Myeloperoxidase-mediated toxicity of the industrial contaminant, 6-PPD, in vitro

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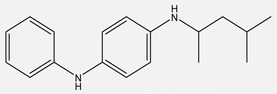
Introduction

Abstract

Purpose: 6-PPD (N-[1,3-Dimethylbutyl]-N'-phenyl-p-phenylenediamine) quinone has been recently reported as highly toxic to coho salmon and potentially toxic to other aquatic organisms. The possible toxicity of 6-PPD quinone's parent compound, 6-PPD, an industrial antiozonant, in the human body, remains unknown. The enzyme myeloperoxidase (MPO) in neutrophils, is known to produce radical products with chemical agents and so its role in 6-PPD-mediated toxicity was investigated. **Methods:** UV-visible spectrometry assay, LC-MS, cellular viability assay in HL-60 cell line was assessed with Cell-TiterGlo® viability assay kit and Electron paramagnetic resonance (EPR) analysis. **Results:** UV-Vis analysis of MPO heme site showed that the addition of MPO resulted in minor spectral change of 6-PPD oxidation whereas the addition of GSH resulted in greater spectral change indicating a possible GS-6-PPD conjugate formation which rapidly increased in the presence of H_2O_2. LC/MS results showed the formation of multiple products, including the possible GS-6-PPD conjugates, and N-dealkylated metabolites which could potentially induce cytotoxicity. The IC_{50} of 6-PPD in HL-60 cells was 2.039 µM. EPR data of MPO mediated 6-PPD oxidation showed a concentration-dependent relationship between 6-PPD and GS radical signal intensity in the presence of H_2O_2.**Conclusion:**6-PPD's oxidation can induce radical products formation and GSH conjugation in the presence of MPO. In addition, 6-PPD induces toxicity in MPO-rich HL-60 cells. Our results suggest that MPO could be a possible mediator of 6-PPD' toxicity in humans. A potential relationship between 6-PPD's oxidative metabolites and toxicity mechanism requires further investigation.

Background

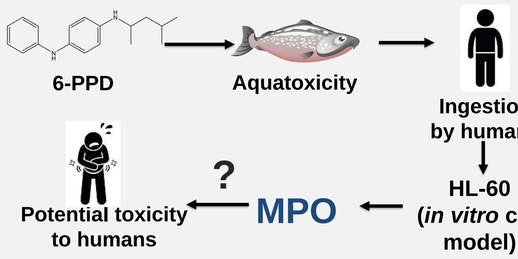
- 6-PPD (N-[1,3-Dimethylbutyl]-N'-phenyl-p-phenylenediamine) is an antiozonant that is added frequently to rubber products such as tires for preventing degradation due to heat and fatigue.[1]



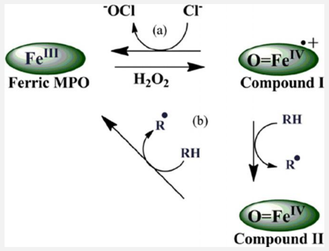
Structure of 6-PPD (N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine)

- Recent findings show 6-PPD is highly toxic to aquatic organism such as salmon while there is a lack of mechanistic study in human.[2]

Rationale



- Myeloperoxidase (MPO) in neutrophils is known for generating free radicals via metabolizing xenobiotics which may mediate 6-PPD toxicity via generate reactive metabolites



Peroxidation and chlorination cycle associated with Myeloperoxidase [3]

- There is a need to identify the reactivity of 6-PPD and the possible mechanism which may induce toxicity.

Goal/Hypothesis

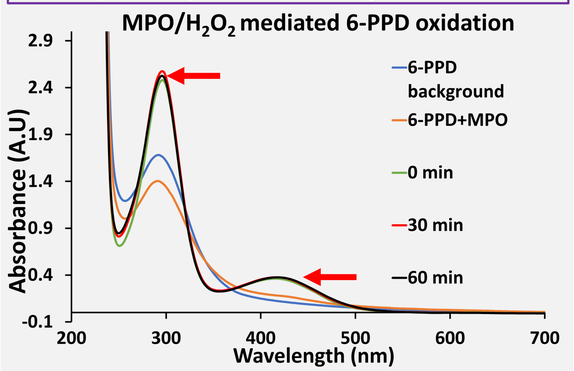
- **The aim of this study is to identify the role of MPO in possible toxicity of 6-PPD in human cell line.**
- **We hypothesize that MPO-mediated 6-PPD oxidation will induce toxicity via the generation of reactive metabolites.**

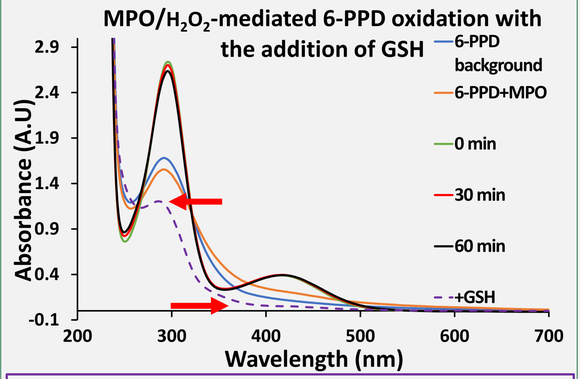
Methods

- **UV-Vis spectrometry analysis of MPO activity on 6-PPD**
- **Liquid chromatography-mass spectrometry analysis of metabolites formed by MPO-mediated metabolism**
- **Cellular ATP viability assay with HL-60 cell line (MPO rich)**
- **EPR analysis of 6-PPD reactive metabolites induced glutathionyl radical**

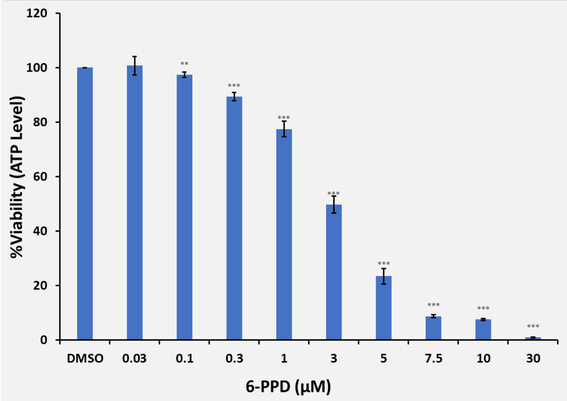
Results

UV-Vis analysis of MPO activity catalyzes spectral changes of 6-PPD





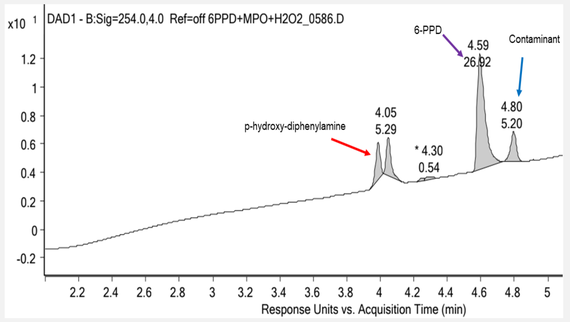
Cellular viability assay (ATP level)



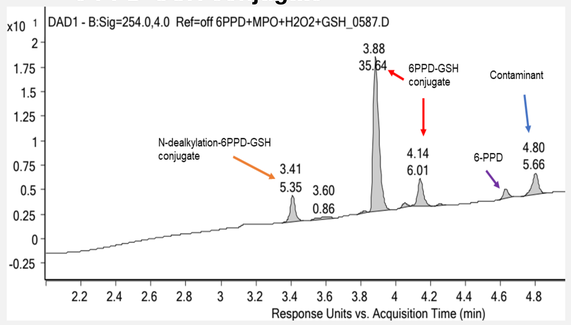
Results

LCMS chromatograms of 6-PPD metabolites (proposed)

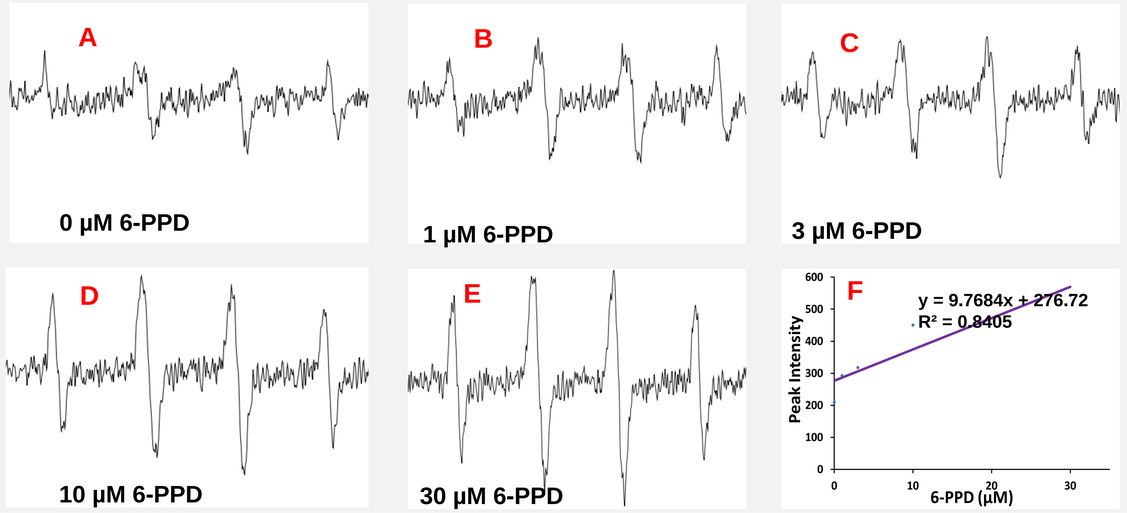
p-hydroxy-diphenylamine



6-PPD-GSH conjugate & N-dealkylated-6-PPD-GSH conjugate



EPR analysis of Glutathionyl radical produced by MPO/H2O2 mediated 6-PPD metabolites



Conclusion & Future studies

- **MPO mediates the metabolism of 6-PPD and generates metabolites via facilitating GSH conjugation and dealkylation**
- **6-PPD both decreased cell viability of HL-60 cells (IC_{50} ≈ 2.039µM) and increase GS radical formation in a concentration dependent manner. A potential relationship between cytotoxicity and formation of free radical needs further investigation.**
- **In the future, we will investigate MPO mediated 6-PPD metabolism effect on GSH depletion, mitochondrial potential change, MPO's peroxidation and chlorination cycle**

References

1. Abele M, Albrecht KD, Eholzer U, Fries H, Langner G-P, Hofmann W (1977) KautschuckChemikalien und Zuschlagstoffe. In Ullmanns Encyklopädie der technischen Chemie, 4. Aufl., Weinheim, Verlag Chemie, Bd. 13, 637 – 670
2. Tian, Z., Zhao, H., Peter, K.T., Gonzalez, M., Wetzel, J., Wu, C., Hu, X., Prat, J., Mudrock, E., Hettinger, R., Cortina, A.E., Biswas, R.G., Kock, F.V.C., Soong, R., Jenne, A., Du, B., Hou, F., He, H., Lundeen, R., Gilbreath, A., Sutton, R., Scholz, N.L., Davis, J.W., Dodd, M.C., Simpson, A., McIntyre, J.K., and Kolodziej, E.P. 2021. A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon. Science 371(6525): 185. doi: 10.1126/science.abd6951.
3. PSS288. Potent Reversible Inhibition of Myeloperoxidase by Aromatic Hydroxamates - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Normal-catalytic-cycling-of-myeloperoxidase-The-native-state-of-the-enzyme-ferric-MPO_fig1_258314677 [accessed 6 May, 2022]

Acknowledgment

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