Review

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Sex and Gender Differences in Iron Chelation

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Abstract: Background/Objectives: In the absence of physiological mechanisms to excrete exceeding iron, the administration of an iron-chelation therapy is necessary. Age and hormones have an impact on the absorption, distribution, metabolism, and excretion of medications used to treat iron excess, resulting in notable sex and gender-related variances. **Methods**: Here we aimed to review the literature on sex and gender in iron overload assessment and treatment. **Results**: The development of iron chelators has shown to be a successful therapy for lowering the body's iron levels and averting the tissue damage and organ failure that follows. Numerous studies describe how individual factors can impact the chelation treatment, potentially impact the therapeutic response, and/or result in inadequate chelation or elevated toxicity; however, most of the data do not consider male and female as different group and, particularly, effect of woman hormonal variation were never considered. **Conclusions**: An effective iron chelation treatment should take into account sex and gender differences.

Keywords: iron overload; deferoxamine; deferiprone; deferasirox; luspatercept; personalized medicine.

1. Introduction

Women and men respond differently to treatments: this mainly depends on physiological, anatomical, and hormonal characteristics. The existence of the differences in therapeutic agent pharmacokinetics and pharmacodynamics influences the response to treatments. Although this was already known since 1932, the year in which the first report on the gender difference in the pharmacology of barbiturates in rats is reported, full awareness of the relevance of the role of gender pharmacology only came at the end of the last century [1-5]. By pharmacokinetics we mean the study of the four phases of a medicine transition in our body: absorption, distribution, metabolism, and elimination. These four stages are primarily influenced by age and hormones, thus showing significant differences related to sex. Pharmacodynamics, on the other hand, indicates the effect of a therapeutic agent on bodies and studies the biochemical and physiological effects and their mechanism of action. There are numerous pharmacodynamics differences depending on sex, mainly mediated by hormones, genes, and the environment. While, however, the pharmacokinetic differences are simpler to analyze, the pharmacodynamics differences are more difficult to detect [1]. But both should deserve a worthy study in the preclinical phase in terms of gender differences or the resulting clinical phase will be limited and approximate.

Here we aimed to review the literature on sex and, if possible, gender in iron overload assessment and treatment.

2. Iron Metabolism

Iron is a necessary trace element for many living organisms biological functions. Most of the iron content in mammals bodies is used for Heme synthesis and therefore erythropoiesis, but a modicum is also transported to peripheral tissues. Here, iron can be used in different ways depending on the needs of the specific cell: it can be brought directly to mitochondria and other sites of utilization, or it can be stored as a ferritin-bound form. The concentration and localization of iron in mammals' bodies must be correctly balanced, as a quantitative anomaly of this element, such as iron deficiency, is the cause of IDA (iron deficiency anemia), one the most common worldwide diffused condition [6].

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The etiology of anemia is multifactorial and complex, and the major contributor to the burden of this disease is iron deficiency, which is responsible for approximately 50% of anemia cases. According to recent data, 13% of the world population is affected by IDA, which corresponds to about 850 thousand people. Conversely, iron overload could have cytotoxic effects and cause tissue damage. Specifically, siderosis (also named secondary hemochromatosis) and Hereditary Hemochromatosis are the diseases characterized by severe iron overload, the first being mainly represented by Hemoglobinopathy patients iron overload, the latter by Hfe Hemochromatosis, whose frequency is very high in Caucasian population (allelic frequency 5-10% in Celtic background populations) [7,8] .

Complex molecular pathways strive to obtain iron homeostasis, characterized by a balance between iron intake from the diet via duodenal enterocytes, iron usage and iron recycling via macrophages from senescent red blood cells, while an iron reserve is predominantly maintained in hepatocytes [9,10]. Dietary iron is mainly represented by Heme-iron, in which the metal is part of the Heme porphyrin, that is found in meat, poultry, and seafood, with considerable bioavailability (25–30% of this form is absorbed), and non-heme iron, present in vegetables, of which only 1-10% is absorbed [11]. During digestion, Heme containing proteins present in the meat, Hemoglobin and Myoglobin, are released in the stomach low pH environment and Heme is made available by the action of proteases in stomach and intestine. Heme enters into enterocytes through HCP1 and/or two other putative transporters, the Feline leukemia virus subgroup C receptor 2 (FLVCR2) and the Heme carrier protein 1/Proton-coupled folate transporter (HCP1/PCFT). Once in the cytoplasm, Heme is metabolized by heme oxygenase (HO) and iron is released, joining the labile iron pool (LIP) present in the cytoplasm. A fraction of intact heme can be released directly into the blood stream via heme transporter FLVCR1. FLVCR1 exports cytoplasmic heme, and it can export heme into the lumen during increased cellular heme content to protect from heme toxicity [12].

Non-Heme iron is present in the body in two forms: ferric iron Fe^{3+} and ferrous iron Fe^{2+}. The two forms are employed in different mechanisms, and they are readily converted in the alternative form by oxidases and reductases, a mechanism that is mandatory for iron to be transported across the cell's membrane. Dietary non-Heme iron in reduced by a duodenal enterocyte specific reductase called Duodenal Cytochrome B (DCYTB), therefore allowing its passage from the intestinal lumen to the enterocytes via the iron importer DMT1, where it joins the LIP and if need reaches again the bloodstream via the iron exporter Ferroportin 1 present on the basolateral membrane of the duodenal cells [13]. In the bloodstream, Fe^{2+} is rapidly converted into Fe^{3+} via the ferroperoxidase Heph (Hephaestin) and is taken up by free Tf (Transferrin) to be transported to the tissues [14]. Tissues cells expressing TfR1 (Transferrin Receptor 1) on their plasma membranes introduce the holo-transferrin ($Tf\text{-}2Fe^{3+}$)/Tfr1 complex through clathrin-mediated endocytosis and the iron is released from Tf in endosome, reduced by Six-transmembrane epithelial antigen of the prostate 3 (Steap3) and released to the cells' cytosol via a DMT1 specific isoform. Inside the cells cytoplasm, LIP can be used to produce iron-sulfur clusters and to synthesize Heme in the mitochondria, to be included in iron-containing proteins or it can be stored by being bound to Ft (Ferritin) [15]. If cellular iron amount starts to increase the metal is exported through Ferroportin 1. This intracellular iron homeostasis is finely tuned by the so-called IRE/IRP post-transcriptional regulatory system [15].

The master regulator of body iron homeostasis is a small hormone called Hepcidin (Hepc), codified by HAMP gene [16]. Hepcidin is produced mainly by the liver and its hepatic expression is regulated by body iron demand. In fact, a high demand during iron deficiency reduces HAMP gene expression, while high iron levels stimulate it. Hepcidin binds the iron exporter Ferroportin 1 (Fpn1) and the complex is internalized and degraded, hampering the release of iron absorbed by the enterocytes and its export from stores. Therefore, in iron deficiency condition Hepc amount is reduced, Fpn1 internalization and degradation is decreased, and more iron can be released from intracellular stores and be used and vice versa. A higher expression of HAMP, and the consequent iron entrapment in the cells, can also be observed in response to inflammation and infection. This is thought to occur because lowering the iron levels during an infection can adjuvate the body host's defense mechanism, as it hampers the pathogen's metabolism [17]. Hepcidin expression is regulated by several effector proteins that exert a positive or a negative effect on Hepc synthesis [18]. In martial

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physiologic condition, Hepc amount in plasma and urine is consistent but in further and rapidly raises as a response to an increased iron availability. This change is molecularly mediated by a compound of proteins involved in a signal transduction pathway. Briefly, Hfe (Hereditary hemochromatosis protein) physiologically binds to TfR1, using certain binding sites that overlap with Tf/Tfr1 binding sites. In case of increased iron levels, Tf saturation raises and Tf-Fe2 forces out Hfe, binds TfR1 and the complex is internalized. Hfe disassociated from TfR1 and binds two other proteins: Hjv (Hemojuvelin) and TfR2 (Transferrin Receptor 2). This complex is able to activate the common BMP-SMAD1/5/8 pathway. A second signal pathway is mediated by BMP2 and BMP6 (Bone Morphogenetic Proteins 2 and 6), whose expression is dependent on iron levels, causing their increased synthesis in an iron-abundance situation. These proteins interact with BMP Receptor (BMPR) and hemojuvelin (Hjv), forming a complex that activates the SMAD pathway. Specifically, this consists in inducing the phosphorylation of SMAD (Small Mother Against Decapentaplegic) regulatory proteins SMAD1, SMAD5, SMAD8, that in turn binds SMAD4. This complex is then translocated to the nucleus, where it induces HAMP gene transcription. On the other hand, Hepcidin expression is downregulated in conditions of iron deficiency. The main effector of this negative regulation is Matriptase 2 (or transmembrane serine protease 6, TMPRSS6), that senses iron levels and cleaves Hjv, suppressing its action. This blocks the activation of BMP-SMAD1/5/8 pathway, ending in a decreased HAMP gene transcription [15]. Lastly, erythropoietic activity, hypoxia and inflammation influence Hepcidin regulation. Erythroferrone (Erfe) contributes to Hepcidin inhibition if erythropoiesis is compromised, while Platelet-derived growth factor-BB (PDGF-BB) has the same effect on Hepcidin in hypoxic conditions [19]. In inflammatory conditions, Interleukin-6 (IL-6) is a pro-inflammatory cytokine that induces the HAMP promoter [16].

3. Iron Level Measurement

Serum ferritin should reflect the organic iron deposit levels. It is measured by a blood collection and it allows frequent monitoring. However, the results can be influenced by several factors, including infections and inflammations [20].

Biopsy is the most precise direct method for the assessment of hepatic hemosiderosis. It allows to evaluate:

- the quantitative determination of siderosis;
- the pattern of metal accumulation in hepatocytes and Kupffer cells;
- the evaluation of inflammation, fibrosis and any cirrhosis.

Hepatic siderosis is measured by cytochemical staining for iron (Perls method), according to the Scheuer gradation (grades I-IV). The pattern of martial overload at the level of hepatocytes, Kupffer cells, sinusoids and the main structures of portal spaces can also be evaluated [21].

Many ultrasound elastography approaches have been developed [22]. Transient elastography, performed using a FibroScan device, uses a low-frequency pulsed excitation able to generate shear waves in liver tissue. The shear waves velocity has been related to the tissue stiffness [23]. The comparison between the METAVIR classification (from F0, healthy liver state, to F4, the most severe stage of fibrosis [24]) and stiffness values, reported by the Fibroscan system, shows an excellent correlation [25]. The rate of successful measurements was calculated as the ratio between the number of those validated and total measurements [23]. The results were expressed as a median value of the total measurements in kPa. Values < 7.0 kPa are indicative for not significant fibrosis [26].

The Superconducting Quantum Interference Device (SQUID) is based on the physical properties of ferritin and hemosiderin (pigment containing iron, consisting of ferritin molecules and other structural elements aggregates, which is found in liver, spleen and bone marrow). This non-invasive method provides a LIC value that can be completely overlapped with that obtained with liver biopsy, but does not allow the assessment of inflammation and fibrosis. SQUID determines iron concentration in liver and spleen [27].

Magnetic Resonance Imaging (MRI) is a non-invasive technique based on nuclear magnetic resonance; to date, quantification of iron with MRI is considered the standard of care in diagnosis and monitoring of iron overload diseases [28]. It is extremely sensitive in assessing iron concentration

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and distribution throughout the body, therefore not only in liver, but also in other organs, including heart. Currently there are several MRI methods available [29]. T2* MRI imaging is accomplished using a breath-hold multiple echo gradient echo pulse sequence to acquire a series of images with increasing echo times. These sequences can be used in liver and heart. The value of T2* =20 ms corresponds to the lower limit of normality; lower is the T2* value and higher can be the risk for serious, and sometimes fatal, cardiac event in a short time [30,31]. Considering liver, MRI T2* normal values are ≥ 6.3 ms [32].

It is very well established that the amount of iron varies in the two sexes and according to life period, so much so that, in humans, serum iron (SI) and ferritin (sFt) physiological ranges are different between men and women (Table 1).

Table 1. Serum iron levels in adult males and females, newborns and children. [1] Tables may have a footer.

	Serum Iron (mcg/dL)	Serum Ferritin (ng/mL)
Adul males	65-176	12-300
Adult females	50-170	12-150
Newborns	100-250	25-200
Children (6m-15y)	50-250	7-140

Furthermore, females have relevant iron oscillation due to their reproductive metabolism during menstrual cycles and after the menopause. A recently published paper on a significant cohort of healthy subjects reports that while the serum ferritin values are higher in young males compared to age-matched females, this difference lessens during ageing. Moreover, in women during the perimenopause period, ferritin levels soar with age, while this increase is milder in postmenopausal condition [33].

4. Hepcidin Levels Measurement

Soon after the determination of Hepcidin as a pivotal regulator of iron metabolism and the finding of this small peptide in urine and plasma [34,35], several attempts to determine Hepc amount in these biological fluids and to correlate it to the subject's iron metabolism have been undertaken. Nowadays, the widely used methodologies are enzyme-linked immunosorbent assay (ELISA) and Surface-Enhanced Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (SELDI-TOF-MS). Specifically, using a set ELISA kit, it has been demonstrated a good correlation among urine and serum Hepc amount as well as serum Ft values. Furthermore, it has been highlighted that the Hepc amount has a circadian variation that parallels the one of SI [36]. Lastly, it has been demonstrated in animal models that Hepcidin quickly responds to inflammation irrespective of the anemic condition of the animals [37]. Sex differences in serum Hepcidin level have been unraveled on a considerable number of healthy subjects participating to the Nijmegen Biomedical Study [38]. Determining Hepc amount in the males and females groups stratified by 5 years interval of age, Hepc amount remains mainly constant in males while Hepcidin concentrations in women mildly increase through menopause (4.1nM median for women younger than 55 years and 8.5nM for women 55 years of age and older). Anyway, it must be underlined that serum Hepcidin concentration has significant variability among subjects, so that the accepted reference ranges are quite wide.

5. Animal Models

Concerning the animal models typically used to investigate iron overload and iron chelation, differences related to the sex of the animals were not taken into account. However, we decided to briefly list the models used in the study of iron overload. Rodent models, like mice or rats, can be treated to iron overload via nutritional manipulation or genetic mutation. High-iron diets or iron compound injections can cause excessive iron buildup in a variety of tissues, simulating iron overload illnesses in humans. These animal models allowed to study the pathophysiology of iron overload and assess the effectiveness of iron chelation therapy [39]. Knockout mice, missing in the genes involved in iron manage and homeostasis, helped researchers to understand the molecular pathways driving

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iron overload and to create targeted therapeutics [40]. Non-human primates, such as baboons and macaques, additionally be utilized as models for iron overload and chelation research. These animals have physiological and genetic similarities to humans, making them pertinent to translational study. Non-human primate models give a more precise characterization of iron metabolism, chelation therapy, and potential adverse effects by mimicking human physiology [41]. The zebrafish (Danio rerio) has developed as a well-known model organism for biomedical research. They have similar metabolic routes to humans and might be manipulated to produce iron overload circumstances. Zebrafish models allow researchers to analyze the consequences of iron overload, test prospective chelators, and probe the underlying molecular pathways in an advantageous and high amounts way [42]. These animal models have certainly brought advantages for studying the pathophysiology of iron overload diseases, evaluating the effectiveness of iron chelation therapy and understanding the underlying molecular pathways. They enable researchers to explore the impacts of iron overload at several levels, including tissue pathology, gene expression, and metabolic parameters, therefore advancing the development of tailored therapies for iron-related illnesses. Concerning the rodent models discussed above, mutant mice models have been generated to replicate iron excess diseases. Mice with specific mutations in iron metabolism genes, such as Hfe, hepcidin, or transferrin receptors, can be used to explore the molecular processes behind iron excess and chelation [39]. Additionally, high-iron dietary regimens helped scientists to monitor iron consumption and investigate the effects on long-term iron excess. Dietary models are beneficial because they accurately represent dietary iron overload reported in some human groups, such as those with genetic hemochromatosis or heavy iron supplementation. In recent years, genetic approaches such as gene knockout or knockdown have been employed to change different genes related in the iron metabolism processes. By altering genes involved in iron absorption, storage, and transport. These models allow for the investigation of specific molecular targets and processes involved in iron homeostasis and chelation [40]. Scientists can utilize these distinct animal models to explore various aspects of iron overload, such as its origins, processes, development, and potential therapies. These models enable in-depth research into iron metabolism, chelation therapy, and the evaluation of new therapeutic methods to address iron excess illnesses.

6. Chelation Therapy

In the absence of physiological mechanisms to excrete exceeding iron, the administration of an iron-chelation therapy is necessary, and this results in a negative net iron balance [43-45]. While for genetically iron overloaded patients the routine therapy consists in periodical phlebotomy to normalize serum iron parameters [46], iron chelation is applied to the majority of diseases with secondary iron overload. Chelation is a chemical reaction in which a metallic atom, acting as Lewis acid, is bound by a chelating reagent, through more than one coordinating bond. The structure of the resulting compound constitutes a very stable complex which sees the central atom surrounded by the chelator. The chelator is often a polydentate binder (specifically, bidentate, tridentate, etc.). Once chelated, the metal loses its characteristics and then it can be eliminated linked to the chelator. The goal of chelation therapy in beta-thalassemia patients is to bind and remove iron from the body; it must also try to satisfy other important needs [47]: the elimination rate must be equal to or greater than iron input rate with the transfusion, so it is important that the therapy allows a flexible dosage, it must provide 24-hours chelation coverage, to avoid the iron accumulation and thus to prevent the adverse effects of its overload; this translates into the need of a molecule with a long half-life, the route and timing of administration (treatment regimen) must guarantee the maximum adherence to therapy, the number of days in which patients receive the chelation therapy is more important than the total dose taken during treatment; thus, the exposure length of chelation therapy is crucial, the treatment-related adverse effects must be minimal. The main drugs currently used in iron chelation therapy are deferoxamine, deferiprone, deferasirox (DFX) and luspatercept.

6.1. Deferoxamine

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Deferoxamine (Desferal®) was the first commercial iron chelator, approved in 1970. It is a large hexadentate molecule with a short half-life (20-30 minutes), which binds iron with high affinity; Fe-deferoxamine complex is eliminated in urine and faces [48]. Deferoxamine intake requires a slow parenteral infusion of 8-12 hours, 5 to 7 times a week. This therapy regimen has a significant impact on adherence and many patients do not get the full benefits from therapy and die early. Moreover, a 24-hours chelation coverage is not possible with the use of deferoxamine due to its short half-life [47]. The deferoxamine-iron chelate is charged and does not readily enter and leave cells. Eventually, this drug leads to many side effects, such as local reaction at the site of infusion, hypoacusis, ocular toxicity, retarded growth and skeletal changes [49,50]. It has been determined that treatments that target iron chelation with deferoxamine and iron dependent cell death (ferroptosis) [51] suppression with Edaravone, a medication that is clinically licensed for the treatment of ischemic stroke, are therapeutically promising [52]. Diabetes increases the risk of ferroptosis since it modifies several pathways implicated in the condition. Iron chelation prevents stroke-induced vasoregression, blood-brain barrier breach and neuronal damage in male diabetic rats [53,54]. Li et al, in a study on the impact of deferoxamina therapy on poststroke outcomes in female rats with and without diabetes observed the following sex-related differences [55]: drug induced deficits in fine motor skills in male control rats but not in female control rats, working memory deficits at baseline are more pronounced in female rats than in males, poststroke vascularization was not seen in female diabetic rats as opposed to male diabetic rats, poststroke vasoregression was not seen in female diabetic rats as opposed to male diabetic rats and deferoxamina decreased vascular volume and surface area indices in females but increased them in males. On the other hand, in male diabetic rats, the drug reduced poststroke microglial activation. Remarkably, in diabetic female rats, deferoxamina enhanced anti-inflammatory phenotype, suggesting a variety of microglial morphological and functional processes explain the effects of therapy in both male and female rats. Always considering diabetes, research on brain microvascular endothelial cells (BMVECs) from both male and female participants showed that when cells are exposed to high glucose levels as opposed to normal glucose, ferroptosis happens in response to hypoxia [56]. Iron-responsive element-binding protein 2 (IREB2), a hallmark of ferroptosis, was notably expressed at considerably higher levels in male cells than in female ones. Only male cells that were susceptible to the ferroptosis inhibitor Fer-1 showed decreased glutathione peroxidase expression when ferroptosis was induced with erastin. The cytoprotective effects of deferoxamina on male and female BMVECs treated with hemin were likewise shown in other tests. However, in hemin-treated cells, the cytoprotective effects of the drug showed minor sex-dependent variations linked to the expression of glutathione peroxidase and important proinflammatory proteins. Lastly, the combined provocative in vitro results imply that iron chelation may be harmful in non-diabetic circumstances. All things considered, the BMVEC results corroborated the in vivo evidence demonstrating the therapeutic effectiveness of iron chelation in both males and females.

6.2. Deferiprone

The first oral chelator, deferiprone (Ferriprox®) is a bidentate chelator (3 molecules bond an iron ion), approved in 1987; the Fe-deferiprone complex is not loaded and therefore can easily cross the membranes, allowing a rapid removal of the iron accumulation from cells [48]. It is taken orally 3 times a day and has a half-life of 3-4 hours; thus, as deferoxamine, it is not able to guarantee a 24-hours chelation coverage. Furthermore, treatment with deferiprone has been correlated with rare, but severe, agranulocytosis, mild neutropenia, abdominal discomfort and erosive arthritis [49,57,58]. A study on deferiprone-induced agranulocytosis reported that agranulocytosis and neutropenia appeared to be dose independent and three times more frequent in females than males [59]. Bellanti and colleagues indicated a statistically significant gender effect on the volume of distribution, which may ultimately affect peak plasma concentrations, in contrast to data from non-compartmental analysis [60]. Furthermore, deferiprone dose adjustment is advised for individuals with decreased creatinine clearance, according to simulated scenarios. For patients with mild, moderate, and severe renal impairment, doses of 60, 40, and 25 mg kg−1 are suggested based on creatinine clearance values of 60–89, 30–59, and 15–29 ml min−1, respectively. To conclude, their work highlights the need for

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additional research on the pharmacokinetics of deferiprone in young children and toddlers, for whom pharmacokinetic data does not yet support current dosing recommendations. Combination therapy with deferiprone and deferoxamine has been investigated for the removal of cardiac iron and the normalization of its body stores [61]. Moreover, it has been demonstrated to reverse anomalies of glucose metabolism and enhance gonadal function more successfully, as it is more effective in lowering the total body iron burden [62]. Oral glucose tolerance tests revealed considerably lower mean glucose levels in patients receiving combo medication. Men and women gonadal function and fertility improved, and some patients were able to conceive successfully [62]. According to research on animals, deferiprone is teratogenic and embryotoxic. It is recommended that women of reproductive age receiving deferiprone therapy either refrain from becoming pregnant or, if they intend to become pregnant, switch to a different iron chelator. Deferiprone should also be avoided by nursing moms [63].

6.3. Deferasirox

The urgent need for an effective and safe once-daily orally administered iron chelator leads to the expedited approval of deferasirox (DFX) [64,65]. DFX is a tridentate iron chelator, composed of two molecules forming a stable complex with an iron ferric atom (Fe3+). The lipophilic active molecule (ICL670) is highly bound to protein, above all albumin. It induces a mean net iron excretion per day of 0.119 mg Fe/Kg body (10 mg/Kg/day DFX dose), 0.329 mg Fe/Kg body (20 mg/Kg/day DFX dose) and 0.445 mg Fe/Kg body (40 mg/Kg/day DFX dose) mg Fe/Kg body, classified under the clinical relevant range (0.1-0.5 mg/Kg/day) [66]. DFX chelating properties are: high and specific affinity for Fe3+, oral bioavailability, facilitating use in pediatric patients, high efficiency and effectiveness, long half-life (8-16 hours), which determines a chelating coverage of 24 hours, allowing a once-daily dosage, flexible therapeutic regimen and, generally, well tolerated. DFX was approved as a first-line therapy for blood-transfusion-related iron overload by the Food and Drug Administration (FDA) in 2005 and the European Medicines Agency (EMA) in 2006. It is indicated in patients aged ≥ 2 years and with chronic iron overload due to transfusion-dependent or non-transfusion-dependent thalassemia or anemia. The EMA guidelines states that DFX treatment should only be initiated after the transfusion of ≥ 100 mL/kg of red blood cells (e.g. ≥20 units for an individual weighing 40 kg) or when serum ferritin levels are > 1000 µg/L [67]. The recommended initial daily dose is 20 mg/Kg, except for those with a higher iron burden (30/mg/Kg), taken on an empty stomach at least 30 minutes before food. To achieve therapeutic goals, dose adjustments in steps of 5-10 mg/Kg/day, up to 40 mg/Kg/day, can be made [68]. The tablet can be dissolved in water, orange juice or apple juice and any residue can be resuspended in a smaller volume of drink. DFX should be taken on an empty stomach, no less than 30 minutes before taking food [48]. DFX is mainly metabolized in liver (glucuronidation) and eliminated through hepatobiliary excretion in faces [65,69-72]. UDP-glucuronyltransferase 1A1 (UGT1A1) is the main UGT isoform responsible for DFX glucuronidation [64,73-76]; in vitro studies showed the role of cytochrome-P450 (CYP) 1A1, 1A2 and to a lesser extent 2D6 enzymes [69]. Particularly, UGT transforms DFX in M3 (acyl glucuronide) and M6 (2-O-glucoronide) metabolites; the 6% of the pro-drug is metabolized by CYPs to M1 (5-hydroxy DFX) and M4 (5'-hydroxy DFX), respectively [72]. DFX and metabolites are mostly excreted in bile through multidrug resistance protein 2 (MRP2, also known as ABCC2) [69]; breast cancer resistance protein (BCRP1, also known as ABCG2) may influence drug toxicity [73]. Chirnomas et al. defined as inadequate responders patients with a rising ferritin trend over 3 consecutive months, at least one higher than 1500 ng/mL, or a rising LIC, documented by biopsy or non-invasively and on a dose of more than 30 mg/Kg per day of DFX; instead, adequate responders have a ferritin trend below 1000 ng/mL (evaluating the interval between the LIC at the beginning and at the end of the study) documented declining liver iron burden by MRI or biopsy and a DFX administration of 30 mg/Kg per day or less [77]. Based on Chirnomas efficacy definition, we determined an efficacy DFX concentration at the end of dosing interval (Cthrough) threshold of 20,000 ng/mL and an area under the curve (AUC) concentrations efficacy (360 µg/mL/h) and non-response (250 µg/mL/h) cut-offs [78,79]. DFX is well tolerated by adults and children with different chronic anemia. Phase II and III

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studies described and defined the clinical safety profile of DFX in patients of all ages, including patients younger than 2 years [80]. The most common adverse effects are gastro-intestinal disorders (diarrhea, abdominal pain, nausea and vomiting) and rash (mild, moderate or severe). In one third of patients treated a non-progressive increase in serum creatinine values, proportional to the chelating dose, was observed, which resolves spontaneously and decreases with the reduction of drug dose. Recent studies also report cases of: cytopenia (agranulocytosis, neutropenia and thrombocytopenia), acute renal injury (also in pediatric patients), hepatic toxicity, adverse effects involving hearing and sight. The length of treatment period does not seem to correlate with the increase in adverse effects [47]. Considering drug pharmacokinetics, De Francia and colleagues observed difference between sexes: females had mean DFX Cthrough of 16.79 ± 17.46 μg/ml, higher than value reported for males, 12.90 ± 13.49 μg/ml [75]. In contrast to previous authors, Mattioli et al. indicated that gender has no effect on plasma concentrations, but we did discover a trend toward an inverse relationship between DFX and age [81]. Given that the drop in DFX clearance may be more than 20%, a close monitoring of liver and kidney functions in DFX-treated patients is necessary [82]. This decrease could put kids at higher risk for drug-induced toxicities, which would seriously impair the activity and function of already-damaged organs. Adding a bodyweight-based allometric scaling exponent of 0.75 to the population pharmacokinetic model may improve the prediction of drug clearance by accounting for differences in organ functioning [83]. This approach has been questioned by several studies, though, as the exponent value in children can range from 0.6 to 1.11 [84]. In infants, toddlers, and kids, for example, such variability could be between 0.50 and 1.20 [82].

6.4. Luspatercept

Luspatercept, a recombinant fusion protein and erythroid maturation agent, is the last approved drug to treat individuals with transfusion-dependent anemia brought on by lower-risk myelodysplastic syndrome or β-thalassemia. It binds and inhibits some transforming growth factor-β superfamily ligands, such as growth differentiation factor 11. This interferes with Smad2/3 signaling, which is markedly increased in disease situations when erythropoiesis is unsuccessful [85]. Therefore, through the differentiation of late-stage erythroid precursors, or normoblasts, luspatercept stimulates erythroid maturation in the bone marrow [85]. Moreover, it promotes erythropoiesis and reduced Smad2/3 signaling [86,87]. Over a dose range of 0.125–1.75 mg/kg, luspatercept demonstrated linear pharmacokinetics, first-order absorption and elimination [85,88]. The maximum drug concentration and AUC in serum increased roughly proportionately with increasing dose [85]. The median time to reach the maximum drug concentration was around seven days and after three doses (a total of nine weeks), a steady state was attained with an accumulation ratio of about 1.5 [85]. In patients with myelodysplastic syndromes, the mean half-life in serum was approximately 13 days, while in those with β-thalassemia, it was around 11 days [85]. There was a mean apparent total clearance of 0.52 L/day and 0.44 L/day. Luspatercept will be eliminated in urine since its molecular mass is greater than the glomerular filtration size exclusion threshold and it will be broken down into amino acids in a variety of tissues [85]. Luspatercept pharmacokinetics seem not significantly impacted by patient age, sex, race, β-thalassemia genotype or ring sideroblasts status, splenectomy status, mild to moderate kidney impairment or specific baseline laboratory values [85,88]. Body weight is the only clinical measure that coincides with the volume of distribution and clearance, which supports the body weight-based dosing strategy [88]. A pediatric study will evaluate the pharmacokinetic profile in younger patients because this may be a problem in extreme body weights, especially in pediatric population [89]. In healthy postmenopausal women, luspretercept enhanced hematological parameters (increased RBC, hemoglobin, and hematocrit levels) [90]. Thirty-two healthy postmenopausal women participated in a Phase I trial in which they were given two subcutaneous doses of either luspatercept (0.0625–0.25 mg/kg) or a placebo (3:1 randomization) separated by two weeks. Curiously, this study cohort was selected because osteoporosis may be well treated with luspatercept and other comparable compounds (such as sotatercept). Furthermore, it was unclear how the hypothalamic-pituitary-gonadal axis activin signaling might be inhibited [91]. Assessing the safety, tolerability, pharmacokinetics, and pharmacodynamic consequences of

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increasing luspatercept dosage levels was the aim of this experiment. Three cohorts of eight patients each were created from the 24 patients, and they were given three increasing doses (0.0625, 0.125, and 0.25 mg/kg). During the research, there were no serious side effects and luspatecept was well tolerated. Beginning seven days after the injection and continuing for many weeks after therapy, a dose-dependent rise in hemoglobin concentration was noted in the treated patients. 83.3% of participants in the highest dose group (0.25 mg/kg) experienced a hemoglobin rise of ≥1.0 g/dl. Notably, luspatercept action may seriously harm embryonic development, as demonstrated by animal studies; for this reason, it must be avoided during pregnancy. The drug should be avoided during breastfeeding because it is secreted into the milk of nursing rats and is passed through the placenta of pregnant rats and rabbits [92]. This is likely to happen in humans as well [93,94]. The use of luspatercept in men and women of reproductive age must be accompanied by appropriate contraceptive counselling and pregnancy planning because of these reasons, which make it completely inappropriate during pregnancy.

7. Conclusions

Patients with sickle cell disease, thalassemia syndrome, and myelodysplastic syndromes who need daily transfusions may suffer from chronic iron overload. One of siderosis harmful impacts is the increasing destruction to organs and tissues, which impairs their ability to operate. The development of iron chelators has shown to be a successful therapy for lowering the body's iron levels and averting the tissue damage and organ failure that follows. Numerous studies describe how individual factors can impact the chelation treatment, potentially impact the therapeutic response, and/or result in inadequate chelation or elevated toxicity; however, most of the data do not consider male and female as different group and, particularly, effect of woman hormonal variation were never considered. Moreover, gender differences of the enrolled patients should be highlighted to track the drugs levels to maximize and ascertain the best dosage for every patient.

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