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PHARMACOKINETICS AND DISPOSITION

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Magnesium pyridoxal-5'-phosphate glutamate, "A vitamin B₆ derivative", does not affect lipoprotein levels in patients with familial hypercholesterolaemia

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Abstract *Objective:* In this double-blind, randomized, placebo-controlled, dose-finding study we assessed the short-term efficacy and safety of increasing dosages of magnesium pyridoxal-5'-phosphate glutamate (MPPG) compared to placebo in patients with familial hypercholesterolaemia (FH). Twenty-three patients of either sex, over the age of 18 years and suffering from heterozygous FH, were treated with MPPG for a period of 16 weeks.

Results: Baseline characteristics and lipoprotein profiles of the patients were comparable in the two treatment groups. Overall compliance was 90%. Neither after the first 8 weeks treatment period with 450 mg MPPG daily nor after the second 8 weeks treatment period with 600 mg MPPG daily were statistically significant changes in plasma total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol or triglyceride levels observed between the treatment and placebo groups. Plasma levels of lipoprotein (Lp)(a), apolipoprotein (apo) A₁, apo B₁₀₀, very low density lipoprotein (VLDL) cholesterol and VLDL triglyceride also did not change.

Conclusion: Although it has been demonstrated that MPPG improves lipoprotein levels in patients with different forms of dyslipidaemia, MPPG is not effective for the treatment of FH patients.

Key words Familial Hypercholesterolaemia, Magnesium pyridoxal-5'-phosphate glutamate

Introduction

Pyridoxine (vitamin B₆) deficiency may decrease hormone-sensitive lipase activity, consequently increasing plasma cholesterol levels in experimental animals and promoting an acceleration of atherosclerosis [1–4]. Supplementation of large doses of vitamin B₆ in humans revealed a consistent, but modest decrease in plasma cholesterol levels, although regression of atherosclerosis was never conclusively demonstrated [5].

Magnesium pyridoxal-5'-phosphate glutamate (MPPG), a recently developed vitamin B₆ derivative, has been extensively investigated with regard to its influence on lipoprotein levels. However, the overall methodology of these studies was often flawed, i.e. they were open labelled, uncontrolled and not randomized or analysed for independent end point assessments. Treatment studies with different dosages of MPPG in heterogeneous patient populations suffering from diverse secondary hyperlipidaemias revealed reductions of total cholesterol levels ranging from 7.5% to 25%, and of plasma triglyceride levels from 20% to 40%, sometimes in conjunction with a 10–30% increase in high-density lipoprotein (HDL) cholesterol levels [6, 7]. For example, patients with a secondary dyslipidaemia due to chronic renal insufficiency showed a 26% reduction of plasma cholesterol levels upon treatment with 150 mg MPPG per day. The latter represents the only randomized and placebo-controlled study with MPPG to date [8].

Since most studies with MPPG did not fulfil the generally accepted standards for clinical investigation, patients were not homogeneous with respect to their lipoprotein disorder, and results, in our opinion, were not conclusive, we decided to design a double-blind, randomized, placebo-controlled, dose-finding pilot

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study in order to verify previous findings and to establish the short-term efficacy and safety of two dosages of MPPG compared to placebo in a well-defined patient population, i.e. patients with definite classical familial hypercholesterolaemia (FH) [9, 10].

FH is a dominantly inherited disease, which predisposes to early atherosclerosis of coronary, cerebral and peripheral arteries [11]. The underlying molecular defect consists of mutations in the gene coding for the low-density lipoprotein (LDL) receptor protein, which results in a reduction or even absence of its function and causes an increase in plasma LDL cholesterol in these patients [12, 13]. Lipid-lowering therapy usually consists of inhibition of cholesterol synthesis with a 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitor and interference of intestinal bile absorption by using bile-acid-binding resins [14]. Although normalization of plasma total and LDL cholesterol cannot always be achieved, one may expect an average reduction of 30–40% and up to 50% in some patients, respectively, upon administration of high doses of reductase inhibitors [15, 16]. Since pharmacological therapy in FH patients is usually indicated for a lifetime, both efficacy and safety are important issues. The safety of MPPG does not seem to be in question, but the efficacy as hitherto mentioned certainly is. We present here the data of this study with MPPG in FH heterozygotes.

Materials and methods

Patients

Twenty-three patients of either sex, over the age of 18 years and suffering from heterozygous FH, were included in this study. All patients were enrolled from the Amsterdam Lipid Research Clinic at the Academic Medical Centre of the University of Amsterdam and the Slotervaart Training Hospital affiliated to the University of Amsterdam. The inclusion criteria were a diagnosis of FH, based on an LDL cholesterol level consistently above the 95th percentile for age and sex, while already on a lipid-lowering diet. Furthermore, one or more of the following typical signs for FH had to be present: tendon xanthomas, xanthelasmata or an arcus cornealis before the age of 45 years, a positive family history of hypercholesterolaemia or a history of premature atherosclerosis before the age of 50 years.

Patients with plasma triglyceride levels above $2.7 \text{ mmol} \cdot \text{L}^{-1}$ or LDL cholesterol levels below the 95th percentile and patients with type I, III, IV or V hyperlipidaemia, diabetes mellitus, anorexia nervosa, kidney, liver or thyroid disease were excluded. A history of drug or alcohol abuse, concurrent use of other lipid-lowering drugs or drugs that might interfere with lipoprotein metabolism were not included.

The protocol was approved by the institutional review board of both hospitals and written informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

Study design

The design of the study was double-blind, randomized, placebo-controlled and dose-finding, evaluating the short-term efficacy and

safety of two dosages of MPPG compared to placebo in 23 patients with FH. Before randomization to either treatment with MPPG or placebo, patients passed through a diet and placebo period of 4 weeks each, after withdrawal of all lipid-lowering medication. Patients adhered to a standard lipid-lowering diet. All patients received dietary advice at visits to the clinic at weeks 0, 8 and 16. Patients randomized to the MPPG group were treated with 150-mg MPPG tablets, 3 times daily during the first 8 weeks of the active treatment period and with 150 mg MPPG tablets, 4 times a day during the second 8 weeks.

Efficacy and safety measurements

Clinical visits were scheduled at weeks –4, 0, 4, 8, 12 and 16. Fasting plasma total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride levels were determined at each visit, while apo A₁, apo B₁₀₀ and lipoprotein (Lp)(a) were measured at weeks 0, 8 and 16, as well as lipoprotein analysis by preparative ultracentrifugation, i.e. HDL₂ cholesterol, HDL₃ cholesterol, VLDL cholesterol and VLDL triglycerides. Lp(a) was determined by an electro-immuno-diffusion procedure using a monospecific polyclonal goat antiserum calibrated on immunostandard serum [17, 18], and VLDL cholesterol and triglycerides were determined after separation by ultracentrifugation in a Cs chloride solution and subsequently quantified enzymatically in the supernatant [19]. Furthermore, HDL₂ and HDL₃ cholesterol subfractions were quantified, after precipitation of VLDL and HDL cholesterol by heparin and manganese chloride followed by separation by ultracentrifugation in a KBR gradient, by an enzymatic colorimetric procedure using cholesterol esterase and cholesterol oxidase [20, 21]. Plasma LDL cholesterol levels were calculated using the Friedewald equation [22]. Blood samples were obtained after an overnight fast and prior to breakfast. All plasma lipid and lipoprotein analyses were performed at the central laboratory of the Lipid Research Clinic Amsterdam at the Slotervaart Training Hospital. This laboratory is a member of the Centre for Disease Control (CDC) approved Lipid Reference Laboratory Network of the University Hospital Dijkzicht, Rotterdam, The Netherlands.

Safety laboratory assessment was performed at every visit, including routine haematological and biochemical parameters and urinalysis. A complete medical history was taken and a physical examination performed on the first and final clinical visits, whereas vital signs were recorded on every visit. Smoking, drinking and exercise habits were documented and patients did not change these habits during the study. Drug compliance was encouraged and evaluated by means of tablet counts at every visit.

Statistical analysis

Analysis of variance (ANOVA) for repeated observations was used for data analysis, which does allow comparison of two treatment groups with regard to multiple time points. Lipid parameters were analysed using the change from baseline to week 12, in contrast to haematological and chemistry parameters, which were analysed as absolute numbers. Both an intention-to-treat analysis and an efficacy analysis were performed to evaluate the results of the study.

Results

Twenty-three patients entered the treatment period of the study and were randomized to treatment with either MPPG or placebo. Twelve patients were treated with

Table 1 Patient characteristics

	Active treatment group	Placebo group
Number of patients	10	9
Age	33.6	33.9
Body mass index	23.1	22.8
Sex (male/female)	1/9	5/4
Coronary vascular disease	0	0
Cigarette smokers	2	3
Mean number of cigarettes	22.5 per day	11.5 per day
Alcohol consumers	7	7
Mean units of alcohol	7 per week	4.5 per week
Exercise	8	7
Mean duration of exercise	2.4 h per week	3.1 h per week
Oral contraceptives	5	3

MPPG and 11 patients received placebo. Four patients did not complete the study. Three patients refused further cooperation after randomization, two patients after the week 0 visit and one patient after the week 8 visit. One patient, asymptomatic and with a normal EKG previous to the study, developed symptoms of cardiac ischaemia after the week 12 visit. This patient, who represents the only adverse experience observed in this study, underwent a coronary artery bypass graft procedure and was withdrawn from the study. Both an intention-to-treat analysis and an efficacy analysis were performed and yielded similar results. Therefore, only the efficacy analysis will be presented here, which consisted of ten patients receiving MPPG and nine patients receiving placebo.

Baseline characteristics

Baseline characteristics of the 19 remaining study patients are shown in Table 1. Mean age at randomization was 33.7 years for both groups. Thirteen women and six men participated in the study, divided between the treatment and placebo groups as one to nine and five to four, respectively.

There were no changes with regard to smoking, alcohol consumption and physical exercise before and during the study. Eleven patients were treated with an HMG CoA reductase inhibitor before entering the study. All patients discontinued their lipid-lowering treatment 8 weeks before randomization. Eight female patients used oral contraceptives and one patient was treated with acetylsalicylic acid before entering the study. Concurrent medication remained unchanged during the treatment period. Twelve patients had pathognomonic signs for familial hypercholesterolaemia, i.e. an arcus lipoides corneae, tendon xanthomas or xanthelasmata. All patients had EKG results within normal limits before and after the treatment period. Overall compliance was 90%, calculated by means of tablet counts.

Efficacy results

Lipid values before and after two treatment periods are shown in Table 2. Mean baseline plasma total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride levels were 9.94, 7.90, 1.33 and 1.30 mmol · l⁻¹, respectively, and were not statistically different in the two treatment groups. Neither after the first treatment period with a daily dosage of 450 mg MPPG, nor after the second treatment period with a daily dosage of 600 mg MPPG, were changes in plasma total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride levels statistically significant within and between the treatment or placebo groups. Plasma levels of Lp(a), apo A₁, apo B₁₀₀, VLDL cholesterol and VLDL triglycerides also showed no statistically significant differences.

Safety results

None of the study subjects experienced adverse effects possibly or definitely related to the study treatment. Routine laboratory safety parameters, including those reflecting liver and kidney function, did not change significantly during the study (data not shown).

Discussion

In our study, patients suffering from heterozygous FH were treated with MPPG to determine its short-term efficacy and safety. Although very well tolerated, not even a trend of a beneficial effect of MPPG on lipoprotein levels could be demonstrated. The statistical test used, the ANOVA test, predicted similar results if larger numbers of patients had been included. These poor results are in contrast to the results of some of the previous studies with pyridoxine and MPPG in human and animal models. In an 8-week study, extremely high dosages of MPPG were administered to rabbits on a cholesterol-enriched diet. The control group consisted of animals fed an identical diet. Although in our opinion not conclusive, MPPG was reported to retard hypercholesterolaemia in these experiments. In the same study, reduction of calcium deposition and macroscopic regression of atherosclerotic lesions in the aortic wall were observed [23]. Studies pertaining to human dyslipidaemia were only reported for the treatment with MPPG in secondary lipid disorders, showing encouraging results [8]. Nevertheless, assuming that the dosages of MPPG were sufficient and the compliance of the patients was adequate, only the type of lipoprotein disorder or the length of the treatment period could be responsible for the poor results observed in our study.

It has been clearly demonstrated that vitamin B₆ reduces plasma homocysteine levels, by stimulation of

Table 2 Lipid and lipoprotein mean plasma levels and mean percentage changes

	Baseline Mean (SEM)	Week 8		Week 16	
		450 mg Mean (SEM)	% change Mean (SEM)	600 mg Mean (SEM)	% change Mean (SEM)
Total cholesterol ($<5.2 \text{ mmol} \cdot \text{l}^{-1}$)					
MPPG	10.8 (0.86)	10.6 (0.96)	-1.7 (3.07)	9.91 (0.62)	-5.8 (4.17)
Placebo	9.03 (0.77)	8.90 (0.77)	-1.3 (2.84)	8.79 (0.59)	-1.5 (3.42)
LDL cholesterol ($<3.2 \text{ mmol} \cdot \text{l}^{-1}$)					
MPPG	8.55 (0.93)	8.58 (0.93)	1.4 (3.02)	8.10 (0.61)	0.7 (7.55)
Placebo	7.19 (0.77)	7.06 (0.78)	-1.4 (3.50)	7.00 (0.58)	-0.7 (4.00)
HDL cholesterol ($>1.00 \text{ mmol} \cdot \text{l}^{-1}$)					
MPPG	1.32 (0.11)	1.43 (0.12)	8.9 (3.35)	1.24 (0.09)	-4.7 (3.67)
Placebo	1.34 (0.06)	1.36 (0.09)	1.7 (4.32)	1.28 (0.10)	-4.6 (4.81)
Triglycerides ($<2.3 \text{ mmol} \cdot \text{l}^{-1}$)					
MPPG	1.44 (0.22)	1.30 (0.28)	-9.5 (6.29)	1.27 (0.22)	-12.3 (5.10)
Placebo	1.14 (0.15)	1.05 (0.14)	-4.7 (8.99)	1.13 (0.15)	1.9 (7.81)
Apolipoprotein B ₁₀₀ ($0.6\text{--}1.3 \text{ g} \cdot \text{l}^{-1}$)					
MPPG	2.51 (0.32)	2.81 (0.28)	18.9 (9.38)	2.92 (0.24)	23.5 (7.76)
Placebo	2.11 (0.13)	2.21 (0.20)	10.0 (7.50)	2.37 (0.17)	14.0 (6.55)
Apolipoprotein A ₁ ($0.9\text{--}1.8 \text{ g} \cdot \text{l}^{-1}$)					
MPPG	1.31 (0.08)	1.42 (0.06)	10.3 (5.25)	1.19 (0.05)	-7.2 (5.11)
Placebo	1.25 (0.06)	1.31 (0.07)	5.8 (6.18)	1.25 (0.09)	1.1 (7.35)
Lipoprotein (a) ($<50 \text{ g} \cdot \text{l}^{-1}$)					
MPPG	39.3 (12.9)	32.6 (12.0)	-6.5 (15.3)	25.9 (11.9)	-31.8 (8.66)
Placebo	33.1 (14.4)	33.6 (17)	-6.4 (6.05)	25.6 (10.9)	-19.1 (8.62)

cystathionine synthase [24]. Hyperhomocysteinaemia is an independent risk factor for the development of atherosclerosis. It might be hypothesized that the reported anti-atherogenic effect of MPPG is the result of reduction of plasma homocysteine levels. In our study, however, plasma homocysteine levels were not measured; therefore this hypothesis remains open for research.

In conclusion, this double-blind, randomized, placebo-controlled, dose-finding pilot study clearly demonstrates that 16 weeks treatment with increasing dosages of MPPG is not effective in the treatment of patients suffering from FH. On the other hand, promising results in other studies with MPPG, although methodologically often incorrect, justify further investigation, but only in a prospective, randomized controlled fashion.

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