EFFECT OF POLICOSANOL (20 mg/d) ON THE FUNCTIONAL RECOVERY OF PATIENTS WITH ISCHEMIC STROKE: A ONE YEAR STUDY

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ABSTRACT. Stroke is a leading cause of mortality and disability. Policosanol has been effective in brain ischemia models and previous clinical studies suggested that policosanol (20 mg/d) + standard aspirin (AS) therapy had benefits versus placebo +AS given for 6 and 12 months to patients with recent ischemic stroke. The objectives of this study investigate whether policosanol, added to AS therapy within 30 d of stroke onset, is better than placebo + AS for the long-term recovery of ischemic stroke subjects. This study was randomized, double-blind, placebo-controlled. Patients with a modified Rankin Scale score (mRSs) 2 to 4, within 30 d of onset, were randomized and consumed policosanol/AS or placebo/AS, for 12 months. The primary outcome was mRSs reduction. Decreases of low-density lipoprotein-cholesterol (LDL-C), total cholesterol and increases of high-density lipoprotein-cholesterol (HDL-C) levels were secondary outcomes. Thirty-eight patients (mean age: 66 years) included in the study. Policosanol/AS decreased significantly mean mRSs from the first interim check-up (1.5 months) ($p < 0.01$) to final of treatment. In adition, policosanol/AS significantly lowered LDL-C, total cholesterol and increase HDL-C versus placebo/AS. Treatments were well tolerated. There were 15 withdrawals, five due to severe adverse events. It is conclude the long-term (12 months) administration of policosanol/AS given after suffering ischemic stroke showed to be better than placebo/AS in improving functional outcomes at 3 and 12 months when used among patients with ischemic stroke of moderate severity.

RESUMEN. El ictus es una causa principal de mortalidad y discapacidad. El policosanol ha sido eficaz en modelos de isquemia cerebral y estudios clínicos previos sugieren que la terapia estándar con aspirina (AS)+policosanol (20 mg/d) durante 6 y 12 meses produce beneficios versus placebo+AS en pacientes con un reciente ictus isquémico. El objetivo de este estudio consistió en investigar si el tratamiento a largo plazo con policosanol, añadido a la terapia con AS, dentro de los 30 d posteriores a un ictus isquémico, es mejor que el placebo+AS en la recuperación de los sujetos. Este es un estudio aleatorizado, a doble ciegas, controlado con placebo. Pacientes que sufrieron un ictus en los 30 d previos y con un puntaje de 2 a 4 en la escala neurológica de Rankin modificada (mRSs), se distribuyeron aleatoriamente y recibieron policosanol/AS o placebo/AS, durante 12 meses. La variable primaria de eficacia fue la reducción del puntaje mRSs. La reducción del colesterol transportado por lipoproteínas de baja densidad (LDL-C) y el colesterol total, así como el incremento del colesterol transportado por lipoproteínas de alta densidad (HDL-C) fueron consideradas variables secundarias de eficacia. Treinta y ocho pacientes (edad media: 66 años) fueron incluidos en el estudio. El tratamiento con policosanol/AS disminuyó significativamente el puntaje mRSs desde el primer chequeo intermedio (1,5 meses) ($p < 0.01$) hasta el final del tratamiento. Además, el tratamiento policosanol/AS disminuyó significativamente las concentraciones de LDL-C y colesterol total e incrementó las concentraciones de HDL-C. Los tratamientos fueron bien tolerados. Quince pacientes causaron baja del estudio, cinco debido a eventos adversos severos. Se concluye que el tratamiento a largo plazo (12 meses) con policosanol/AS es más efectivo que el tratamiento con placebo/AS en la recuperación funcional de los pacientes que han sufrido un ictus isquémico de moderada gravedad.

INTRODUCTION

Stroke results from the sudden interruption of blood flow to a brain region that impairs the energy supply to the central nervous system. Hypoxia is the main cause of central nervous system damage in stroke. Although neurons and

glial cells are also affected in the penumbra, neurons are more vulnerable because they depend on the oxidative metabolism of glucose for energy. Most strokes (70 - 80 % of cases) are ischemic on nature. [1,2]

Ischemic stroke remains as a leading cause of mortality, and is the main cause of disability worldwide. About half of stroke survivors remain with physical or cognitive impairment that severely affect their physical and social functions. Also, stroke implies a high cost to patients, families and health systems.[3,4]

Control of modifiable stroke risk factors, such as hypertension, dyslipidemia, diabetes, cigarette smoking and obesity are key measures to prevent recurrent strokes.[5]

Up to date, aspirin remains the gold standard of antiplatelet therapy for stroke recovery and prevention, and several studies and meta-analyses support the merits of antiplatelet drugs in stroke prevention by lowering platelet function, which reduces thrombotic complications of atherosclerosis.[6-9]

On its side, reduction of low-density lipoprotein-cholesterol levels has been shown to be relevant not only for stroke prevention, but also for improving functional outcomes after stroke, a key matter for reducing the disability after stroke.[10-13]

Policosanol, a mixture of 8 high molecular weight sugarcane wax alcohols,[14] has been shown protective effects in experimental brain ischemia,[15-17] and clinical studies have found coherent results.[18-23]

Two double-blind, placebo-controlled studies demonstrated that policosanol 20 mg/d + AS 125 mg/d given for 6 months improved the neurological recovery as compared to placebo + AS in patients with recent (≤ 30 d) ischemic stroke.[18,19] Also, a shorter duration (three months) study demonstrated that policosanol, was as effective as atorvastatin (20 mg/day), for improving the functional outcome in stroke patients treated with AS.[20] Likewise, open long-term (five years) studies found that policosanol added to AS was associated to a very good neurological recovery among sufferers of ischemic stroke.[21,22]

Previous long-term (12 months) administration of policosanol/AS given after suffering ischemic stroke was show to be better than placebo/AS in improving functional outcomes at 3 and 12 months when used among patients with ischemic stroke of moderate severity.[23]

In light of these facts, this study was undertaken to investigate whether policosanol added to AS within 30 d of stroke onset, is better than placebo + AS for the long-term recovery of ischemic stroke subjects.

MATERIALS AND METHODS

Study design

Patients who suffered recent ischemic stroke (≤ 30 d before recruitment) and gave their informed written consent enrolled at external visits of the Carlos J. Finlay Hospital (Havana, Cuba). The independent Ethics Committee of the Hospital approved the study protocol.

All participants underwent clinical history and full clinical examination. As part of healthy life style measures, we advised patients to start or continue on a low-sodium and low fat diet and strongly recommend stop smoking.

Eligible patients were randomized to policosanol (20 mg) + AS (policosanol/AS) or placebo + AS (placebo/AS) for 12 months and attended to control visits at 1.5, 3, 6, 9 and 12 months on treatment. Patients underwent general examination and neurological assessment at each visit, laboratory analyses at baseline and at 6 and 12 months on therapy, meanwhile we controlled treatment compliance and adverse experiences (AE) at each visit post randomization.

Study patients

Enrolled patients were ambulatory men and women over 35 years of age who had had an ischemic stroke (diagnosed by a neurologist) within the 30 d prior to enrolment.

The study protocol defined stroke as the occurrence of focal clinical signs of central nervous system dysfunction of vascular origin that lasted for at least 24 h. Ischemic stroke was confirmed through clinical assessment and computerized axial tomography performed within the following 48 h after stroke onset.

Patients were then eligible for randomization if they had a modified Rankin Scale score (mRSs)[24] of 2, 3 or 4. The exclusion criteria included suspected or confirmed haemorrhagic stroke, atrial fibrillation, other cardiac sources of embolism, subarachnoid haemorrhage, diastolic hypertension ≥ 110 mm Hg, cardiac valve diseases, history of myocardial infarction, instable angina or revacularisation surgery within the six months prior to the trial and previous consumption of policosanol.

Treatment

Patients consumed policosanol (20 mg)/AS or placebo/AS once daily with the breakfast for 12 months. Keeping in mind that randomised controlled trials support the use of daily doses of AS (75-150 mg) for the prevention of vascular events in high-risk patients we used 125 mg/d.[7-9]

Good treatment compliance, assessed through counts of remainder tablets and patient's interviews, was to consume at least 85 % of the scheduled tablets per period. Antiplatelet or lipid-lowering drugs were not allowed to use during the study.

Study outcomes

The primary outcome of this study was functional outcome measured by the mRSs, which assesses the outcome with scores that range from 0 to 6 (0 no symptoms; 1 no relevant disability despite symptoms, able to conduct all usual activities; 2 slight disability, unable to carry out all previous activities but able to conduct self-assistance; 3 moderate disability requiring some help, but able to walk without assistance; 4 moderate severe disability, unable to walk without assistance, and unable to attend body needs without assistance; 5 serious disability; bedridden, incontinent, and requiring constant care and attention; and 6 death).[25, 26]

We assumed to obtain a higher rate of cases with a favourable stroke outcome (mRSs ≤1) than in the placebo/AS group. In addition, reduction of mean mRSs with poli/AS should be greater than with placebo/AS.

Significant reduction of low-density lipoprotein-cholesterol (LDL-C), total cholesterol and significant increases of high-density lipoprotein-cholesterol (HDL-C) levels were secondary outcomes.

Lipid profile and blood safety indicators

Venous blood samples were taken following a fasting of 12 h. Plasma was separated from red blood cells by centrifugation at 4°C and 2000 x *g* for 10 min, and aliquots were immediately taken. Laboratory analyses were performed within the next eight hours after blood drawing.

Serum levels of total cholesterol, triglycerides, HDL-C and blood biochemistry indicators (alanine amino transferase - ALT, aspartate amino transferase-AST, glucose, creatinine) were determined using reagent kits (Roche, Basel, Switzerland) in a Hitachi 719 autoanalyzer (Tokyo, Japan) of the clinical laboratory of the Medical Surgical Research Centre. LDL-C values were calculated by using the Friedewald equation.[27]

Safety and tolerability assessment

Safety and tolerability indicators included laboratory and physical examination data (weight, pulse rate, diastolic and systolic arterial pressure), and AE reports. Study protocol defined an AE as any undesirable experience, absent at hospital discharge or worsened thereafter, happening in a patient, independently if it could be or not related with the therapy. AE were classified as mild, moderate or serious according to their intensity. Mild AE should not require stopping of study medications or specific treatment of the AE, moderate AE should require the withdrawal of study medications and/or treatment of the AE, while serious AE (SAE) should lead to patient hospitalization and/or to death.[28]

Statistical Analysis

We analysed the data on an intention-to-treat basis, including those of all patients who underwent randomization. A sample size of 40 patients (20 subjects/treatment group), assuming a 10 % of premature withdrawals, approximately 45 patients should be enrolled.

Continuous values were compared with the t test for paired (within group comparisons) and independent (between group comparisons) samples, and the Bonferroni´s test was used to adjust significances from repeat comparisons.[29] Categorical data were compared with the Fisher Exact probability test. All p values were two-sided. A value of $\alpha = 0.05$ was assumed for statistical significance. Comparisons were done with the Statistics software for Windows (USA).

RESULTS

Population characteristics

Of enrolled patients, 38 (mean age: 66 years) (18 men, 20 women) were eligible for randomization and 23 completed the study. Fifteen patients (8 from placebo/AS, 7 from policosanol/AS) discontinued prematurely the trial. Five withdrawals three from the placebo/AS group and two from policosanol/AS group, were serious adverse events (4 recurrent stroke, 1 intestinal oclusion-surgery). Other withdrawals were not AE-related: Ten patients (5 placebo/AS and 5 policosanol/AS) discontinued because of protocols violations, change of localization and unwillingness to follow-up.

Baseline characteristics were well balanced in the two groups (Table 1). The most frequent (≥ 20 %) risk factors at baseline were hypertension (78.9 %), family history of ictus (50.0 %), hypercholesterolemia (31.6 %), smoking (28.9 %), diabetes mellitus (23.7 %) and coronary disease (21.1 %). Concomitant therapy was also well matched in both groups, the most frequent being the angiotensin converting enzyme inhibitors (ACEI) (31.6 %), diuretics (28.9 %), β-blockers (15.8 %) and oral hypoglycemic agents (15.8 %).

Table 1. Baseline characteristics of study population

Characteristics	**Poli/AS (n = 19)**	**Placebo/AS (n = 19)**	**Total (n = 38)**
Age (years) (X ± SD)	64 ± 11	68 ± 9	66 ± 10
Body mass index (kg/m^2) (X ± SD)	25.8 ± 3.1	25.1 ± 3.6	25.4 ± 3.3
Women n (%)	9 (47.4 %)	11 (57.9 %)	20 (52.6 %)
Men n (%)	10 (52.6 %)	8 (42.1 %)	18 (47.4 %)
Hypertension n (%)	15 (78.9 %)	15 (78.9 %)	30 (78.9 %)
Family history of ictus	9 (47.4 %)	10 (52.6 %)	19 (50.0 %)
Hypecholesterolemia n (%)	6 (31.6 %)	6 (31.6 %)	12 (31.6 %)
Smoking n (%)	7 (36.8 %)	4 (21.1 %)	11 (28.9 %)
Diabetes mellitus n (%)	6 (31.6 %)	3 (15.8 %)	9 (23.7 %)
Coronary artery disease n (%)	3 (15.8 %)	5 (26.3 %)	8 (21.1 %)
Alcoholism n (%)	5 (26.3 %)	3 (15.8 %)	8 (21.1 %)
Concomitant therapy (≥ 5 %)			
At least 1 concomitant therapy n (%)	15 (78.9 %)	15 (78.9 %)	30 (78.9 %)
ACEI	6 (31.6 %)	8 (42.1 %)	14 (31.6 %)
Diuretics	4 (21.1 %)	7 (36.8 %)	11 (26.9 %)
β-blockers	2 (11.1 %)	4 (21.1 %)	6 (15.8 %)
Oral hypoglycemic drugs	4 (21.1 %)	2 (11.1 %)	6 (15.8 %)

(X ± SD) mean ± standard deviation. Poli policosanol (20 mg/d), AS aspirin (125 mg/d), mRSs Modified Ranking Scale score, ACEI angiotensing converting enzyme inhibitors
All comparisons were not significant

Effects on stroke functional outcomes

During the study drug compliance was 90 % and similar in both groups. Table 2 lists the effects on functional stroke scales. Treatment with policosanol/AS decreased mean mRSs significantly from the first interim check-up ($p < 0.000$ 1 vs placebo/AS). The treatment effect did not wear off, even improved, after long-term therapy versus placebo/AS) when the net decrease versus placebo/AS was 88.4 %.

Table 2. Effects on the neurological recovery assessed through the functional stroke scale

Treatment	**Baseline**	**1.5 months**	**3 months**	**6 months**	**9 months**	**12 months**	**Changes % vs baseline**
			Modified Rankin Scale score (mRSs) (X ± SD)				
Placebo/AS	1.84 ± 1.30	1.58 ± 1.22	1.53 ± 1.26	1.37 ± 1.21	1.37 ± 1.21	1.37 ± 1.21	-25.5
Policosanol/AS	1.79 ± 1.08	1.21 ± 1.03*	1.05 ± 1.13*	0.95 ± 0.97^{*+}	0.95± 0.97^{*+}	0.95 ± 0.97^{*+}	-88.4^{+}

(X ± SD) mean ± standard deviation, AS aspirin
$^{*}p < 0.01$ Comparisons with baseline (Wilcoxon test for matched samples, Bonferroni adjustment)
$^{+}p < 0.05$ Comparisons with placebo (Man Whitney U Test)

Effects on lipid profile

All lipid variables were similar in both groups at randomization. No significant changes occurred in the placebo/AS group. Policosanol/AS decreased persistently and significantly LDL-C, final reduction was 29.3 % ($p < 0.05$ vs baseline, $p < 0.05$ vs placebo/AS), and the same happened with total cholesterol (final decrease of 14.9 %. In turn, the treatment significantly increased HDL-C by 13.1 % (Table 3). Policosanol/AS failed to modify triglycerides.

Table 3. Effects on lipid profile (X ± SD)

Treatment	Baseline	12 months	Changes (%)
	LDL-C (mmol/L)		
Placebo/AS	2.93 ± 0.84	3.67 ± 1.09	+25.2
Policosanol/AS	3.45 ± 1.16	2.44 ± 0.93^{*+}	-29.3^{+}
	Total cholesterol (mmol/L)		
Placebo/AS	4.71 ± 0.92	5.47 ± 1.02	+16.1
Policosanol/AS	5.16 ± 1.35	4.39 ± 1.17^{*+}	-14.9^{+}
	HDL-C (mmol/L)		
Placebo/AS	1.05 ± 0.24	0.89 ± 0.20	-15.2
Policosanol/AS	0.99 ± 0.33	1.12 ± 0.44^{*+}	+13.1^{+}
	Triglycerides (mmol/L)		
Placebo/AS	1.96 ± 1.05	2.47 ± 0.57	+26.0
Policosanol/AS	2.01 ± 1.03	2.23 ± 2.21	+10.9

(X ± SD) mean ± standard deviation, AS aspirin LDL-C low-density lipoprotein-cholesterol, HDL-C high-density lipoprotein-cholesterol $^{*}p < 0.05$ Comparisons with baseline (Wilcoxon test for matched samples) $^{+}p < 0.05$ Comparison with placebo/AS (Mann Whitney U test)

Safety and tolerability

According to the effects on physical and blood safety indicators, treatments were safe and well tolerated (data not shown for simplicity). The treatment policosanol/AS did not modify physical or blood safety indicator versus placebo/AS.

Three patients from the placebo/AS group and two patients from policosanol/AS group reports serious adverse event (4 recurrent stroke, 1 intestinal oclusion-surgery). In addition, six patients (5 placebo/AS and 1 policosanol/AS) moderate or leve experienced (insomnia, gastric discomfort, chest pain, behavioural troubles, asthenia).

DISCUSSION

The aim of the present study was to investigate long-term (12 months) administration of policosanol (20 mg/d) added to conventional AS therapy. The treatment was able to provide sustained and relevant benefits over placebo/AS on the functional outcome of patients who suffered a recent ischemic stroke of moderate severity. The long-term effects of policosanol/AS on ischemic stroke outcome, assessed throughout both the mRSs, were better than those of placebo/AS.

Study patients were randomized within 30 d of the onset of the ischemic stroke, so that the effects of policosanol/AS cannot be interpreted as effects on the acute stroke, but on the further recovery step. Following the recommendations for ischemic stroke management, all patients received AS early on their admission in stroke unit and followed on this thereafter.[7-9] Our study population was restricted to have 2 to 4 mRSs values for lowering the influence of variable stroke severity on the results. Study patients had not been received policosanol before being randomized, so that they were technically virgin to study treatment.

The strength of the study includes that it was randomized, double-blinded and placebo-controlled, with all patients receiving AS, first-line therapy recommended after non-cardioembolic ischemic stroke. Since both groups were homogeneous at baseline the effects here found can be attributable to policosanol/AS therapy. In particular, the mean mRSs were comparable in the two groups. Also, the fact that treatment compliance was very good (≥ 85 %) and comparable in both groups supports the validity of the present results.

Baseline characteristics of study patients match well with stroke epidemiological data. The mean age of patients, and the high frequency of concomitant morbidities were consistent with common stroke risk factors. In addition to AS, consumed by all patients, the most frequent concomitant drugs were ACEI, diuretic and β-blockers but such consumption, coherent with the prevalence of hypertension, was also similar in the two groups, so that we discard the potential influence of concomitant therapy to the present results.

We assessed the effects on stroke outcome by measuring the functional status and degree of functional dependence of the patients with the mRSs, scales used widely to assess post-stroke functional impairment and disability. In particular, mRSs is the clinical outcome tool most widely used for stroke recovery in clinical studies.[24-26]

The present results confirms that the addition of policosanol to conventional AS therapy after hospital discharge should help the neurological recovery post-ischemic stroke. This concept is supported by the proportion of policosanol/AS patiens who acchieved a good stroke outcome (mRSs≤1) at study completion and the mean reduction (88.4 % versus placebo/AS) of mRSs, the primary study outcome, as compared to placebo/AS. These results are consistent with the efficacy of policosanol/AS demonstrated in previous randomized, double-blind controlled studies in which the control group received placebo/AS.[18,19]

Also, keeping in mind the neurological improvement at 12 weeks after stroke in the NINDS rt-PA study (11-13 % reduction of mRSs) despite the patients were treated as soon as within the first hours of acute stroke, we should consider that the results achieved with policosanol/AS were clinically meaningful.[30]

In addition, policosanol/AS reduced significantly LDL-C (29.3 %) and total cholesterol (14.9 %), and increased HDL-C (13.1 %). Although some trials have failed to find lipid/lowering effects of other policosanol tablets, the lipid-modifying effects here seen are coherent with previous data in post-stroke patients,[18-23] and with the general lipid-lowering profile of policosanol.[31-36]

The mechanism(s) whereby policosanol may help to improve stroke recovery are beyond the objective of this study. Nevertheless, antiplatelet effects of policosanol[37-40] should be responsible, at least partly, of the benefits of policosanol/AS therapy on stroke outcomes over the conventional AS therapy. In such regard, a previous six months clinical study conducted in patients who had suffered ischemic stroke demonstrated that the antiplatelet efficacy of policosanol/AS was better than that of placebo/AS.[19] A recent study demonstrated that it inhibits cyclooxygenase 1 (COX-1) activity *in vitro*, which makes rationale that it may inhibit platelet aggregation.[41]

Also, cholesterol-lowering effects of policosanol (LDL-C and total cholesterol decrease, HDL-C increase) may contributed to the benefits of policosanol/AS on stroke outcomes since LDL-C reduction and HDL-C increase are linked to stroke recovery and prevention.[11,42] In this sense, other lipid lowering drugs such as statins, lower the stroke risk in different population subsets.[11,20] Greater reductions in stroke risk are associated with higher LDL-C decreases.[11] In a large meta-analysis, statin therapy at stroke onset was associated with improved outcome.[43]

Recent data have shown that pretreatment with statins, hypercholesterolemia or both in ischemic stroke patients could have neuroprotective effects with reduced neurological deficits at presentation, lower early death and dependency rate, thus increasing the chances for good outcome.[44]

Moreover, Policosanol (20 mg/d) and atorvastatin (20 mg/d), administered for 12 weeks within the next 30 d after stroke onset, were similarly effective for improving the functional outcome in patients with recent ischemic stroke, all treated with AS.[20]

The cholesterol-lowering activity of policosanol involves the inhibition of cholesterol synthesis by regulating HMG-CoA reductase through activation of AMP kinase,[45,46] the main regulatory kinase for HMG-CoA reductase. Policosanol treatment of hepatoma cells increased AMP-kinase phosphorylation, providing a clue by which it might down-regulate HMG-CoA reductase activity and decrease cholesterol synthesis without directly inhibiting the enzyme, AMP-kinase.[45] Further studies demonstrated that metabolic transformation of very long chain alcohols to fatty acids is needed for the suppression of cholesterol synthesis, presumably by increasing cellular AMP levels.[46] In turn, the mechanism(s) responsible of HDL-C elevation by policosanol have not been demonstrated. Recent studies have proven that policosanol enhances HDL functionality improving anti-glycation, anti-apoptosis, and cholesteryl ester transfer inhibition in vitro.[47] A study conducted in hyperlipidemic zebrafish demonstrated that policosanol supplementation displayed lipid-lowering and HDL-C-elevating effects associated with in vitro anti-apoptotic activities.[48]

In agreement with previous studies, policosanol/AS was safe and well tolerated. Nevertheless, this study present limitation that influence in the results: First, the number of patients included were insufficient according to proposed the protocol, because to difficulty with tomography equipment. Second, the number of withdrawals was relatively high, but the long-term studies in patients with ischemic stroke show the variable frequency of withdrawals (11.8 - 52.0 %),[49] and the frequency of SAE was high in this study (4 recurrent stroke and 1 intestinal oclusion-surgery) was acording the range established in long-term studies in patients with stroke (3.0 - 20.9 %).[50]

The conjugations of both limitations (number the patients insufficient and number of withdraw) reduce of clinical relevance to the present results. Others randomized, double-blind, controlled studies including larger sample size are required to confirm these results.

CONCLUSIONS

Long-term (12 months) administration of policosanol/AS given after suffering ischemic stroke were shown to be better than placebo/AS in improving functional outcomes at 3 and 12 months when used among patients with ischemic stroke of moderate severity. Further multicentric, randomized, double-blind, controlled studies including larger sample size are required to confirm these results.

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