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The Fine Print:

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Desferal®*

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Paper II

Abbreviations

AlO Aluminoxamine
DFO Deferrioxamine
ESR Electron Spin Resonance
FDA Federal Drug Administration
FO Ferrioxamine
ROS Reactive Oxygen Species

* Desferal® is a registered Trademark for Novartis Pharma AG. Basel, Switzerland.

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Abstract

Deferrioxamine (DFO) (Desferal®) is currently the mostly used iron chelator in treatment of iron overload. Deferrioxamine prevents reactive oxygen species (ROS) generation by inhibiting the Fenton reaction by strongly complexing the ferric ion. Desferal® (Deferrioxamine mesylate) (DFO) the only Federal Drug Administration (FDA) approved drug used to remove toxic levels of iron from systemic circulation. This short review will summarize some of its biochemical properties, biological and some clinical properties.

1. Introduction

Desferal® is an excellent chelating agent for Fe^{+3}. It is currently being used clinically to treat iron-overload patients [1]. Apart from iron, Desferal® can bind several other transition metals, but with much lower stability constants [2]. Since Desferal is able bind transition metals in such a way that it inhibits their catalytic activity [3,4], this chelator has been used extensively for *in vitro* experiments to remove metal ions from the reaction system.

Desferal® forms complexes predominantly with ferric iron and with trivalent aluminium ions: the complex formation constants are 10^{31} and 10^{25}, respectively. The affinity of Desferal® for divalent ions such as Fe^{+2}, Cu^{+2}, Zn^{+2}, Ca^{+2} is substantially lower (complex formation constants 10^{14} or below). Chelation occurs at a 1:1 molar basis, so that 1 g Desferal® can theoretically bind 85 mg ferric iron or 41 mg Al^{+3} [5].

2. Biochemistry and Reactions

The primary amino group of Desferal® does not participate in the coordination of iron (Figure 1) and contributes to the low lipophilicity of this iron chelator because it is protonated at physiological pH [6].

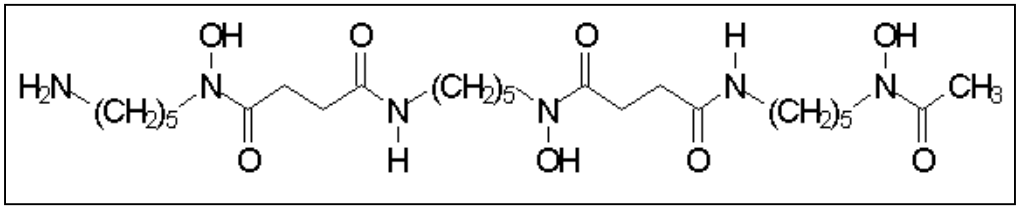


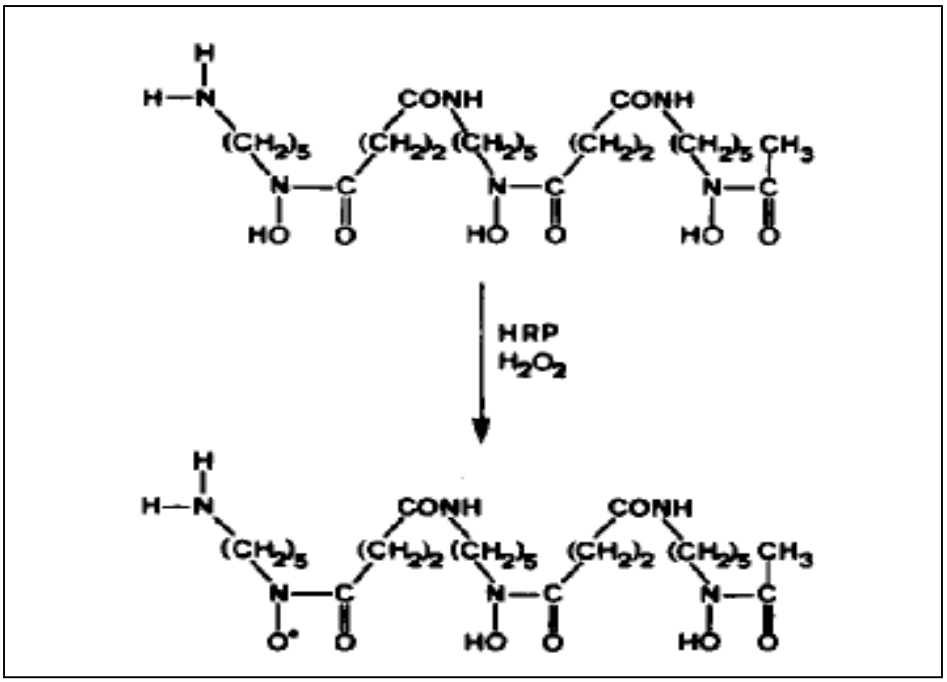
Figure 1 Structure of Desferal®. Adapted from [8]

Desferal® chelating action of Fe^{+3} prevents the Fenton reaction (reaction 1) and the production of the hydroxyl radical ($HO^{\bullet}$), which have a wide spectrum of tissue damage from DNA to lipid peroxidation, also it prevents the Haber-Weiss reaction (reaction 2) [7].

$$Fe^{+2} + H_2O_2 \longrightarrow Fe^{+3} + HO^{\bullet} + OH^{-} \quad (1)$$

$$Fe^{+3} + O_2^{-} \longrightarrow Fe^{+2} + O_2 \quad (2)$$

There have also been reports that the hydroxyl ($HO^{\bullet}$) and superoxide anion ($O_2^{\bullet -}$) radicals are capable of reacting with Desferal®, and the production of nitroxide free radical form of Desferal® [9]. As mentioned above, the hydroxyl radical has previously been shown to react with Desferal with a diffusion-controlled rate (k = 1.3 x10^{10} M^{-1} s^{-1}) indicating that it is a powerful hydroxyl radical scavenger [10]. Scheme 1 shows the formation of Desferal® radical in a system where hydrogen peroxide (H_2O_2) and horseradish peroxidase (HRP) were use to generate the radical.



Scheme 1 Formation of Desferal® free radical in system containing hydrogen peroxide (H_2O_2) and horseradish peroxidase (HRP). Adapted from [10].

As mentioned above there is a large discrepancy between the Fe^{+2} and Fe^{+3} binding to Desferal®, this difference result from the large shift in reduction potential of Fe^{+3} from +0.77 V for ionic iron to –0.44 V for Desferal®-bound Fe^{+3} [11].

Desferal® also know as an inhibitor for iron catalyzed lipid peroxidation, or in other word, it works as an antioxidant, but on the other hand, it could act as a prooxidant. In a study by Miller D.M and coworker showed that the binding of Fe^{+3} from biologically relevant iron chelates by Desferal® is relatively slow process, requiring minutes to hours for complete binding to happen. They also demonstrate that Desferal® is an inhibitor of iron catalyzed lipid peroxidation, but under appropriate conditions, Desferal® also stimulated lipid peroxidation. This prooxidant effect of Desferal® was suggested to be related either to the slow rate of Fe^{+3}

chelation, in case of Fe^{+3} dependent lipid peroxidation, or to rapid Fe^{+2} autoxidation, yielding H_2O_2, in case of lipid peroxidation catalyzed by autooxidation of Fe^{+2} (reaction 3)[12].

$$2\text{Desferal} - Fe^{+2} + O_2 + 2H^{+} \longrightarrow 2\text{Desferal} - Fe^{+3} + H_2O_2 \qquad (3)$$

3. Detection of Desferal® Radical.

Electron Spin Resonance (ESR) is used to detect nitroxide free radical of Desferal®. radical-generating system. 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 the neighboring CH_2 group giving the 9-line spectra. The unusually small nitrogen coupling of this nitroxide is characteristic of a neighboring carbonyl group. Figure 2 shows a example of this spectrum, in a system contains hydrogen peroxide [13].

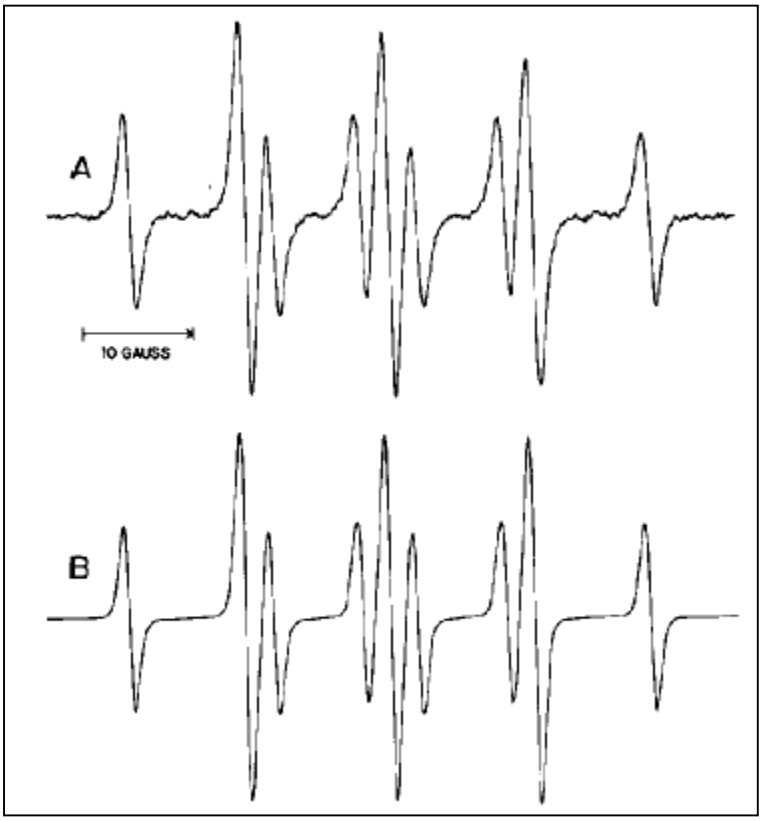


Figure 2 EPR spectrum of the nitroxide free radical generated by one-electron oxidation of Desferal®. (A) The solution contained 1 mM Desferal, 0.2 mg/mL horseradish peroxidase and 100 μM hydrogen peroxide in 100 mM phosphate buffer (pH 7.4) containing 0.1 mM DTPA. Spectrometer settings: microwave power, 21 mW; modulation amplitude, 0.2 G; sweep rate, 0.1 G/s; time constant, 1 s. (B) Computer simulation of A, a^N = 7.85 G and a^H = 6.35 G. Adapted from [13].

4. Biology and Physiology

Owing to its chelating properties, Desferal® is capable of taking up free iron, either in plasma or in cells thereby forming the complex ferrioxamine (FO). Urinary iron excretion of FO is predominantly a reflection of iron derived from plasma turnover whereas faecal iron reflects mainly intrahepatic iron chelation. Iron may be chelated from ferritin and haemosiderin but is relatively slow at clinically relevant concentrations of Desferal®. Desferal®, however, does not remove iron from transferrin or from hemoglobin or from other heme containing substances.

Desferal® can also immobilize and chelates aluminum, forming an aluminoxamine (AlO) complex [14].

Four main biotransformation reactions were found to occur with Desferal®: transamination and oxidation yielding an acid metabolite, beta-oxidation also yielding an acidmetabolite, decarboxylation and N-hydroxylation yielding neutral metabolites [15].

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