



# Exploration of the Binding of Hydroxocobalamin with Sulfur Containing Compounds

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Patrick C Ng, MD

Maj, USAF, MC

Director, USAF En route Care Research Center

59<sup>th</sup> MDW/ST Office of the Chief Scientist

U.S. Army Institute of Surgical Research



U.S. AIR FORCE



# Disclaimer Statement

- The views expressed are those of the authors and do not reflect the official views or policy of the Department of Defense or its Components. The experiments reported herein were conducted according to the principles set forth in the National Institute of Health Publication No. 80-23, Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966, as amended.

# Background

- Hydrogen sulfide and Methanethiol are highly toxic chemicals
- There is no FDA approved antidote for hydrogen sulfide and methanethiol toxicity
- Cobalt containing compounds such as cobinamide have shown efficacy as an antidote for different toxic chemicals

# Objectives

- Create a methanethiol and hydrogen sulfide toxicity model *in vitro*
- Explore then mechanism of cobalt containing compounds such as hydroxocobalamin (HOC) or aquo-hydroxycobinamide (AHC) as a counter measure for these toxins *in vitro*

# Methods (HOC)

- 100 milligrams of hydroxocobalamin (HOC) dissolved into 200 mL of water in a 250mL Erlenmeyer flask with an stir bar
- Physiologic pH adjusted to (7.0-7.5) using diluted sodium in dropwise fashion
- Under a fume hood, an equimolar amount of sodium hydrogen sulfide (SHS) or sodium thiomethoxide (STM) was added and mixed on a stir plate
- Sample analyzed at 30 minutes via LC/MS to detect if sulfur complexed with hydroxocobalamin

# Methods (AHC)

- ~70-100 milligrams of aquohydroxycobinamide (AHC) was dissolved in 5 mL of water in a 20 mL scintillation vial with a stir bar
- SHS or STM added under fume hood, volume determined to afford a full equivalent of thiol over 30 minutes
- System placed on stir plate
- Samples taken at 10-minute intervals and analyzed via LC/MS

# Results

- Hydroxocobalamin (HOC)
  - Reaction with a single equivalent of sodium hydrogen sulfide (SHS) consumed 50.9% of the HOC after 30 minutes
  - Analysis of the solution at 330 minutes showed that no significant further reaction occurred (13.092  $\mu\text{g}/\text{mL}$  to 12.952  $\mu\text{g}/\text{mL}$ ).
  - This indicates HOC is capable of reacting with two equivalents of SHS

# Results

- Aquohydroxycobinamide (AHC)
  - Reaction with a single equivalent of SHS resulted in 44.0% consumption of the AHC. Addition of a second equivalent of SHS consumed 89.0% of the starting AHC.
  - AHC reacted with one equivalent of sodium thiomethoxide resulted in the area count of AHC decreasing by 39.8% after 30 minutes. Addition of a second equivalent of sodium thiomethoxide consumed 57.7% of the starting AHC.

# Conclusion

- The intent of this experiment was to establish that thiols indeed react with the cobalt complexed inside a porphyrin ring, such as HOC or AHC
- In the case of HOC, only the reaction with equimolar sodium hydrogen sulfide gave a clear indication that a reaction had occurred and only one-half of the HOC had been converted
- The reactions using AHC and either sodium hydrogen sulfide or sodium thiomethoxide proceeded to 40-44% conversion

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# Questions