Experimental study

Lipid disorders

Effects of a nutraceutical combination of monacolin, γ-oryzanol and γ-aminobutyric acid on lipid profile and C-reactive protein in mice

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Abstract

Introduction: The aim of this study was to evaluate the efficacy of two nutraceutical agents aimed to improve lipid profile in a sample of mice.
Material and methods: Fifty mice were randomly divided into four groups. Control mice were fed a standard diet (SD), while the other three groups were fed with a high-fat diet (HFD) for 4 weeks. At the end of the run-in period, mice fed with the SD continued to follow the SD, while mice fed with the HFD were divided into three groups: one continued with the HFD, one continued to follow the HFD + a blend of natural components derived from rice and fermented rice (monacolin K 3%, γ-oryzanol, and γ-aminobutyric acid) (S1), and the other one followed the HFD + one nutraceutical containing monacolin K 3% alone for 24 weeks.
Results: The results showed that mice treated with HFD + S1 and HFD + S2 had lower levels of TC compared to mice fed with the HFD alone ($p < 0.01$, and $p < 0.05$, respectively). Moreover, mice treated with HFD + S1 had lower TC and LDL-C levels compared to mice fed with HFD + S2 ($p < 0.05$). Mice treated with HFD + S1 or S2 had lower Tg levels compared to mice fed with the HFD ($p < 0.05$).
Conclusions: We can conclude that a combination of monacolin K 3%, γ-oryzanol, and γ-aminobutyric acid is more effective than monacolin K 3% alone in reducing the negative effects of a HFD in a sample of specific pathogen-free mice.

Key words: diet, lipid profile, mice, monacolin K, nutraceutical.

Introduction

Cardiovascular disease, and in particular coronary heart disease (CHD), is the main cause of mortality in developed countries [1]. In atherosclerosis development, cholesterol, and in particular low-density lipoprotein (LDL) cholesterol, plays the main role: usually atherosclerosis begins with endothelial damage caused by high blood pressure, smoking, or high cholesterol. The cornerstone of hypolipidemic treatment should be appropriated lifestyle modifications and statin treatment, or 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors [2].

Statins are demonstrably effective in reducing cholesterol [3], but they also have some potential adverse effects, including muscle pain with creatine phosphokinase (CPK) elevation, fatigue and weakness, and liver transaminases increase [4].

The incidence of adverse events is dose related; the higher the dose of statin is, the more frequent can be the adverse events. Because of those adverse events, patients may seek alternative therapies to manage their hypercholesterolemia, including newer hypolipidemic therapies such as PCSK9 inhibitors, even if their use is limited by the high cost.

For all these reasons, in recent years, the nutraceutical market has rapidly increased. Nutraceuticals are dietary supplements. They contain a concentrated form of a presumed bioactive substance originally derived from a food, but now present in a nonfood matrix, and used to enhance health in dosages exceeding those obtainable from normal foods [5]. Several substances have shown positive benefits for lipid profile, such as *Berberis aristata*, omega-3, krill oil, and cinnamon [6–11].

In particular, in this study we focused our attention on two nutraceutical combinations aimed to improve lipid profile, one called Rossopuro Forte (S1), a blend of natural components derived from rice and fermented rice (monacolin K 3%, γ-oryzanol, and γ-aminobutyric acid) developed by Giellepi S.p.A. (Lissone, Monza MB, Italy) and one called Rossopuro (S2) containing monacolin K 3% alone.

In this context, we planned a study to evaluate the efficacy and safety of the two nutraceutical agents in a sample of C57BL/6J mice fed with a standard diet (SD), or high-fat diet (HFD), or HFD with the addition of S1 or S2. The primary outcomes were the effects of these nutraceutical combinations on the lipid profile; the secondary outcomes were considered the changes in C-reactive protein (CRP).

Material and methods

Study design

Principles of good laboratory animal care were followed and animal experimentation was in compliance with specific national and international laws and regulations.

The authorization to use animals in Biogem laboratories (Ariano Irpino, Avellino, Italy) was obtained from the Italian Health Authority. The care and husbandry of animals were in accordance with European Directive no. 2010/63 and with the Italian Regulatory system (D.L. vo no. 26, March 4th, 2014). All parts of this study concerning animal care have been approved by the official Biogem veterinarian.

Animals

Specific pathogen-free 4-week-old male C57BL/6J mice purchased from Charles River Laboratories International, Inc. were allowed to acclimate for 1 week before use. Mice were allowed access to food and water ad libitum. The animal room was maintained at a temperature of 20 ±2°C, humidity of 45 ±10% and a 12-h light/dark cycle. Their care and handling were in accordance with the provision of the European Union Council Directive 2010/63 UE, recognized and adopted by the Italian Government (Decree No. 26/2014).

Treatments

Mice were randomly separated into four groups. Control mice were fed a standard diet (SD) (D12450B; Research Diets, Inc., New Brunswick, NJ, USA), while the other three groups of mice were all fed a high-fat diet (HFD) (D12492; Research Diets, Inc.) for 4 weeks, in a run-in period. The composition of each diet is listed in Tables I and II. At the end of the run-in period, mice fed with the SD diet continued to follow the SD, while mice fed with the HFD were divided into three groups: one group continued with the HFD, one continued to follow HFD + S1, and the other one continued to follow HFD + S2 for 24 weeks.

Assessments

Body weight was recorded twice a week and food intake three times a week. At the end of the experiment, blood collected from the posterior vena cava and plasma was prepared for biochemical analysis. We evaluated at the baseline and after 24 weeks the following parameters: weight, blood glucose, total cholesterol (TC), triglycerides (Tg), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), C-reactive protein (CRP).

The levels of TC, Tg, HDL-C, and LDL-C in plasma were measured using a Beckman Coulter AU5421 (Beckman Coulter, Fullerton, CA, USA).

C-reactive protein were assayed using an ELISA kit (Boster Biological Technology Co., Ltd., Pleasanton, CA, USA) according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using the unpaired t-test or an analysis of variance (ANOVA) to analyze continuous laboratory measurements and to perform comparisons among the study groups. (Graph Pad Prism 6). Data are presented as mean ± standard deviation. For all statistical analyses, $p < 0.05$ was considered statistically significant.

Table I. Standard diet description with 10% kcal % fat

Product # D12450B	**g (%)**	**kcal (%)**
Protein	19.2	20
Carbohydrate	67.3	70
Fat	43	10
Total		100
kcal/g	3.85	
Ingredient	**g (%)**	**kcal (%)**
Casein, 30 Mesh	200	800
L-cystine	3	12
Corn starch	315	1260
Maltodextrin 10	35	140
Sucrose	350	1400
Cellulose, BW200	50	0
Soybean oil	25	225
Lard*	20	180
Mineral Mix S10026	10	0
Dicalcium phosphate	13	0
Calcium carbonate	5.5	0
Potassium citrate, 1 H_2O	16.5	0
Vitamin Mix V 10001	10	40
Choline bitartrate	2	0
FD&C Yellow Dye #5	0.05	0
Total	1055.05	4057

**Typical analysis of cholesterol in lard = 0.72 mg/g, cholesterol (mg)/4057 kcal = 14.4, cholesterol (mg)/kg = 13.6.*

Table II. High-fat diet description with 60% kcal % fat

Product # D12492	**g (%)**	**kcal (%)**
Protein	26.2	20
Carbohydrate	26.3	20
Fat	34.9	60
Total		100
kcal/g	5.24	
Ingredient	**g (%)**	**kcal (%)**
Casein, 30 Mesh	200	800
L-cystine	3	12
Corn starch	0	0
Maltodextrin 10	125	500
Sucrose	68.8	275.2
Cellulose, BW200	50	0
Soybean oil	25	225
Lard*	245	2205
Mineral Mix S10026	10	0
Dicalcium phosphate	13	0
Calcium carbonate	5.5	0
Potassium citrate, 1 H_2O	16.5	0
Vitamin Mix V 10001	10	40
Choline bitartrate	2	0
FD&C Blue Dye #1	0.05	0
Total	773.85	4057

**Typical analysis of cholesterol in lard = 0.72 mg/g, cholesterol (mg)/4057 kcal = 216.4, cholesterol (mg)/kg = 279.6.*

Results

Study sample

A total of 50 mice were enrolled in the study. Of these, all mice completed the study. The characteristics of the mice population at study entry are shown in Table III.

Body weight

As shown in Table III, at the end of the study, animals fed with the HFD had a larger body weight increase compared with animals fed with the SD ($p < 0.05$), without differences among the group treated with HFD and the groups treated with HFD and S1 or S2.

Blood glucose

No variation of BG was recorded during the study among groups.

Lipid profile

After 24 weeks, mice fed with the HFD alone showed an increase of TC ($p < 0.05$) compared to the group fed with the SD. However, mice treated with HFD + S1 and HFD + S2 showed lower levels of TC compared to mice fed with the HFD alone ($p < 0.01$ vs. HFD at 24 weeks for HFD + S1, and $p < 0.05$ vs. HFD at 24 weeks for HFD + S2). Moreover, mice treated with HFD + S1 had lower TC levels compared to mice fed with HFD + S2 ($p < 0.05$ vs. HFD + S2 at 24 weeks). A similar trend was recorded for LDL-C, while no variations were recorded for HDL-C during the study. Triglycerides recorded after 24 weeks of the HFD were higher compared to mice fed with the SD. Moreover, mice treated with HFD + S1 or S2 had lower Tg levels compared to mice treated with the HFD at 24 weeks ($p < 0.05$ for both), without any significant difference between S1 and S2 groups.

Table III. Effect of HFD + S1 and HFD + S2 compared to SD or HFD on metabolic parameters during the period of evaluation

Group	Time (days)							
	Baseline				24 weeks			
	SD	HFD	HFD + S1	HFD + S2	SD	HFD	HFD + S1	HFD + S2
N	12	12	13	13	12	12	13	13
M/F	6/6	6/6	7/6	6/7	6/6	6/6	7/6	6/7
Weight [g]	29.4 ±2.7	35.2 ±5.6*	34.7 ±4.2*	36.8 ±6.1*	30.2 ±3.1	38.7 ±6.8*	36.5 ±5.9*	38.3 ±6.4*
BG [mg/dl]	77.8 ±6.1	81.4 ±8.5	79.3 ±6.9	75.4 ±5.8	81.8 ±8.9	84.6 ±9.4	76.1 ±6.0	73.4 ±5.1
TC [mg/dl]	103.5 ±13.4	117.0 ±14.6*	114.2 ±12.3*	119.8 ±16.1*	98.0 ±9.7	121.6 ±20.9*	91.4 ±8.6*£^	102.1 ±12.1°
LDL-C [mg/dl]	16.0 ±2.1	20.7 ±2.9	20.2 ±2.5	20.4 ±2.6	12.7 ±1.6	17.5 ±2.7*	10.1 ±1.8*£^	15.2 ±1.9°
HDL-C [mg/dl]	111.2 ±14.5	122.2 ±19.1	123.4 ±19.4	124.6 ±20.7	92.4 ±3.9	118.3 ±17.1	121.9 ±18.9	122.5 ±19.7
Tg [mg/dl]	110.4 ±21.6	136.4 ±26.9*	138.7 ±28.2*	140.9 ±31.7*	115.7 ±22.8	127.3 ±23.6*	101.3 ±13.6°	108.4 ±17.5°
CRP [mg/l]	3.5 ±1.9	4.0 ±2.2*	4.1 ±2.3*	3.9 ±2.1*	3.8 ±2.1	7.0 ±3.3*	3.6 ±2.5£^	4.3 ±2.5*°

*Data are mean ± standard deviation. *p < 0.05 vs. SD at baseline; $p < 0.01 vs. SD at baseline; °p < 0.05 vs. HFD at 24 weeks; £p < 0.01 vs. HFD at 24 weeks; ^p < 0.05 vs. HFD + S2 at 24 weeks. SD – standard diet, HFD – high-fat diet, HFD + S1 – high-fat diet + Rossopuro FORTE, HFD + S2 – high-fat diet + Rossopuro.*

C-reactive protein

After 24 weeks, mice treated with the HFD had higher levels of CRP compared to mice treated with the SD. However, mice treated with HFD + S1 and HFD + S2 showed lower levels of CRP compared to mice fed with the HFD alone ($p < 0.01$ vs. HFD at 24 weeks for HFD + S1, and $p < 0.05$ vs. HFD at 24 weeks for HFD + S2). Moreover, mice treated with HFD + S1 had lower CRP levels compared to mice fed with HFD + S2 ($p < 0.05$ vs. HFD + S2 at 24 weeks).

Discussion

In our study we observed a better effect of the combination of monacolin K, γ-oryzanol, and γ-aminobutyric acid compared to monacolin K alone, probably due to the synergic effect of the various components. Monacolin K has been extensively studied. Several intervention studies have demonstrated that red yeast rice containing 5 to 10 mg of monacolin K lowers elevated LDL-C levels by about 22% to 27% [12–14]. The European Food Safety Authority considers that a daily intake of 10 mg of monacolin K from red yeast rice contributes to the maintenance of a normal LDL-C plasma level. Less known is γ-oryzanol. Rice (*Oryza sativa* L.) bran is a by-product produced in processing rice. Traditionally, rice bran, produced as a by-product during milling, is considered as waste. However, it is a fact that rice bran comprises up to 10% of rough rice and is a rich natural source of important antioxidant polyphenolics, flavonoids, phytic acid, vitamin E and γ-oryzanol. Gamma-oryzanol is an anti-oxidant compound associated with decreases in plasma cholesterol, cholesterol absorption, and platelet aggregation [15]. Oryzanol has also been used to treat hyperlipidemia and disorders of menopause and to increase the muscle mass [16]. On the other side, γ-aminobutyric acid is one of the many nutritional components in brown rice and pre-germinated brown rice with slight germination. It has been reported that pre-germinated brown rice rich in γ-aminobutyric acid effectively reduced glucose levels in diabetic rats [17]. γ-Aminobutyric acid also showed anti-anxiety effects [18] and positive effects against chronic renal failure [19]. γ-Oryzanol was also suggested to show blood cholesterol lowering [20] and anti-oxidant effects [21].

In conclusion, the study showed that a nutraceutical combination containing monacolin K 3%, γ-oryzanol, and γ-aminobutyric acid is more effective than monacolin K 3% alone in reducing the negative effects of a HFD in a sample of specific pathogen-free 4-week-old male C57BL/6J mice. These data are really interesting, and are a good starting point for future studies in humans. Of course, nutraceuticals cannot replace an appropriate lifestyle, but should be complementary, and used before starting traditional therapy in low cardio-metabolic risk patients. We also need

to consider that an improvement of lipid profile does not always translate into a net cardiovascular benefit for the patient. Studies examining "hard" clinical endpoints are necessary to prove the efficacy of a hypolipidemic treatment, because at present nutraceuticals lack such major studies.

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Conflict of interest

Dr. Rosario Russo is employed by Giellepi S.p.A. Health Science, Lissone, Italy. The other authors declare that they have no conflict of interest. No writing assistance was used in the production of this manuscript.

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