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ROLI I ANTIOXIDANTËVE NË MENAZHIMIN E FAKTORËVE PROATHEROGJEN DHE PROTROMBOTIK

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Përmbledhje

Hyrje

Sëmundja e arterieve koronare që njihet ndryshe edhe si sëmundje ishemike e zemrës është shkaktari kryesor i vdekjeve në ditët e sotëshme në vendet e zhvilluara. Qëllimi i këtij studimi është që të ofrojë udhëzime parandaluese bazuara në evidencat e fundit mbi rëndësinë e antioksidantëve natyrorë tek pacientët me patologji koronare përmes monitorimit të vlerave të trimetilaminëoksidazës (TMAO), metabolitit pro-atherogjen e protrombotik, të Low density lipoprotein (LDL-së), Proteinës C-Reaktive (PCR-së) duke testuar *in vivo* efikasitetin e një nutraceutiku me bazë ekstraktet polifenolike të resveratrolit përgatitur nga bërsitë e rrushit (*Vitis vinifera* L.)

Metodologjia. Për realizimin e këtij studimi double-blind placebo kontroll u zgjodhën 60 pacientë vullnetarë të rastësishëm mbipeshë që plotësuan kriteret e pranimit duke respektuar Deklaratën e Helsinkit dhe Bazuar në Komitetin e Etikës. Forma e administrimit të nutraceutikut për pacientët ishte per os, në formën e tabletave të standartizuara me ekstraktet polifenolike të rrushit 400 mg, një herë në ditë larg ushqimit, ndërkohë grupi placebo të njëjtën formë farmaceutike por me maltodextrinë për një periudhë 3 muajore.

Rezultatet. Pas administrimit dhe monitorimit të nutraceutikut për një periudhë 90 ditore, u vu re një ulje e niveleve plazmatike të TMAO (trimetilamineoksidave) si dhe ox-LDL (loë density lipoprotein) dy ndër markuesit kryesorë të patologjive koronare me $-49.78\% \pm 0.2$ ($p < 0.0001$), dhe $-43.12\% \pm 0.1$ respektivisht krahasuar me grupin placebo si dhe një ulje e nivele të Proteinës C-Reaktive me 0.2 mg/dcl ose në masën 39% e cila lidhet me inflamacionin kronik endotelial të arterive koronare.

Përfundimet. Këto rezultate premtuese hedhin dritë për rëndësinë e antioksidantëve dhe përbërësve natyralë si dhe për zhvillimin e testeve të mëtejshme për të formuluar suplemente efektive në tregun e industrisë farmaceutike.

Fjalë çelës: *patologji koronare, trimetilamineoksidave, resveratrol, produkte natyrale, antioksidant.*

THE ROLE OF ANTIOXIDANTS IN THE MANAGEMENT OF PROATHEROGENIC AND PROTHROMBOTIC FACTORS

Abstract

Introduction. Coronary artery disease, also known as ischemic heart disease, is the leading cause of death in developed countries nowadays. The purpose of this study is to provide preventive guidelines based on the latest evidence on the importance of natural antioxidants in patients with coronary pathology by screening the values of trimethylamine oxidase (TMAO), the pro-atherogenic and pro-thrombotic metabolite, of low-density lipoprotein (LDL), C-Reactive Protein (PCR) by evaluating *in vivo* the efficacy of a nutraceutical based on polyphenolic extracts of resveratrol prepared from grapes pomace (*Vitis vinifera* L.)

Methodology. For the realization of this double-blind placebo control study, 60 random overweight volunteer patients were selected who met the acceptance criteria respecting the Declaration of Helsinki and Based on the Ethics Committee. The form of administration of the nutraceutical for the patients was per os, in the form of standardized tablets with polyphenolic extracts of grapes 400 mg, once a day away from meal, while the placebo group received the same pharmaceutical form but with maltodextrin for a period of 3 months.

Results. After the administration of the nutraceutical for a period of 90 days, a decrease in the plasma levels of TMAO (trimethylamine oxides) and ox-LDL (low density lipoprotein), two of the main markers of coronary pathologies, was observed by $-49.78\% \pm 0.2$ ($p < 0.0001$), and $-43.12\% p < 0.0001 \pm 0.1$ respectively compared to the placebo group as well as a decrease in C-Reactive Protein levels by 0.2 mg/dcl or to the extent of 39% which is related to chronic endothelial inflammation of the coronary arteries.

Conclusions. These promising results highlight the importance of antioxidants and all-natural products as well as trigger for further research to formulate effective supplements in the market of the pharmaceutical industry.

Key Words: *Coronary disease, trimethylamine oxides, resveratrol, natural products, antioxidant.*

Introduction

Atherosclerosis is among the main causes of mortality worldwide. Several risk factors have been associated with this chronic artery disease, including hypertension, dyslipidemia, obesity, diabetes and cigarette smoking (1). However, recently trimethylamine-N-oxide (TMAO) has been recognized as novel proatherogenic factor closely involved in atherosclerosis development and progression, beyond the other historically known risk factors (2-3). TMAO is a bioactive gut microbiota-derived metabolite produced from the metabolism of food components, mainly dietary choline, betaine, and carnitine. The clinical interest for this metabolite derives from emerging evidence suggesting its role in pathophysiology of cardiovascular diseases (CVD) (with particular interest in atherosclerotic risk) (4-5) and other chronic pathological conditions. It is widely reported in literature that TMAO is closely linked to oxidative stress (OxS), as it is a highly oxidant and reactive molecule (16). With OxS refers to an unbalance between production and clearance of oxidants, mainly reactive oxygen species (ROS) (6). It has been reported that various pathological conditions, including obesity, diabetes, hypertension or dyslipidemia, are responsible for increasing levels of ROS. Considering this evidence, thus, the use of therapeutic strategies aimed to contrast the OxS is desirable, although no specific antioxidant treatment has been recommended to prevent cardiovascular complications (7). The aim

of the study was to evaluate the effect of a novel nutraceutical formulation based on grape pomace polyphenolic extract in reducing the serum levels of TMAO and oxLDL in humans.

Methods

Study Population and Protocol

This study was designed as a monocentric, double-blind, randomized, placebo-controlled, 2-arm parallel-group trial. The study duration was 14 weeks: subjects underwent 2-week run-in period (with administration of capsules containing only excipients as placebo), followed by 8 weeks of intervention (active or placebo), and 4 weeks of follow-up. The examinations were performed in an outpatient setting. Clinic visits and blood sampling were performed after 12 h of fasting at weeks 0, 2, 6, 10, and 14. Subjects aged 18–75 years with body mass index (BMI) ≥ 18.5 kg/m² were eligible for enrolment. The study was approved by the committee (protocol 123512 of 18/06/2018) and carried out in accordance with the Helsinki declaration of 1964 (as revised in 2000).

Study Treatment

The nutraceutical treatment consisted of 400 mg of a polyphenol extract obtained from Aglianico cultivar grape. For the polyphenol extract production, grapes were extracted with water (50°C), and the solution was filtrated and concentrated and underwent a spray-drying process with maltodextrins as support (5–15%) to obtain a fine microencapsulated powder. The polyphenol profile was evaluated by High-Performance Liquid Chromatography-diode array detector (HPLC-DAD, Jasco Inc., Easton, MD, USA). TMAO serum levels were quantified by the LC-MS method described by Annunziata et al (8). Serum levels of oxidized low-density lipoproteins (oxLDL) as oxidative stress-related biomarker was carried out on an automated analyzer (Free Carpe Diem, Diacron International, Grosseto, Italy) using relative commercial kits (Diacron International).

Results

TMAO-Reducing Effect of nutraceuticals were monitored in all study participants at run-in, before starting treatment (baseline), after 4-week treatment with nutraceuticals or placebo, after 8-week treatment with both nutraceuticals or placebo and after 4-week follow-up. TMAO serum levels significantly reduced after 4-week nutraceuticals treatment, from 2.91 ± 2.30 μ M to 1.10 ± 1.17 μ M (-49.78% , $p < 0.0001$), and after 8-week nutraceuticals treatment (0.53 ± 0.53 μ M, -75.85% , $p < 0.0001$). No significant changes were observed in the control group after treatment period with placebo.

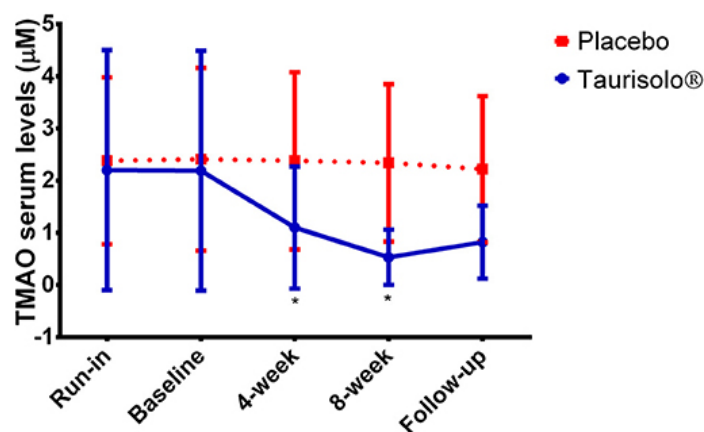


Figure 1. Graphical representation of the TMAO serum levels at the different time points. Values are

expressed as mean $\pm$ standard deviation. $p < 0.05$ was considered as significant.

	Baseline	t-4wk			t-8wk		
	Mean $\pm$ SD	Mean $\pm$ SD	$\Delta\%$ (vs. t0)	p (vs. t0)	Mean $\pm$ SD	$\Delta\%$ (vs. t0)	p (vs. t0)
Men ($n = 66$)	2.57 $\pm$ 2.71	1.43 $\pm$ 1.41	-44.23	<0.0001	0.50 $\pm$ 0.38	-80.59	<0.0001
Women ($n = 42$)	2.11 $\pm$ 1.53	1.21 $\pm$ 0.94	-42.89	<0.0001	0.61 $\pm$ 0.67	-71.22	<0.0001

Statistical significance was calculated with Student's *t*-test; $p < 0.05$ was considered as significant.

Table 1. Serum levels of TMAO before and after 4-week and 8-week treatment with nutraceutical

Serum levels of oxLDL were monitored in all study participants at run-in, before starting intervention period (baseline), after 4-week and after 8-week treatment with nutraceutical or placebo and after 4-week follow-up (Figure 2). No significant changes were observed in the control group after treatment period with placebo.

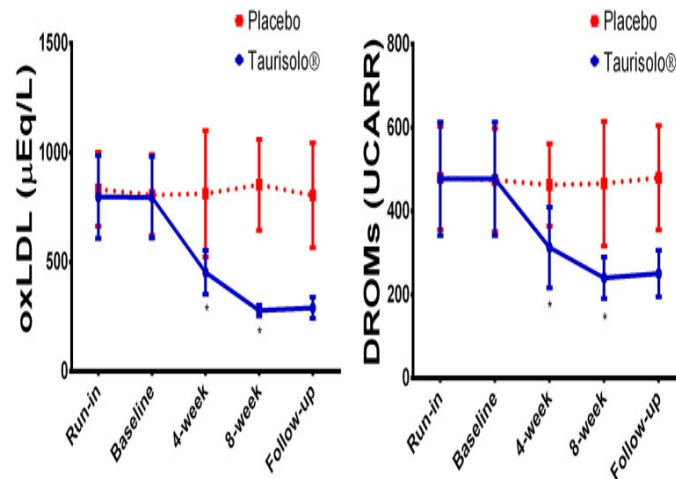


Figure 2. Graphical representation of the oxidative stress-related biomarkers serum levels at the different time points. Values are expressed as mean $\pm$ standard deviation.

Discussion

The first aim of our study was to prove that chronic administration of nutraceuticals based of polyphenolic extract reduced TMAO serum levels in a large number of subjects. TMAO is a novel proatherogenic factor playing a crucial role in development and progression of atherosclerosis via different mechanisms (9). Overall, this evidence clarifies the role played by TMAO in atherosclerotic risk and suggests this compound as a potential target for management of atherosclerosis. In addition to the TMAO-reducing effect, we also investigated the ability of nutraceuticals to reduce oxLDL as OxS-related biomarkers, since OxS is historically recognized as one of the main risk factors for CVD. Besides the well-established antioxidant potential of polyphenols, several studies reported the ability of polyphenols to contrast atherosclerosis progression via reduction of LDL oxidizability (10). In line with both this evidence and data herein reported, this study demonstrated the ability of chronic treatment with nutraceuticals based antioxidants to reduce circulating oxLDL in overweight/obese subjects. Interestingly, our correlation analysis demonstrated that TMAO serum levels correlated positively with circulating oxLDL at baseline and after 4- and 8-week treatment. However, further clinical trials investigating the effects of nutraceutical on plaque reduction and atherosclerosis progression in high-risk patients are needed, and currently ongoing.

Reference

1. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, et al. Heart disease and stroke statistics'2017 update: a report from the American Heart Association. *Circulation*. (2017) 135:e146–603. doi: 10.1161/CIR.0000000000000485
2. Zhu Y, Li Q, Jiang H. Gut microbiota in atherosclerosis: focus on trimethylamine N-oxide. *AP-MIS*. (2020) 128:353–66. doi: 10.1111/apm.13038
3. Shi X, He J, Lin M, Liu C, Yan B, Song H, et al. Comparative effectiveness of team-based care with a clinical decision support system versus team-based care alone on cardiovascular risk reduction among patients with diabetes: Rationale and design of the D4C trial. *Am Heart J*. (2021) 238:45–58. doi: 10.1016/j.ahj.2021.04.009
4. Kaze AD, Santhanam P, Musani SK, Ahima R, Echouffo-Tcheugui JB. Metabolic dyslipidemia and cardiovascular outcomes in type 2 diabetes mellitus: findings from the look AHEAD study. *J Am Heart Assoc*. (2021) 10:e016947. doi: 10.1161/JAHA.120.016947
5. Rostomian AH, Tang MC, Soverow J, Sanchez DR. Heterogeneity of treatment effect in SPRINT by age and baseline comorbidities: The greatest impact of intensive blood pressure treatment is observed among younger patients without CKD or CVD and in older patients with CKD or CVD. *J Clin Hypertens (Greenwich)*. (2020) 22:1723–6. doi: 10.1111/jch.13955
6. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med*. (2013) 368:1575–84. doi: 10.1056/NEJMoa1109400
7. Zhang Y, Wang Y, Ke B and Du J (2021) TMAO: how gut microbiota contributes to heart failure. *Transl Res* **228**, 109–125
8. Annunziata G, Maisto M, Schisano C, Ciampaglia R, Narciso V, Hassan STS, et al. Effect of grape pomace polyphenols with or without pectin on TMAO serum levels assessed by LC/MS-based assay: a preliminary clinical study on overweight/obese subjects. *Front Pharmacol*. (2019) 10:575. doi: 10.3389/fphar.2019.00575
9. Ivashkin V, Kashukh YA. Impact of L-carnitine and phosphatidylcholine containing products on the proatherogenic metabolite TMAO production and gut microbiome changes in patients with coronary artery disease. *Vopr Pitan*. (2019) 88:25–33. doi: 10.24411/0042-8833-2019-10038
10. Pignatelli P, Menichelli D, Pastori D, Violi F. Oxidative stress and cardiovascular disease: new insights. *Kardiol Pol*. (2018) 76:713–722. doi: 10.5603/KP.a2018.007