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Original Article

Phosphoethanolamine and omega-3 in patients with asthma

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Abstract: The effect of omega-3 (n-3) in asthma has been inconclusive. One explanation for it may be the low incorporation of these fatty acids in clinical studies. Phosphoethanolamine (PEtn) can increase the synthesis of phosphatidylethanolamine, which can, in turn, increase the incorporation of n-3 in cell membranes. The aim of this study is to evaluate the effect of synthetic PEtn in patients with asthma who are receiving n-3. This randomized, double-blind, placebo-controlled study was carried out over a two month period by using spirometry, the Asthma Control Test questionnaire (ACT) and medicine intake. Forty-one patients with asthma were studied. Twenty-one patients received n-3 daily (1.080 mg of EPA, 720 mg of DHA) and 800 mg of PEtn (PEtn group), and twenty patients received the same doses of n-3 and placebo (control group). All patients continued receiving their conventional treatment for asthma. The hospital ethics committee approved the study. Five patients of each group required systemic corticosteroids, being the total consumption, smaller in the PEtn group (127.4 mg of prednisone/patient versus 416.0 mg of prednisone/patient in the control group, *p*-value = 0.0269). There were no significant differences in the changing of ATC and FEV1, as well as in the intake of formoterol or budesonide between the groups. In this study, patients who received phosphoethanolamine and omega-3 needed a smaller dose of systemic corticosteroid for asthma control than patients who only received omega-3. However, as the trial was conducted on a small scale, more studies are necessary.

Keywords: N-3, omega-3, phosphoethanolamine, phosphatidylethanolamine, asthma

Introduction

Although epidemiological and experimental studies have demonstrated an anti-inflammatory effect of omega-3 (n-3), clinical studies in asthma have presented inconsistent results. One of the possible explanations for these discordant findings may be the low incorporation of n-3 into the cell membrane in clinical studies, because in clinical studies, the doses of n-3 are shorter or smaller than in epidemiological and experimental studies [1-14].

Omega-3 is incorporated into cell membranes by phospholipids. Among phospholipids, phosphatidylethanolamine (PtdEtn) exerts a fundamental role in the n-3 incorporation, because, besides having n-3 in its molecule, PtdEtn is the substrate for the synthesis of other phospholipids with long polyunsaturated fatty acids, such as phosphatidylcholine (PtdCho) with long-polyunsatureted fatty acid. While arachidonic acid (AA) is predominantly incorporated in PtdCho, long-chain n-3 is predominantly incorporated in PtdEtn, mostly 22:6 docosahexaenoic acid (DHA). In the FtdEth synthesis, there is selectivity for DHA, and it has been attributed to the specificity of the enzyme that binds the phosphoethanolamine (PEtn) to the diacylglycerol. This enzyme, CDP-ethanolamine: 1,2-diacylglycerolethanolamine-phosphotransferase, shows a specificity for 16:0-22:6-diacylglycerol [15-20].

It is important to be highlighted that the synthesis of FtdEtn has a limiting step, that is, the phosphorylation of ethanolamine to form PEtn, which is the phosphate radical of this phospho-

Table 1. Baseline Characteristics of the Patients

	Total (N = 41)	PEtn Group (N = 21)	Control Group (N = 20)
Age (y)-mean (SD)	48.5 (11.1)	48.6 (9.7)	48.5 (12.4)
Female sex-n (%)	33 (80.4)	18 (85.7)	15 (75.0)
Smoking-n	0	0	0
Atopic symptoms-n (%)	31 (75.6)	16 (71.1)	15 (75)
ACT-mean (SD)	15.4 (4.5)	15.6 (4.4)	15.2 (4.8)
FEV1 (l)-mean (SD)	1.79 (0.60)	1.78 (0.73)	1.81 (0.43)
% Predicted-mean (SD)	68.5 (17.6)	68.9 (19.9)	68.1 (15.4)
Formoterol dose (mcg/day)-mean (SD)	20.6 (9.8)	21.4 (8.6)	19.8 (11.1)
Budesonide dose (mcg/day)-mean (SD)	829.2 (353.7)	761.9 (352.2)	900.0 (364.1)

Definition of abbreviations: ATC = Asthma Control Test; FEV1 = forced expiratory volume in the first second.

lipid. This limiting step of FtdEtn synthesis may be overcome by the supplying of ready-made PEtn [21, 22].

As the providing of ready-made PEtn can, theoretically, improve the synthesis of FtdEtn, and as this phospholipid synthesis is selective for n-3, mainly DHA, our hypothesis is that providing synthetic PEtn and n-3, it is possible to improve the incorporation of n-3 in cell membranes, improving inflammatory processes such as asthma. This study aimed to evaluate the clinical effects of PEtn in patients with asthma who are being supplemented with n-3.

Method

Study oversight

Study Design was conducted at School of Medical Sciences, of the State University of Campinas. The protocol of the study was approved by the human ethics committees of the State University of Campinas (IRB 00002737), and all participants gave written informed consent. The study was conducted in accordance with the International Conference on Harmonization guidelines for Good Clinical Practice and the principles of the Declaration of Helsinki.

The capsules of synthetic PEtn 400 mg (2-aminoethyl dihydrogen phosphate, in solid form, with calcium, magnesium and zinc) were produced by the Institute of Chemistry, University of São Paulo, São Carlos, Brazil. These capsules were donated to study, as well as the omega-3,500 mg capsules.

All the authors were involved in each stage of the manuscript development, made the decision to submit the manuscript for publication, and take responsibility for the accuracy and completeness of the data and analyses.

Patients

This study was composed of asthmatic patients assisted at Hospital das Clinicas of State University of Campinas, in the city of Campinas, Brazil. Eligible participants were 18 years of age or older, who had not received systemic corticosteroid and who had not the dose of inhaled corticosteroid changed during the four weeks before the beginning of the study.

Study procedures

A total of 41 eligible patients was randomly assigned in a 1:1:1 ratio to receive either 12 omega-3 500 mg capsules (equivalent to 1.080 mg of eicosapentaenoic acid-EPA, 720 mg of DHA) plus two synthetic PEtn 400 mg capsules per day (PEtn group), or to receive the same dose of omega-3 plus two amide capsules per day (control group). Patients and clinicians were blind to treatment allocation. These treatments lasted two months, and all patients continued to receive conventional treatment prescribed by physicians of the ambulatory department.

Monthly visits were performed. Consumption of medicines for asthma treatment was registered. The questionnaire on quality of life "Asthma Control Test" (ACT), with the Brazilian Portuguese version, was carried [23, 24]. Spirometries were performed before and after treatment, according to American Thoracic Society criteria [25]. It was used the MasterScope spirometer, Jaeger, by Viasys Healthcare (Germany).

Table 2. Variation of ATC, FEV1 and inhaled medications in PEtn and Control groups

	FEtn Group	Control Group
ATC-D0, mean (SD)	15.6 (4.4)	15.25 (4.8)
ATC-D60, mean (SD)	20.7 (3.9)	17.8 (6.1)
FEV1 (l)-D0, mean (SD)	1.78 (0.73)	1.81±0.43
% Predicted-mean (SD)	68.9 (19.9)	68.1 (15.4)
FEV1 (l)-D60, mean (SD)	1.94±0.83	1.86±0.55
% Predicted-mean (SD)	74.0 (20.7)	69.7 (15.3)
Formoterol (mcg/day)-D0, mean (SD)	21.4 (8.6)	19.8 (11.1)
Formoterol (mcg/day)-D60, mean (SD)	23.7 (4.8)	19.8 (8.9)
Budesonide (mcg/day)-D0, mean (SD)	761.9 (352.2)	900.0 (364.1)
Budesonide (mcg/day)-D60, mean (SD)	819.0 (244.1)	820.0 (303.6)

Definition of abbreviations: ATC-D0 = Asthma Control Test at beginning of the study; ATC-D60 = Asthma Control Test at the end of the study; FEV1-D0 = forced expiratory volume in the first second, at beginning of the study; FEV1-D60 = forced expiratory volume in the first second, at the end of the study.

Study outcomes

The primary study outcome was the changing in spirometric values. Secondary outcomes were changing in ACT, changing in the consumption of medicine for asthma treatment and adverse effects of the treatment.

Statistical analysis

Linear mixed models were fitted to the data, including the effects of group (PEtn and control), time point, and a random effect by patient, as the two observations of the same patient are correlated. The following response variables were analyzed: ACT score, forced expiratory volume in the first second (FEV1), formoterol and budesonide intake. An analysis of variance model was also fitted to the total of prednisone taken by patients who needed systemic corticosteroids during the study [26-28].

Results

Forty-one patients were included. The data of the groups of patients are shown in **Table 1**. Patients from the PEtn group presented a larger increase in FEV1 during the study (from 1.79±0.74 liters to 1.94±0.84 liters versus from 1.81±0.43 liters to 1.86±0.55 liters in control group), but the difference between the groups was not statistically significant (P = 0.1628).

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ACT score presented an increase in both groups, being bigger in the PEtn group (from 15.62± 4.43 to 20.71±3.99 versus from 15.25±4.85 to 17.85±6.17 in the control group), but the difference was not statistically significant between the groups (P = 0.2068. **Table 2**).

All patients continued using inhaled formoterol and budesonide, as prescribed by their physicians. The consumption of these medicines did not present statistically significant differences during the study, neither for formoterol (P = 0.5297) nor budesonide (P = 0.2961. **Table 2**).

Ten patients needed systemic corticosteroid for controlling asthma, five patients in each group. In this subgroup of patients, the consumption of systemic corticosteroid was significantly smaller in PEtn subgroup (127.40± 42.82 mg of prednisone/patient versus 416.00±234.79 mg of prednisone/patient in control subgroup (P = 0.0269. **Table 3**).

The prevalent side effect was gastric intolerance: four patients of each group, being one patient had improvement of stomachache after stopping alendronate intake. Two patients of each group had weight alteration. Three patients in the placebo group had, respectively, headache, pneumonia, and hair loss (**Table 4**).

Discussion

One possible explanation for disappointing results in clinical studies with n-3 in asthma may be the low incorporation of n-3 into the cell membrane. Our hypothesis is that providing PEtn together n-3 might, it could be expected an improvement in n-3 incorporation, with a consequent improvement in the inflammatory process of asthma.

Our outcomes showed a significant effect only in a subgroup of patients, those need systemic

Table 3. Characteristics of patients who need systemic corticosteroids during the study

	Total	PEtn Subgroup	Control Subgroup
Total No.	10	5	5
Age (y), mean (SD)	46.8 (10.3)	48.6 (12.2)	45 (9.0)
Male: female (% male)	2:8 (20)	5:0 (0)	2:3 (25)
Smoking, n	0	0	0
Atopic symptoms, n (%)	8 (80)	4 (80)	4 (80)
ACT-D0, mean (SD)	14.5 (5.7)	14.8 (4.4)	14.2 (7.2)
ACT-D60, mean (SD)	16.2 (6.6)	18.4 (4.8)	14.0 (7.9)
FEV1-D0, (l), mean (SD)	1.68 (0.47)	1.43 (0.53)	1.92 (0.23)
% Predicted, mean (SD)	65.2 (21.9)	62.5 (26.8)	67.9 (18.5)
FEV1-D60, (l), mean (SD)	1.69 0.60)	1.50 (0.27)	1.89 (0.80)
% Predicted, mean (SD)	64.5 (18.7)	65.9 (21.2)	63.2 (18.3)
Formoterol (mcg/day)-D0, mean (SD)	21.0 (11.7)	20.4 (11.6)	21.6 (13.1)
Formoterol (mcg/day)-D60, mean (SD)	28.2 (8.0)	25.2 (2.6)	31.2 (10.7)
Budesonide (mcg/day)-D0, mean (SD)	740 (377)	680 (268)	680 (268)
Budesonide (mcg/day)-D60, mean (SD)	860 (353)	800 (489)	1.040 (357)
Total dose of prednisone (mg/patient), mean (SD)	272.7 (221.3)	127.4 (42.8)	416.0 (234.7)

Definition of abbreviations: ATC-D0 = Asthma Control Test at beginning of the study; ATC-D60 = Asthma Control Test at the end of the study; FEV1-D0 = forced expiratory volume in the first second, at beginning of the study; FEV1-D60 = forced expiratory volume in the first second, at the end of the study.

Table 4. Adverse effects

	PEtn Group (N = 21)	Control Group (N = 20)
Stomachache, n (%)	1 (4.7)	1 (5)
Heartburn, n (%)	1 (4.7)	1 (5)
Náusea, n (%)	2 (9.5)	2 (10)
Constipation, n (%)	0	1 (5)
Diarrhea, n (%)	0	1 (5)
Weight loss, n (%)	2 (9.5)	1 (5)
Weight gain, n (%)	0	1 (5)
Headache, n (%)	0	1 (5)
Pneumonia, n (%)	0	1 (5)
Hair loss, n (%)	0	1 (5)

corticosteroids for the asthma control. This subgroup probably consists of more severe patients. The effect seen in this subgroup is due to two hypotheses: the symptoms of asthma are intense and variable in this subgroup, affecting more the systemic corticosteroids prescription. Another hypothesis is the treatment, in fact, might have a visible effect only in more severe asthma. This finding suggests the subgroup that received n-3 and PEtn might have a less intensive inflammation in the airways than subgroup that received only n-3 and placebo.

There are some limitations in this study such as the small number of patients and the lack of a more precise methodology to assess the asthma inflammation. This study could be improved with the measurement of specific inflammatory biomarkers for asthma [29]. Furthermore, n-3 incorporation was not studied. This incorporation could be evaluated by specific methods, such as the measurement of plasmatic EPA and DHA for acute changes in intake, and the measurement of platelet and mononuclear cell EPA and DHA, which relate to long-term intake of n-3 [30].

In conclusion, in this study, patients who received phosphoethanolamine and omega-3 needed a smaller dose of systemic corticosteroid for asthma control than patients who only received omega-3. However, as the trial was conducted on a small scale, more studies are necessary to consider this combination as a possible modality of asthma treatment.

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Disclosure of conflict of interest

We have got a patent: “Combi-preparation of phosphoethanolamine and omega-3-fatty acid for treating bronchial asthma and COPD”.

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