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Effects of Oral Glutathione Supplementation on Systemic Oxidative Stress Biomarkers in Human Volunteers

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Additional article information

Abstract

Background

The tripeptide glutathione (GSH) is the most abundant free radical scavenger synthesized endogenously in humans. Increasing mechanistic, clinical, and epidemiological evidence demonstrates that GSH status is significant in acute and chronic diseases. Despite ease of delivery, little controlled clinical research data exist evaluating the effects of oral GSH supplementation.

Objectives

The study objectives were to determine the effect of oral GSH supplementation on biomarkers of systemic oxidative stress in human volunteers.

Design

This was a randomized, double-blind, placebo-controlled clinical trial.

Setting/location

The study was conducted at Bastyr University Research Institute, Kenmore, WA and the Bastyr Center for Natural Health, Seattle, WA.

Subjects

Forty (40) adult volunteers without acute or chronic disease participated in this study.

Intervention

Oral GSH supplementation (500 mg twice daily) was given to the volunteers for 4 weeks.

Outcome measures

Primary outcome measures included change in creatinine-standardized, urinary F2-isoprostanes (F2-isoP) and urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG). Changes in erythrocyte GSH concentrations, including total reduced glutathione (GSH), oxidized glutathione (GSSG), and their ratio (GSH:GSSG) were also measured by tandem liquid chromatography/mass spectrometry. Analysis of variance was used to evaluate differences between groups.

Results

There were no differences in oxidative stress biomarkers between treatment groups at baseline. Thirty-nine (39) participants completed the study per protocol. Changes in creatinine standardized F2-isoP (ng/mg creatinine) (0.0±0.1 versus 0.0±0.1, *p*=0.38) and 8-OHdG (μg/g creatinine) (-0.2±3.3 versus 1.0±3.2, *p*=0.27) were nonsignificant between groups at week 4. Total reduced, oxidized, and ratio measures of GSH status were also unchanged.

Conclusions

No significant changes were observed in biomarkers of oxidative stress, including glutathione status, in this clinical trial of oral glutathione supplementation in healthy adults.

Introduction

Reduced glutathione (GSH) is a low-molecular-weight, water-soluble tripeptide, composed of the amino acids cysteine, glutamic acid, and glycine.[1] GSH is an important antioxidant and plays a major role in the detoxification of endogenous metabolic products, including lipid peroxides, and xenobiotic compounds including pollutants, heavy metals, and drugs.[2,3] Intracellular GSH exists in both the oxidized disulfide form (GSSG) or in reduced (GSH) state; the ratio between GSH and GSSG is held in dynamic balance depending on many factors including the tissue of interest, intracellular demand for conjugation reactions, intracellular demand for reducing power, extracellular demand for reducing potential, and the complex interplay between several regulatory enzymes including glutathione peroxidase (GPx), glutathione-*S*-transferase (GST) and γ-glutamyltransferase (GGT).[1] In the absence of adequate GSH concentrations, numerous oxidative and nitrosative reactive intermediates persist, including superoxide, hydroxide, peroxide, and peroxynitrite radicals, which all can lead to modification of cellular macromolecules including lipid membranes (measured *in vivo* by F2-isoprostanes) and DNA adduct formation (measured *in vivo* by 8-hydroxy-2′-deoxyguanosine [8-OHdG]).[1,4]

As oxidative and nitrosative processes continue, and cellular modification increases, physiologic function becomes altered secondary to impaired cellular messaging.[4] As such, there is considerable overlap in the pathogenesis of metabolic disease, environmental toxicity, and physiologic aging. Supporting this concept, total glutathione concentration appears to declines with aging, as demonstrated in both rat models of aging and in human aging (particularly after age 45 in humans), possibly due to a reduced ability to synthesize GSH.[5–7] Suboptimal GSH concentration has been associated with aging-related induction of oxidized and glycated proteins, similar to metabolic disease.[4] Furthermore, reduced glutathione concentration and/or a disproportionate ratio of GSH:GSSG has been associated with a number of diseases, including cancer, human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), hepatitis, type 2 diabetes, Parkinson's disease, and cystic fibrosis.[2,8–14] Although no experimental research to date has demonstrated disease prevention from GSH supplementation or other strategies to specifically increase GSH, observational research suggests increased dietary glutathione intake has been associated with reduced risk for oral cancer.

Because of the theoretical benefits of maintaining antioxidant defenses to combat various acute and chronic diseases, reducing the consequences of aging and reducing tissue damage from exposure to environmental oxidants, therapeutic approaches to increase systemic and/or tissue-specific glutathione concentrations are of considerable investigational interest. Erythrocyte GSH (RBC GSH) serves as a convenient biological reserve in which to measure GSH status, and several valid measurement methods are available, with enzymatic recycling being the most sensitive.[15–18] Importantly, the reactive nature of GSH causes challenges in sample stability and necessitates correct sample collection methods.[19] The bioavailability of orally administered cysteine is believed to be the rate-limiting step for the synthesis of glutathione,[10] although the amino acids glutamate/glutamine are also integral to GSH synthesis.[11,20] Most studies aimed to increase GSH concentration have used glutathione precursors, such as *N*-acetylcysteine or sodium salts of GSH,[2] based on the assumption that oral reduced GSH is poorly absorbed and/or oxidized in the gastrointestinal tract. However, to lesser degrees, intravenous, intramuscular, intrabronchial (i.e., nebulized), and intranasal glutathione administrations have all been used to increase circulating or tissue-specific GSH levels.[12,13,21]

Minimal experimental evidence has demonstrated significant increases in blood or intracellular levels of GSH through oral supplementation with reduced GSH. Animal *in vivo* data suggest that oral GSH is absorbed in rats.[6,7] Specifically, Hagen et al. demonstrated a doubling of plasma GSH (15–30 μmol/L) within 120 minutes after oral administration in rats,[7] and demonstrated dietary GSH is absorbed through a principal absorption site in the jejunum.[7] However, the absorption of GSH in humans has not been adequately demonstrated, and may prove more challenging. The human gastrointestinal tract contains significant amounts of the enzyme GGT, which recycles GSH precursors and may prevent significant intact absorption of GSH *per se* from oral supplementation. Few human clinical trials have evaluated the effects of oral GSH supplementation. Witschi et al. administered a single oral dose of up to 3 g of GSH to seven healthy subjects and did not observe an increase in blood GSH levels, concluding "it is not feasible to increase circulating GSH to a clinically beneficial extent by the oral administration of 3 g of GSH."[22] Yet three studies have evaluated the impact of reduced GSH supplementation combined with oxidative chemotherapeutic agents in patients with various cancers[23–25]; all three trials demonstrated reduced adverse effects of the chemotherapy without negative effects on the effectiveness of the regimen, suggesting some physiologic effect from oral dosing. Unfortunately GSH status was not measured in any of these trials.

In order to evaluate the impact of longer term, oral GSH supplementation on GSH status and *in vivo* oxidation status, this study performed the first randomized, double-blinded, placebo-controlled trial of orally administered GSH in healthy, adult humans. Because GSH status is dynamic, the outcome measures were extended beyond GSH status alone to include 8-OHdG and F2-isoprostanes (F2-isoP), in case a change in oxidation status was exemplified by evidence of reduced oxidation products rather than a change in GSH or GSH:GSSG ratio (i.e., improvement in tissue-level oxidative stress not represented by RBC indices). It was hypothesized that there would be measurement of a direct increase in RBC GSH concentration, or an increase in GSH:GSSG ratio, or a reduction in at least one biomarker of oxidation products (i.e., 8-OHdG or F2-isoP).

Experimental Procedure

Study design

A randomized, double-blind, placebo-controlled trial in health human adults was conducted. Oral glutathione capsules (500 mg twice per day) or placebo capsules (dicalcium phosphate, 500 mg twice per day) were self-administered 15 minutes before breakfast and dinner for 4 weeks. Outcome measures included 8-OHdG, F2-isoP, and RBC GSH, GSSG, and GSH:GSSG and were determined at baseline and following 4 weeks of supplementation.

Participants

Forty (40) healthy, nonsmoking men (*n*=7) and women (*n*=33) aged 21–62 years (mean=40.7±11.8) with body–mass index ranging from 19 to 29 kg/m^2 provided written informed consent participated in the study. Clinical procedures were performed at the Bastyr Center for Natural Health (Seattle, WA). Eligibility was established with a telephone questionnaire followed by a clinical examination to obtain height, weight, blood pressure, and screening hematology and blood chemistry. All enrolled participants were weight stable, free from acute or chronic diseases, and did not eat a diet or take medications that might interfere with outcome markers including antioxidant supplements, high intake of dietary polyphenols including coffee, or anti-inflammatory medications. This clinical trial was reviewed and approved by the Institutional Review Board of Bastyr University (Kenmore, WA).

Study drug

L-Glutathione was provided by Kohjin Co. Ltd., Japan. Kohjin's L-glutathione has qualified for GRAS (*G*enerally *R*ecognized *A*s *S*afe) status. Kosher and Halal certified, Kohjin's GSH is manufactured through a fermentation process using Food and Drug Administration–approved nongenetically modified torula yeast in a current Good Manufacturing Practices–certified laboratory facility. GSH and placebo pills were encapsulated by Natural Factors, Coquitlam, British Columbia, Canada. The batch of GSH used in this study was analyzed to verify physical, chemical, and microbiological parameters. The GSH study drug contained no less than 98% reduced glutathione, <1.4% oxidized glutathione, and was free of microbial contamination and heavy metals.

Blood and urine sample collection

Blood samples were collected by venipuncture after an overnight fast according to standard laboratory procedures. Red blood cells were washed, lysed, and mixed (<10 minutes) with a precipitating acid solution to minimize spontaneous oxidation of GSH.[19] Samples were placed on ice and stored at −70°C for batch analysis; all samples were measured within 90 days of collection. First morning urine samples were collected by the participants at home, brought to the clinic and frozen (-70°C). All urines samples were assayed in batches for 8-OHdG and F2-isoP. Urinary creatinine was measured from the same urine samples in order to standardize measurements.

Urinary 8-hydroxydeoxyguanosine and F2-isoprostane measurements

Urine samples were analyzed by Genox Corp. (Baltimore, MD) using a competitive enzyme-linked immunosorbent assay (ELISA) for quantification of both 8-OHdG and F2-isoP. Briefly, after thawing, 50-μL samples and standards were added to 8-OHdG conjugate plates followed by the primary antibody solution. Samples were incubated for 1 hour at 37°C. Plates were washed and a secondary antibody solution was applied for 1 hour at 37°C. After washing, 100 μL of a chromagenic substrate (3,3′5,5′-tetramethylbenzidene) was added and allowed to react for 15 minutes. Optical density of each sample was measured at 490 nm and compared to a standard curve to determine final concentration.[19]

Red blood cell glutathione measurement

Glutathione was measured using enzymatic recycling methodology.[18] Samples were analyzed in triplicate by Kronos Laboratory (Phoenix, AZ) for total GSH, GSSG, reduced GSH, and the ratio (GSH:GSSG).

Dietary intake

A food frequency questionnaire (FFQ) was completed at the beginning of the study. Dietary intake of cysteine, glutamate, and methionine was quantified based on FFQ at the Fred Hutchinson Cancer Research Center, Seattle, WA. Dietary intake was measured for possible adjustment purposes in our final analysis.

Adverse events

Possible adverse events were recorded using the Monitoring of Side Effects Scale questionnaire (DSHS Form 10-334) completed at baseline, week 2, and week 4.

Adherence

Adherence was assessed by interview and pill count at week 2 and 4 clinical visits.

Data analysis

Data were analyzed using SPPS version 15.0 (SPSS Inc., Chicago, IL). The distributions of all outcome variables were confirmed to be normally distributed, allowing analysis of variance and paired *t* tests to evaluate the primary endpoint(s) of differences in the mean change in urinary 8-OHdG, urinary F2-isoP, and RBC GSH:GSSG from baseline to week 4 compared between the active and placebo groups. Secondary endpoints included changes in RBC GSH and GSSG concentrations and adverse events. Changes in routine blood chemistries including electrolytes, hepatic, and renal functioning were also measured and compared between groups to confirm safety.

Results

Thirty-nine (39) participants completed the study; 1 participant was lost to follow-up after her baseline visit. Because our primary analyses related to mechanistic efficacy rather than effectiveness, baseline data from the 1 withdrawal was not included and final data were not estimated, rather these data were omitted from the final analysis. Subjects were generally similar between the two groups (Table 1).

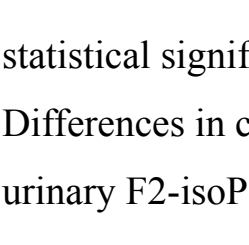

Table 1.
Baseline Characteristics of Trial Participants

Differences between groups for observed changes in primary or secondary endpoints were without notable trend and did not reach statistical significance (Figs. 1–3). Differences in creatinine-standardized urinary F2-isoP between baseline and week 4 were unchanged following supplementation (0.0±0.1 ng/mg creatinine versus 0.0±0.1 ng/mg creatinine; *p*=0.38). Similarly, differences in 8-OHdG were not statistically significant between treatment groups (-0.2±3.3 μg/mg creatinine versus 1.0±3.2 μg/mg creatinine, *p*=0.27).

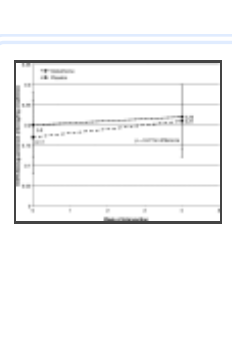

FIG. 1.
Effect of oral glutathione (GSH) on urinary 8-hydroxy-2′-deoxyguanosine. This figure demonstrates no effect of oral GSH supplementation at 500 mg twice daily for 4 weeks on