NATURAL ANTI-PLATELET AGENTS

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Summary

Background: Antiplatelet agents are useful as antithrombotic drugs. **Aims:** To show that natural products could provide new alternatives for anti-platelet therapy. The analysis of the effects of the Policosanol, a by-product from sugar cane wax, in clinical assays is an example. **Methods:** The following experimental designs have been performed in order to assess the anti-platelet effect of: double blind randomized versus placebo clinical assay of Policosanol (1 mg/day x 12 weeks) in hypercholesterolemic patients. A cross-over randomized double blind versus placebo clinical assay of Policosanol (10 mg/day x 7 days) in type II diabetic patients was the second experimental design. A descriptive study on platelet reactivity of atherosclerotic patients consuming Policosanol, aspirin or both was performed too. The decrease of platelet aggregation in platelet-rich plasma induced by physiologic stimuli (ADP. collagen or epinephrine) was the end point of the pharmacological effect. **Results:** Platelet aggregation was significantly lower in hypercholesterolemic and diabetic patients treated with Policosanol than in those in the placebo groups. Furthermore, there were not statistical difference among the groups of atherosclerotic patients who were taking Policosanol, aspirin or both with respect to the percentages of responders to the treatments with ADP, collagen and epinephrine-induced platelet aggregation below the reference normal range, suggesting the similarities of Policosanol and aspirin with regard to the anti-platelet efficacy and mechanism of action. **Conclusion:** These results suggest that Policosanol may be a useful natural alternative for anti-platelet therapy.

Key words: Platelet Aggregation, Natural Product, Policosanol, Hypercholesterolemia, Atherosclerosis, Diabetes mellitus

Introduction

Anti-platelet therapy is recommended in high-risk of atherothrombotic diseases and in all subjects with personal history of acute atherothrombotic events (myocardial infarction, stroke, peripheral vascular death) (1). . However, the preventive efficacy of current anti-platelet drugs (aspirin, ticlopidine, dypiridamol and clopidogrel) is still low. Therefore, the identification of new anti-platelet agents is a current matter for research (2).

Chemical compounds bearing acetyl, imidazol, pirazolidine, and hydroxyl groups are potentially active as inhibitors of platelet aggregation. In fact, the anti-platelet actions of plant metabolites, which carry such chemical groups, have been demonstrated (3). Therefore, plants are potential natural sources for anti-platelet drugs.

Policosanol is a mix of high molecular weight alcohols, mainly octacosanol, which is obtained from sugar cane (*Sacharum officinarum*, L.) waxes. Policosanol lipid-lowering action has been reported (4-7). Its chemical composition brought about the hypothesis that it could be a natural inhibitor of platelet aggregation. In fact, Policosanol anti-platelet potential has been demonstrated in experimental animals (8-12). Furthermore, Policosanol 180 and 360 mg/kg were as effective as aspirin 300 mg/kg to prevent the mortality of mice induced by an intravenous injection of collagen (unpublished results).

Clinical assays on healthy volunteers have provided promising evidences (13-17). This effect has been demonstrated also in patients with risk factors for atherothrombosis (17-21).

This work is aimed to summarize our results on the effect of Policosanol on platelet aggregation in patients with high-risk for atherothrombosis.

Patients and Methods

The following experimental designs were carry out. The Ethics Committee of the National Institute of Angiology and Vascular Surgery approved these protocols. All patients gave their informed consent to participate in the assays.

Effect of Policosanol (1 mg/day) on platelet aggregation in patients with type II hyperlipoproteinemia.

A randomized double blind versus placebo study of Policosanol versus placebo was done. Forty-five patients suffering from Type II hyperlipoproteinemia (29 female and 20 male, age 40 to 55 years) were included in this study. Serum concentrations of total cholesterol and LDL- cholesterol higher than 5.7 and 3.37 mmol/L respectively were included in the study.

People under anti-platelet therapy were not included in the study. The treatments consisted of one tablet containing placebo or Policosanol (1 mg) / day, during twelve weeks. Counting the tablets in the bottles controlled adhesion to the treatments. Platelet aggregation was determined before and after the treatments.

Effect of Policosanol on platelet aggregation in diabetic patients.
A randomized crossed over double blind assay of Policosanol versus placebo was performed. Seventeen patients (9 female and 8 male, age 59 to 63 years) with type II Diabetes mellitus were included in this assay. Those under anti-platelet regimens were not included in the study. Patients were advised not to take any drug with known anti-platelet effects during the assay. Patients consumed one tablet containing placebo or Policosanol (5 mg) twice a day, during seven days, followed by a four-week washout period, Afterwards, they took the opposite treatment, during a seven-day period. Therefore, some of them consumed Policosanol during the first stage of treatment and the others during the second one. Counting the tablets in the bottles controlled the adhesion to the treatments. Platelet aggregation was measured before and after each period of treatment

Responses of atherosclerotic patients to the anti-platelet effects of Policosanol, aspirin or both drugs combined.
A descriptive study on platelet reactivity to physiologic stimuli in patients with high risk of atherothrombotic acute events was performed. This study was aimed to assess the responses of an open unselected population of patients to the anti-platelet effect of Policosanol and to compare them with the responses to aspirin and Policosanol plus aspirin. The study included 129 patients with any of the risk factors for athrombosis, who attended the Service on Hypercoagulability of the National Institute of Angiology between November 2000 and October 2003. The patients had systematically consumed Policosanol, aspirin or both combined every day during at least one month before the evaluation of platelet reactivity against three physiological stimuli.

Subjects were considered responders to the anti-platelet therapy when platelet aggregation was lower than 50 %, taking in account that this is the lowest value of our laboratory normal reference ranges for ADP, collagen and epinephrine-induced platelet aggregation in platelet-rich plasma.

Blood Collection
Blood samples were obtained from an antecubital vein through disposable plastic syringes, avoiding stasis and after 12 h fasting. Blood was anticoagulated with 0.1 volume of sodium citrate 3.8 % and centrifuged at 150 x g during 5 min to obtain platelet-rich plasma (PRP). The remainder was centrifuged at 1000-x g to obtain platelet-poor plasma (PPP). Platelet counts in PRP was adjusted to 200 –250 x 10^9/L with autologous PPP.

Assessment of platelet aggregation

Platelet aggregation in PRP (22) was measured in an Elvi 840 aggregometer. Two hundred microliters of PRP were added to an aggregometer cuvette and allowed to equilibrate at 37 ^{o} C for 2 min before adding 20 µL of ADP, collagen or Epinephrine (Sigma Chem Co.) 3,2 µmol/L, 3,0 µg/mL y 10^{-5} mol/L respectively. The percentage of platelet aggregation was determined as the Increase of light transmission through the PRP.

Dalmer Laboratory, Havana City, Cuba, supplied tablets containing placebo or Policosanol.

Statistical Analysis

Changes within groups were statistically analyzed by the Wilcoxon′ s test for paired data. Comparisons between groups were performed by ANOVA.

Results

Effect of Policosanol on platelet aggregation in patients with type II hyperlipoproteinemia

There was a statistically significant decrease of ADP-induced platelet aggregation in Policosanol but not in placebo group (Table1).

Table 1. Platelet aggregation in patients with type II hyperlipoproteinemia, before and after the treatments with Policosanol or Placebo.

		ADP-induced platelet aggregation (%)	
GROUP	**N**	**BEFORE**	**AFTER**
POLICOSANOL	23	51. ± 4.6	33.0 ± 2.6 *
PLACEBO	22	42.9 ± 3.8	62.1 ± 4.2 NS

The data are the mean ± s.e.m. ; * $p < 0,05$ by the Wilcoxon′ s test.

Effect of Policosanol on platelet aggregation in patients with type II Diabetes mellitus

ADP-induced platelet aggregation was significantly reduced after the ingestion of Policosanol, but not after placebo intake. However collagen-induced platelet aggregation was not modified by the treatments (Table 2).

Table 2. Platelet aggregation in patients with type II Diabetes mellitus before and after the treatments with Policosanol or Placebo.

	ADP-induced platelet aggregation (%)	
GROUP	**BEFORE**	**AFTER**
POLICOSANOL	58.56 ± 5.21	19.62 ± 5.83 **
PLACEBO	47.54 ± 4.46	48.89 ±5.55 NS
	Collagen-induced platelet aggregation (%)	
GROUP	**BEFORE**	**AFTER**
Policosanol	41.12 ± 5.20	45.49 ± 3.55 NS
Placebo	45.05 ± 7.55	42.20 ± 5.35 NS

The data are the mean ± s.e.m.; ** $p < 0.01$ Wilcoxon′s test, n = 17

Responses of atherosclerotic patients to the anti-platelet effects of Policosanol, aspirin or both drugs combined.

Sixteen patients were taking Policosanol (20 mg/day), 66 were under treatment with aspirin (250 mg/day) and 47 were consuming Policosanol (20 mg/day) plus aspirin (250 mg/day).

Table 3 shows that there were not differences between the three groups of treatment with respect to the percentages of responders. This result was obtained with all the platelet stimulation stimuli used.

Table 3 Percentages of responders to the anti-platelet effects of Policosanol, aspirin or both drugs combined.

		PLATELET AGGREGATION STIMULUS		
GROUP	**N**	**ADP**	**Collagen**	**Epinephrine**
ASPIRINA	66	29.6	61.1	46.7
POLICOSANOL	16	30.8	53.8	41.7
POLICOSANOL AND ASPIRIN	47	25.6	55.9	53.6

There were not statistical significant differences between the groups of treatments, within each platelet aggregation stimulus column after the statistical analysis by ANOVA test for $p < 0.05$.

Discussion

Hypercholesterolemia, Diabetes mellitus and history of acute atherothrombotic events are risk factors for atherothrombosis. Anti-platelet agents are recommended to patients with high-risk Policosanol inhibited platelet aggregation.

Policosanol inhibited platelet aggregation in patients with high-risk for atherothrombosis. The study on hypercholesterolemic patients showed that a dose as low as 1 mg/day was able to provoke a decrease of platelet reactivity to ADP.

The evidence on the inhibition of platelet aggregation in diabetic patients support the possible utility of this drug to decrease the platelet hyperreactivity associated to Diabetes mellitus. The effects of this natural drug on two different platelet activation pathways (23-24) were assessed in this assay. ADP but not collagen-induced aggregation was affected, thus, suggesting that Policosanol selectively inhibits ADP-induced platelet activation pathway and that, perhaps, higher doses of the drug would be needed to inhibit collagen-induced platelet aggregation.

This study have provided the first evidences of Policosanol ability to reach the therapeutic goal of the anti-platelet therapy (to keep platelet aggregation below 50 %) and its similarities with aspirin with regard to the percentages of responders in an open unselected population.

Collagen-induced aggregation was the most susceptible platelet stimulus to the actions of Policosanol, suggesting that the impairment of platelet thromboxane A2 production is involved in the Policosanol mechanism of anti-platelet action, such as have been proposed (11,16).

The synergism between Policosanol and aspirin (8, 15) could not be demonstrated in this assay, since the lack of information about the individual responses of patients to each drug before taking them combined. Nevertheless, the hypothesis that such a synergism could have functioned in some patients has not been rejected.

General considerations

These studies have demonstrated that:

1- Patients with different risk factors for atherothrombosis are susceptible to the anti-platelet action of Policosanol.
2- This effect may be obtained with a range of doses from 1 to 20 mg/day, though 20 mg/day is the dose most frequently used in the clinical practice.
3- The daily intake of Policosanol may achieve the goal of keeping platelet reactivity below the reference normal range in patients with high risk for atherothrombosis.
4- There are Inter-individual differences between patients with respect to the responses to anti-platelet effect of Policosanol, aspirin and both combined. This

and previous findings (25) suggest that the effect of anti-platelet agents on platelet reactivity should be monitored.

5- Physicians should be aware of the anti-platelet action of Policosanol when they are going to prescribe this drug to subjects with congenital or acquired hypocoagulability.

Conclusion

These results suggest that Policosanol may be a useful natural alternative for anti-platelet therapy.

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