

Review Article

THERAPEUTIC ADVANCEMENTS IN MANAGEMENT OF IRON OVERLOAD–A REVIEW

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ABSTRACT

Removal of excess of free iron, produced under certain physiological conditions in human body, has always been of great concern for therapists worldwide. A number of naturally occurring and synthetic ligands are being explored for maximal iron decorporation, enhanced patient compliance and minimal side effects. Siderophores are the most important class of currently used chelators and few of them are approved for treating iron overload. This review focuses on the developments taking place in the field of iron overload treatment to find the most novel, efficient and safest iron decorporating agent. The importance of many natural and synthetic iron scavengers, having potential for clinical application in the treatment of iron overload and their associated side effects, are discussed along with the currently followed methods. Special emphasis is given to review the status of new ligands, combination therapy and methodologies adopted for overcoming limitations of existing therapeutic agents for treatment of different iron overload disorders.

**Keywords:** Iron chelation, Iron overload, Phlebotomy, Siderophores, Deferasirox.

INTRODUCTION

Iron is an essential and most abundant transition metal in human body. Iron overload is a condition of excess iron stores in different parts of body. Since very little iron is excreted from the body normally, it becomes a potentially toxic heavy metal in case of accidental ingestion, transfusional siderosis (e. g.  $\beta$ -thalassemia, sickle cell disease) or hereditary conditions (e. g. hereditary hemochromatosis) leading to the production of destructive free radicals [1, 2] and life threatening complications such as cirrhosis, hepatocellular cancer, diabetes and heart diseases [3]. According to the Institute of Medicine of the National Academy of Sciences, Washington, the tolerable upper level of iron intake for healthy adult males and females is 45 mg/day [4].

Transferrin (Tf) (fig. 1) is a freely circulating natural iron chelator in human blood responsible for uptake of free iron after intestinal absorption and storing it as intracellular ferritin or hemosiderin. In normal humans, Tf is only 25-30% saturated with iron while in iron-overload it becomes completely saturated, leading to the appearance of non transferrin bound iron (NTBI) in blood and tissues.

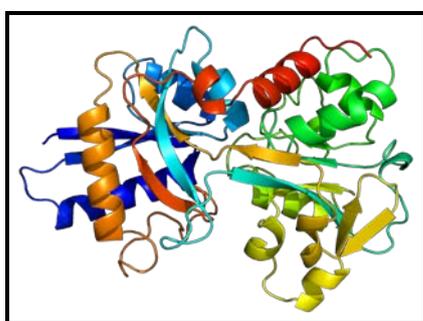


Fig. 1: Structure of protein transferrin (<http://en.wikipedia.org/wiki/Transferrin>)

Efforts are being made since long time for effective diagnosis and management of free iron in human body. Phlebotomy and chelation therapy, either alone or in combination, are currently the most widely used clinical practices to reduce iron overload. Lot of work is being done all over the world to find the most suitable therapeutic agent having enhanced efficacy and reduced toxicity so as to overcome the limitations of commercially available iron decorporating agents.

Iron toxicity

The overburden of iron in body leading to the production of NTBI and tissue iron are commonly called as 'free iron'. Free iron ( $Fe^{+3}$ ) in body acts as antioxidant because of its ability to gain and loose electrons. The highly reactive free radicals generated by free iron could damage vital organs and tissues of body (fig. 2), by reacting with surrounding biomolecules [5-7], as indicated by increase in biomarkers in iron overloaded thalassemia and sickle cell patients [8].

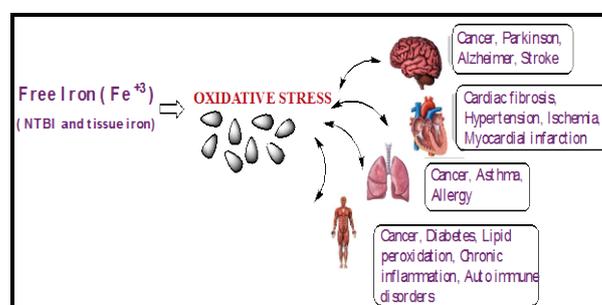


Fig. 2: Effect of oxidative stress on vital organs of human due to free blood circulating and tissue iron

These oxygen free radicals may be utilized by cancer cell for growth and indefinite division [9]. Iron toxicity is also associated to lipid peroxidation [10], increased plasma histamine and serotonin in patients suffering from hemochromatosis, sickle cell disease, thalassemia, myelodysplastic syndrome, African iron overload, Atransferrinemia, sideroblastic anemia etc.

Classical method of iron decorporation-phlebotomy

Decorporation of any toxic substance from human body has been an important practice since the ancient times. The ancient medical practitioners used to remove the blood borne toxins by removal of excess blood from patient's body [11]. This clinical process of drawing large volume of blood from patient's body is called phlebotomy. It is the simplest, safest and most inexpensive treatment for excess iron decorporation [12]. The procedure involves venipuncture in which blood is removed from veins of patient's arm, through a narrow bore needle, for a period of approximately 30-45 minutes. Before initiating the procedure, the

practitioner must be aware of the patient's serum ferritin levels (men  $\geq 300 \mu\text{g/l}$  & women  $\geq 200 \mu\text{g/l}$ ).

Studies have suggested that decrease in serum ferritin level is the most reliable indicator for monitoring the progress of therapeutic phlebotomy instead of transferrin saturation or serum iron [13]. Initially the procedure is done till the serum ferritin levels reach 20% of the initial value after which the therapy should be done at regular intervals [14]. Therapy should be discontinued when serum ferritin level drops below 20-50 $\mu\text{g/l}$ . Twice weekly phlebotomies may be suggested for heavily iron-loaded patients. Phlebotomy procedure was found to mobilize iron from body stores when patients have iron level  $>1000 \mu\text{g/ml}$  were treated every 1-2 week with 500 ml of blood removed during each session depending on body weight [15]. As compared to women, men usually require twice the phlebotomy units to induce iron depletion [14].

Phlebotomy has been found beneficial in case of iron overload problems occurring due to periodic blood transfusions such as  $\beta$ -thalassemia and sickle cell anemia [16, 17]. Prolonged phlebotomy at a rate of 6-ml/kg-body weight, for several years in  $\beta$ -thalassemia patients, was found to be highly effective to mobilize excess iron from 48 patients under study [18]. Russell and coworkers used a combination of hydroxyurea treatment and phlebotomy to increase fetal hemoglobin and reduce the iron overload and observed decreased serum ferritin from average level of 3134 ng/ml to 617 ng/ml as well as non recurrence of strokes in sickle-cell affected children [19]. They suggested the combined treatment to be a better option rather than commonly used siderophores that suffers numerous side effects. A team of Chinese workers also found considerable decrease in serum ferritin levels among phlebotomy treated patient groups [20]. Bone marrow transplantation is adopted as a treatment procedure to cure thalassemia [21] and phlebotomy performed in such cases has helped patients to get relieved from the iron overload that persisted in their bodies for many years after treatment. Erythropoietin-assisted phlebotomy was found beneficial for thalassemia patients suffering from iron overload due to allogenic hematopoietic stem cell transplantation [22-24].

Therapeutic phlebotomy is the preferred treatment for *hereditary hemochromatosis* patients. Iron overload in such patients was also found to be associated with hyperlipidemia [25] as a result of mutation in HFE gene [26] and phlebotomy procedure was found to be highly effective for such patients [27]. Cardiac function was greatly improved in patients with primary hemochromatosis by undergoing aggressive phlebotomy [28-31]. Repeated phlebotomy has shown a drastic decrease in spleen ferritin level in iron overloaded rat models [32]. Eleven patients with chronic liver disease and associated iron overload showed an improvement in cytolysis when 300 ml of blood was removed from their bodies for five consecutive weeks [33]. Therapeutic phlebotomy showed resolution in some observed complications of iron overload such as elevated hepatic serum aminotransferases [34], hepatomegaly [35], cutaneous hyperpigmentation [36], hyperferritinemia [37] and rarely improves certain others such as diabetes mellitus [38, 39], cardiomyopathy [40] and refractory arrhythmia [41]. Regular therapeutic phlebotomy has resulted in an increase in longevity as well as quality of life [42]. Regular phlebotomy is suggested as the ultimate treatment for patients with end-organ damage due to iron overload [43].

However iron overload resulted hepatic cirrhosis cannot be resolved by therapeutic phlebotomy leaving the patients at higher risk for liver cancer [36]. Disadvantages associated with the procedure involve exacerbation of joint pains, difficulty to operate on young children, difficult methodology, excessive pain and non-compliance (Fig.3). Several physiological and psychological therapies are given for pain relief to patients undergoing therapeutic phlebotomy such as hot and cold packs, distraction and relaxation [44]. According to the French Superior Health Security, at-home phlebotomy should be recommended for hereditary hemochromatosis patients to reduce health care expenditures and improve patient compliance [45].

#### Current methods of iron decorporation

Repeated phlebotomy is often required to treat iron overload in patients with thalassemia [46] that leads to in compliance and

extreme pain. Also the therapy cannot be given to patients who are hemodynamically unstable and suffer congestion problems related to heart [47]. Therefore drug therapy is commonly prescribed to such patients. Currently drug therapy involving chelation of free iron from blood has become a routine practice for treating iron overloaded patients. The best way to mobilize a toxic metal out of the body is to chelate it with a specific ligand and wash off the stable ligand-metal complex through excretory system of body. Such ligands are mostly organic molecules called as chelating agents, chelators or sequestering agents that have a very high specificity for certain metal ions. Till now researchers have suggested a number of natural and commercially prepared ligands that can be used as iron chelators (fig. 4).

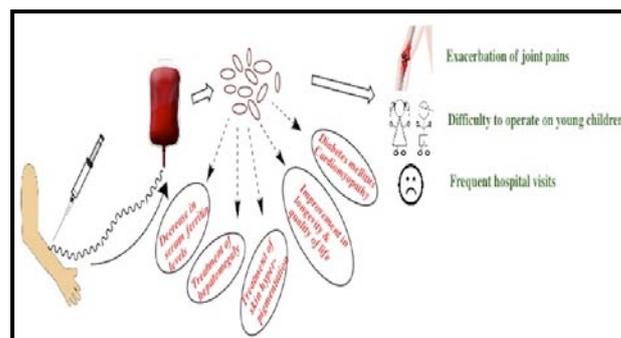


Fig. 3: Advantages and side effects of Phlebotomy procedure for iron decorporation

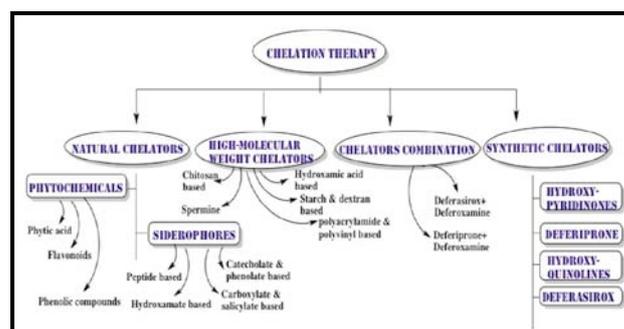


Fig. 4: Classification of chelators commonly used in iron chelation therapy

#### Natural chelators

The naturally occurring chemicals in plants are called phytochemicals. Many of these phytochemicals have been used over a long time and found to bind iron. Therefore workers are trying to explore certain natural agents as iron chelators in humans.

#### Phytochemicals

Phytic acid (InsP<sub>6</sub>, myo-Inositol hexaphosphate) which is the storage component of most cereal grains and seeds [48] has been found to inhibit free radical formation in a ferric iron standard assay and was effective similar to the commonly used chelator *Deferoxamine mesylate* [49]. Spinach, beans and nuts have significant levels of oxalic acid that inhibits iron absorption. An administration of wheatgrass juice of a week old leaves daily for six months reduced the iron burden in myelodysplastic syndrome patients [50]. The catechins (flavonoids) such as epigallocatechin gallate and epicatechin gallate are pharmacologically active components of green tea (suggested by many natural medicine practitioners) that form a complex with ferric ions and aid in the chelation of NTBI [51] as well as penetrate the brain barrier and remove the abnormally accumulated iron on dying neurons in certain neurodegenerative disorders such as Parkinson's disease [52]. The flavonoids and

phenolic compounds being the active components of plant extracts have been suggested to possess iron-chelating properties [53, 54] by acting as the free radical terminator [55]. Mohammad Ali and coworkers prepared extract from a series of plant parts and determined the amount and chelation properties of phenols and flavonoids present in them [56]. They found that plants showing highest content of these compounds also showed the highest iron chelating properties. Silybin (200 mg/kg), a flavonoid derived from the herb milk thistle (*Silybum marianum*), has been found to reduce elevated iron levels in kidney [57-59]. The iron chelating ability of curcumin and its diacetyl derivatives was found to lie in the enolic form of  $\beta$ -diketo moiety [60]. Later the orally available glycosyl derivatives of curcumin were prepared by reducing the molecular weight and hydrophilicity for enhanced absorption through small intestine [61]. Though a number of Ayurveda preparations are prescribed for treating iron overload yet none of them is currently approved for routine clinical application.

### Siderophores

Siderophores are low molecular weight (500-1000Da) iron sequestering agents produced by certain microbes in response to the utilization of insoluble form of iron present in soil [62, 63]. They exhibit strong specificity towards ferric iron. The research in this field began around six decades ago. The first siderophores to be chemically identified were amino acid conjugates of 2,3-dihydroxybenzoic acid i. e. itoic acid (2,3-dihydroxybenzoylglycine) [64]. Since then more than 500 different types of siderophores have been isolated and characterized [65, 66]. Siderophores play an important role not only to treat iron overload [67,68] but also to keep balance in the dietary iron intake and excretion in body fluids [69, 70]. Siderophores used in human medicine are classified according to the chemical nature of their metal binding functionalities as under:

#### Hydroxamate based siderophores

These hydroxamic acid containing siderophores are highly hydrophilic and can permeate membranes by forming neutral tris complex with ferric ion [71, 72]. More and more new compounds are being explored under this category due to their good iron chelating properties. Hydroxamates are poorly absorbed through the gastrointestinal tract and are therefore delivered through the parenteral route [73, 74]. Typical tri-hydroxamate siderophores are ferrichromes, fusarinines and ferrioxamines out of which only ferrioxamines are widely used for treating iron overload associated with a number of diseases in humans. It comprises of a group of trihydroxamate siderophores produced by actinomycetes [75]. Since 60 years the most common and effective therapeutic agent belonging to this group is desferrioxamine (DFO), which is an FDA approved hexadentate chelator for treatment of iron toxicity in human subjects [76-78] and is commercially marketed in the form of its methane-sulfonate salt. Before DFO, DTPA and dimeceprol were used [79, 80] and DTPA was found less effective as compared to DFO [81].

DFO is a colourless crystalline substance produced by the fungi *Streptomyces pilosus* [82]. It is impermeable through cell membrane and is taken up by cells through endocytosis for targeting primarily the excess iron present in liver and spleen. DFO has always been the drug of choice for iron decorporation due to its capacity to chelate iron effectively from ferritin and slowly from transferrin also [83-85]. A combination of DFO with pyrophosphate was suggested to mobilize iron from transferrin which otherwise was not possible with DFO alone [86,87]. DFO is effective not only in treating NTBI but also chelates hepatocellular iron for removal through urine and bile [88]. Free DFO has a very limited stability in plasma and gets metabolized even at very low temperature storage. In-vitro radioactive studies have confirmed that ferrioxamine complex formed after binding of DFO to iron stabilizes the unbound form of the drug in patient's plasma samples [89].

In the late 1960s it was realized that intramuscular DFO removed only limited amount of iron and hence ascorbic acid should be administered along with the chelator [90, 91]. Smaller doses of ascorbate (100-200 mg/day) given along with DFO chelated iron through urine as well as faeces [92]. DFO therapy was also found

beneficial in improving cardiac function of patients suffering from iron overload disorders due to severe congestive cardiomyopathy [93]. Long-term DFO therapy is capable to prevent the severe complications of transfusional iron overload and to improve significantly the life expectancy of thalassemia patients [94-96].

However DFO is not orally available and has a short blood circulation time (5-10 minutes) [97] due to which it is administered as painful continuous infusion for 8-12 h atleast 5 days/week [98, 99] and hence is poorly patient compliant. DFO therapy is expensive [100] with less than 10 mg iron removal in a single session. According to a recent FDA report, DFO treatment in 196 patients in age group of 10-59 years showed serious side-effects such as renal failure, gastritis, anaemia, decreased folate concentration and 6% of them even suffered the persisting iron overload (<http://www.ehealthme.com>). The chelator has been found unsuccessful in removing small increases in iron in rheumatoid arthritis patients [101]. Still DFO therapy is the choice of prescription for iron-overloaded patients today in view of it being FDA approved and currently most worked ligand.

#### Catecholate and phenolate based siderophores

Catechol-based ligands are the most powerful known siderophores [102]. Enterobactin is a cyclic tricatecholate siderophore capable of rapid removal of iron from human transferrin [103] as well as possess greater in-vitro affinity for iron than transferrin [104]. But catechol subunits are somewhat air-sensitive [105] and therefore require protection by methyl, benzyl or acetyl groups. Its trimeric backbone gets easily hydrolyzed at physiological pH and also has very low aqueous solubility [106]. Phenolic groups play a crucial role in iron chelation [107]. Cranberries are the richest source of total phenolic content among fruits [108] and quercetin present in cranberries is found to possess strong iron-binding properties in physiological conditions due to the presence of three iron-binding motifs [109]. Desferriothicin, a tridentate chelator of ferriothicin discovered in 1980s, is a subgroup of the ortho substituted phenolate class of iron chelators. It was the first orally available chelator capable of efficiently complexing with FeIII in 2:1 ratio in both iron overloaded rodent and primate models. Desferriothicin was however found to be highly nephrotoxic and therefore research is being done to prepare its analogs with similar oral activity but reduced toxicity. A few analogs prepared include Deferitrin, which underwent phase II clinical trials but was found to show severe renal toxicity [110]. FBS0701, an orally available member of the desazadesferriothicin class of siderophore related tridentate chelators has demonstrated equal iron chelation ability to deferasirox and appears less toxic and is currently under phase II trial [111]. R-apomorphine used in late stage Parkinson disease therapy shows strong iron chelation and antioxidant properties due to its catechol structure [112].

#### Carboxylates and salicylates

This class of siderophores contains hydroxyl and carboxyl functional groups as donor for iron chelation. Rhizoferrin was found to form 1:1 iron complex as DFO and parabactin [113]. A series of aminocarboxylate chelators designed with a pendant aromatic group resulted in strong iron chelation as well as potent protective effect against oxidative damage [114].

#### Peptidic siderophores

In view of the oral inactivity of DFO, pyoverdinin type of peptidic siderophores isolated from *Pseudomonas* species were reported to be insensitive to proteases and thus suggested as oral chelators for iron overload disorders [115,116]. The two pyoverdinin-PyA (from *Pseudomonas aeruginosa* ATCC15692) and PyF (from *Pseudomonas fluorescens* CCM2798) were found to be as effective as DFO in iron-loaded rat hepatocyte cultures due to inhibited release of aspartate aminotransferase and lactate dehydrogenase enzymes. Both chelators decreased the intracellular iron level and increased the concentration of the metal in the culture medium. Later two bidentate chelators-hydroxypyridin-4-ones (CP20 and CP94) were also demonstrated as orally active chelators in iron overloaded animals and were found to be equally effective as the subcutaneous DFO [117]. However a recent comparison of efficacy of pyoverdinin,

pyochelin and DFO to decorporate iron from ferritin showed DFO to be most effective in iron mobilization [118] and inhibition of lipid peroxidation [119] followed by pyoverdinin and pyochelins.

### Synthetic chelators

Owing to the expense and inconvenience associated with commonly used chelators such as deferoxamine or ferrichromes, a search for new and effective oral iron chelators has given rise to many synthetically produced compounds that possess improved pharmacological properties. Synthetic siderophores play an important role not only in our understanding of how natural siderophores function but also in the development of new improved therapeutic agents [120, 121]. Such chelators require easy and cheap production and must possess high specificity and high binding constant [122, 123] for specific metal ions. In 1960s, ferrioxamineB was the first siderophore to be synthesized to elucidate its chemical structure. Baret and coworkers proposed a series of tripodal iron sequestering agents based on *o*, *o'*-dihydroxybiaryl that were water soluble (due to sulfonation) and possessed well hydrolytic and oxidation stability [124]. The complexation of these agents with Fe<sup>3+</sup> in water showed similar results as the hydroxamate siderophores, but were poorer than the catecholate siderophores proposed by Raymond and coworkers.

### Hydroxypyridinones

Hydroxypyridinones are bidentate, cyclic hydroxamic acid containing oral iron chelating ligands and currently the most important class of chelators being explored. In the late 20<sup>th</sup> century it was suggested that highly hydrophilic polydentate compounds are mostly orally bioavailable and can act as effective chelators [125]. In this respect a series of hydrophilic ligands based on kojic acid and substituted linkers were prepared (L4-L8) and strongest iron chelating activity was observed with L8 expected to remove iron from Tf in further studies [126]. On the other hand lipophilic hydroxypyridinone 'CP094' was prepared that readily suppressed NTBI as well as process of lipid peroxidation due to iron overload in sickle cells as compared to DFO [127]. Clinical studies on tridentate oral ligand pyridoxal isonicotinoyl hydrazone (PIH) suggested its easy permeation through cell membrane [128, 129] and highest selectivity for Fe III at lower doses [130]. A novel group of hybrid and less toxic oral chelators, the 2-pyridylcarboxaldehyde isonicotinoyl hydrazone (PCIH) analogues and pyridylcarboxaldehyde 2-thiophenecarboxyl hydrazone (PCTH) were found two times more effective than DFO or PIH in Tf iron removal from primary myocardiocyte cultures and mice [131, 132]. A range of 3-hydroxypyridin-4-ones (CP102, CP117, CP41) were designed to target the hepatocellular low molecular weight iron pool and were found to mobilize completely the intrahepatic chelatable iron pool in rats [133]. Two lipo-hydrophilic hexadentate 3,4-hydroxypyridinone based compounds were prepared by attaching variable length alkyl-amino arms to tricarboxylic molecular scaffold of KEMP through amide linkage [134] so as to completely wrap free iron and form a stable complex [135]. Many researchers have reviewed the clinical applications and design of hydroxypyridinones for use in iron overload [136, 137]. N, N-bis(2-hydroxybenzyl) ethylenediamine-N,N-diacetic acid (HBED) is a hexadentate synthetic aminocarboxylate ligand that binds to iron in 1:1 ratio [138, 139]. The small size and lipophilicity of phenolic groups maintained ferric iron in redox inert ferrous form thus reducing the free radical toxicity [140]. Galey and coworkers synthesized pro-drugs which got activated by reactive oxygen species into ones with high iron chelation ability and found them effective against in-vitro oxidative injury [141]. The monoethyl and dimethyl ester [142] containing pro-drugs of HBED have been reported to possess great oral bioavailability. However the toxicity of the molecules was observed due to presence of two coordinating N-atoms rendering the molecules to exhibit high affinity for ZnII ions. In order to increase iron chelation ability of 1-hydroxy-2-pyridinones, their tetra-dentate derivatives with amino-bisphosphonate moiety were prepared [143]. A Canadian researcher recently claimed to produce a new novel compound similar to deferiprone for treatment of iron storage in brain tissues of Parkinson affected patients [144]. The low molecular weight bi- and tri-ligands have been prepared for greater

efficacy and higher oral bioavailability but are found potentially more toxic than hexadentate ligand [145] and therefore more research is required to prepare safer ligands.

### Deferiprone (Ferriprox®)

Alternatively deferiprone is also known as CP2/11/Kelfer. It is a hydrophilic small molecular weight hydroxypyridone (3-hydroxy-1, 2-dimethylpyridin-4-one) and the first oral iron chelator introduced in Europe in early 1980s that eliminates iron through faeces [146]. Among various hydroxypyridinones discovered, the 3-hydroxy-4-pyridinones are found most effective at physiological pH [147]. Large volume of work was done on this oral chelator between 1990-2000 till deferasirox was found more suitable alternative. Deferiprone is a neutral molecule that readily enters into cell membrane and forms 1:3 iron-chelator complexes. It has a half-life of 160 minutes [148]. The drug could efficiently remove myocardial iron compared to hepatic iron [149,150]. Many groups indicated in their study, greater reduction in iron overload induced cardiac failure due to deferiprone treatment [151, 152]. In a recent study, sixty patients who received thrice daily oral deferiprone (total dose 75 mg/kg/day), showed great enhancement in left-ventricular ejection function as compared to other 180 patients who received deferoxamine alone [153]. No deaths were seen in fifty-five thalassemia patients treated with deferiprone alone or eighteen patients with deferiprone-DFO combination, as compared to eleven cardiac arrests in DFO alone treated patients [154]. The drug has been found to cross blood brain barrier efficiently and is under phase II clinical trials for its safe and efficient use in treating neurodegenerative disorders such as Parkinson's disease.

However few case reports of deferiprone therapy indicated death due to fatal congestive heart failure with no other known etiology [155, 156]. In a recent case study of a 30-year-old thalassemia patient, the switching from DFO to deferiprone resulted in decrease in serum ferritin levels but also produced seizures after five months of treatment leading to permanent discontinuation of deferiprone treatment [157]. Therefore seizures may be one of the potential adverse effects of deferiprone therapy. Though the drug proved effective in treating iron overload of thalassemia patients [158-160], it also exhibited severe side effects of neutropenia and agranulocytosis and therefore remained as second-line defense only for which deferoxamine treatment remained inadequate [161-163]. Severe side effects such as arthropathy have been observed at dose of 100 mg/kg/day [164]. Deferiprone is found effective in MDS patients but is not licensed in USA and European countries on account of its associated toxicity. Usual doses of treatment are very high (75 mg/kg/day) as compared to DFO (20-40 mg/kg/d) and deferasirox (20-30 mg/kg/d).

### Deferasirox (ICL670)

Commercially called as Exjade, deferasirox is a tridentate, synthetic oral iron chelator prepared from salicylic acid, salicylamide and 4-hydrazinobenzoic acid and is proposed to serve the patients who are either non-compliant or show intolerance to DFO. It was approved by FDA in USA in 2005 and Europe in 2006 and is under phase II/III clinical trials for iron decorporation in humans [165]. Deferasirox binds to iron in 2:1 ratio and is lipophilic with half life of 8-12 hours so that it has to be administered only once in a day. An affordable single dose of deferasirox per day was found highly tolerable as well as efficient by iron-overloaded patients of sickle cell anaemia [166] and other iron overload disorders [167, 168]. More than 80% of drug is excreted from fecal route while renal excretion was only eight percent [169]. Deferasirox was effective in reducing serum ferritin level in 166 patients suffering from non-transfusion dependent thalassemia with only minor side effects such as nausea, body rashes and diarrhea [170] and increased the quality of life by reducing morbidity and mortality among thalassemia patients [171]. A study on 175 iron loaded myelodysplastic syndrome (MDS) patients was done with dose range 20-30 mg/kg/day, depending on the frequency of blood transfusions, and found that deferasirox showed well-defined and manageable safety profile [172]. In their later study and others [173-174], it was observed that one-third of the patient volunteers did not achieve satisfactory iron balance at doses  $\geq 30$  mg/kg per day. A group of workers found increase in

toxicity of deferasirox if dose was increased from 30 to 40 mg/kg/d [175]. Deaths in older patients with MDS have been reported who were administered higher doses of deferasirox [176]. More recently it is suggested that instead of a single high dose, twice-daily doses of deferasirox should be administered to patients with chronic iron overload [177].

Several authors have reviewed the development and current status of oral deferasirox [178, 179]. A one-year long study in about six hundred thalassemia patients with iron overload, who contraindicated DFO treatment, was done by European Medicines Agency and observed encouraging results with deferasirox [180]. The drug was found effective in treating iron overload in patients suffering from MDS [181-183],  $\beta$ -thalassemia [184, 185], hereditary hemochromatosis [186], sickle cell disease [187, 188] and allogeneic hematopoietic cell transplantation [189]. However deferasirox was found inefficient in few pediatric patients who underwent frequent blood transfusions during and after high-dose chemotherapy with autologous stem cell transplantation [190]. A phase II clinical study on the same confirmed the poor tolerability and GI toxicity of deferasirox [191].

Many workers have suggested that deferasirox will soon replace the conventional chelation therapy with DFO but the fact that it is contraindicated in patients in advanced stage of hematologic disorders is also unavoidable [192]. Of more importance is that safety data on deferasirox prescribed doses is quite limited and need to be worked upon extensively before it is recommended as first-line treatment against DFO [193].

### Hydroxyquinolines

Hydroxyquinoline is an oral bidentate chelator that forms five-membered ring structure with iron [194]. It is lipophilic and readily enters into cell membrane and BBB. VK-28 and its derivatives such as M-10, M-30 are hydroxyquinoline based iron chelators having potency similar to DFO [195]. Their ability to cross BBB has been used for treating neurodegenerative disorders. Hydroxyquinoline based antibiotic such as clioquinol (5-chloro-7-iodo-hydroxyquinoline) chelates iron [196], though not very specifically, but is being explored for possible use in iron reduction in Parkinson disorder [197].

### Chelator combination therapy

Many practitioners suggest sequential or combined regimen with two or more chelators to effectively overcome the iron overload associated with many diseases instead of single drug therapy. There is a great deal of work on the combination therapy with deferiprone and DFO since 1990s based on same or different days therapy and many of them are under clinical trials [198-200]. A 7-15 day study on five transfusion-dependent patients showed that instead of increasing deferiprone dose from 75 mg/kg to higher values, a combination of subcutaneous DFO and oral deferiprone could be more effective with reduced toxicity [201]. Few studies have suggested that the combination of deferiprone and deferoxamine is effective if given on same day [202, 203] while others indicated dose dependent chelator combinations to be given on different period of days [204, 205]. The synergistic effect of chelator combinations is expected to minimize the toxicity and inconvenience associated with monotherapy.

### High molecular weight iron chelators

Though DFO therapy is currently the most commonly practiced treatment, yet it is costly and unaffordable by a large percentage of patients living in under-developed and developing countries. Short circulation time, patient non-compliance and toxicity issues associated with DFO treatment has led to the development of high molecular weight chelators. Iron binding polymers have a great potential at therapeutic level to bind iron irreversibly and form stable and non-toxic complexes. The first of these attempts included inclusion of hydroxamic acids onto polymers to obtain a high stability constant for iron. Hydroxamic acids are long known for their powerful ability to selectively bind iron. In this regard a series of water-soluble acrylic polymers with hydroxamic acid bearing side chains (PHAs) were prepared which proved highly selective for iron with reduced

circulation time [206]. But these polymers were non-biodegradable and could lead to intoxication followed by deposition. Ten years later Meshchanov & coworkers suggested that 30-35 hydroxamic acids per hundred monomer units of polymer increase the ability of PHAs to eliminate body iron as well as to undergo biodegradation [207]. To enhance patient compliance, the development of effective and highly selective oral chelators is must. Therefore orally active drugs based on chitosan [208-209] and pectin derivatives [210] were prepared, that got approval by FDA for iron chelation. The adhesive property of chitosan based microspheres functionalized with catechol or hydroxyl-carboxylic acid was used to prepare orally active and therapeutically efficient iron chelators [211]. A team of workers claimed to produce hydrogels based on conjugate of dihydroxybenzoic acid on polyamine vehicles such as polyacrylamide and polyvinyl alcohol that were ten times more effective than any of the current therapeutics [212]. Various other chelators such as desferriethiol and hydroxypyridinones were also covalently linked to polyamine backbone such as spermine and showed increased permeation of the chelator into cells. Taking into account the selectivity and specificity of DFO for iron, the drug was bound to high molecular weight polymeric vehicles through its amino group. This approach led to the development of high molecular weight chelators having enhanced blood retention times and reduced toxicity. Dextran was proposed as a biodegradable and biocompatible matrix to which DFO was covalently bonded [213]. Hydroxyl ethyl starch-conjugated deferoxamine (HES-DFO) is currently under Phase Ib clinical trial (40SD02) as a novel long-lasting formulation [214].

### CONCLUSION

Iron overload is a common problem all over the world. Workers from all over the world are involved in finding the best suitable therapeutic agent that can treat the iron overload and its associated trauma. The active involvement of a number of researchers to find the best suitable iron overload treatment may increase the availability of therapeutic options for patients. A number of iron chelators are approved for treatment of iron overload associated to thalassemia, cancer, asthma etc and others are being improved to get the patients relieved from iron overload with reduced toxicity and maximum efficacy. Though a number of agents, natural or synthetic, are currently available or are being screened to reduce the iron overload, yet the development of a best ligand is challenge in the area of drug discovery and development. The treatment of chronic iron overload is challenge to the modern practitioners, which exposes them to great dilemma regarding management of the problem.

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### CONFLICTS OF INTERESTS

The authors declare that they have no conflicts of interest

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