Feasibility of determining oxidative biomarkers in healthy human subjects after oral Vitamin C administration

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Background

Post-operative atrial fibrillation (POAF) is the most prevalent arrhythmia following cardiac surgery, increasing mortality rates, stroke rates, and ICU length of stay. Some small interventional trials have shown a decrease in POAF following administration of antioxidant Vitamin C (ascorbic acid). The pharmacokinetics and pharmacodynamics of ascorbic acid in this population is unknown. Feasibility of measuring serum concentrations of ascorbic acid and other biomarkers of POAF in human subjects must be assessed to later investigate the broader effects of ascorbic acid in the cardiac surgery population.

Research Question

Determine the feasibility of measuring serum concentrations of oxidative biomarkers related to POAF including malondialdehyde (MDA), NADP+ to NADP ratio, reduced to oxidized glutathione ratio (GSH:GSSG), and plasma protein nitrotyrosine in healthy human subjects.

Oxidative Biomarker Reactions

Tyrosine reacts with peroxynitrite free radical to create nitrotyrosine.

Tyrosine

H2O2

NO

Nitrile Oxide

ONO

Peroxynitrite

3-Nitrotyrosine

In the presence of oxidative stress, NADPH is oxidized by GSSG

H2O2

GSH

MDA

Lipid peroxidation results in MDA production

Measurement of ascorbic acid in human plasma using spectrophotometric detection

Measurement of MDA in human plasma using spectrophotometric detection

Methods

Healthy human subjects were recruited and screened for inclusion. Baseline blood samples were drawn in ethylenediaminetetraacetic acid (EDTA) tubes after an 8 hour fast. Participants then received 20mg/kg ascorbic acid orally, and blood samples were drawn at hours 2 and 4. Samples were chilled and centrifuged at 1000 g. Aliquots of plasma and red blood cells (RBCs) were then stored at -80°C. A lipid peroxidation assay kit was used to measure MDA. Samples were reacted with thiobarbituric acid to produce a color readable at 532 and 553nm. Analysis of alternate biomarkers will be conducted for each sample. Glutathione will be measured in RBCs using high-performance liquid chromatography (HPLC), nitrotyrosine will be measured using an ELISA kit, and NADP+/NADPH will be measured using HPLC with fluorescence detection.

Results

Five subjects consented and participated. The average MDA concentration at baseline was 0.102 nmol/µL (SD 0.0075), 0.098 nmol/µL (SD 0.0075) at 2 hours, and 0.102 nmol/µL (SD 0.004) at 4 hours. Glutathione, NADP+/NADPH and nitrotyrosine assays will be completed over the next few months.

Conclusion

Determination of serum concentrations of MDA using a Lipid Peroxidation Assay Kit is feasible in human subjects administered oral vitamin C, and can be applied to determine the pharmacodynamic response of MDA to vitamin C in cardiac surgery patients. Further analysis must be completed to determine the feasibility of measuring serum concentrations of glutathione, nitrotyrosine, and NADP+/NADPH.

Future Plans

Conduct a pilot pharmacokinetic and pharmacodynamic study of ascorbic acid based off the data obtained from the completion of the current study

Conduct a larger pharmacokinetic and pharmacodynamic study for the prevention of oxidative stress in the cardiac surgery population

Conduct dose finding/validation studies

Conduct clinical intervention trials using the dosing strategy to determine potential reduction in POAF and reduce the risk of mortality, stroke, length of stay, and healthcare costs