Evaluation of the abilities of hemoprotein reductants to attenuate rhabdomyolysis-induced renal failure

Phillip H. Behrens IV, William Zachert, Irene Zagol-Ikapitte, Olivier Boutaud, and L. Jackson Roberts, II
Vanderbilt University, Nashville, TN

Introduction

Hemoglobin and myoglobin (Mb) are hemoproteins that cause severe oxidative damage as a result of redox cycling that generates radical species that induce lipid peroxidation and oxidative injury. Lipid peroxidation catalyzed by these hemoproteins is responsible for the oxidative injuries associated with rhabdomyolysis, subarachnoid hemorrhage, sickle cell disease, malaria, and myocardial injury followed by reperfusion. Thus, inhibitors of hemoprotein redox cycling might be used to effectively treat a wide variety of clinical diseases. Rhabdomyolysis is a condition of severe damage to skeletal muscle which causes myoglobin to be released into the blood stream and deposited in the kidney. Renal oxidative injury ensues when redox cycling between ferrous Mb (Fe2+) and ferric Mb (Fe3+) generates lipid based radical species. Rhabdomyolysis-induced renal failure is attributed to 7% of all cases of renal failure in the United States.

Clinical treatment of severe rhabdomyolysis-induced renal failure is alkalinization by administering bicarbonate. Increasing pH effectively decreases lipid peroxidation by stabilizing the ferryl heme. Recent studies show that therapeutic doses of acetaminophen (AcM) better attenuate rhabdomyolysis-induced renal failure by reducing ferryl Mb to ferric Mb (1). However, AcM is toxic to the liver and kidney at higher concentrations. Novel compounds have been synthesized that inhibit hemoprotein induced lipid peroxidation in vitro. It is believed that these compounds will be more effective than AcM at reducing the kidney damage that accompanies rhabdomyolysis.

Objectives

> Induce rhabdomyolysis that leads to significant kidney damage in Sprague-Dawley rats.
> Administer novel hemoprotein reductant compounds by injection following induction of rhabdomyolysis.
> Analyze urine, plasma, kidney cortex, and kidney medulla to assess the level of renal function and oxidative damage.

Methods

### Treatments

- **Standard treatment:** Acetaminophen (25 mg/kg and 50 mg/kg) intraperitoneal injections were given to eight rats with rhabdomyolysis. At 24 hr post-rhabdomyolysis, rats were sacrificed via cardiac puncture to collect blood plasma and kidney samples. Urine was collected 24 hr pre-rhabdomyolysis and 6 hr and 22 hr after induction of rhabdomyolysis.

- **Experimental groups:** Treated with AcM (25 mg/kg and 50 mg/kg), in rats with rhabdomyolysis treated with pyridinol derivative #64 (#64 25 mg/kg and #64 50 mg/kg), in rats with rhabdomyolysis treated with dimethylpyridinol #56 (#56 25 mg/kg and #56 50 mg/kg).

### Experimental Groups

- **Experimental Groups:**
  - **Normal (Control):** Normal rats
  - **Acetaminophen:** Rats treated with Acetaminophen
  - **Dimethylpyridinol #56:** Rats treated with Dimethylpyridinol
  - **Pyridinol derivative #64:** Rats treated with Pyridinol derivative

### Analysis

#### Urinary F2-Isoxprostanates

F2-Isoxprostanates are a byproduct of non-enzymatic free radical reactions that indicate the level of oxidative damage. Redox cycling between ferrous and ferric myoglobin in renal tubules catalyzes the formation of F2-Isoxprostanates. A standard assay for measuring F2-Isoxprostanates was performed followed by extraplotation of mass spectrometer chromatograms.

- **Effect in normal rats:**
  - No treatment
  - Acetaminophen (25 mg/kg and 50 mg/kg)
  - Pyridinol derivative #64 (#64 25 mg/kg and #64 50 mg/kg)

- **Due to small experimental group sizes, values were not statistically significant.**

- **Creatinine clearance.** Due to small experimental group sizes, values were not statistically significant.

### Results

#### Rhabdomyolysis

- **Creatinine:** A breakdown product of creatine phosphate in muscle. It is normally produced at a fairly constant rate and is filtered by the kidneys. Creatinine levels from the urine and is the basis for diagnosis of rhabdomyolysis (2). The amount of creatinine in the urine was determined by SI spectrophotometry.

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### Conclusions

- **Methods of rhabdomyolysis induction produced sufficient oxidative and renal damage.**
- **Treating rhabdomyolysis with acetaminophen and hemoprotein reductants may decrease oxidative damage and improve kidney function.**
- **Study must be continued to determine which treatment best attenuates rhabdomyolysis-induced renal failure.**

### Future Directions

- **Increase experimental group and control group sizes to increase precision.**
- **Analyze F2-Isoxprostanates from blood plasma, kidney cortex and medulla.**
- **Based on further results, possibly submit a drug for testing and eventual use in humans with rhabdomyolysis-induced renal failure.**

### References

2. [Vanderbilt University, Nashville, TN](http://www.vanderbilt.edu)

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Works Cited