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Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

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Desferrioxamine (DFO)

By

Ling Xiao

B-180 Medical Laboratories

Free Radical and Radiation Biology program

The University of Iowa

Iowa City, IA 52242-1181

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Abbreviations

DFO, desferrioxamine, Desferal®
$O_2^{\cdot -}$, superoxide
$HO^{\cdot}$, hydroxyl radical
ESR, electron spin resonance

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Abstract

Desferrioxamine (DFO) is a strong Fe(III) chelator, and is used clinically to treat iron overload diseases. DFO acts as antioxidant to prevent damage caused by superoxide anion or hydroxyl radicals. The primary mechanisms of DFO antioxidant role is that DFO forms a complex with Fe(III) to inactivate Fe(III), thus inhibiting the potential to generate $HO^{\cdot}$ via the Fenton reaction. Furthermore, DFO can directly react with superoxide anion and hydroxyl radical to act as a free radical scavenger. However, DFO can produce nitroxide free radical through one-electron oxidation reaction, and hence cause lipid peroxidation and enzyme inactivation, especially at high concentrations. This nitroxide free radical has been identified by electron spin resonance (ESR). And efforts have been given to generate other DFO derivatives without the toxicity while keeping iron chelating ability. This review focuses on the biochemical properties and the mechanisms of DFO as an agent in treating iron overloaded diseases as well as the biological relevant of DFO.

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Introduction

Desferrioxamine (DFO) is a hydroxamate type of siderophore derived from the microorganism, *Streptomyces pilosus* [1]. It is available as Desferal®, a commercial name for the methanesulfonate salt of DFO. DFO has a high affinity and specificity for ferric iron and is used clinically to treat iron overload diseases such as acute iron poisoning and β-thalassemia [2]. The $O_2^{\cdot -}$ can be formed in almost all aerobic organisms and $HO^{\cdot}$ can be generated from $O_2^{\cdot -}$ together with H_2O_2 via the Haber-Weiss reaction, which usually requires iron as a catalyst [3]. DFO has been shown to be able to inhibit this reaction, thus blocking generation of $HO^{\cdot}$ from $O_2^{\cdot -}$ by forming a unreactive DFO-Fe(III) complex [4]. Furthermore, DFO was shown to be an $HO^{\cdot}$ scavenger [5]. However, DFO has been shown to promote lipid peroxidation at high concentration through interaction between DFO and $O_2^{\cdot -}$ and $HO^{\cdot}$ to form the DFO nitroxide radical, which caused some damages to the cells [6, 7]. Nevertheless, DFO is still a very useful agent to treat iron overload diseases. This review focuses on the structure, chemical reactivity, and biological relevance of DFO.

Structure of DFO

DFO is the principal product of the various sideramines obtained from *Streptomyces pilosus* [1]. DFO consists of one molecule of acetic acid, two molecules of succinic acid, and three molecules of 1-amino-5-hydroxylamino-pentane (Figure 1, adapted from [1]). The organic units of DFO are interlinked to from a chain, in which three hydroxamic groups are inside and one free amino group at the end. The free amino group is capable of reacting with organic and inorganic acids to from DFO salts.

1Mol CH_3-COOH Acetic Acid

2Mol HOOC-$(CH_2)_2$-COOH Succinic acid

3Mol NH_2-$(CH_2)_5$-NH)H 1-Amino-5-hydroxylamine-pentane

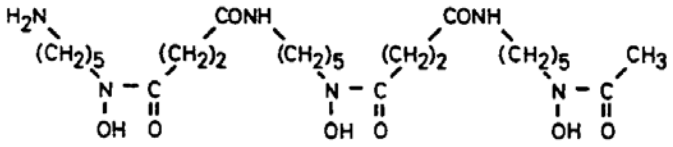

FIGURE 1. Desferrioxamine B.

Physical and chemical properties of DFO

DFO is colorless and water-soluble substance, which reacts slightly with alkali. DFO remains stabilized for eighty-four hours in a buffer solution at pH 6.1, but decomposes slowly at rate of 10% in fourteen hours in a buffer at pH 1.1. If it incubated with gastric juice of pH 2.5 at 37 ºC, it disintegrates at the rate of 4% h^{-1}. DFO has a molecular weight of 597 Da, and 1 molecule binds 1 molecule of Fe(III) ions [8]. DFO can act as scavenger of $HO^{\cdot}$ and $O_2^{\cdot -}$ [5]. Paradoxically, DFO has been shown to promote lipid peroxidation, especially at high doses [6].

Iron(III) chelator

DFO is a very strong and specific iron(III) chelator. The stability constants for various DFO-metal complexes compared with other chelating agents are listed in table 1. The higher the stability constant is, the more stable the complex is. The highest constant is 10^{31}, which is for iron(III)-DFO complex. DFO showed no significant affinity to ferrous ions.

Adapted from [1].

TABLE 1
STABILITY CONSTANTS OF SOME CHELATING AGENTS

Metallic ion	Desferrioxamine	EDTA	DTPA	Transferrin
Fe^{3+}	10^{31}	10^{25}	10^{29}	10^{27}; 10^{29}
Ca^{2+}	10^{2}	10^{11}	10^{10}	
Mg^{2+}	10^{4}	10^{9}	10^{9}	
Sr^{2+}	10	10^{9}	10^{10}	
Zn^{2+}	10^{11}	10^{16}		
Ni^{2+}	10^{10}	10^{19}	10^{20}	
Co^{2+}	10^{11}	10^{16}	10^{19}	
Cu^{2+}	10^{14}			
Fe^{2+}	~ 10^{10}			

The mechanisms of DFO in preventing iron overloading diseases

The generated $O_2^{\cdot-}$ in the cell has many deleterious effects, and in presence of H_2O_2, it will give rise to the highly reactive $HO^{\cdot}$. There is no evidence that $O_2^{\cdot-}$ will react directly with H_2O_2, but in presence of iron salt will results in $HO^{\cdot}$ through following reactions [3]:

$$\text{Fe(III) complex} + O_2^{\cdot-} \longrightarrow \text{Fe(II) complex} + O_2 \qquad (1)$$

$$\text{Fe(II) complex} + H_2O_2 \longrightarrow \text{Fe(III) complex} + OH^- + HO^{\cdot} \qquad (2)$$

DFO twines itself around the iron and attaches to the latter by means of its three hydroxamic acid groups to form ferrioxamine complex (Figure 2). The iron is surrounded by a shell of organic material, and this complex is very stable and cannot undergo redox cycling, thus preventing iron-

catalyzed $OH^{\cdot}$ formation or lipid peroxidation [4].

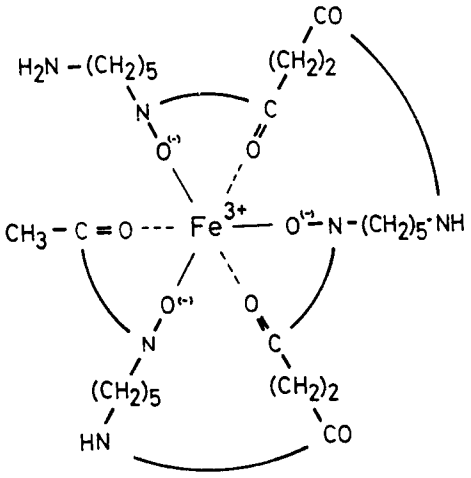

Figure 2. The schematic structure of DFO-Fe(III) complex (adapted from [1])..

Furthermore, it was shown that DFO could act as a scavenger of $HO^{\cdot}$ and $O_2^{\cdot -}$ to inhibit oxygen-mediated damage [6, 7]. $HO^{\cdot}$ reacts rapidly with thiocyanate to produce $^{\cdot}(SCN)_2^{-}$. By observing the ability of DFO and ferrioxamine to compete with thiocyanate for $HO^{\cdot}$, rate constants for their reaction with this radical can be determined [5]. Figure 3A shows a typical result for DFO and Figure 3B shows a result for ferrioxamine [5]. From duplicate experiments rate constants for the reaction of both compounds with $HO^{\cdot}$ were found to be $(1.3 \pm 0.1) \times 10^{10}$ M^{-1} s^{-1}, indicating that that they are powerful scavengers of $HO^{\cdot}$ [5]. And reaction of ferroxamine with $HO^{\cdot}$ led to release of Fe(III) from the DFO-Fe(III) complex [5]. And this process followed first order kinetics and was independent of the rate of initial reaction [5].

In addition, DFO was also able to react with $O_2^{\cdot -}$ generated by the action of xanthine oxidase on xanthine to act as scavenger of $O_2^{\cdot -}$ [8]. And the rate constant of the reaction between DFO and $O_2^{\cdot -}$ is approximately $1.3 \times 10^6 dm^3$ mol^{-1} s^{-1} at physiological pH [9].

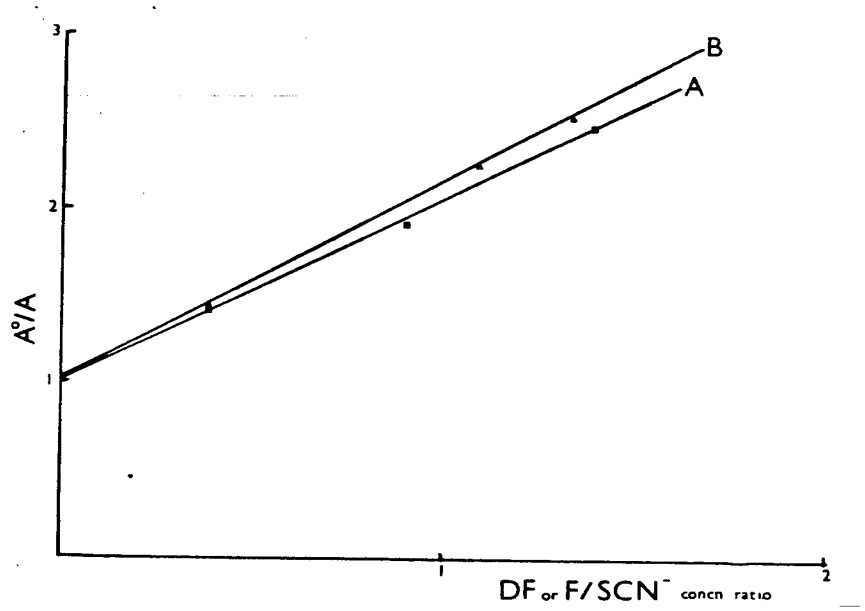

Figure 3. Determination of rate constant for the reaction of DFO and ferrioxamine by competition with thiocyanate. The absorbances at 480 nm of irradiated thiocyanate solution with (A) and without (A°) DFO and ferrioxamine was measured and are related by the equateion

$$A^{\circ}/A = 1 + K_c/K_t \times [\text{chelator}]/[\text{thiocyanate}]$$

Thus plotting A°/A against the ratio of concentrations allows calculations of the rate constant K_c. Kt is taken as 1.1 × 1010 M-1 s-1. Plot (A) shows data for DFO and plot (B) for ferrioxamine.

Formation of the nitroxide radical by DFO

In spite of the antioxidant roles of DFO, the prolonged clinical use of DFO is far from benign. And paradoxically, DFO has been shown to promote lipid peroxidation at higher concentration [10]. The toxicity might be due to the formation of DFO radicals. This nitroxide free radical from DFO can be produced by various peroxidases, or by ionizing, or UV irradiation [11, 12]. The following diagram shows the formation of a nitroxide radical of DFO (Figure 4). It should be noted that DFO has three hydroxamic acid groups and due to the structural similarities of the environment surrounding the nitroxide groups, it is not possible to determine which hydroxamic acid group of DFO is under attack. Thus, the scheme shows one possible radical structure that produces the spectrum they observed [13]. Furthermore, the DFO nitroxide radical

formed from the interaction between DFO and superoxide anion and hydroxyl radical has been shown to inactivate alcohol dehydrogenase [12].

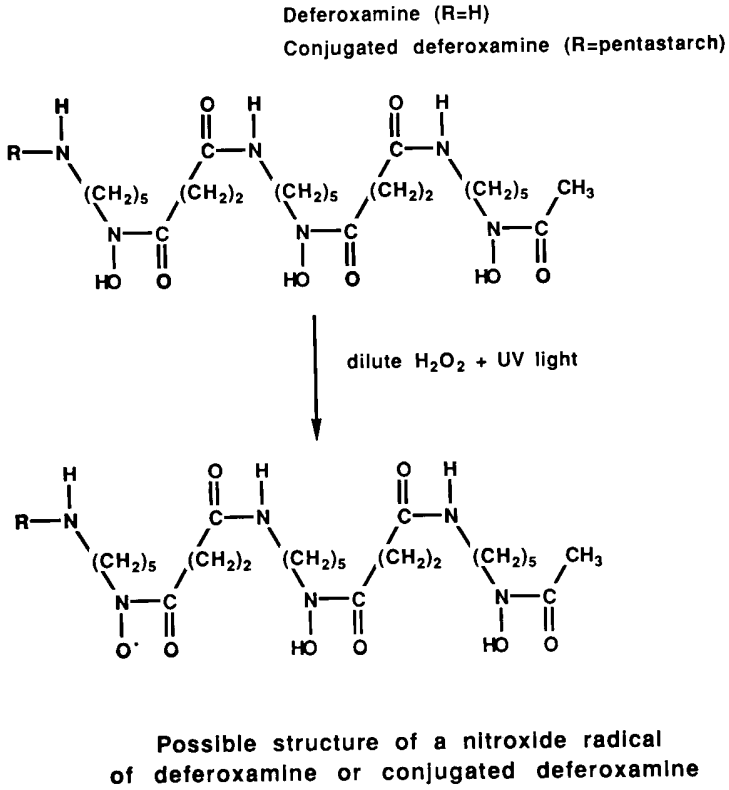

Figure 4. Diagram showing one possible form of a DFO nitroxide radical [13].

This nitroxide radical of DFO is the one-electron oxidation product of DFO, which has been successfully identified by electron spin resonance (ESR) (Figure 5). The oxidation of Desferal leads to a stable nitroxide free radical with a g factor of 2.0065. An acetyl nitroxide nitrogen coupling (a^N = 7.85 G) is split by two protons (a^H = 6.35 G) from a neighboring CH_2 group [6].

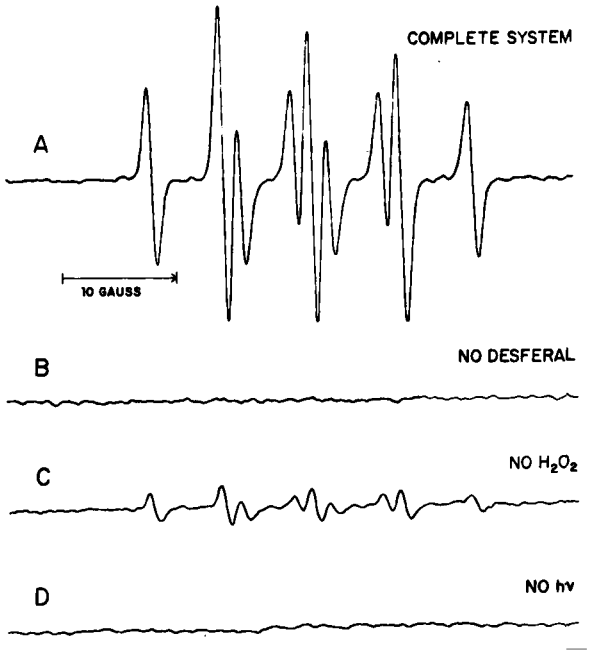

Figure 5. ESR spectrum of the nitroxide free radical obtained upon reaction of the hydroxyl radical with DFOl [6]. (A) Photolysis of a 0.1 mM hydrogen peroxide solution containing 1 mM Desferal in 100 mM phosphate buffer (pH 7.4). (B) Same as A, but in the absence of Desferal. (C) Same as A, but without H_2O_2 added. (D) Same as A, but with lamp off. Spectrometer settings: microwave power, 21 mW; modulation amplitude,0.53 G; sweep rate, 0.1 G/s; time constant, 2 s.

Summary

DFO has high affinity and specificity for Fe(III). It is used major in iron overloading diseases clinically. DFO play antioxidant role by direct chelating Fe(III) and superoxide anion and hydroxyl radical scavenger. But it has some cytotoxicity due to the nitroxide free radical from one electron oxidation of DFO. And it is not very clear whether this nitroxide radical is generated *in vivo*.

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