Clinical research

Effects of a nutraceutical combination of fermented red rice, liposomal berberine, and curcumin on lipid and inflammatory parameters in patients with mild-to-moderate hypercholesterolemia: an 8-week, open-label, single-arm pilot study

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Abstract

Introduction: The present open-label, single-arm pilot study sought to evaluate the effects of a nutraceutical combination containing fermented red rice, liposomal berberine, and curcumin on lipid and inflammatory parameters in patients with mild-to-moderate hypercholesterolemia.
Material and methods: Forty patients with mild-to-moderate hypercholesterolemia received the nutraceutical combination containing fermented red rice, liposomal berberine, and curcumin, once a day for 8 weeks. The study outcomes included changes from baseline in lipid (total cholesterol (TC), low-density-lipoprotein cholesterol (LDL-C), oxidized low-density lipoprotein (oxLDL), high-density-lipoprotein cholesterol (HDL-C), and triglycerides (TG)) and inflammatory parameters (high-sensitivity C-reactive protein (hs-CRP) and tumor necrosis factor-α (TNF-α)).
Results: Compared with baseline, the nutraceutical combination produced a statistically significant reduction of TC (–20.4%, $p < 0.05$), LDL-C (–27.6%, $p < 0.05$), oxLDL (–23.2%, $p < 0.05$), and TG (–17.9%, $p < 0.05$). We also observed a reduction from baseline for hs-CRP (–15.4%, $p < 0.05$) and TNF-α (–14.3%, $p < 0.05$). The treatment was well tolerated and none of the patients discontinued treatment due to adverse effects. No cases of myalgia or musculoskeletal system disorders were observed.
Conclusions: The nutraceutical combination of fermented red rice, liposomal berberine, and curcumin improves lipid profile and reduces markers of inflammation in low-risk dyslipidemic patients, with potential implications for primary prevention of cardiovascular disease.

Key words: fermented red rice, berberine, curcumin, nutraceutical, hypercholesterolemia, primary prevention.

Introduction

The causal relationship between dyslipidemia – particularly increased serum low-density-lipoprotein cholesterol (LDL-C) – and cardiovascular disease (CVD) – including coronary heart disease, cerebrovascular disease, and peripheral arterial disease – is well established [1, 2]. Statins

– the most commonly used lipid-lowering drugs, which act as inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA) – are highly effective for secondary prevention of CVD [3]. Unfortunately, approximately one third of statin users discontinue therapy in the first 12 months for several reasons, including adverse reactions (e.g., muscle pain with increased creatinine phosphokinase (CPK) levels, weakness, fatigue, and elevated liver enzymes) [4]. Consequently, the use of statins for primary prevention of CVD is rarely encouraged in clinical practice [5]. Owing to their higher tolerability, lipid-lowering nutraceuticals are increasingly being used as a viable alternative to statins for primary prevention of CVD in subjects with mild-to-moderate hypercholesterolemia [6] as well as for metabolic syndrome management [7]. Specifically, lipid-lowering effects have been advocated for nutraceuticals containing fermented red rice [8], berberine [9], and curcumin [10].

The lipid-lowering effects of fermented red rice have been attributed to the presence of monacolins which – similar to statins – act as reversible inhibitors of HMG-CoA reductase [11]. The administration of 10 mg/day of monacolin K from fermented red rice has been approved by the European Food Safety Authority for the maintenance of normal blood cholesterol concentrations [12]. Berberine – an isoquinoline alkaloid isolated from *Berberis aristata* – has been shown to lower lipid levels through HMG-CoA reductase-independent mechanisms. Accordingly, the lipid-lowering effects of berberine are mediated by: 1) the upregulation of LDL receptor (LDLR) expression on hepatocytes [13] and 2) the reduction of hepatic cholesterol and triglycerides (TG) synthesis through the activation of adenosine monophosphate-activated protein kinase (AMPK) [14]. Unfortunately, the bioavailability of orally administered berberine is generally poor because of its low intestinal absorption and significant first-pass metabolism [15]. In this context, strategies aimed at increasing the absorption rate of berberine (including liposomal formulation) are expected to improve its lipid-lowering properties. Finally, curcumin is a dietary polyphenol with lipid-lowering, antioxidant, anti-proliferative, and anti-inflammatory properties [16, 17] – especially against tumor necrosis factor-α (TNF-α) [18].

Starting from these premises, the present 8-week open-label, single-arm pilot study was designed to evaluate the effects of a nutraceutical combination containing a mixture of fermented red rice (monacolin K 10 mg), liposomal berberine (47.2 mg), and curcumin (50 mg) (Berbered Plus; Biodue, Tavarnelle Val di Pesa, Italy) on lipid and inflammatory parameters in low-risk subjects with mild-to-moderate hypercholesterolemia.

Material and methods

Patients

This 8-week uncontrolled pilot study had a single arm, open-label design. A total of 40 Caucasian subjects aged between 18 and 75 years with mild-to-moderate hypercholesterolemia – defined as LDL-C levels between 3.0 and 4.7 mmol/l or 115 and 180 mg/dl, total cholesterol (TC) levels between 5.2 and 6.8 mmol/l or 200–260 mg/dl, and TG levels < 2.8 mmol/l or 250 mg/dl – were enrolled within a cardiovascular primary prevention program [19]. We excluded subjects who suffered from 1) any known cardiac or pulmonary disease; 2) hypertension (blood pressure > 140/90 mm Hg or taking anti-hypertensive medication); 3) diabetes mellitus/impaired fasting glucose (fasting blood glucose > 6.1 mmol/l or taking hypoglycemic drugs); and 4) angina pectoris [19]. Additional exclusion criteria were as follows: use of lipid-lowering drugs or dietary supplements in the 8 weeks before enrollment; pregnancy or breastfeeding; documented intolerance to one or more components of Berbered Plus; previous cardiovascular events; familial dyslipidemia; positive family history of CVD; hepatic or muscular disorders; and inability to provide written informed consent. Suitable patients identified from a review of laboratory records were contacted personally or by phone for inclusion. All subjects expressed their willingness to participate in the study after a full explanation of its goals and procedures. Participants were encouraged to maintain their usual lifestyle (both in terms of diet and physical activity) throughout the 8-week study period, during which no other medications or food supplements were allowed. The study protocol followed the tenets of the Declaration of Helsinki and was approved by the local Institutional Review Board. Written informed consent was obtained from all participants.

Study procedures

At baseline, all subjects underwent physical examination and anthropometric measurements (including body mass index, waist circumference, and hip circumference). Berbered Plus was self-administered once a day (one tablet after dinner) for 8 weeks. Tolerability was assessed by asking patients about any signs or symptoms of systemic adverse reactions. The outcomes of interest were the changes from baseline to week 8 in lipid (TC, LDL-C, oxidized low-density lipoprotein (oxLDL), high-density-lipoprotein cholesterol (HDL-C), and TG) and inflammatory parameters (high-sensitivity C-reactive protein (hs-CRP) and TNF-α). Biochemical analyses of serum lipids were performed on a Hitachi-912 Auto Analyzer (Hita-

chi, Mannheim, Germany) using kits supplied by Roche Diagnostics (Mannheim, Germany), the only exception being OxLDL levels, which were determined using an immunoassay (Mercodia AB, Uppsala, Sweden). Hs-CRP was measured using a latex-enhanced immunonephelometric assay on a BN II analyzer (Dade Behring, Newark, DE, USA). The intra- and interassay coefficients of variation (CsV) were 6.2% and 2.8%, respectively. Concentrations of TNF-α were quantified using a commercially available immunoassay (Titer-Zyme EIA kit; Assay Designs, Ann Arbor, MI, USA) according to the manufacturer's instructions. The intra- and interassay CsV were 7.4% and 9.3%, respectively.

Data analysis

Owing to the exploratory nature of the study, the sample size was not based on any statistical calculation. No patient was lost to follow-up. Continuous variables were expressed as means ± standard deviations, whereas categorical data are given as counts. Changes in lipid and inflammatory variables from baseline to week 8 were tested for significance using the paired Student's *t*-test. All calculations were performed with the SPSS 20.0 statistical package (IBM, Armonk, NY, USA). A p-value < 0.05 (two-tailed) was considered statistically significant.

Results

The general characteristics of the 40 study patients are summarized in Table I. Variations of lipid and inflammatory parameters over the 8-week study period are depicted in Table II. Compared with baseline, the nutraceutical combination produced a statistically significant reduction of TC (–20.4%, $p < 0.05$), LDL-C (–27.6%, $p < 0.05$), oxLDL (–23.2%, $p < 0.05$), and TG (–17.9%, $p < 0.05$). We also observed a reduction from baseline for hs-CRP (–15.4%, $p < 0.05$) and TNF-α (–14.3%, $p < 0.05$). Berbered Plus was well tolerated and none of the patients discontinued treatment due to adverse reactions. No cases of myalgia or musculoskeletal disorders were observed.

Discussion

The current pilot study demonstrates that a nutraceutical combination of fermented red rice, liposomal berberine, and curcumin improves lipid profile and reduces markers of inflammation in low-risk patients with mild-to-moderate hypercholesterolemia, with potential implications for primary prevention of CVD.

Several previous investigations have shown that monacolin K 10 mg from fermented red rice – either alone or in combination with berberine – may produce reductions of LDL-C in the 20–35% range [20, 21]. In the current study, we achieved an LDL-C reduction of approximately 28% from baseline, which was accompanied by a significant decrease in oxLDL (–23.2%). Notably, growing evidence indicates that OxLDL may play a key role in atherosclerosis initiation and development [22].

Table I. General characteristics of the study participants

Variable	Value
Males/females	20/20
Age [years]	49.8 ±5.9
Body mass index [kg/m^2]	27.1 ±1.0
Abdominal circumference [cm]	89.8 ±2.7
Waist circumference [cm]	86.4 ±2.0
Hip circumference [cm]	98.6 ±2.9
Serum creatinine [µmol/l]	82 ±11
Systolic blood pressure [mm Hg]	125 ±10
Diastolic blood pressure [mm Hg]	78 ±7
Fasting plasma glucose [nmol/l]	4.7 ±0.6
Current smoking, yes/no	5/15

Table II. Variation of lipid profile and inflammatory markers with Berbered Plus

Biochemical variable	Baseline	8 weeks	*P*-value
TC [mg/dl]	225 ±21	179 ±18	< 0.05
LDL-C [mg/dl]	152 ±12	110 ±13	< 0.05
OxLDL [U/l]	69 ±10	53 ±8	< 0.05
HDL-C [mg/dl]	43 ±4	45 ±5	NS
TG [mg/dl]	123 ±32	101 ±24	< 0.05
Hs-CRP [mg/l]	1.3 ±0.5	1.1 ±0.5	< 0.05
TNF-α [ng/ml]	1.4 ±0.7	1.2 ±0.6	< 0.05

TC – total cholesterol, LDL-C – low-density-lipoprotein cholesterol, OxLDL – oxidized low-density-lipoprotein, HDL-C – high-density-lipoprotein cholesterol, TG – triglycerides, hs-CRP – high-sensitivity C-reactive protein, TNF-α – tumor necrosis factor-α, NS – not significant.

Although we cannot exclude that the decrease in OxLDL levels can be at least in part explained by the reduction of LDL-C *per se*, we speculate that the antioxidant effects of highly bioavailable liposomal berberine coupled with those of curcumin can contribute to the observed decrease in OxLDL. The reductions in TC and TG observed in our study were similar to those previously reported for similar nutraceutical combinations [20, 21].

Further to the favorable modifications in terms of lipid profile, we also observed significant anti-inflammatory effects for the nutraceutical combination under study, with both hs-CRP and TNF-α levels being significantly reduced after 8 weeks. Hs-CRP is an acute-phase reactant that independently predicts the risk of coronary heart disease, cerebrovascular disease, and peripheral arterial disease in apparently healthy individuals [23]. Similarly, TNF-α is a macrophage-derived proinflammatory molecule that can serve as a biomarker of increased cardiovascular risk [24]. However, caution should be exercised when making inferences on the impact of nutraceuticals on low-grade subclinical inflammation (as effect sizes are modest and baseline inflammatory activity is typically low). We hypothesize that both liposomal berberine and curcumin may exert hs-CRP- and TNF-α-lowering effects – which can depend on their bioavailability. It is also noteworthy that berberine may attenuate oxLDL-induced inflammation by stimulation of macrophage autophagy [25].

Another positive result of the study was the observation that the nutraceutical combination was well tolerated, with no cases of myalgia or musculoskeletal disorders being evident. Based on these observations, we believe that its use for primary prevention in individuals at low cardiovascular risk is promising and can offer inherent advantages in terms of tolerability compared with statins.

Our results need to be interpreted in the context of several major limitations. First, our research had a pragmatic pilot, open-label design and lacked a placebo arm. We are aware that the study design is suboptimal and that a placebo-controlled trial would have provided more scientifically robust results. However, randomization and the use of a placebo arm were hampered by financial constraints. Because resources to conduct a placebo-controlled randomized clinical trial were lacking, our data should be viewed as preliminary and hypothesis-generating. The small sample size and the short observational period require further confirmation of our findings in independent samples. Second, it can be argued that most of the lipid-lowering effects observed in the current study can be attributed to monacolin K (10 mg) and that both liposomal berberine and curcumin may be underdosed. While this possibility cannot be ruled out, it should be noted that: 1) we used a liposomal form of berberine (which is expected to increase bioavailability) and 2) even 15 mg of curcumin given three times per day may exert significant lipid-lowering effects [26]. Third, only two biomarkers of inflammation were examined, and further research is needed to clarify the potential anti-inflammatory effects of the nutraceutical combination. Fourth, we did not measure serum liver and muscle enzyme levels. As such, clinical safety data obtained in our study should be interpreted cautiously.

These caveats notwithstanding, our pilot study demonstrates that a nutraceutical combination of fermented red rice, liposomal berberine, and curcumin may reduce lipid (TC, LDL-C, oxLDL, TG) and inflammatory parameters (hs-CRP, TNF-α) in subjects at low cardiovascular risk. Our findings may have implications for primary prevention of CVD as well as for statin-intolerant individuals [27].

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Conflict of interest

This study was partly funded by 2E Science (Robbio, Italy), a privately held biomedical research organization of which Enzo Emanuele is the major shareholder.

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