DUNEDIN STUDY CONCEPT PAPER FORM

Provisional Paper Title: Peroxiredoxins as markers of redox homeostasis in human blood cells: application to a large population study

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P.I. Sponsor: N/A
(if the proposing author is a student or colleague of an original PI)

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Please describe your proposal in 2-3 pages with sufficient detail for helpful review.

Objective of the study:

Oxidant stress causes irreversible damage to proteins, lipids and nucleic acids, and increased oxidative damage is detectable in elderly individuals. However, this may be a late event linked to disease processes, rather than a significant contributor to the underlying biology of ageing.

The longitudinal Dunedin Study provides an opportunity to monitor oxidative stress in a large population, starting in mid-life before the onset of age-associated chronic disease. Phase 45 included the collection of blood samples from consenting participants specifically for measuring markers of oxidative stress.

Peroxiredoxins are abundant proteins in human cells that form part of the cellular defence mechanism against oxidative stress. Peroxiredoxins play an important role in removing the hydrogen peroxide generated by various metabolic processes, and are constantly active. During the processing of hydrogen peroxide, peroxiredoxins form oxidised dimers that can be detected by western blotting. We have pioneered assays to quantify peroxiredoxin oxidation in cells and tissues, and have shown that these proteins are extremely sensitive to disturbances in redox homeostasis (1). We hypothesize that oxidised peroxiredoxins are a more sensitive marker of underlying oxidative stress than traditional end-point assays of oxidative damage.

To apply this technology to human population studies, we have developed a protocol to measure the redox status of peroxiredoxins in platelets and red blood cells obtained from freshly drawn blood. We can also challenge red blood cells with hydrogen peroxide to monitor how quickly the peroxiredoxins are recycled back to their basal redox state. This provides a readout of the effectiveness of cellular antioxidant defence systems for each person sampled.

Protocols were established in pilot studies to optimize blood processing and the challenge of isolated red blood cells with hydrogen peroxide. These protocols were then applied to all blood samples obtained during phase 45. In this paper we will undertake statistical analysis of the population data, with particular focus on technical variation and the first-ever population distribution for these novel biomarkers. We will also investigate the impact of sample processing and storage time, and the contribution of having different people undertaking the processing and analysis. Finally, we will examine the relationship between each of the oxidative stress markers, including comparison of peroxiredoxin oxidation to plasma protein carbonyls and allantoin. Both of these plasma markers are commonly used end-point markers of oxidative damage.

Data analysis methods:

Data will be assessed for outliers related to data collection (i.e., hemolysis, replicates differ by more than a pre-defined threshold). Sensitivity analyses will be performed by calculating summary statistics before and after outliers are removed. Coefficients of analytical variation (CVA) will be calculated at the individual level, then averaged to give a pooled CVA. Replicates will be averaged to give one measure per individual for subsequent analyses. Spearman's rank-order correlation will be used to evaluate collinearity of variables (with categorical variables represented by dummy variables), and correlation between oxidative stress markers. We will then use multiple linear regression to investigate relationships between laboratory parameters (i.e., storage time, processor/analyst, hematological measures) and the untransformed/transformed dependent variables. Model assumptions and performance will be assessed by diagnostic plots and appropriate measures. Analysis and data visualization will be performed in RStudio with additional packages.

Variables needed at which ages:

Prx3 age 45
Prx2 basal age 45
Prx2 challenge age 45
Protein carbonyls age 45
Allantoin age 45
Haematological measures age 45

Significance of the Study (for theory, research methods or clinical practice):

Peroxiredoxins have not been used as biomarkers of oxidative stress in population studies, but we propose they will be more sensitive than traditional end-point markers of oxidative damage. Here we will present our newly developed method to measure the basal state of peroxiredoxins in erythrocytes and platelets, as well as a modification that assesses the response of cells to an oxidative challenge. We want to publish details on the optimization of these methods before using the data to assess the relationship between oxidative stress and other variables measured within the Dunedin Study.

References:

1. Poynton R.A. and Hampton M.B. Peroxiredoxins as biomarkers of oxidative stress. Biochim. Biophys. Acta 1840:906-912, 2014.