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Long-term efficacy and tolerability of a multicomponent lipid-lowering nutraceutical in overweight and normoweight patients

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Abstract

The short-term lipid-lowering activity of some nutraceuticals is well known, however there is a lack of knowledge about their long-term effect, especially regarding insulin-resistance parameters and biomarkers of vascular health. This study is a 12-month follow-up randomised clinical trial carried out on 269 non-smoker hyperlipidaemic patients in primary prevention for cardiovascular disease; 214 of them (129 men and 85 women) completed the trial with good compliance. None of the subjects were diabetics or had been treated with antihyperlipidaemic drugs. Seventy-nine normoweight subjects (Group 1) were treated with a combined nutraceutical monakolin-berberine-policosand (MBP) mixture associated with a standardised therapeutic lifestyle (TLS) (as defined in the third Adult Treatment Panel of the National Cholesterol Education Program), 85 overweight subjects were treated with MBP-TLS (Group 2) and 50 overweight subjects only with intensified TLS (Group 3). Efficacy parameters (Body Mass Index, fasting plasma glucose, fasting plasma insulin, Homeostasis Model Assessment index, metalloproteinase-2 (MMP-2), MMP-9, tissue inhibitor of metalloproteinase-1 (TIMP-1), TIMP-2, total cholesterol, low-density lipoprotein-C, high-density lipoprotein-C, triglyceride) were evaluated every 4 months. Educational reinforcements were also planned every 4 months. This study demonstrated the long-term efficacy and safety of a combined nutraceutical added to a TLS in overweight and normoweight dyslipidaemic subjects. Berberine- and red yeast rice-based nutraceuticals showed efficacy in body weight reduction and insulin-sensitivity promotion in non-diabetic hyperlipidaemic patients. Favourable effects of a combined nutraceutical in combination with a TLS were observed on biomarkers of vascular remodelling in overweight and normoweight dyslipidaemic subjects.

Introduction

Increasing evidence supports the antihyperlipidaemic efficacy of some nutraceuticals [1]. However, the use of full-dose nutraceuticals entails some tolerability concerns, because they could have statin- or metformin-like side effects. The use of a combination of nutraceuticals with different but synergic mechanisms of action at lower and safer dosages appears to be an interesting alternative. In particular, recent data support the efficacy of a MBP mixture as a lipid-

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lowering drug. The MBP mixture was developed and clinically tested after evaluation of the lipid-lowering efficacy of different mixtures of its components: monakolins and policosanols [2, 3] and berberine and monakolins [4]. Finally, MBP consumed in conjunction with a standard Mediterranean healthy diet seems to be able to reduce low-density lipoprotein (LDL)-C and triglyceride (TG) by a mean of 20% in different kinds of patients [5]. This effect has obvious positive considerations for metabolic syndrome management and patients with high risk of cardiovascular disease [6]. On the other hand, MBP has been proven to have some direct protective vascular effects, similar to pharmacological lipid-lowering agents, such as improvement of endothelial dysfunction [7]. The MBP tolerability has also been confirmed in elderly hypercholesterolaemic subjects [8] and in patients previously intolerant to more than one statin [9]. However there is no published data on its long-term effects on lipid parameters and other parameters related to cardiovascular disease risk. In this context we planned a 12-month trial to evaluate the long-term efficacy of the MBP mixture and its effect on insulin-resistance-related parameters.

Methods

This is a 12-month, partially randomised clinical trial. We consecutively enrolled 269 non-smoker, non-diabetic, pharmacologically untreated hyperlipidaemic patients in primary prevention for cardiovascular disease.

Group 1 included 79 normoweight subjects treated with a combined nutraceutical containing berberine 500 mg, policosanols 10 mg and monakolins 3 mg/dose (MBP) combined with a standardised therapeutic lifestyle (TLS, as suggested by the third Adult Treatment Panel of the National Cholesterol Education Program); 135 overweight patients were randomised to be treated with MBP-TLS (Group 2) or with placebo-TLS (Group 3).

The efficacy parameters were evaluated every 4 months. Educational reinforcements were also planned every 4 months.

Systolic (1st phase) and diastolic (5th phase) blood pressure readings were measured to the nearest even digit using a standard mercury manometer (Erkameter 3000, ERKA, Bad Tolz, Germany), with a large cuff size where the arm circumference was greater than 33 cm. Three readings were recorded on the right arm of the seated participant following a minimum of 10 minutes rest in a quiet room and the average of the second and third readings was defined as the subject's blood pressure. Measurements were always taken by the same investigator in the morning before daily drug intake (i.e., ~24 h after dosing).

All plasma parameters were determined after a 12-h overnight fast. Venous blood was taken between 08:00 and 09:00 and samples were kept on ice prior to spinning. Plasma was obtained by addition of Na_2-EDTA, 1 mg/ml, and centrifuged at 3000 g for 15 min at 4°C. Immediately after centrifugation plasma samples were frozen and stored at –80°C for no more than 3 months. Plasma glucose was assayed using the glucose-oxidase method (GOD/PAP, Roche Diagnostics, Mannheim, Germany) with intra- and inter-assay coefficients of variation (CsV) <2% [10]. Plasma insulin was assayed with a Phadiaseph Insulin RIA (Pharmacia, Uppsala, Sweden) using a second antibody to separate the free and antibody-bound ^{125}I-insulin (intra- and inter-assay CsV 4.6 and 7.3%, respectively) [11]. Total cholesterol (TC) and TG levels were determined using a fully enzymatic technique on a clinical chemistry analyser (HITACHI 737; Hitachi, Tokyo, Japan) [12, 13]. Intra- and inter-assay CsV were 1.0 and 2.1 for TC determination, and 0.9 and 2.4 for TG determination, respectively. High-density lipoprotein (HDL)-C level was measured after precipitation of plasma apo B-containing lipoproteins with phosphotungstic acid; intra- and inter-assay CsV were 1.0 and 1.9, respectively. LDL-C level was calculated using the Friedewald formula [14, 15]. Homeostasis Model Assessment (HOMA) index was calculated using the formula fasting plasma insulin (FPI) (mcU/ml)×fasting plasma glucose (FPG) (mmol/l)/22.5, as described by Matthews and coworkers [16].

Homocysteine was measured by a modified procedure of Araki and Sako [17], with high-pressure liquid chromatography and fluorescence detection. The intra-assay variation of the method was 2.5%.

C-reactive protein was measured with a validated high-sensitivity assay [17] on a BN™ II nephelometer (Dade Behring Inc., Newark, DE, USA). The intra- and inter-assay CsV were <4% and <2%, respectively, with a detection limit of 0.20 mg/l.

Metalloproteinase (MMP)-2, MMP-9, tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 levels were determined by a two-site ELISA method using commercial reagents (Amersham Biosciences, Uppsala, Sweden). The intra- and inter-assay CsV for measuring MMP-2 levels were 5.4% and 8.3%, respectively. The intra- and inter-assay CsV to evaluate MMP-9 levels were 4.9% and 8.6%. The intra- and inter-assay CsV for measuring TIMP-1 levels were 9.3% and 13.1%, respectively, while those for measuring TIMP-2 levels were 5.4% and 5.9%, respectively [18].

Compliance was considered acceptable when higher than 90% (i.e., the patient consumed at least 90% of the prescribed pills).

The study was carried out in agreement with the Helsinki declaration and all participants signed an informed consent.

Statistical analysis was carried out on the data obtained from subjects with acceptable compliance to the proposed treatments. All the data were sampled in a database and statistically analysed with the help of SPSS 19.0, version for Windows. A full descriptive analysis has been carried out as well as a normality distribution test for continuous variables. ANOVA and ANCOVA analyses were then done to detect differences vs. baseline values and among treatment groups respectively. A *p* level of less than 0.05 was considered significant for all tests.

Results

Of 269 subjects enrolled, 214 completed the trial with good compliance: 129 men and 85 women. No adverse drug event was reported during the trial. No patients withdrew from the study because of side effects. The main characteristics of the enrolled patients are shown in Table 1.

	Normoweight MBP+TLS		Overweight MBP+TLS		Overweight TLS	
	Mean	SD	Mean	SD	Mean	SD
BMI	26.80	0.87	26.95	0.86	24.17	0.99
SBP	135.08	5.60	134.35	6.21	133.24	5.31
DBP	85.16	5.30	86.25	6.09	84.09	6.82
PP	49.92	6.11	48.11	7.07	49.15	6.69
TC	219.80	15.19	218.26	14.43	213.52	16.98
LDL-C	135.70	15.93	134.58	15.23	135.98	18.91
HDL-C	38.48	3.78	38.64	4.46	38.97	4.27
TG	228.08	41.36	225.20	42.72	192.82	44.39
FPG	110.02	11.26	109.58	12.03	92.22	10.25
FPI	11.44	3.95	11.49	4.34	7.47	3.14
HOMA	3.15	1.24	3.17	1.37	1.73	0.83
HCYS	11.58	2.96	11.41	2.76	10.79	2.77
FBG	371.66	54.91	369.28	48.30	366.92	40.11
hsCRP	2.22	0.36	2.05	0.31	1.85	0.43
MMP-2	1106.94	108.28	1106.94	75.73	877.90	67.42
MMP-9	136.33	48.53	137.67	51.77	124.35	52.11
TIMP-1	303.64	51.83	306.55	50.29	316.58	47.50
TIMP-2	98.97	5.56	99.43	3.36	100.27	3.61

BMI= Body Mass Index, SBP= Systolic Blood Pressure, DBP= Diastolic Blood Pressure, PP= Pulse pressure, TC= Total Cholesterol, LDL-C= Low-density Lipoprotein Cholesterol, HDL-C= High-density Lipoprotein Cholesterol, TG= Triglycerides, FPG= Fasting Plasma Glucose, FPI= Fasting Plasma Insuline, HOMA= Homeostastis Assessment Index, HCYS= Homocysteine, FBG= Fibrinogen, hsCRP= high-sensitivity C-reactive protein, MMP= Metalloproteinase, TIMP= Tissue Inhibitor of Metalloproteinase

Table 1 Baseline characteristics of the enrolled patients

The overweight subjects treated with TLS experienced a significant improvement in TC (–23±17 mg/dl, $p<0.05$), LDL-C (–6±22 mg/dl, $p<0.05$), HDL-C (+2±6 mg/dl, $p<0.05$) and TG (–97±51 mg/dl, $p<0.05$).

The overweight subjects treated with MBP-TLS experienced a significant improvement in TC (–42±17 mg/dl, $p<0.05$), LDL-C (–27±21 mg/dl, $p<0.05$), HDL-C (+5±6 mg/dl, $p<0.05$) and TG (–103±54 mg/dl, $p<0.05$).

The normoweight subjects treated with MBP-TLS experienced a significant improvement in TC (–38±20 mg/dl, $p<0.05$), LDL-C (–29±26 mg/dl, $p<0.05$), HDL-C (+5±6 mg/dl, $p<0.05$) and TG (–71±56 mg/dl, $p<0.05$).

TC, LDL-C and HDL-C improved significantly more in Group 2 than in Group 3, TC and LDL-C more in Group 1 than in Group 3, and TG more in Group 2 than in Group 1 ($p<0.05$) (Fig. 1-4).

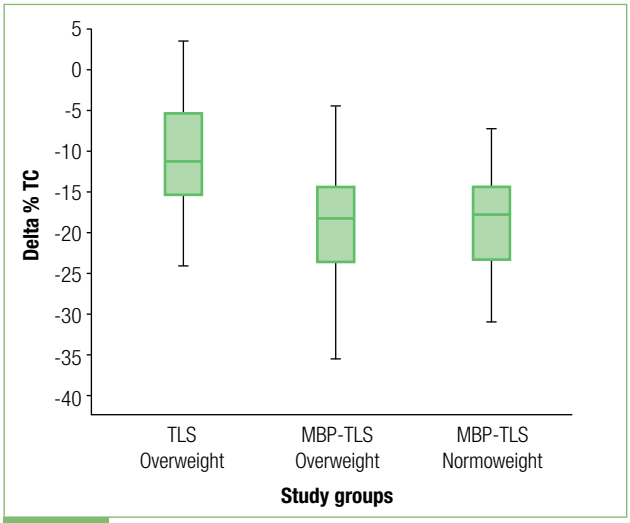

Figure 1 Long-term effect (12 months) of life-style (TLS) improvement and TLS plus nutraceuticals in normoweight and overweight patients enrolled in the trial on TC plasma level

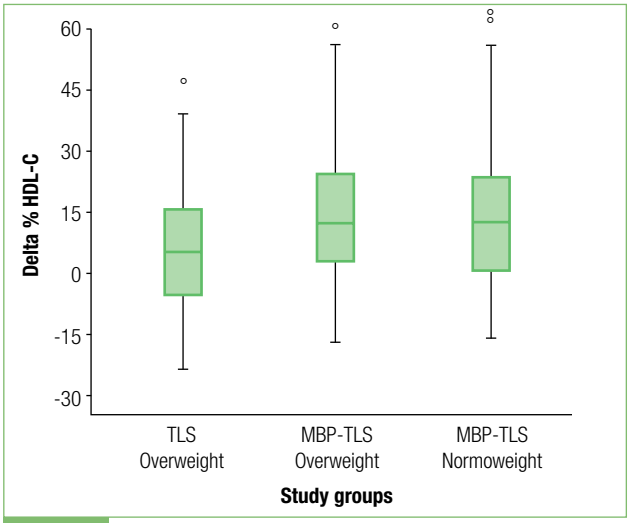

Figure 2 Long-term effect (12 months) of life-style (TLS) improvement and TLS plus nutraceuticals in normoweight and overweight patients enrolled in the trial on HDL-C plasma level

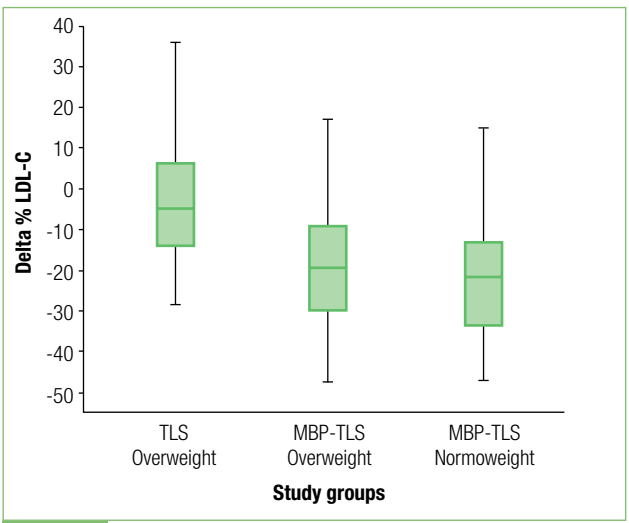

Figure 3 Long-term effect (12 months) of life-style (TLS) improvement and TLS plus nutraceuticals in normoweight and overweight patients enrolled in the trial on LDL-C plasma level

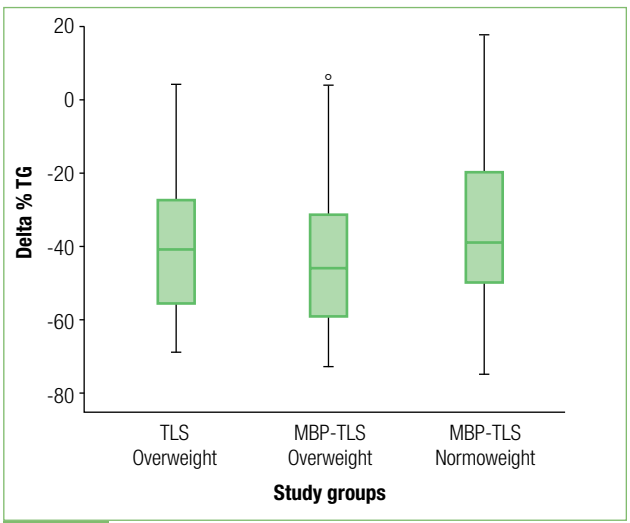

Figure 4 Long-term effect (12 months) of life-style (TLS) improvement and TLS plus nutraceuticals in normoweight and overweight patients enrolled in the trial on TG plasma level

The overweight subjects treated with TLS experienced a significant improvement in MMP-2 (–257±322 ng/ml, $p<0.05$), MMP-9 (–43±53 ng/ml, $p<0.05$), TIMP-1 (–61±41 ng/ml, $p<0.05$) and TIMP-2 (–6.6±8.0 ng/ml, $p<0.05$).

The overweight subjects treated with MBP-TLS experienced a significant improvement in MMP-2 (–419±295 ng/ml, $p<0.05$), MMP-9 (–85±57 ng/ml, $p<0.05$) and TIMP-2 (–1.5±6.5 ng/ml, $p<0.05$).

The normoweight subjects treated with MBP-TLS experienced a significant improvement in MMP-2 (–279±255 ng/ml, $p<0.05$), MMP-9 (–69±54 ng/ml,

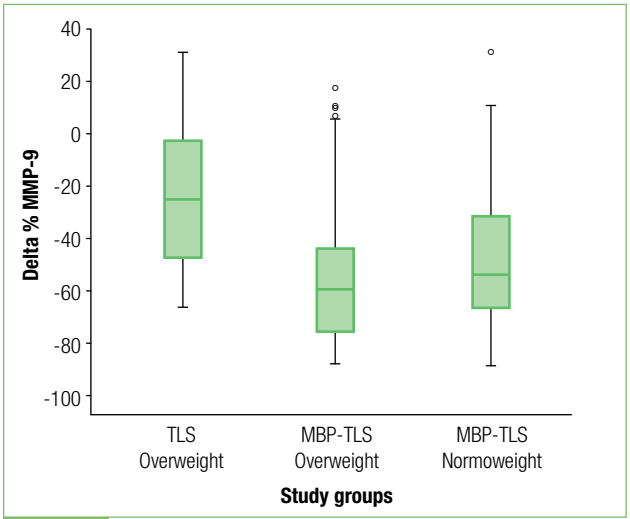

Figure 5 Figure 5 – Long-term effect (12 months) of life-style (TLS) improvement and TLS plus nutraceuticals in normoweight and overweight patients enrolled in the trial on MMP-9 plasma level

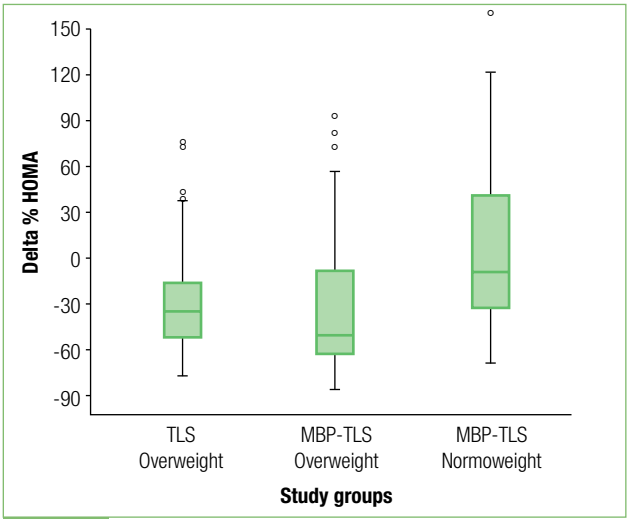

Figure 6 Long-term effect (12 months) of life-style (TLS) improvement and TLS plus nutraceuticals in normoweight and overweight patients enrolled in the trial on HOMA index level

$p<0.05$), TIMP-1 (–31±74 ng/ml, $p<0.05$) and TIMP-2 (–1.9±6.5 ng/ml, $p<0.05$).

All parameters improved significantly more in Group 2 than in Group 3, while MMP-9, TIMP-1 and TIMP-2 improved more in Group 1 than in Group 3. No significant difference was observed in improvement between Groups 1 and 2 (Fig. 5).

The overweight subjects treated with TLS experienced a significant improvement in Body Mass Index (BMI) (–0.3±0.1 kg/m^2, $p<0.05$), FPG (–11±13 mg/dl, $p<0.05$), FPI (–3.5±4.2 mU/ml, $p<0.05$) and HOMA index (–1.2±1.3, $p<0.05$).

The overweight subjects treated with MBP-TLS experienced a significant improvement in BMI (–0.6±0.8 kg/m^2, $p<0.05$), FPG (–21±15 mg/dl, $p<0.05$), FPI (–4.3±4.8 mU/ml, $p<0.05$) and HOMA index (–1.6±1.5, $p<0.05$).

The normoweight subjects treated with MBP-TLS experienced a significant improvement in BMI (–0.3±0.1 kg/m^2, $p<0.05$) and HOMA index (–0.2±0.8, $p<0.05$), but not in FPG and FPI (Fig. 6).

BMI and FPG improved significantly more in Group 2 than in Groups 1 and 3 ($p<0.05$), while FPG decreased significantly more in Group 3 than in Group 1 ($p<0.05$).

Discussion

The long-term efficacy and overall safety of red yeast rice has been clearly demonstrated in Asian subjects in the large Chinese Coronary Secondary Prevention Study [19]. Our results only confirm its continuous lipid-lowering effect after 12 months of treatment also in Caucasian subjects. Because red yeast rice has a statin-like mechanism of action [20, 21], an improvement in vascular remodelling biomarkers, such as metalloproteinases, was expected.

The short-term lipid-lowering effect of berberine has also been clearly demonstrated in animal models and in clinical trials carried out in Asian hypercholesterolaemic subjects. With this trial we confirmed its long-term effect in Caucasian dyslipidaemic subjects as well [22]. The berberine effect on glucose homeostasis and insulin resistance has been widely demonstrated in *in vitro* and animal models [23, 24]. A significant antidiabetic effect has also been observed in the short term in Asian subjects affected by type 2 diabetes, however the high dosages employed (1.5 g/day) were often associated with gastrointestinal disorders [25, 26]. In our long-term study carried out with a low, well-tolerated dosage

of berberine (500 mg/day), we confirmed the insulin-sensitising effect of berberine in Caucasian subjects, more evident in overweight patients.

This study has some limitations. The main one is the lack of a total double-blind randomisation, associated with a lack of a placebo-treated normoweight control group. Moreover, efficacy and safety evaluation were systematically carried out only after 12 months of treatment. This was partly due to the aim of the study, which was to verify the long-term effects of the MPB mixture in a clinical setting. Regarding the investigated parameters, there were relatively few, but we selected those we were accustomed to evaluating in our research practice in order to maintain a high level of standardisation. Also, we tested the MBP mixture effects but not the single component effects. On the one hand, the subject of the study was the nutraceutical mixture; on the other hand, the effect of the three components had already been largely characterised in the available literature.

In conclusion, in our study, the long-term consumption of a combination of monascus-policosanol-berberine nutraceuticals is associated to a significant reduction in LDL-cholesterolaemia, triglyceridaemia, insulin resistance-related parameters and vascular remodelling parameters, more evident in overweight dyslipidaemic patients. Further studies on instrumental biomarkers of vascular health are needed to evaluate the health effects of this product on intermediate outcomes.

Conflict of interest

Dr. Cicero acted as R&D consultant for Rottapharm-Madaus. The other Authors have no direct or indirect conflict of interest in the publication of this paper.

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