl-6-aminohexyl)-3-hydroxy-2-methylpyridin-4-one (CM1) with promising studies in thalassaemia patients that will be extended to be included in future clinical trials.

Administration of Fe chelators is not only used in idiopathic Fe overload pathologies, but also in the treatment of diseases that lead to symptoms associated with the accumulation of Fe in excess, as well. In this regard, Rosa et al. [43] suggested that the milk derivative bovine Lactoferrin (bLf), a multifunctional glycoprotein that possesses high homology and identical functions with human Lf could be used in the treatment of pregnant women with hereditary thrombophilia and suffering from anemia of inflammation, when administered under fasting conditions. Cui et al. [44] reported that the oral Fe chelator DFP is protective against retinal degenerations associated with oxidative stress, such as in glaucoma (a progressive neurodegenerative process affecting the retinal ganglion cells and the optic nerve) acting as a Fe- chelator.

Figure 1 briefly summarizes the main Fe-complexes and Fe-chelators that are currently being used both, in the treatment of Fe deficiencies, and Fe overload conditions; either in scientific studies or in the treatment of clinical situations. The information included in Table 1, is aimed to show examples of the chelators used (or proposed to be used) in human health that has been studied in terms of the ability of the complexes that they form with Fe to generate oxidative stress. Moreover, the chelators DFO, DFP, DFS, and CM1 are employed to treat patients with Fe overload, and they are aimed to sequester cellular Fe and favored its excretion. Thus, the formed Fe-complex is expected to be not capable of generating oxidative stress. The effectiveness of the chelator in these treatments not only depends on its ability to take Fe but, in forming Fe complexes without the possibility of catalyzing reactive species. Extensive laboratory research is still essential to select the optimal chelator for application in therapeutic strategies in clinical treatments.

DISCUSSION AND CONCLUSIONS

Even though it is widely recognized that Fe toxicity is associated to its ability of catalyzing free radical reactions, not all the Fe forms are equally efficient. Mostly Fe forming the LIP is the most efficient Fe fraction in acting as a free radical promoter. In the treatment of Fe deficiencies, as it was summarized here, many options are available for clinical protocols. However, the administration of Fe-complexes to the patients should be carefully monitored, since Fe-overload could appear. This condition must be treated, as other pathologic clinical situations involving Fe overload. Therapeutic strategies are designed to chelate either Fe from the LIP, or Fe loosely bound to Ft to avoid Fe-related oxidative damage.

Basic biochemical knowledge is critical to successfully achieve the goal of improving the protection of the health of the individual, and the optimization and effectiveness of medical procedures for the Fe overload-dependent diseases. Different strategies have been taken to fulfill this goal. Since Ft was described by Murray-Kolb et al. [48] as an efficient source of Fe, Liu et al. (49) suggested that opening Ft pores to enhance Fe chelation, could lead to faster removal of Fe from Ft during Fe overload. However, even though new chelators that did not alter the global properties of the Ft pores in the native structure were proposed [49], they did not prosper as much as the current Fe chelators therapies that are mainly targeted to Fe outside the cells or in the LIP.

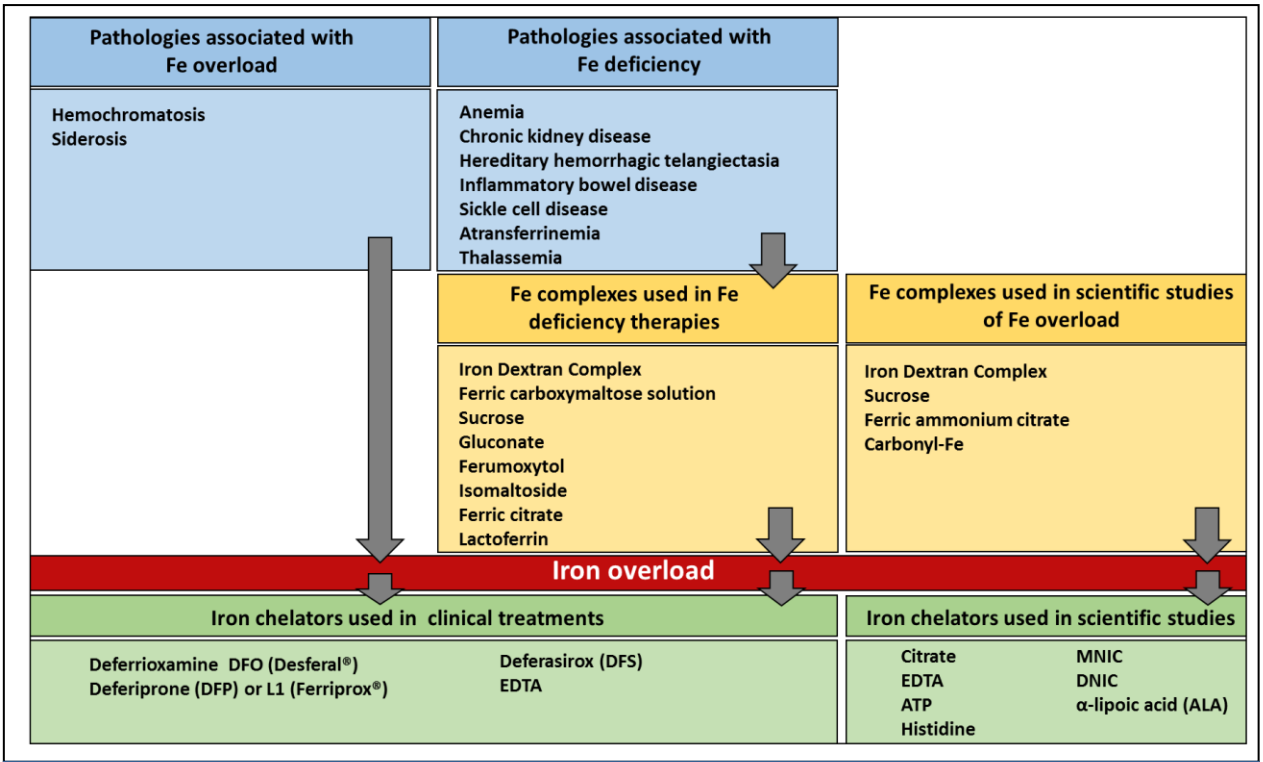

Figure 1: Fe complexes and Fe chelators used in the treatment of Fe deficiencies and under Fe overload conditions either in scientific studies or in the treatment of the clinical pathologies.

Table 1: Examples of chelators used (or proposed to be used) in clinical medicine, and their effect on oxidative status.

Chelator	Chemical formula	Clinical use	Oxidative information
EDTA 2-[2[bis(carboxymethyl)amino]ethyl-(carboxymethyl)amino]acetic acid	$C_{10}H_{16}N_2O_8$	EDTA chelation therapy is used in alternative medicine but it is not supported by the highest quality of evidence [36].	Fe-EDTA inhibits lipid peroxidation, and increases hydroxyl radical production [46].
DFO N-[5-[[4-[5-[acetyl(hydroxy)amino]pentylamino]-4-oxobutanoyl]-hydroxyamino]pentyl]-N'-(5-aminopentyl)-N'-hydroxybutanediamide	$C_{25}H_{48}N_6O_8$	In Fe overdose, HH either due to multiple blood transfusions or an underlying genetic condition [4]. Treatment for spinal cord injury and intracerebral hemorrhage [45]. Cardiomyopathy can be reversed with the intravenous administration [39]. In MDS and SCD patients wich require red blood cell transfusions [40,31].	Fe-DFO complex does not catalyze neither lipid peroxidation nor radical generation [46]. It reduced Fe burden in MDS patients measured by reduction in serum Ft content and plasmatic LIP content [40].
DFP 3-hydroxy-1,2-dimethylpyridin-4-one	$C_7H_9NO_2$	SCD patients require long-term chronic transfusion [31]. Protective against retinal degenerations associated with oxidative stress [44].	Antioxidant effects in vivo, in vitro and in clinical studies. Lipid peroxidation inhibition and increase in glutathione levels in the liver of iron loaded mice [47]. Reduction of excess toxic iron in the brain, ataxic gait and neuropathy in general, in Friedreich ataxia patients [47].
DFS 4-[3,5-bis(2-hydroxyphenyl)-1,2,4-triazol-1-yl]benz	$C_{21}H_{15}N_3O_4$	In low or intermediate-1-risk MDS [40].	Reduced Fe burden measured by reduction in serum Ft content and plasmatic LIP content [40].
CM1 1-(N-acetyl-6-aminohexyl)-3-hydroxy-2-methylpyridin	$C_{14}H_{22}N_2O_3$	In thalassaemia patients [42].	CM1 is able to remove non transferrin bound iron from the ser

In designing Fe chelators for clinical application different aspects should be taken into account. Not only the chelating chemical aspects (such as metal selectivity, complex stability and redox activity) but also, pharmacological aspects (such as administration-routes, the ability of the compound to reach the target site and toxicity) should be considered. As it was previously reviewed [4], hydroxamic acids, aminocarboxylates, catechols, hydroxypyridones, pyridoxal isonicotinoyl hydrazone, desferrithiocins, triazoles, dialkylhydroxypyridinones, and hydroxypyridinone derivatives were explored to find Fe chelators with optimal performance when used for clinical treatments. However, the complex scenario generated by Fe nature seems responsible for the lack of critical advances on this matter over the last decade. Despite the efforts, DFO and DFS are the only products currently actively used in therapeutics [50]. DFO was introduced in the 1970s, and constitutes the most widely Fe-chelator used in therapy. DFO shows a poor intestinal absorption and a short half-life in plasma (5-10 min) [51]. Previously, the treatment with DFO formulation required parenteral infusion during 8-12 h for 5-7 days each week [52] in a dose 40-50 mg/kg/day. To improve the comfort of the patient other protocols of administration of the Fe-chelator were tested. Gattermann et al. [53] in a study enrolling 341 patients reported a DFO formulation, that once-daily oral DFO dose (20-40 mg/kg/day) reduced serum Ft, liver Fe concentration and labile plasma Fe levels in patients with transfusion-dependent anemias, including MDS. Thus, this is the treatment currently in use. Deep understanding of the biochemical mechanisms of Fe-dependent cellular reactions, and of the Fe chelators and the Fe complexes formed, are strongly linked to clinical responses. The focus in the equilibrium of these many aspects should help to find new and efficient drugs and/or to characterize the impact of DFO in morbility and mortality in patients with specific pathologies, such as MDS.

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