Diamine oxidase (DAO) supplement reduces headache in episodic migraine patients with DAO deficiency: a randomized double-blind trial.

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Received 23 March 2017 Accepted 4 January 2018

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This study is listed on the **ISRCTN registry** with trial ID ISRCTN10091019.

Abbreviations:

DAO: Diamine oxidase

IHS: International Headache Society

IQR: interquartile range

HDU: Histamine Degrading Units

NPRS: Numeric Pain Rating Scale

ABSTRACT

Background & Aims: Histamine intolerance is a disorder in the homeostasis of histamine due to a reduced intestinal degradation of this amine, mainly caused by a deficiency in the enzyme diamine oxidase (DAO). Among histamine related symptoms, headache is one of the most recorded. Current clinical strategies for the treatment of the symptomatology related to this disorder are based on the exclusion of foods with histamine or other bioactive amines and/or exogenous DAO supplementation. The aim of this study was to assess the efficacy of a food supplement consisting of DAO enzyme as a preventive treatment of migraine in patients with DAO deficiency through a randomized double-blind trial.

Methods: 100 patients with confirmed episodic migraine according to current International Headache Society (IHS) criteria and DAO deficiency (levels below 80 HDU/ml) were randomized in two groups. One group received DAO enzyme supplementation and the other received placebo for one month. Clinical outcomes assessed were duration and number of attacks, perception of pain intensity and adverse effects during treatment. The use of triptans was also recorded.

Results: Great variability was found in the duration of migraine attacks reported by placebo and DAO groups. A significant reduction (p=0.0217) in hours of pain was achieved in patients treated with DAO supplement, with mean durations of 6.14 (±3.06) and 4.76 (±2.68) hours before and after treatment, respectively. A smaller reduction without statistical signification was also observed for this outcome in the placebo group, from 7.53 (±4.24) to 6.68 (±4.42) hours. Only in DAO group, a decrease in the percentage of patients taking triptans was observed. The number of attacks and the scores of pain intensity showed a similar reduction in both groups. No adverse effects were registered in patients treated with DAO enzyme.

Conclusions: Migranous patients supplemented with DAO enzyme during one month significantly reduced the duration of their migraine attacks by 1.4 hours. No statistically significant reduction was found in placebo group before and after treatment. The reduction of pain hours observed in placebo group (0.9 hours) could explain the lack of significant differences between both study groups. One month of DAO supplementation has demonstrated a positive trend in the improvement of migraine but more studies with a longer treatment period are needed to better assess the efficacy of DAO supplementation.

Clinical trial registration number ISRCTN10091019; www.isrctn.org.

Keywords: Diamine oxidase (DAO); DAO supplementation; Histamine; Histamine intolerance; Migraine**.**

1. INTRODUCTION

Histamine is a bioactive amine with essential physiological activities, which can also be found in some common foods in a wide range of concentrations (1,2). Histamine from diet is principally metabolized in the digestive tract by diamine oxidase (DAO), regulating its presence in the systemic circulation (3). DAO deficiency could be one of the main causes of histamine intolerance, an alteration in the homeostasis of histamine, which results in a reduced intestinal degradation, and its subsequent increase in plasma (3,4). DAO deficiency may be congenital; resulting from genetic mutations in DAO gene (chromosome 7q36) that code for an altered protein with low enzymatic activity (5-7), or acquired by certain pathologies that limit DAO secretion, especially in inflammatory or degenerative intestinal disorders (8-10), or by enzymatic blockade by some commonly used drugs (4,5). Multifaceted clinical symptoms associated with histamine intolerance include headaches, skin reactions such as urticaria and pruritus, gastrointestinal disorders as flatulence, diarrhea, nausea and abdominal pain, sneezing, rhinorrhea, arrhythmias, hypotension and muscle aches (4,11). Headache is one of the symptoms most frequently related to histamine intolerance. Reduced DAO activity has been described by several clinical studies in patients diagnosed with some pathology such as atopic eczema, chronic urticaria, chronic abdominal pain or inflammatory bowel diseases (9,10,12-15). A recent study also reported a high prevalence (87%) of DAO deficiency in a group of 137 patients diagnosed with migraine (16).

Migraine is a neurological and disabling pathology with a multifactorial etiology that can negatively impact both family and work activities. Its prevalence in Spanish population has been estimated to be 12% (17). Physiopathological mechanisms supporting the onset of migraine are complex, but several pathways have been described to explain the association

between histamine and headache. In the nervous system, certain neurons synthesize histamine in the posterior-basal hypothalamic nuclei, an area recently postulated as the locus of diverse primary headaches due to increased activity detected during the prodromal phases of migraine attacks (18). Although it was first thought that histamine did not cross the blood-brain barrier, it seems that it may stimulate hypothalamic activity through the circumventricular organs, which lack this barrier (18). For this reason, high plasmatic histamine levels could originate an increase of histamine in hypothalamus. Moreover, neurogenic inflammation involves the release of histamine, which, in turn, promotes the release of substance P and the gene-related peptide, both closely linked to the pain process in migraine patients (19). On the other hand, histamine could also induce a vascular headache, since its plasmatic increase would provoke a release of nitric oxide upon stimulation of H1R receptors found in intracranial arteries (4).

DAO deficiency is responsible of plasmatic histamine accumulation, thus hypothetically could be one of the migraine triggers. The aim of this study is to assess the efficacy of DAO supplementation as a preventive treatment in migraine patients with DAO deficiency through a randomized double-blind trial.

2. METHODS

2.1. Subjects

A double-blind randomized study was carried out with 100 patients with confirmed episodic migraine diagnosis according to current International Headache Society (IHS) criteria (20) and DAO deficiency.

A total of 139 participants diagnosed with migraine were recruited by the Headache Unit of the Hospital General de Catalunya (Sant Cugat del Vallès, Barcelona). After DAO activity determination, 119 of them were eligible candidates to be included in the study (migraine diagnosis and DAO deficiency). Finally, 100 patients were selected by applying additional criteria. Inclusion criteria were the age between 18 and 65 years old and 4 to 14 migraine episodes/month for a minimum of six months prior to study start. Exclusion criteria were: the onset of migraine over 50 years old, the diagnosis of other kind of headache in the same patient, pregnancy and the following of a preventive treatment for episodic migraine during three months prior to the study.

DAO activity was determined from plasma samples with an enzymatic immunoassay method (D-HIT, Sciotec, Austria) after an 8-hour fasting period. Serum DAO levels below the cut-off value of 80 HDU/ml were considered as DAO deficient.

2.2. Outcome measures

Clinical outcomes assessed were duration and number of migraine attacks, perception of pain intensity and adverse effects during treatment. The duration of migraine attacks was measured by the number of hours of pain. Pain intensity was assessed by the Numeric Pain Rating Scale (NPRS), a 10-point grading scale where 0 represents absence of pain and 10 is the worst possible pain.

Other complementary information has been also recorded, such as adverse effects during treatment and the use of specific analgesic drugs, specifically the intake of triptans during migraine attacks. Data about the number of triptans in each migraine attack was recorded per patient, classifying their intake as low (less than 5 capsules/month), moderate (between 6 and 10 capsules/month) and high (more than 10 capsules/month).

2.2. Study protocol

Figure 1 provides details of the study design. In baseline consultation (C0) patients received a first diary to record data related to the outcome measures during one month prior to the study. After one month, all patients were scheduled for the first consultation (C1) and submitted the completed first diary. Patients were then randomized in two groups (DAO and placebo) and received a second diary to record the information requested during the month of treatment, which they submitted in a second consultation (C2).

Randomization in two groups was double-blind using the RANUNI procedure (SAS, v. 6.12, SAS Institute, Cary, NC, USA). One group received DAO enzyme supplementation (n=50) and the other placebo (n=50). Stratification considering age and sex was applied to assure similarity in baseline characteristics of both groups. The treatment consisted in the oral administration of 2 capsules of DAO supplement or placebo 20 minutes before breakfast, lunch and dinner. Each capsule contained 4.2 mg of porcine kidney protein extract with 7% of DAO with an enzymatic activity of 10,000 HDU/ml. The content of the capsules was microencapsulated with a gastroresistent shellac coating. Placebo consisted of microcrystalline cellulose and gelatin capsules with the same form, size and color than DAO ones and was provided by the same

manufacturer. Treatment compliance was verified by counting the remaining capsules for each patient.

Table 1 shows the characteristics (sex, age and DAO activity) of all patients included in both study groups.

The Ethics Committee of the Hospital General de Catalunya approved the study and all participants signed an informed consent form.

2.4. Statistical analysis

The size of the sample was calculated assuming a 5% type 1 error rate and a desired statistical power of 80%. Taking into account a minimal detectable mean difference of 1.25 attacks/month with a SD of 2, a total of 82 subjects would be needed. Based on an expected 15% dropout rate, a total of 100 patients (50 per group) were included to assure that at least 82 subjects would complete the full study.

T-test was used to evaluate differences within groups before (C1) and after (C2) treatment and between groups (placebo and DAO) in migraine attack duration (hours), number of attacks, and pain scales. Chi-squared test was used to determine significant differences in patients taking triptans. Probability values of $P<0.05$ were accepted as significant. Statistical analysis was performed using a SPSS for Windows, version 22 (Chicago, IL).

3. RESULTS

No significant differences in treatment compliance were observed between both groups ($p=0.543$), with a mean (SD) of remaining capsules of 17 (20.6) and 19.9 (24.7) in placebo and DAO group, respectively.

Great variability was found in the duration of migraine attacks reported by placebo and DAO groups, with some extremely high values recorded (**Figure 2).** Values exceeding that obtained by the formula P_{75} + 3·IQR (IQR: interquartile range; P_{75}-P_{25}) were statistically considered as extreme outlier values, and the corresponding patients were excluded, resulting in a final sample of 82 subjects, 43 in the placebo and 39 in the DAO group.

Table 2 shows the duration of migraine attacks, as the mean of pain hours for each patient (total number of pain hours/number of attacks) before and after treatment. Before treatment, mean (SD) values were 7.5 hours (4.2) and 6.1 hours (3.1) for placebo and DAO group, respectively. A statistically significant reduction in the duration of pain was obtained in DAO group after one month of treatment (p=0.0217). Although to a smaller extent, a reduction in this outcome was also observed in placebo group, but without statistical signification. Regarding the difference between groups, patients treated with DAO supplement reached a higher reduction of migraine attack duration (1.4 hours) than in placebo group (0.9 hours), although with a lack of statistical signification. When considering the number of attacks, significant reduction was found both in DAO group (2.67 attacks, p=0.0004) and in placebo group (2.16 attacks, p=0.0059). Similar reductions in the frequency of migraine attacks were achieved in both study groups. Regarding pain intensity, similar scores were found before and after the treatment in both groups (Table 2).

No adverse effects were registered in patients with DAO supplementation. Only one patient of the placebo group described gastrointestinal troubles and subsequently abandoned the study.

Figure 3 shows the percentage of patients who used triptans before and after the treatment with placebo or DAO. A reduction of patients taking triptans was observed in DAO group

whereas in placebo group there was an increase in patients with triptans intake. **Figure 4** illustrates the intake of triptans before and after one month of treatment with placebo or DAO for each of the patients that reported consumption. In fact, among individuals treated with DAO, 44% of them reduced triptans intake (5 reported a total withdrawal), 30% remained unchanged and 26% increased the intake of these drugs. On the contrary, an increase in triptans intake in the placebo group was recorded in 57% of the individuals. However, in neither case the association was statistically significant.

4. DISCUSSION

To our knowledge, this is the first double-blind randomized trial to assess the effect of DAO supplementation in migraine patients. Currently the main measure to prevent or mitigate the symptomatology related to histamine intolerance by DAO deficiency is the dietary management avoiding foods with high contents of histamine or other bioactive amines also metabolized by DAO, such as putrescine and cadaverine, as well as foods described as histamine-releasing (4,5,21). Several intervention studies have demonstrated the effectiveness of histamine restrictive diets, some of them specifically focused on headache, with positive results in most of the patients involved in each study (12,13,22,23). However, the wide and variable distribution of histamine in foods together with other additional restrictions (histamine-releasing foods and presence of other bioactives amines) makes difficult the diet adherence and increases the risk of nutritional imbalances. The use of DAO supplementation could be an alternative to a histamine-free diet or be useful to make easier its compliance.

In our double-blind randomized clinical study, DAO enzyme supplementation consisted in porcine kidney extract microencapsulated with a shellac gastroresistant coating that guaranteed its resistance in the pH of the stomach and subsequently its hydrolysis by pepsin. Intestinal conditions allow the release of the unaltered enzyme that will potentially resist the proteolytic attack of trypsin and chymotrypsin. In fact, Federico et al. (24) reported a high in vitro stability of DAO of plant origin in the presence of trypsin, with 100% of the enzymatic activity during the first 45 minutes.

The administration of the DAO supplement was able to significantly reduce the mean duration of migraine attacks by 1.4 hours (from an initial mean value of 6.1 hours). The duration of migraine attacks has been used as an efficacious measure to assess the clinical and quality of life impact of the treatment (25-27). On the other hand, the effect of this supplementation in the migraine frequency could not be established because both study groups showed a significant reduction greater than 2 monthly attacks. Moreover, the lack of significance observed between groups could be explained by the limited duration of one month of treatment. A longer intervention period might confirm the trend observed in the current study about the effects of DAO supplementation on migraine improvement.

Regarding pain intensity, no differences were found based on the NPRS score reported by the subjects from both groups. The usefulness of this scale has been questioned in patients with long-term pain because they tend to score between 4 and 6 (golden section) (28). Likewise, the use of triptans (allowed in this study), could cover up the real perception of pain.

Patients with DAO deficiency could be stratified as reduced DAO activity level (40-80 HDU/ml) and markedly reduced DAO activity level (<40 HDU/ml). In this study, only few individuals

showed a markedly reduced DAO activity, with 7 and 4 patients in the placebo and DAO group, respectively. The reduced number of subjects with markedly reduced DAO activity, which did not allow the stratification of these patients, together with the fact that all subjects received the same dose of treatment, could diminish the strength of the current study. Markedly reduced DAO activity patients could require a higher dose to reach the improvement of symptomatology.

A trend to diminish the triptans intake was observed in DAO group, with some patients that abandoned the use of triptans. A reduction of the number of patients with low and moderate intake of these drugs was also observed. The reduction in triptans intake could indicate a decrease in the intensity of pain during migraine crisis. This trend was not observed in the placebo group where the intake of triptans increased in comparison with the baseline.

There are few studies about the efficacy of DAO supplementation in the improvement of headache and other symptoms associated with histamine intolerance (29,30), and all of them with a lower number of patients involved. A randomized double-blind crossover provocation study (29), using histamine containing and histamine-free tea in combination with DAO capsules or placebo in 39 patients with histamine intolerance revealed that two capsules of DAO supplementation reduced the appearance of headache, skin reactions, and respiratory and gastrointestinal disorders in comparison with placebo. Recently, Manzotti et al. (30) also observed that DAO supplementation for a period of at least two weeks was associated with a reduction of one or more of the reported symptoms in 13 out of 14 patients with histamine intolerance. When DAO supplementation was associated with low-histamine diet a higher reduction of symptoms was reported in comparison to the diet alone. However, in this study no placebo group was considered.

In the current trial the efficacy of DAO supplementation was assessed after only one month of treatment, so probably, a longer treatment period could lead to a great improvement. Moreover, no complementary dietary intervention was considered, and then it is possible that DAO supplementation together with histamine free-diet could enhance the results of the treatment. Likewise, in further studies it would be of interest to segregate patients in different grades of DAO deficiency. Therefore, more randomized placebo-controlled studies, preferably with a cross-over design, are needed to assess the benefits of DAO supplementation for a longer treatment period, and the combined effect of DAO supplement together with histamine free diet, which would make diets less restrictive and improve patients quality of life.

Acknowledgements

The authors' responsibilities were as follows — JIC, MCVC, MLG and LSS: designed the research; JIC and AD: conducted research; OCB, MLM and MCVC: analyzed data and performed statistical analysis; JIC, OCB, MLM and MCVC: wrote the paper; and all authors: critically reviewed the manuscript for important intellectual content and read and approved the final manuscript.

Conflict of Interest

None of the authors reported a conflict of interest related to the study.

Sources of support: DR Healthcare (Barcelona, Spain) has provided DAO supplement and placebo capsules. Oriol Comas-Basté is a recipient of a doctoral fellowship from the University of Barcelona (APIF 2015).

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Table 1. Characteristics (sex, age and DAO activity levels) of all patients included in both placebo and DAO groups.

VARIABLE		PLACEBO N=43	DAO N=39	TOTAL N=82
Sex (number of patients)	Female	36	32	68
	Male	7	7	14
Age (years old)	Mean (SD) min-max	43.6 (11.0) 23.8-64.9	40.8 (10.9) 18.4-61.2	42.3 (11.0) 18.4-64.9
DAO activity (HDU/ml)	Mean (SD) min-max	54.8 (11.3) 18.6-79.6	53.3 (11.2) 22.6-79.0	54.1 (12.9) 18.6-79.6

SD: standard deviation; min: minimum; max: maximum; HDU : histamine degrading units.

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Table 2. Duration, number and pain intensity of migraine attacks in placebo and DAO groups before (C1) and after one month of treatment (C2).

			PLACEBO N = 43	**DAO N = 39**	P [a]
DURATION OF ATTACKS (HOURS)	Before treatment (C1)	Mean (SD) min - max	7.53 (4.24) 0 - 21	6.14 (3.06) 1.8 - 15	
	After treatment (C2)	Mean (SD) min - max	6.68 (4.42) 0 - 16.5	4.76 (2.68) 0 - 11	
		P[b]	0.3034	0.0217	
	C2-C1	Mean (SD) min - max	-0.85 (5.35) -15.2 – 10.5	-1.38 (3.60) -15.0 – 6.6	0.6040
NUMBER OF ATTACKS/MONTH	Before treatment (C1)	Mean (SD) min - max	9.26 (5.21) (0 - 20)	10.18 (4.44) (1 - 20)	
	After treatment (C2)	Mean (SD) min - max	7.09 (4.97) 0 - 20	7.51 (4.97) 0 - 19	
		P[b]	0.0059	0.0004	
	C2-C1	Mean (SD) min - max	-2.16 (4.88) -15.0 – 8.0	-2.67 (4.26) -15.0 – 5.0	0.6172
PAIN INTENSITY (NPRS SCORE)	Before treatment (C1)	Mean (SD) min - max	5.62 (1.57) 0 - 9	5.27 (1.43) 1.4 - 7.9	
	After treatment (C2)	Mean (SD) min - max	4.88 (2.31) 0 - 8.5	5.07 (2.02) 0 - 8.6	
		P[b]	0.0885	0.5117	
	C2-C1	Mean (SD) min - max	-0.71 (2.67) -7.8 – 7.8	-0.21 (1.98) -7.0 – 2. 0	0.3424

SD: standard deviation; min: minimum; max: maximum; NPRS: Numeric Pain Rating Scale.

[a] p-value for the statistical difference between placebo and DAO groups; [b]: p-value for the statistical differences between before (C1) and after (C2) treatment.

Figure legends

Figure 1. Schematic representation of the study design.

Figure 2. Box plot for the duration of migraine attacks (in hours of pain) in placebo and DAO groups one month before (C1) and after one month of treatment (C2). The bottom and top of the box (interquartile range) are the percentile 25 and the percentile 75, respectively. Central line represents the median. Lines extending vertically from the boxes (whiskers) indicate variability outside the interquartile range. Outliers are plotted as circles and extreme outliers as asterisks.

Figure 3. Percentage of patients who used triptans before (C1) and after one month of treatment (C2) with placebo or DAO. The triptans intake classification was: no intake, low (1-5 capsules/month), moderate (between 6-10 capsules/month) and high (more than 10 capsules/month).

Figure 4. Number of capsules of triptans required before (C1) and after (C2) one month of treatment with placebo (A) or DAO supplementation (B) for each of the patients that reported consumption. Red line means an increase, green line means a decrease and black line means no change in the triptan intake after one month of treatment.

RECRUITMENT OF PARTICIPANTS

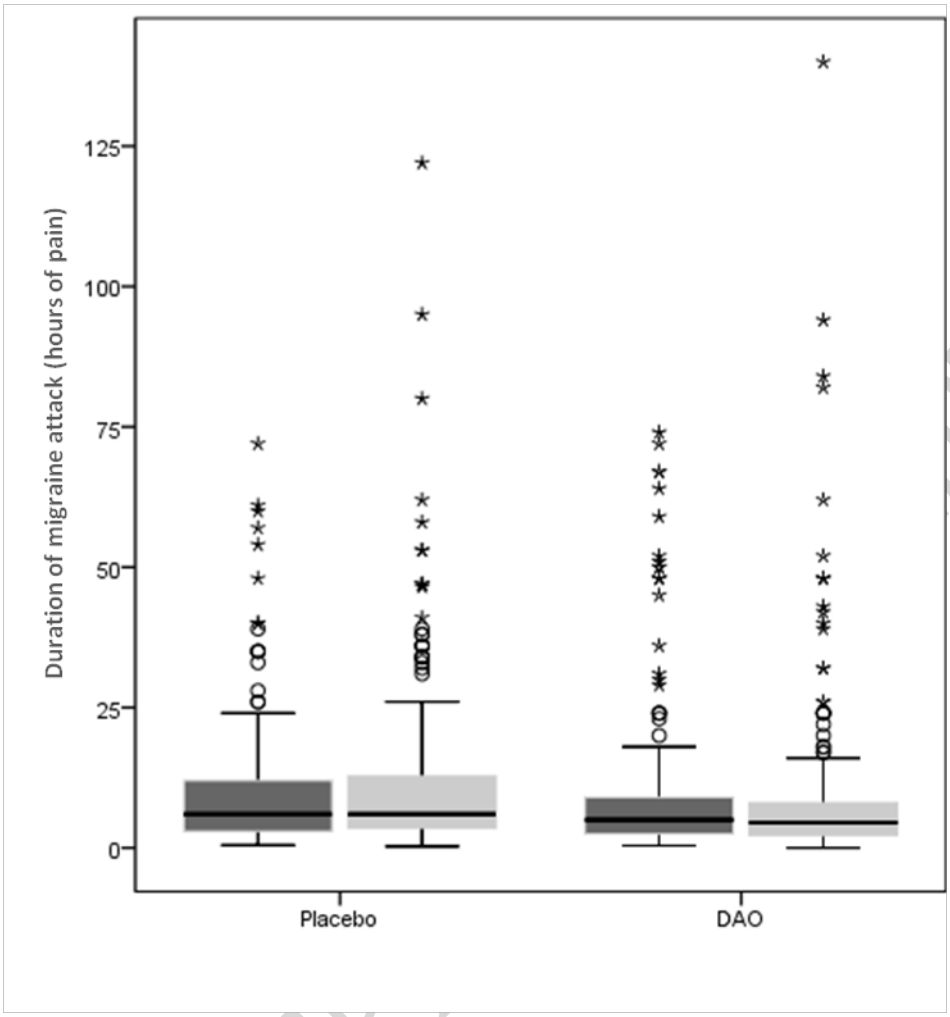
- From a pool of 139 patients diagnosed with migraine at the Headache Unit of the Hospital General de Catalunya (Sant Cugat del Vallès, Spain), 119 with DAO deficiency were eligible candidates to be enrolled in the study. By applying additional selection criteria, 100 subjects were finally included.

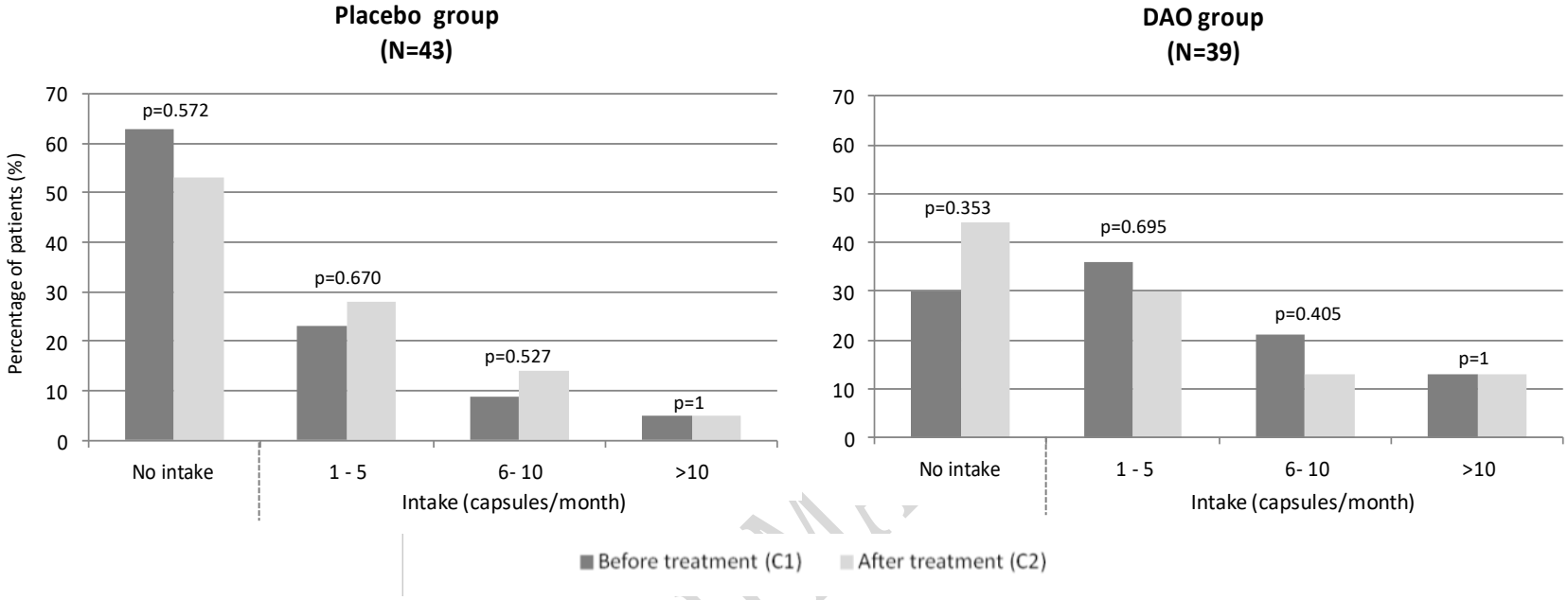
STUDY PROTOCOL

- **Baseline consultation**: a first diary was given to each patient to record data related to outcome measures during the month prior to treatment start.
- **Consultation 1 (C1)**:
  - The completed first diary was submitted and a second diary was given to record all information related to outcome measures during the month of treatment.
  - Patients were randomized in two groups: DAO (n=50) and placebo (n=50).
- **Consultation 2 (C2)**:
  - The completed second diary was submitted and treatment compliance was checked by counting the remaining capsules.
  - One patient from the placebo group abandoned the study.

DATA ANALYSIS

- Patients with migraine attack durations statistically considered as extreme outliers were excluded for data analysis, resulting in a total of 82 subjects.




Placebo group
(N=43)
DAO group
(N=39)
Percentage of patients (%)
70
60
50
40
30
20
10
0
p=0.572
p=0.670
p=0.527
p=1
p=0.353
p=0.695
p=0.405
p=1
No intake
1 - 5
6- 10
>10
Intake (capsules/month)
Before treatment (C1)
After treatment (C2)