Study of Potential Drug for Alzheimer's Disease: Small Organic Molecules, 1,5-DHN and TMPyP Inhibit Amyloid-β peptide Aggregation and Quench Hydroxyl Radicals

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Abstract

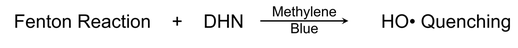
Alzheimer disease (AD) is recognized as the six leading cause of the death in the United States. As of now, there is no cure for this fatal disease. The current treatment methods can only temporarily slow the worsening of symptoms. Research data suggested that an excess generation of hydroxyl radical in the brain causing the aggregation of Amyloid-β (Aβ) peptide which is considered to be responsible for Alzheimer's disease. Thus, there is a pressing need to find a suitable drug which can quench hydroxyl radicals effectively and stop or slow down the formation of aggregation of Aβ peptide. The primary objective of the project is to find out a dual functional drug which can quench the reactive hydroxyl radicals produced in the brain and prevent Aβ peptide chains to come close to form Aβ peptide aggregate at the same time. A small organic molecule 1,5-dihydroxynapthalene (DHN) was found to quench hydroxyl radical at a rate of 3 X 10^{-4} s^{-1}. An independent experiment suggested that it intercalated efficiently into the Aβ peptide chains. Upon addition of meso-tetra(N-methyl-4-pyridyl)porphyrin tetrachloride (TMPyP) with DHN, a strong synergistic effect in quenching the hydroxyl radical and intercalating into the Aβ peptide chains was observed. This data suggests that DHN or DHN & TMPyP are the potential drug for Alzheimer disease treatment.

Introduction

Approximately, 200,000 Americans under the age of 65 are suffering from Alzheimer disease today and, unfortunately, there is currently no cure. Researchers believe that the cause of Alzheimer's disease is mostly due to the aggregation of Amyloid-β (Aβ) peptide.[1] Generally, the aggregation of Aβ is initiated and enhanced when the balance between oxidants and antioxidants is disrupted in the brain.[2] This unbalancing process between antioxidant and oxidant is called oxidative stress. Oxidative stress can occur when there is an increase in free radical concentrations within the brain. Research studies indicated that the major source of free radicals in our brain is due to the reduction of molecular oxygen in water. It has been proven that oxygen gas is reduced into superoxide radical, which further reduced into hydrogen peroxide. Hydrogen peroxide is then subsequently reduced into a highly reactive hydroxyl radicals,[2] this reactive oxygen species (ROS) can disrupt lipids membranes, proteins, nucleic acids,[3] via a chain of irreversible oxidative reactions. Thus, our interest is to develop drugs and/or methods for (1) quenching/trapping excess produced hydroxyl radicals into non-toxic products and (2) stopping or slowing down the formation of aggregation of amyloid β (Aβ) peptide.

Methods

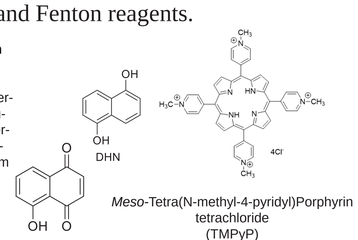
1. Quenching of hydroxyl radical generated *in situ* via Fenton reaction where DHN and methylene blue are used as hydroxyl radical quenchers.



2. Optimization of [DHN] concentration for hydroxyl radical quenching reactions.
3. DHN, TMPyP, and Juglone effect on Amyloid-β aggregation
4. Study hydroxyl radical effects on Amyloid-β aggregation using TMPyP, DHN, and Fenton reagents.

Amyloid-β protein (1 - 42) sodium salt

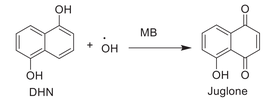
H_2N-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-OH sodium salt



Meso-Tetra(N-methyl-4-pyridyl)Porphyrin tetrachloride (TMPyP)

Results

1. Quenching of hydroxyl radical generated *in situ* via Fenton reaction where DHN and methylene blue (MB) are used as hydroxyl radical quenchers.



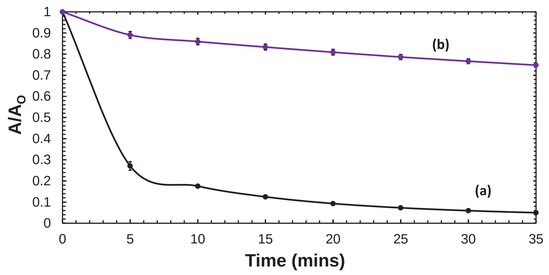


Figure 1: A comparison of absorption spectra of methylene blue at 664 nm in the presence of (a) reactive oxygen species and (b) reactive oxygen species and DHN.

2. Optimization of [DHN] for determining the slowest rate in which methylene blue is oxidized

Table: Methylene blue oxidation rate constants for various concentrations of DHN

Solution	Rate Constant (s^{-1})	R^2
Methylene Blue only	3.1×10^{-3}	0.9162
1.0×10^{-6} M DHN	3.3×10^{-3}	0.8406
1.0×10^{-5} M DHN	1.2×10^{-3}	0.9093
1.2×10^{-4} M DHN	3.0×10^{-4}	0.8754
1.0×10^{-3} M DHN	6.0×10^{-5}	0.9408

Results

3. DHN, TMPyP, and Juglone effect on Amyloid-β aggregation observed by UV-Vis spectroscopy

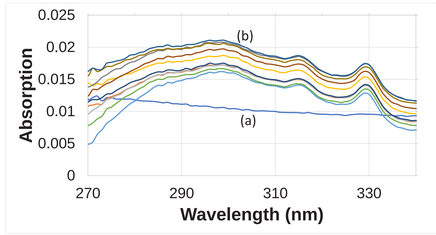


Figure 2: Absorption spectra of DHN (301 nm) in the presence of amyloid-β. (a) 1 x 10^{-5} M DHN and (b) DHN with 8 μM amyloid-β over time. Spectra was recorded every 10 minutes for an hour.

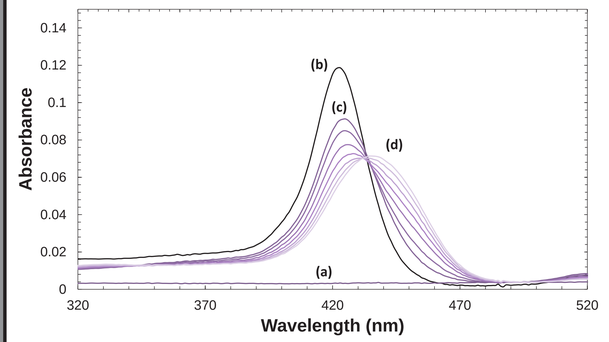


Figure 3: Absorption spectra of TMPyP (423 nm) in the presence of amyloid-β. Spectra was recorded every 10 minutes for an hour. (a) 8 μM amyloid-β (b) 6 μM TMPyP (c) TMPyP and Aβ at 0 mins (d) TMPyP and Aβ at 60 mins.

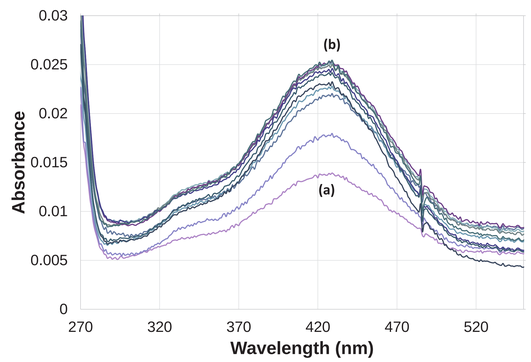


Figure 4: Absorption spectra of Juglone (429 nm) in the presence of amyloid-β. (a) 5 x 10^{-5} M Juglone and (b) Juglone with 8 μM amyloid-β over time. Spectra was recorded every 10 minutes for an hour.

4. Hydroxy radical effects on Amyloid-β aggregation observed using UV-visible spectroscopy and TMPyP

Results

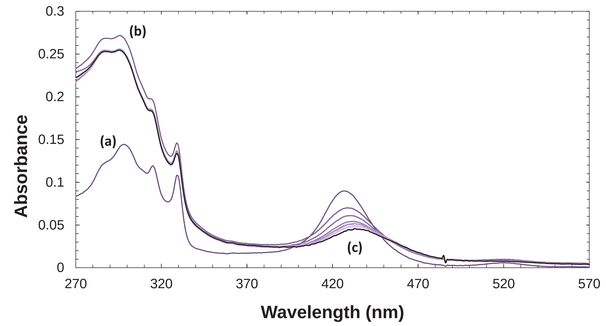


Figure 5: Absorption spectra of DHN (301 nm) and TMPyP (423 nm) in the presence of amyloid-β and Fenton reaction. (a) 1.4 x 10^{-4} M DHN, 6 μM TMPyP and 8 μM amyloid-β (b) DHN, TMPyP, Aβ and 3 mM Fe(II) (c) DHN, TMPyP, Aβ with Fenton Reaction recorded over time. Spectra was recorded every 10 minutes for an hour.

Conclusion

- The oxidation of methylene blue (3.1 x 10^{-3} s^{-1}) was drastically reduced in the presence of DHN (3.0 x 10^{-4} s^{-1}). These results suggest that DHN could be a possible option for slowing the rate of amyloid-β aggregation.
- The absorption spectra revealed that DHN successfully intercalated into the peptide chains and can prevent or slow down amyloid-β aggregate formation. Synergistic effect observed upon addition of TMPyP with DHN.
- Figure 5 shows a relatively slow decrease and right shift in the TMPyP peak at 423 nm. This is indicative of a TMPyP and Aβ aggregation interaction. However, in the presence of DHN, the absorption spectra of TMPyP decreases indicating TMPyP is selectively reacting with hydroxyl radicals. Further investigation is need to understand what is occurring during the reaction.

Future Work

- Analyze atomic force microscopy imaging of Amyloid-β aggregates in the presence of DHN.
- Assessment of conformational changed in the presence of TMPyP and DHN by circular dichroism spectroscopy.
- Observer biomolecule interactions using surface plasmon resonance.

Acknowledgements

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