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Two new conjugates of desferrioxamine with 5-aminolevulinic and γ-aminobutyric acids bind intracellular iron in *C. elegans*

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Iron overload is harmful and potentially neurotoxic condition because it leads to a wide range of undesired reactions, such as DNA and lipid oxidation.[1] Desferioxamine (DFO) is a clinically used iron chelator with low permeability to physiological barriers, including the blood-brain barrier (BBB). Aiming to improve its permeability, we employed a strategy of conjugating DFO (**I**) to a few molecules permeable to BBB and studied these conjugates. Since GABA-DFO (**II**, with γ-aminobutyric acid) have shown promising results, we conjugated DFO with 5-aminolevulinic acid (ALA). ALA-DFO (**III**) was obtained in two steps:[2] initially, Boc-ALA-DFO (**IIIa**) was formed from DFO and Boc-ALA using activating reagents EDC and HOBt; then, Boc removal was achieved in a 50% TFA/DCM solution. The formation of **III** and **IIIa** was confirmed by LC/ESI-MS (m/z 674.41 and m/z 774.57 related to $[M+H]^+$, respectively), ^1H and ^{13}C NMR, CHN elemental analysis, FTIR, and UV-vis. Like **II**, conjugate **III** displayed iron-binding ability similar to DFO, showed antioxidant property in iron/ascorbate system, did not remove iron from transferrin, and weakly interacted with human serum albumin. Given the results for **II** and **III**, we conducted studies with *Caenorhabditis elegans*, a convenient model to investigate iron-induced neurotoxicity.[3] Iron overload was simulated through exposure to ferric sodium EDTA, Fe(edta) (LC_{50} = 44 mM). Pre-exposure to 100 and 25 µM of **I**, **II**, **III** and salicylaldehyde isonicotinoyl hydrazone (SIH, **IV**), a lipophilic iron chelator, protected worms from toxicity of Fe(edta). At 18 µM, the same protection was observed only for **II** and **III**. Moreover, **I**-**IV** presented lower lethality than Fe(edta) with post-treatment at 25 µM. However, no protection was observed for **I**-**IV** at 18 µM. In contrast, pre- and post-treatment with ALA and GABA (25 µM) did not decrease the lethality observed for Fe(edta). Finally, the iron binding ability of conjugates in *C. elegans* was assessed with Fe(edta) in calcein-AM-loaded worms. At 100 µM, **I**-**IV** were able to recover the fluorescence of calcein; at 25 µM, only **II** and **IV** showed similar results. Based on these results, we concluded that the conjugation of DFO with ALA and GABA increased DFO permeability, protecting worms from iron overload toxicity. These data also suggest that **II** showed intracellular iron-binding ability, which opens up the possibility of new experiments focusing on this iron-chelating conjugate.

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References

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