# CENTRAL CHOLINERGIC CHALLENGING OF MIGRAINE BY TESTING SECOND-GENERATION ANTICHOLINESTERASE DRUG

# Central Cholinergic Challenging of Migraine by Testing Second-Generation Anticholinesterase Drugs

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**The antinociceptive activity of donepezil, a novel cholinesterase inhibitor, was investigated in the mouse hot plate test. Donepezil (5 to 10 mg $kg^{-1}$ i.p.) induced a dose-dependent antinociception that reached its maximum effect 15 minutes after injection. Donepezil antinociception was prevented by the antimuscarinic drug scopolamine. At analgesic doses, donepezil did not alter gross animal behavior. These results indicate that donepezil is endowed by muscarinic antinociceptive properties, suggesting this compound as a potential therapeutic approach for the treatment of painful pathologies. Therefore, we investigated donepezil's effect in migraine. Donepezil (5 mg per os, evening assumption) was effective as a prophylatic agent in patients suffering from migraine with or without aura by reducing the number of hours with pain, the number of attacks, and the severity of the pain attack. The efficacy of donepezil was compared with that of the β-blocker propranolol (40 mg bid per os), showing higher activity. Response rates of a large-sized open study devoid of entry criteria regarding migraine subtypes suggest the drug as an excellent prophylactic compound for migraine in general practice. Clinical results also indicate that the activation of the cholinergic system can represent a novel prophylactic approach to migraine.**

**Key words: donepezil, migraine, prophylaxis, cholinesterase inhibitor, analgesia, primary pain, central cholinergic system**

**Abbreviations: VAS visual analogue scale**

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It is widely accepted that organisms possess endogenous systems within the central nervous system that inhibit nociceptive transmission. More than 20 years ago, a defective function of analgesia systems, chiefly the serotonergic one, was been indicated to be a crucial event in migraine pathogenesis.[1,2] The hypothesis has been widely supported by the high effectiveness of serotonergic drugs both in prophylaxis and acute treatment of migraine pain. Interaction between cholinergic and serotonergic systems had been demonstrated in several brain areas.[3] Our group recently demonstrated a functional intertwining between serotonin and acetylcholine in migraine. In fact, we observed that the serotonin-like molecule sumatriptan provides a central analgesia that seemingly depends on the activation of central cholinergic system.[4] Several reports have provided evidence for the critical involvement of the cholinergic system in pain inhibitory pathways. The first observation that the cholinesterase inhibitor physostigmine increased the nociceptive pain threshold in man was made more than sixty years ago.[5] Since then, a vast literature has appeared describing the controlling action on secondary, nociceptive pain of both cholinesterase inhibitors and cholinomimetic drugs. Intrathecal and systemic administration of acetylcholinesterase inhibitors has been reported to produce antinociception in several animal species, including mice.[6-9] Unfortunately, first-generation cholinesterase inhibitors such

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as physostigmine are poorly able to cross the blood brain barrier. This problem highly spoiled their availability for the therapy of human central primary pain, including migraine.[10] The recently synthesized second-generation drug donepezil is a potent and selective reversible inhibitor of acetylcholinesterase that is capable of easily crossing the blood brain barrier.[11] Several studies showed that donepezil is a well-tolerated and efficacious agent for the symptomatic treatment of mild to moderately severe Alzheimer's disease.[12] Following the hypothesis that second-generation cholinesterase inhibitors are endowed with central analgesic activity, in the present study we investigated the potential antinociceptive profile of donepezil in mice and in primary, neurogenic human pain. Migraine is amongst the most frequent primary, neurogenic painful syndrome in humans. Currently, great advances have been done regarding symptomatic relief during migraine attacks.[13] Pharmacological progresses to reduce migraine attack frequency and severity are significantly minor. Therefore, migraine prophylaxis treatment is an appealing therapeutic approach. The ideal drug would nearly completely abolish migraine attacks, resolving the patient's symptoms without serious adverse events. To date, this goal is unattainable, with few drugs being more than 50% effective and with patients still requiring acute treatment. On these bases and considering that second-generation anticholinesterase drugs have not yet been investigated as prophylactic agents, we thought it worthwhile to study this novel therapeutic approach by investigating the efficacy of donepezil in migraine prophylaxis.

## METHODS

**Animals.—**Male Swiss albino mice (23 to 30 g) from Morini (San Polo d'Enza, Italy) were used. The mice were housed 15 per cage. The cages were placed in the experimental room 24 hours before the test for adaptation. The animals were fed a standard laboratory diet and tap water ad libitum and were kept at 23°C ± 1°C with a 12-hour light/dark cycle (lights on at 7 a.m.). Animals were used a only once. All experiments were carried out according to the guidelines of the European Community Council for experimental animal care.

**Hot Plate Test.—**The method adopted was described by O'Callaghan and Holtzman.[14] Mice were placed inside a stainless steel container that was set thermostatically at 52.5°C ± 0.1°C in a precision water bath from KW Mechanical Workshop (Siena, Italy). Reaction times(s) were measured with a stopwatch before and 15, 30, and 45 minutes after donepezil administration. The endpoint used was the licking of the fore or hind paws. Those mice scoring less than 12 and more than 18 seconds in the pretest were rejected (30%). To prevent tissue injury, an arbitrary cut-off time of 45 seconds was adopted.

**Clinical Experience Design.—**The same design was followed to perform **Trial I** and **Trial II**. Patients underwent a run-in period to determine eligibility. Patients had to fill in a pain diary during the entire study period. They had to undergo five visits: before and following run-in, before and after the active treatment, and at the end of the follow-up period. During each visit, we also administered the Zung[15] and Wang[16] tests to assess possible changes in psychometric parameters.

*Inclusion Criteria.*—Subjects were healthy except for migraine, which was diagnosed as migraine with aura or without aura according to International Headache Society criteria.[17] they had normal vital parameters and normal blood routine examinations. The trial was conducted in agreement to the Declaration of Helsinki. Prior to the trials, witness informed consent was obtained from each patient who was free to drop out of the study at any point.

*Exclusion Criteria.*—Women who were pregnant, lactating, or not using adequate contraceptive measures were excluded. Patients with gastric-enteric diseases or dysfunction, bradycardia, asthma, heart diseases or dysfunction, epilepsy, psychiatric illnesses, or scores in the Zung or Wang test higher than 40% (cut-off value) were also excluded. Participation in another trial less than 6 months before the study was asserted led to exclusion from this study. Patients did not receive any active pharmacological treatment, with the only exception for acute, abortive antimigraine drugs.

**Subjects.—**In **Trial I**, we enrolled 156 volunteers suffering from migraine without aura (66 men and 90 women; mean age [±SD] 34.6 ± 3.7 years). They were

a well-defined group of migraine patients selected by following the International Headache Society criteria: they were all ailed by migraine for 3 years, with two to five migraine attacks per month, each lasting no longer than 2 days, with a severity scoring from 65 to 45 on a 0 to 100 visual analogue scale (VAS).

After a computer-generated randomization code was obtained by a software technique, patients were entered in descendent sequential order. After a 1 month run-in, a 2-month propranolol therapy regimen (40 mg twice a day, oral route) or a 2-month therapy with donepezil regimen (5 mg, evening assumption, oral route) was randomly assigned. The two groups of patients were matched for sex and age (propranolol-treated group: 32 men and 46 women, mean age 33.8±4.1 years; donepezil-treated group: 34 men and 44 women, mean age 34.2±5.2 years). **Trial II** consisted of a large-sized open study. Three hundred-fifty-seven volunteers (208 women and 169 men, mean age 34.3±2.9 years), 152 suffering from migraine without aura with no less than four migraine attacks per month, 42 affected by migraine with aura associated to migraine without aura with no less than four migraine attacks a month, and 142 characterized by "chronic migraine" (i.e., headache with attacks having all the features specific to migraine without association to moderate daily migrainous headache) were included in the study that consisted of a 1-month run-in period and 2 months of donepezil. A 2-month follow-up period was planned both for trials.

**Evaluation of Efficacy.—**The primary efficacy endpoint was the decrease in the number of hours with migraine pain as well as the mean monthly decrease of severity of migraine pain. The severity was determined on a 0 to 100 VAS by the following score: 100 to 80 = very severe and completely disabling, requiring bed rest; 80 to 65 = severe, severely impaired working ability; 65 to 45 = moderate; impaired working ability; less than 45 = mild, moderately impaired working ability; 0 = no pain. Consumption of acute, abortive antimigraine drugs was also used as a parameter of efficacy in both trials. Time to pain relief was the secondary endpoint. Regarding **Trial II**, "effective pharmacological response" was a crucial parameter to actually judge the effectiveness of the administered treatment.

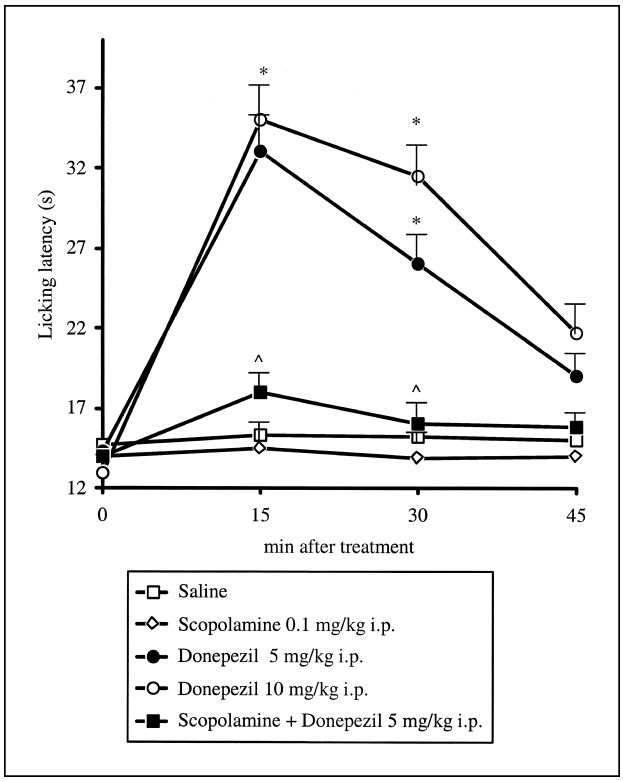
**Evaluation of Safety and Tolerability.—**Heart rate, blood pressure, electrocardiogram, and blood-urine routine examinations were recorded before and after treatment. At the same time, patients have to undergo a careful internal medicine, neurological visit and a psychometric evaluation. Adverse events in relation to the treatment (certain, possible, unlikely) had to be carefully reported in patient's diary.

**Reagents and Drugs.—**The following drugs were used: donepezil (gift of Prof. Nha, Medical School, University of Atlanta for preclinical studies; donepezil hydrochloride, EISAI, Japan for clinical studies), scopolamine hydrobromide (Sigma, St. Louis, MO). For preclinical study, drug concentrations were prepared in such a way that the necessary dose could be administered to animals in a volume of 10 mL $kg^{-1}$ by intraperitoneal (i.p.) injection; the drugs were dissolved in saline solution.

**Statistical Analysis.—**Preclinical experimental results are given as the means ± SEM. Clinical results are given as the means ± SD. An analysis of variance followed by Fisher's protected least significant difference procedure for post hoc comparison, and a paired Student's *t* test were used to verify the significance of differences between two means, respectively, of preclinical and clinical results. The efficacy of donepezil in **Trial II** was also evaluated by means of the expression: 1/(% success rate of the tested drug — % expected success rate of placebo). The arbitrarily rate for placebo was 30% as indicated by the result of a methanalysis we performed on seven placebo-controlled trials for the prophylaxis of migraine, including chronic daily migraine. The result of the expression gives the "expected pharmacological response" scored by the following rule of thumb: 0.05 = effective, 0.03 = very good, and 0.01 = excellent.

## RESULTS

**Antinociceptive Activity of Donepezil.—**The cholinesterase inhibitor donepezil produced a dose-dependent increase in the pain threshold in the mouse hot plate test (5 and 10 mg $kg^{-1}$ i.p.). The antinociceptive effect of donepezil peaked 15 minutes after administration and then slowly diminished. The antinociception induced by donepezil was prevented by the anti-




**Antinociceptive effect of donepezil and its antagonism by scopolamine in a mouse hot-plate test. Each point represents the mean of 10 to 22 mice. Vertical lines show SEM. *$P<0.01$ in comparison with the saline-treated mice; ^$P<0.01$ in comparison with scopolamine-treated mice.**

muscarinic drug scopolamine (0.1 mg $kg^{-1}$ i.p.) (Figure).

**Clinical Trial I.—**Following propranolol treatment, 40 patients reported a 40% to 72% decrease in the number of hours with pain, a 20% to 50% decrease in the number of migraine attacks, and 12% to 40% decrease in migraine severity. Remainder sufferers of migraine reported a benefit ranging from a 30% to 0% decrease versus run-in values regarding the above parameters (Table 1). Four patients dropped out. After donepezil treatment, eight of the treated sufferers of migraine did not report improvement in the severity of the migraine attacks, and another five patients reported a decrease lower than 35% in the number of migraine attacks. Remainders reported a benefit of 35% to 50% in migraine severity, of 35% to 77% in the number of migraine attacks, and of 35% to 80% in the number of hours with pain (Table 1). Three patients dropped out. Donepezil

**Table 1.—Efficacy of Donepezil Versus Propranolol in Patients With Migraine**

| | Number of Migraine Attacks | Hours with Pain | Migraine Severity (VAS 0-100) |
|---|---|---|---|
| Run-in | 3.4 ± 1.2 | 72.9 ± 24.6 | 88.5 ± 1.8 |
| Donepezil | 1.5 ± 0.9* | 27.4 ± 22.5* | 73.8 ± 9.8* |
| Run-in | 3.4 ± 1.2 | 74.1 ± 25.2 | 88.6 ± 11.2 |
| Propranolol | 2.6 ± 1.2* | 49.3 ± 5.7* | 79.8 ± 13.0* |
| Donepezil | 1.5 ± 0.9** | 27.4 ± 22.5** | 73.8 ± 9.8*** |
| Propranolol | 2.6 ± 1.2 | 49.3 ± 25.7 | 79.8 ± 13.0 |

*P < 0.0001 versus corresponding run-in
**P < 0.0001 donepezil versus propranol
***P < 0.002 donepezil versus propranol
All of the patients volunteering in this comparison were diagnosed to suffer from M without aura characterized by a monthly frequency of two to five M attacks lasting no more than 24 hours each. Each value represents the mean ± SD/month.

treatment also induced a significant ($P>0.0001$ versus run-in; $P>0.001$ versus propranol) decrease in the intake of analgesic acute, abortive antimigraine drugs. Time to the onset of amelioration peak value was on days 20 to 24 (mean 22.4 ± 5.1) for donepezil and days 31 to 36 (mean 33.5 ± 3.1) for propranolol ($P<0.0001$). The benefit produced by donepezil lasted up to 35.5 ± 5.9 days of the 2-month follow-up period. The maintenance of benefit was shorter ($P<0.0001$) in the case of propranolol-induced amelioration, which was 28.4 ± 5.7 days.

**Clinical Trial II.—**A total of 346 patients completed the study. The remaining 11 patients dropped out. After 60 days of donepezil treatment, the number of hours with migraine pain, the number of migraine attacks, and pain severity was significantly decreased when compared with run-in values (Table 2). The observed benefit was higher ($P<0.0001$ regarding the number of migraine attacks and the number of hours with pain, $P<0.002$ concerning migraine severity) than that induced by propranol preventive treatment. The result was mirrored by a significant decrease ($P>0.0001$ versus run-in period) in the consumption of acute, abortive antimigraine drugs.

**Table 2.—Efficacy of Donepezil in Patients With Migraine**

| | Number of Attacks | Hours with Pain | Severity (VAS 0-100) |
|---|---|---|---|
| Run-in | 8.29 ± 0.2 | 757.2 ± 27.1 | 78.3 ± 0.8 |
| Donepezil | 3.89 ± 0.2* | 397.9 ± 19.6* | 31.7 ± 1.0* |
| Pharmacological gain | 0.01 | 0.04 | 0.02 |

0.01, excellent; 0.04, effective to very good; and 0.02, very good to excellent.
*$P < 0.001$ versus run-in.
Each value represents the mean ± SD/month. Volunteers entering this open trial underwent no specific entry criteria regarding different migraine subtypes. Thus, a large number of the patients were suffering from chronic migraine (i.e. attacks of migraine without aura associated to daily migrainous headache).

**Table 3.—Trial I: Adverse Effects of Propranolol and Donepezil**

| Donepezil (first week) | Propranolol (first week) |
|---|---|
| Increase REM phase (29 M/35 F) | Nightmares (1 M/2 F) |
| Anxiety (1 M/4 F) | Asthenia (2 F) |
| Nausea (5 F) | Drowsiness (1 M/1 F) |
| Vomiting (1 F) | |
| Sleep-time reduction (3 M/3 F) | |
| Donepezil (remainder period) | Propranolol (remainder period) |
| Increase REM phase (19 M/6 F) | Nightmares (1 M/2 F) |
| Increased self-confidence (13 M/6 F) | Mild asthenia (16 M/18 F) |
| Increase in working hours (13 M/9 F) | Sexual dysfunction (2 M) |
| | Bradychardia (1 M) |

M, men and F, women.

There was no significant difference versus run-in values on days 1 through 5. For days 6 through 17 (mean 13.1 ± 9.4), donepezil induced amelioration (range 20% to 38%) of migraine severity and the number of the attacks. A higher ameliorative effect, ranging from 49% to 53%, regarding the three parameters under observation was been shown on days 17 to 23 (mean 20.2 ± 3.1). This trend increased during days 23 through 28 (mean 24.1 ± 4.9), when it reached the maximum statistical difference that remained unchanged unitl the end of the treatment, as well as for 35.7 ± 6.1 days of the follow-up period. It is noteworthy that donepezil-treated sufferers of chronic migraine experienced a large number of headache free-periods (64.3%; $P < 0.0001$ versus run-in).

**Tolerability.—**No clinically significant change of vital parameters or routine blood and urine examines, and no significant change in Wand and Zung psychometric tests was ever observed following donepezil treatment in either **Trial I** or **Trial II**. During the first treatment week, the three most frequently reported adverse events after oral administration of a 5-mg donepezil hydrochloride tablet were an increase in REM phases, sleep-time reduction, and nausea, as shown in Tables 3 and 4. All of the side effects tended to be subdued during the last days of the first week, i.e., on days 5 through 7 (mean 5.8 ± 3.9). After the first week, donepezil induced side effects that patients perceived as "nonadverse events" (Tables 3 and 4). The three most reported reasons for stopping the use of donepezil tablets were nausea (eight cases: one case in **Trial I** and seven cases **Trial II**), nausea (five cases: one case **Trial I** and four cases **Trial II**), and increased anxiety not evidenced by the Wang test (one case in **Trial II**). All of the subjects who dropped out of the donepezil treatment did it during the first 4 days of treatment. The reasons for dropping out of propranolol treatment (**Trial I**) were prefainting sensation (two cases), asthenia (one case), and brady-

**Table 4.—Trial II: Adverse Events of Donepezil**

| Donepezil (first week) | Donepezil (remainder period) |
|---|---|
| Increase REM phase (n = 201) | Increase REM phase (n = 89) |
| Anxiety (n = 13) | Sleep-time reduction (n = 13) |
| Nausea (n = 27) | Increased self-confidence (n = 51*) |
| Vomiting (1 F) | Increase in working hours (n = 51*) |
| Sleep-time reduction (n = 35) | |

n = number of patients; *The same patients experienced both adverse effects.

chardia (one case), unperceived as an ailment but clinically relevant. These side effects, and others listed in Table 3, emerged clearly following the first 20 days of treatment. The mentioned variation in heart rate was the only observed change in vital parameters following propranolol. No abnormality was observed in body fluid routine examinations and Wang and Zung psychometric tests.

## COMMENTS

Donepezil was able to induce antinociception in mice without producing any visible modification of animal gross behavior. Donepezil antinociception was found to be dependent on cholinergic activation because it was prevented by the nonselective muscarinic antagonist scopolamine at a concentration unable to prevent analgesia induced by nonmuscarinic drugs such as morphine or baclofen,[18] administered at equiactive doses. The present results show that donepezil is able to produce dose-dependent antinociception in mice by potentiating endogenous cholinergic activity. These data indicate a potential employment of a second-generation anticholinesterases to relieve human painful conditions. For this reason, we investigated the effect produced by donepezil in patients suffering from migraine. Clinical experiences consisted both of a comparison with propranolol and a large-sized open study. The latter study was planned to mirror the application of the drug in clinical practice. This led us to eliminate entry criteria regarding migriane subtypes, monthly number of attacks, and overuse of acute antimigraine medications. Both of the clinical experiences gave results showing that donepezil, at a dose of 5 mg/day for 2 months, was endowed with a good-to-excellent therapeutic gain. To our knowledge, this was the first time a second-generation anticholinesterase agent was employed for migraine prophylaxis. In open observations, donepezil induces significant relief as evidenced after the application of an expression capable of nearly eliminating the placebo response. The 2-month duration of donepezil preventive treatment was established in agreement with the golden standards for the Economy of the Ethical Committee of our Interuniversity Centre which fixes limits for expenses (drug price/day $\times$ the number of days in the of treatment period) in migraine treatments. Calculation of time to relief indicated that donepezil seems to be a fast-acting drug. Finally, donepezil appears endowed with high effectiveness and quick onset of pain relief in the prophylaxis of migraine. The drug is well tolerated; indeed, there were no clinically relevant changes in vital parameters, routine body fluid examinations, or in scores of psychometric tests for evaluating depression and anxiety. Adverse events were reported during the first week of treatment. During the following period, side effects were perceived as "positive effects" by the patients. That the drug is well tolerated by patients healthy except for migraine, and its positive dose-dependent analgesic activity in mice suggest the possibility that a gradual increase to 10 mg/day of donepezil can be administered and can ameliorate the here shown pharmacological and therapeutic gain. In previous years,[10] we attempted to cure migraine by using first-generation anticholinesterase drugs endowed with a marked peripheral action. Some relief of migraine was induced only when we administered a large dose, which can partly act at the central nervous system. By increasing the dose, an assumed fan of peripheral cholinergic side effects emerged. This completely spoiled the value of the treatment. That donepezil, known to act at the central nervous system, induces poor peripheral effects suggests the role of central cholinergic analgesia in migraine.

These results and basic science data evidencing the entailing of acetylcholine and serotonin in the treatment of migraine also seem to enlighten a new trend in migraine prophylaxis that focuses on the intertwining of the cholinergic system with other relevant analgesia systems defective in migraine.

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