Nutrition 38 (2017) 41–47

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Medium-term effect of sublingual L-glutathione supplementation on flow-mediated dilation in subjects with cardiovascular risk factors

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ARTICLE INFO

Article history:
Received 23 September 2016
Accepted 26 December 2016

Keywords:
Cardiovascular
Glutathione
Endothelial dysfunction
Oxidative stress

ABSTRACT

Objective: Supplementation of glutathione (GSH) may be a positive strategy to improve the endogenous antioxidant defense required to counteract many acute and chronic diseases. However, the efficacy of GSH treatment seems to be closely related to type of administration, degree of absorption, and increase of its concentrations. The aim of this study was to test a new sublingual formulation of L-GSH, which enters directly the systemic circulation, to assess its efficacy on circulating biochemical markers of hepatic metabolism, lipid profile, and oxidative stress and on peripheral vascular function compared with placebo in patients with cardiovascular risk factors (CVRF).
Methods: We enrolled 16 healthy men with CVRF in a double-blinded, randomized placebo-controlled crossover study. At each visit, blood samples were collected for biochemistry analyses and peripheral endothelial function (reactive hyperemia index [RHI]) and stiffness were measured by Endo-PAT2000.
Results: In the overall population, a decrease in total and low-density lipoprotein cholesterol was highlighted after L-GSH supplementation compared with placebo ($P = 0.023$ and $P = 0.04$, respectively). On the contrary, no difference was observed in RHI and oxidative stress markers between L-GSH and placebo in the study population. However, seven participants with baseline abnormal RHI (≤ 1.67) compared with those with normal RHI showed a significant reduction of arterial stiffness after L-GSH administration, ($P = 0.007$ and $P = 0.037$, respectively).
Conclusions: Supplementation of L-GSH compared with placebo influences the lipid profile of patients with CVRF. Sublingual L-GSH may represent a valid prevention of vascular damage in patients with CVRF and endothelial dysfunction.

Introduction

The strategies to prevent cardiovascular diseases play an important role in the guidelines of different scientific societies [1]. Smoking habits, poor diet, physical inactivity, and stress, together with many other risk factors, may cause the

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http://dx.doi.org/10.1016/j.nut.2016.12.018
0899-9007/

development of atherosclerosis. Smoking habits and hypertension may alter endothelial function (EF) through direct oxidative damage by decreasing nitric oxide availability and affecting the mobilization of endothelial progenitor cells from the bone marrow [2,3].

The antioxidant systems are the main endogenous defense against free radicals, and glutathione (GSH) seems to play an important role in this mechanism. Changes in GSH homeostasis have been implicated in several diseases, such as Parkinson's [4] and Alzheimer's [5]. Low levels of GSH were found in patients with diabetes or metabolic syndrome [6,7] and were identified as an independent indicator of endothelial dysfunction [8]. Supplementation of GSH may improve the endogenous antioxidant defense and may contribute to a decrease in the oxidant tissue damage that occurs in many acute and chronic diseases. Several authors studied the effect of GSH oral administration in acute [9] and medium long-term treatment [10,11] in healthy volunteers or patients. They did not find any beneficial effect in terms of oxidative stress reduction, disease improvement, or both. Moreover, they did not observe any significant increases in circulating levels of GSH, probably because GSH is poorly absorbed in the gastrointestinal tract where high concentrations of γ-glutamyltranspeptidase (GGT) quickly catabolize this molecule. Futhermore, intravenous, intramuscular, and intrabronchial supplementation [12,13] of GSH did not show positive results. On the contrary, the intra-arterial infusion of GSH improves EF in patients with atherosclerosis or cardiovascular risk factors [14] without, however, a concomitant increment in the systemic GSH levels. Thus, the efficacy of GSH treatment seems to be closely related to type of administration, degree of absorption, and local increase of its concentrations.

The present study tested a new sublingual formulation of L-GSH, Oxition, which is able to bypass the gastroenteric tract, thus entering the systemic circulation directly. We assessed the efficacy of medium-term Oxition administration compared with placebo on peripheral vascular function and on circulating biochemical markers of hepatic function, lipid profile, and oxidative stress in men with cardiovascular risk factors (CVRF).

Materials and methods

Oxition bioavailability study

We first verified the bioavailability of 100 mg Oxition (NTCPharma, Milan, Italy) by comparing it with 100 mg L-GSH oral tablets in a cohort of healthy volunteers of both sexes (N = 9). Each dose of sublingual or oral supplementation was administered once in a sequential fashion, with a 1-wk washout period between each administration. Before (T0) and 30 (T1), 60 (T2), and 240 (T3) min after supplementation, a blood sample was collected to measure reduced (R) and total (T = R + oxidized) blood and plasma GSH.

Oxition efficacy study

Sixteen healthy male volunteers (ages 40–60 y) with one or more CVRF, such as smoking (>10 cigarette/d for almost 1 y), arterial hypertension (systolic arterial hypertension ≥140 mm Hg, diastolic arterial hypertension ≥90 mm Hg or both; or receiving antihypertensive treatment) or dyslipidemia (low-density lipoprotein [LDL] >160 mg/dL) not pharmacologically treated, were randomized to sequential allocation to Oxition 100 mg twice daily or placebo.

We enrolled only men to avoid effects on endothelial function related to hormonal fluctuation during the various phases of the menstrual cycle, as reported in the literature [15]. We excluded men with body mass index ≥30 kg/m^2 (obese); diabetes mellitus (fasting glycemia >126 mg/dL); being treated with acetylcysteine or a statin; or supplemented with vitamins, GSH-related molecules, or red rice–derived compounds. Eligible participants were enrolled and sequentially assigned to study treatment following a double-blinded, crossover, randomized controlled experimental design (L-GSH versus placebo) with a 4-wk washout period between the two treatments. Each intervention phase lasted 4 wk, for a total of 12 wk. Thus, two sequences of treatment were obtained: AB group (n = 8) and BA group (n = 8). Study assessments were performed at baseline (visit 0, V0), after 4 wk of treatment with Oxition/placebo (visit 1, V1), after the washout period at week 8 (visit 2, V2), and at the end of the study at week 12 (visit 3, V3).

The protocol was approved by local ethics committee. All patients provided written informed consent to participate. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Endpoints

The primary study endpoint was improvement of endothelium-dependent vasodilation, measured by a noninvasive plethysmographic method and expressed as an increase in reactive hyperemia index (RHI) score after 4 wk of Oxition with respect to placebo. The secondary endpoint was to verify whether Oxition supplementation changes circulating mediators of hepatic function and lipid profile and decreases oxidative stress biomarkers.

Safety was assessed by monitoring adverse events related to the administration of L-GSH or placebo.

Clinical and biochemical assessments

Baseline evaluation included clinical interview, blood pressure and heart rate assessments, vascular function test, blood sampling for glucose, creatinine, GGT, aspartate transaminase, alanine transaminase, total cholesterol (TC), high-density lipoprotein (HDL), LDL, triacylglycerols, and specific biochemical determination of oxidative stress biomarkers (plasma 3-nitrotyrosine, plasma malondialdehyde, and plasma 8-hydroxy-2′-deoxyguanosine [8-OHdG]). GSH and cysteinyl-glycine (CysGly), the main catabolic product of extracellular GSH cleavage, were also measured. At 4-wk follow-up, vital sign assessment, blood sampling for biochemical determinations, and vascular function test were again performed.

Blood and plasma GSH and plasma CysGly were assessed in total and reduced form by high-performance liquid chromatography, as previously described [16].

Table 1
Comparison between Oxition tablets 100 mg and L-GSH oral tablets 100 mg

Variables	T0	T1	T2	T3	*P*-value	Post hoc
Oxition tablets 100 mg						
GSH BT umol/L	741 (689–803)	762 (656–784)	729 (693–769)	718 (681–779)	0.857	-
GSH BR umol/L	412 (369–456)	487 (356–631)	529 (408–563)	524 (469–610)	**0.007**	T0 vs T3
GSH PT umol/L	4.41 (3.40–5.06)	4.19 (3.51–5.11)	4.19 (3.64–5.20)	4.38 (3.78–5.34)	0.050	-
GSH PR umol/L	0.88 (0.74–1.29)	0.81 (0.71–1.11)	0.87 (0.70–1.17)	0.78 (0.70–1.17)	0.522	-
L-GSH oral tablets 100 mg						
GSH BT umol/L	714 (635–785)	748 (611–810)	734 (636–816)	754 (657–827)	**0.048**	-
GSH BR umol/L	421 (384–445)	474 (355–495)	422 (411–487)	468 (399–555)	0.267	-
GSH PT umol/L	3.9 (3.3–4.4)	3.9 (2.9–4.9)	3.7 (3.5–4.4)	3.7 (3.4–4.6)	0.435	-
GSH PR umol/L	0.8 (0.6–0.9)	0.88 (0.8–1)	0.8 (0.8–0.9)	0.8 (0.6–1.1)	0.615	-

GSH BR, glutathione blood reduced; GSH BT, glutathione blood total; GSH PR, glutathione plasma reduced; GSH PT, glutathione plasma total
Data are expressed as median and interquartile range (I-III)
Bold values are statistically significant