Chapter 23. The Current Status of Iron Chelation Therapy

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While iron deficiency is well-known and is in fact the leading cause of anemia in man,[1] the pathological accumulation of excess iron is also widespread. Iron accumulation results from hyperabsorption of dietary iron (primary hemosiderosis) or from transfusion therapy (secondary hemosiderosis). While primary hemochromatosis is a rather rare genetic disease, the thalassemia syndromes (alpha and beta) are much more common. These too are genetic disorders, the defect being an imbalance in the synthesis of the alpha and beta chains of hemoglobin respectively. Patients afflicted with either of the latter syndromes suffer from an anemia which ranges from very mild to life threatening. It is necessary to give frequent blood transfusions to those children whose anemia is severe. Worldwide, it is estimated that 3 million people show clinically significant manifestations of thalassemia. Beta-thalassemia is most common throughout the Mediterranean basin and the Middle East whereas alpha-thalassemia is prevalent in the Far East. The geographical distribution is similar to that of malaria suggesting that, as in the case of sickle cell anemia, the heterozygotes or gene carriers are somewhat protected against the malaria parasite. In some areas heterozygotes make up as much as 30% of the population.[2]

The accumulation of iron is due to the fact that man lacks a physiological means of excreting this metal. The amount of iron present in the body is regulated solely by absorption.[3] This unusual regulatory mechanism probably arose as a result of the need to conserve this essential nutrient for it is relatively difficult to obtain from the diet due to the extreme insolubility of most environmental iron. The acquisition of this element is a problem common to virtually all biological organisms. Hence the multitude of complex mechanisms for sequestration, transport and storage of iron which have been developed by microbes and plants so as to compete effectively for that which is available.[4,5]

In man, iron is stored in various tissues as either ferritin or hemosiderin. Ferritin is a protein consisting of 24 subunits arranged in the form of a shell about a core of precipitated iron oxides. That which is called hemosiderin is believed to be aggregates of ferritin which has been partially digested by lysosomes.[6] Storage of excess iron is necessary to prevent its initiation of free radical reactions within the body. In iron overload, however, de novo synthesis of ferritin is unable to completely protect tissues from cell damage and subsequent fibrosis. Particularly noteworthy are the progressive fibrotic changes in the liver, heart and endocrine organs of iron-overloaded patients.[7] Most untreated

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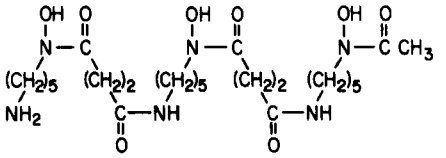
thalassemic patients die of cardiac complications during the first or second decade while those with untreated primary hemochromatosis die in their fifties, primarily of liver failure.

The clinical management of patients with primary hemosiderosis calls for venesection at regular intervals until body stores return to normal. The removal of one unit of blood (500 ml) causes the loss of 250 mg of iron. Phlebotomy of patients with secondary hemochromatosis is obviously precluded since these patients are already anemic and in fact require blood transfusions every few weeks for the maintenance of life. Patients on chronic blood transfusion programs accumulate iron at the rate of 15 to 25 mg per day and thus would be expected to have an excess of approximately 100 g by the age of 20. Until such time as it is possible to treat the primary defect of thalassemia, therapy will most likely depend upon chronic transfusions. Accordingly, the development of an effective pharmacological means of inducing iron excretion is necessary as adjunctive therapy. During the past 20 years, several compounds have been identified as potentially useful iron-chelating drugs and are at different stages of development. Recently, the National Institutes of Health has noted the need for more efficacious drugs and has initiated a program to screen and evaluate iron chelators. Two complementary biological screening procedures are being utilized in the search for new drugs, an *in vitro* technique using Chang liver cells[8] and an *in vivo* assay employing hypertransfused rats.[9]

The compounds that have been studied to date are primarily those having functional groups found in natural products. These include hydroxamic acids and derivatives of 2,3-dihydroxybenzoic acid. These moieties possess a high affinity for ferric ions rendering compounds highly selective for this metal both *in vitro* and *in vivo*. Briefly we shall describe the current status of the iron chelators which have been identified as having potential therapeutic usefulness.



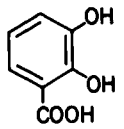
DF

Desferrioxamine - Desferrioxamine (DF) is a hexadentate iron chelator which is produced by the microbe *Streptomyces pilosus*. The three hydroxamic acid groups occupy all six sites in the octahedral coordination sphere of iron. The binding constant of this ligand is 10^{31} reflecting the very high affinity of the hydroxamate moiety for ferric ions. When evaluated in cultured Chang cells, DF is able to prevent the incorporation of iron into ferritin and can induce the mobilization of iron from preloaded ferritin.[10] Moreover, DF causes the excretion of significant amounts of iron when administered intraperitoneally to hypertransfused rats.[9] In spite of this, however, the intramuscular injection of DF into iron over-

loaded patients is incapable of putting them into negative iron balance. Furthermore, the injections are poorly tolerated by the patients and their parents and thus this mode of therapy has been virtually abandoned. A resurgence of interest in desferrioxamine occurred in 1974 when Barry et al.[11] reported that the persistent use of intramuscular DF-therapy over a 7-year period decreased the rate of iron accumulation in thalassemic patients. Reevaluation of DF centered on maximizing its effectiveness via alternative routes of administration. It had been observed by Smith[12] that maximum urinary excretion of iron occurred when DF was given as a slow intravenous infusion. This occurs for two reasons. First, although an iron-overloaded patient has massive amounts of iron in his body stores, most of this iron is unavailable. At any given time, there is only a small pool of iron which is accessible to low molecular weight chelators. When iron is removed from this chelatable pool, it is refilled relatively slowly from sites of iron storage. Second, the circulating half-life of DF is only 76 minutes,[13] thus a significant portion of the chelator is excreted without iron due to prior depletion of the chelatable iron pool. At the time of Smith's observation, the impracticality of administering DF as a continuous infusion precluded evaluation of this mode of therapy. Recent advances in the development of portable infusion pumps have now made this possible. While injection of a single i.m. dose of DF induces the elimination of 14 to 26 mg of iron, continuous i.v. administration of the same dose over 24 hours leads to the excretion of 74 mg of iron, close to that expected on stoichiometric grounds.[14] Similar results have been obtained upon continuous subcutaneous infusion of DF to thalassemics on an outpatient basis.[15,16] Extended clinical evaluation of this mode of administration is currently underway to determine if DF-therapy can significantly delay or, hopefully, prevent the pathological changes associated with iron overload.

Attempts to increase the size of the chelatable pool available to DF have centered upon the use of pharmacological doses of ascorbic acid. It was noted by Bothwell and his co-workers[17] that iron-overloaded patients who had concomitant scurvy showed a decreased response to DF. Administration of ascorbic acid dramatically increased the magnitude of the response. Ascorbic acid therapy has also been shown to increase urinary iron excretion in thalassemic patients treated with DF.[18,19] On the other hand, the advisability of administering DF and ascorbic acid to iron-overloaded patients has been questioned both clinically and theoretically. Apparent cardiac toxicity has been noted in several patients placed on this regimen.[20,21] Moreover, there is justifiable concern about increasing the pool of chelatable iron in the presence of excess ascorbic acid since ferric ascorbate is well known as an initiator of free radical reactions.[22] Thus, most workers are now recommending that patients be replete with ascorbic acid while discouraging its use in pharmacological doses. Nevertheless, a means of increasing the size of the chelatable pool in a safe manner would be a useful adjunct to chelation therapy.

2,3-Dihydroxybenzoic Acid - 2,3-Dihydroxybenzoic acid (2,3-DHB) has been shown to be a potentially useful orally effective iron-chelating agent.[9] Many naturally-occurring iron chelators are conjugates of 2,3-DHB.[23-25] In



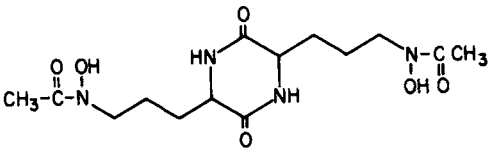
2,3-DHB

the case of these conjugates, both vicinal hydroxyls participate in iron binding while in that of 2,3-DHB itself, mixed complexes may form at physiological pH involving both hydroxyls or the carboxyl group and the adjacent hydroxyl.[26] The oral administration of 2,3-DHB to iron-overloaded rats promotes the excretion of urinary iron in a dose dependent fashion.[9] Preliminary clinical trials in patients with beta-thalassemia showed that iron excretion ranged from 1.4 to 19 mg per day following oral administration of 2,3-DHB.[27] The cause of this variability is not known although there is evidence which suggests that it may reflect the ability of a given person to transform 2,3-DHB into a more active metabolite which has not as yet been identified.[8] Also of note is the fact that the route of iron excretion induced by 2,3-DHB differs in rat and man.[27] In the rat, iron excretion is via the urine whereas in man, only fecal excretion of iron is elevated. A species difference is also observed in the case of DF which promotes predominantly fecal excretion in rats and urinary excretion in man. The basis for such species differences is unknown.

Clinical studies to date suggest that oral administration of 2,3-DHB, like the i.m. administration of desferrioxamine, is not capable of keeping patients in negative iron balance but rather is useful in retarding the rate of iron accumulation.[26] At present, it appears that slow subcutaneous infusion of DF is the only practical way of achieving iron balance in these patients. Another property of 2,3-DHB is its ability to act as a free radical scavenger[29]. As noted above, the tissue degeneration observed in iron overload is believed to result from free radical mediated damage to cellular components. Evidence for this resides in the fact that lipofuscin, which arises via the polymerization of unsaturated lipids, is commonly observed in this disease.[30] However, although 2,3-DHB is quite effective in scavenging radicals *in vitro*,[31] it was not possible to demonstrate decreased radical damage in patients who received 2,3-DHB for a period of one year.[28] Nevertheless, the adjunctive use of free radical trapping agents should decrease the pathology associated with iron overload, thus efforts to find more effective agents seem warranted.

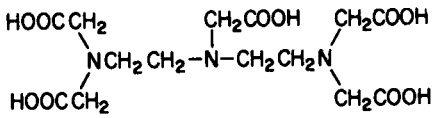
The fact that 2,3-DHB is orally absorbed makes it extremely attractive for use in those parts of the world where the cost of daily parenteral administration of a drug is prohibitive. Attempts to improve the efficacy of 2,3-DHB have not been successful. In a series of structural analogues, none was found to be more effective than the parent compound.[32,32] However, since most of these derivatives contained only a single 2,3-DHB moiety and hence a relatively low binding constant for

iron,[26] a series of compounds containing multiple 2,3-DHB moieties is being prepared in the hope of increasing efficacy while maintaining oral effectiveness.



RA

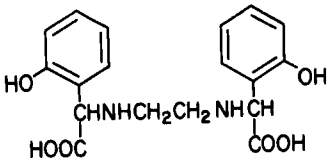
Rhodotorulic Acid - Rhodotorulic acid (RA) is a quadridentate hydroxamic acid produced by the yeast Rhodotorula pilimanae. When this organism is grown in iron deficient medium, it produces in excess of 1 g of RA per liter.[34] This is more than 50 times the amount of DF produced by Streptomyces pilosus. Thus, if clinically useful, RA should be much more economical than DF. On a weight basis, it is more than 2 times as effective as desferrioxamine at inducing iron excretion in hypertransfused rats following i.p. administration.[35] Unfortunately, like DF, rhodotorulic acid is not orally absorbed, hence it must be given parenterally. It differs from the former compound, however, in that it is relatively insoluble in water. This property may prove advantageous. RA is a good candidate for administration as an intramuscular or subcutaneous depot. Administered in this fashion, the drug would be expected to dissolve at a rate more nearly matching that at which the chelatable iron pool is repleted. Pharmacokinetic studies in dogs have revealed the presence of RA in plasma up to 12 hours after an i.m. injection.[36] This contrasts sharply with the rapid loss of DF (half-life 76 minutes) following administration i.m.[13] In the absence of an effective oral chelator, the availability of a repository preparation would eliminate the necessity of portable infusion pumps as a means of delivering a sufficient amount of drug to place patients in iron balance. Clinical evaluation of rhodotorulic acid is currently underway.[36]



DTPA

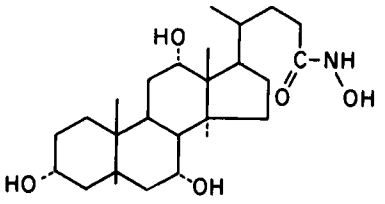
Ethylenediamine Derivatives - The intravenous administration of diethylenetriaminepentaacetic acid (DPTA) was found to be more effective than desferrioxamine in promoting iron excretion by beta-thalassemics.[37] The drug is not specific for iron, however, causing significant excretion of magnesium. This, together with side effects including chills, fever, nausea, vomiting and localized pain at the injection site, has led to abandonment of its

use.[38]



EHPG

Another analogue, ethylenediamine-N,N'-bis-(σ-hydroxyphenylglycine) (EHPG), is more selective and possesses a higher affinity for iron.[39] Nearly 20 years ago, this compound was administered to several iron-overloaded patients and found to promote iron excretion.[40] Unfortunately the work was not followed up until recently when EHPG was found to be the most effective iron chelator in a screen of potential drugs.[41] Of particular interest is the fact that this compound is orally absorbed. In fact, an oral dose of EHPG is more than 75% as effective as an equivalent intraperitoneal dose when administered to hypertransfused rats.[36] Further clinical studies are clearly warranted.



CHA

Cholylhydroxamic Acid - The hydroxamic acid moiety has an unusually high affinity for ferric ions. As noted above, neither DF nor RA is orally absorbed and hence they must be given parenterally. The evaluation of a number of natural and synthetic analogues has revealed that most such compounds are not absorbed from the gut.[32,33] The only orally effective compound found to date is cholylhydroxamic acid (CHA). When evaluated in iron-overloaded rats, cholylhydroxamic acid is as effective as an equivalent parenteral dose of desferrioxamine.[33] Presumably CHA acts as a bile acid and is transported to the liver via the enterohepatic circulation. The iron chelate would then be expected to exit via the bile. Ideally, a non-absorbable iron chelator in the diet, such as a tannin or a polymeric hydroxamic acid,[42] would then remove the iron from the chelate and allow the CHA to be recycled. Pharmacological studies of this compound are now in progress as well as a synthetic program aimed at developing cholic acid derivatives having more than one hydroxamate moiety.[36]

In conclusion, the development of drugs for the purpose of chelating iron is necessary if the sequelae of transfusion therapy are to be avoid-

ed. The rational design of such agents is now a challenge to medicinal chemists. Hopefully, in the years to come, an armamentarium of chelating agents will be available which will allow patients with iron overload to remain in iron balance, free of the complications which currently lead to their early demise.

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