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Original Article

Enalapril improves endothelial function in patients with migraine: a randomized, double-blind, placebo-controlled trial

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Abstract

BACKGROUND: There are increasing evidences of endothelial dysfunction in migraine. The ACE-inhibitors have previously been shown to be effective in migraine prophylaxis. Furthermore, ACE inhibitors have beneficial effects on endothelial dysfunction. We therefore investigated whether Enalapril is effective in endothelial function improvement.

METHODS: In this randomized clinical trial, 10 mg Enalapril daily was compared with matched placebo in 40 patients with migraine for two months. Flow Mediated Dilation (FMD), serum total nitrite and C-reactive protein (CRP) were measured in all patients at the baseline and after 2 months.

RESULTS: Patients' FMD increased in the case group after treatment with Enalapril ($p = 0.002$) while there was no significant change in control group. Total nitrite concentration increased in case group ($p = 0.000$), while there was no significant difference before treatment. There was no significant difference in the CRP concentrations in two groups.

CONCLUSIONS: These results indicate that ACE inhibition can improve endothelial function in patients with migraine, as it has been shown by both FMD and serum levels of nitric oxide. The mechanism could be either that Enalapril limits the angiotensin II-induced production of superoxide radicals which would normally inactivate nitric oxide, or that it may increase bradykinin-mediated nitric oxide release.

KEYWORDS: Endothelium, Migraine Disorders, Nitrites, C-Reactive Protein.

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There are several relations between migraine, endothelial function, and vascular disease. Migraine, particularly migraine with aura, is associated with an increased risk for stroke, and coronary artery disease.[1,2] The endothelial cells have multiple functions, which when changed may provide a common pathophysiology for migraine and vascular disease. Several studies have been shown endothelial dysfunction in patients with migraine.[3]

Endothelial dysfunction is characterized by reduction in bioavailability of vasodilator such as nitric oxide (NO), increase in constricting factors, and subsequent impaired reactivity of the vasculature.[4]

It has been shown that angiotensin-converting enzyme (ACE) inhibition improve endothelial function in several disease states associated with endothelial dysfunction such as diabetes, coronary artery disease (CAD), heart failure, and stroke.[5-11] So, it can also be a promising option in patients with migraine.

ACE inhibitors reduce the production of angiotensin II and prevent its vasoconstrictive effect. Lowering angiotensin II levels also reduces levels of adhesion molecules and inflammation, decreases oxidative stress and prevents endothelial cells apoptosis. Furthermore, ACE inhibitors decrease degradation of

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endothelial bradykinin, which results in vasodilation by stimulating the production of NO and other relaxing factors.[12]

The beneficial effect of ACE inhibition on endothelial function in patients with migraine can be assessed via several methods; the most widely used clinically method is the evaluation of endothelium-dependent dilatations or ischemia-induced flow-mediated dilatation (FMD).[2,13-15]

The purpose of this study was to evaluate the possible role of Enalapril in improvement of endothelial function of patients with migraine.

Methods

Study Populations

Patients were recruited in Isfahan, from neurology outpatient department of Al-Zahra hospital, between July 2008 and June 2009 mainly by office referral. Patients, with diagnosis of migraine without aura in accordance with the International Headache Society criteria (second edition) (IHS-1.1),[16] who agreed to participate after full clarification of the risk and benefits of Enalapril were enrolled in the study.

Patients who had hypertension, diabetes mellitus, coronary artery disease, infectious diseases, known liver or kidney disorders, hypercholesterolemia, hypertriglyceridemia, gynecologic disorders (such as polymenorrhea, cystic ovary disease), morbid obesity (Body Mass Index > 35), current cigarette smoking habit, alcohol or other substances consumption, anemia, sinusitis, tension type headache more than 5 days per month, or less than 5 migraine attacks per month, were not included in this study.

Exclusion criteria included hypersensitivity to ACE inhibitors, pregnancy or lactation, presenting Enalapril complications and use of vitamin B12-containing supplements. All patients have used at least one first-line prophylactic drug without advantage in the past. None of the patients had previously used ACE inhibitors or angiotensin-II receptor blockers.

Study Design

The diagram of patients' participation through the study has been shown in figure 1. In this randomized clinical trial, patients started one month as a baseline period, in which no prophylactic medication was used. After this time patients underwent a complete examination that included: physical examination, blood sampling for biochemistry measurements (NO2/NO3, CRP) and forearm flow mediated dilation (FMD). Patients were randomized to receive 5 mg Enalapril or placebo twice a day for two months with the use of a computer generated randomization list with 11 consecutive balanced blocks of four patients (two Enalapril, two placebo). The placebo was matched with Enalapril tablets in shape, color and size. Patients were allowed to treat their acute migraine attacks with non-steroid anti inflammatory drugs (NSAIDs) or Triptans. During these 2 months the patients advised not to use any drugs without our permission. Every two weeks all patients were evaluated about Enalapril complications and the usage of other medications by an observer blinded to case and control groups. After these 2 months, all patients were recalled and complete examination was done, blood samples for biochemistry measurements (NO2/NO3, CRP) were drawn, and forearm FMD was performed.

In the current study we tested the hypothesis that using 10 mg Enalapril daily would improve endothelial function of patients with migraine according to chemical and functional markers.

The primary outcome was the FMD index which was appraise before and after two months of treatment.

And secondary efficacy measures were the levels of CRP and NO2 and NO3 which were assessed before and after two months of treatment.

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the ethical committee of the Isfahan University of Medical

Sciences. Written informed consent was obtained from all patients participating in the study. This trial is registered with Iranian Registry of Clinical Trials (IRCT), number IRCT138711011570N1.

Biochemical Measurements

Sample Preparation: 5 ml blood samples were obtained from the antecubital vein in supine position before the FMD study. Sera were separated by centrifugation at 4000 rpm for 10 minutes and then were aliquot and stored at -70°C until analysis.

CRP Assay: The serum levels of CRP were determined by ELISA kit according to manufactures' instruction (IBL, Hamburg, Germany). The sensitivity of the assay was less than 1 µg/ml.

NO Assay: The serum level of stable NO metabolites nitrite and nitrate were measured using a colorimetric assay (R&D Systems, Minneapolis, USA) based on Griess reaction as previously described.[11] For nitrite measurement, briefly, after serum addition into wells, sulphanilamide solution was added to all experimental samples, and after incubation, N-1-naphtylethylenediamine dihydrochloride solution was added. Then, absorbance was measured by a microreader in 540 nm wavelength. For nitrate measurement the enzymatic conversion of nitrate to nitrite by nitrate reductase was done. The reaction was followed by colorimetric detection of nitrite as an azo dye product of the Griess Reaction. This assay measures total nitrite by converting nitrate to nitrite. To determine the nitrate concentration, the endogenous nitrite concentration measured from the nitrite assay that was subtracted from the nitrite concentration measured in this procedure.

Flow Mediated Dilation (FMD): A high-resolution B-mode ultrasonographic system (ATL Ultrasound, HDI 5000, Bothell, Washington, USA) with a linear transducer mid-frequency of 7.5 MHz was used to determine FMD of the brachial artery. An expert blinded to case and control groups performed all FMDs. At first, patients laid back at rest for 10 minutes. Then baseline brachial artery diameter was determined by locating probe on 4-5 cm above the antecubital fossa of the non-dominant arm. After that a pneumatic tourniquet of a sphygmomanometer was inflated on the most proximal portion of the forearm to a pressure of 300 mmHg for 5 minutes. The cuff was then released and second scan was taken 30 seconds before and 90 seconds after cuff deflation. Artery diameters were determined with ultrasonic calipers from the leading edge of the anterior wall to the leading edge of posterior wall of the brachial artery at the end of diastolic period. 3 other observers supervised the procedures. Changes in diameter were computed as percentage relative to the baseline diameter. None of the patients were experienced headache during measurements. Also FMD was not measured during menstrual phase in female patients. All the FMDs were performed between 10 A.M. and 12 A.M.

Statistical Analysis

The results are expressed as mean ± standard error (SE). A normal distribution was proven with the test of Kolmogorov-Smirnov. Differences in FMD, serum NO_x and CRP concentrations between case and control groups were analyzed using the independent sample t test and differences within the groups (before and after treating with Enalapril or placebo) were determined by paired sample t test. Differences between the groups were examined by independent sample t test and inside the groups were determined by paired sample t test. P value of less than 0.05 was considered significant. Statistical analyses were performed using SPSS version 16.

Results

The Demographic Information

Forty patients with migraine without aura (IHS-1.1) were enrolled in this study, between July 2008 and June 2009. 6 (15 percents) of them were males and 34 (85 percents) were

female. The mean of patients' age was 34.42 ± 1.82 (with a range of 10-57 years). These patients were randomly divided to 21 and 19 subjects as the case and control group respectively. These people have suffered from migraine headaches for about 74.40 ± 7.54 months.

Endothelial Function Markers

Flow Mediated Dilation (FMD): Flow mediated dilation parameters among the case and control groups are demonstrated in table 1.

As illustrated in table 1, the mean of patients' FMD increased dramatically in the case group after treatment with Enalapril (p = 0.002) but in the control group the changes were not meaningful (p = 0.710).

And also in comparing within groups changes, FMDs were different significantly after treatment (p = 0.004) while that they were similar before the treatment (p = 0.317).

NO2/NO3 Concentration: As summarized in table 2, the NO2/NO3 concentrations increased in the Enalapril treated group and decreased in the control group significantly and also there was no significant difference between the means before treatment (p = 0.305), whereas the difference is very clear after 2 months of treatment (p = 0.000).

CRP Concentration: According to table 2, the CRP concentrations of both groups were not significantly different as the p values were 0.361 and 0.787 before and after treatment, respectively. And also the concentration changes did not differ meaningfully in both groups.

Discussion

In current double blind, placebo controlled, randomized clinical trial, we showed that Enalapril group had better endothelial function according to functional test (FMD) and serum nitrite concentration.

The most important aspect of the endothelial dysfunction is an impaired endothelium-dependent vasodilation, which is mainly due to reduced availability of NO and increased levels of vasoconstrictors such as endothelin and angiotensin II (AT II). This distorted balance induces an increase of oxidative stress, inflammation, and hypercoagulability state. It has been shown that angiotensin-converting enzyme inhibitors (ACEIs) have multiple beneficial effects on endothelial function.

It has been shown that endothelial dysfunction is mediated by impaired vascular reactivity and decreased NO bioavailability; an important factor in normal function of endothelial system.[4] There are many evidences suggesting that patients with migraine have changed reactivity in the systemic as well as cranial vasculature in the interictal period.[17,18] It has been reported that patients with migraine have decreased endothelium dependent function assessed by FMD and increased nitrate-mediated response in their brachial artery.[17]

Evidence of enothelial dysfunction in migraine is increasing. It has been shown that levels of von Willebrand factor (VWF), a reliable endothelial dysfunction biomarker, were significantly higher in patients with migraine than in non-headache controls during the interictal phase.[19,20]

Table1. Comparison of the FMD parameters between case and control groups through the study

Data are mean ± SE

	Case group			Control group			Two groups' comparison	
	Baseline	After treatment	P value	Baseline	After treatment	P value	Baseline p value	After treatment p value
BBD (mm)	3.39 ± 0.15	3.08 ± 0.12	0.039	2.98 ± 0.15	3.03 ± 0.96	0.638	0.071	0.738
PAD (mm)	3.62 ± 0.14	3.60 ± 0.15	0.849	3.26 ± 0.14	3.30 ± 1.02	0.692	0.098	0.128
FMD (%)	7.56 ± 1.60	16.53 ± 1.93	0.002	9.89 ± 1.64	9.06 ± 1.39	0.710	0.317	0.004

FMD: Flow Mediated Dilation; BBD: Basal Brachial Diameter; PAD: Peak Arterial Diameter

Table2. Comparison of the NO2/NO3 and CRP concentrations between case and control groups through the study Data are mean ± SE

	Case group			Control group			Two groups comparison	
	Baseline	After treatment	P value	Baseline	After treatment	P value	Baseline p value	After treatment p value
Nitrate ($NO3^-$)	44.25 ± 3.03	47.74 ± 3.62	0.384	54.89 ± 5.20	41.65 ± 2.39	0.016	0.074	0.100
Nitrite ($NO2^-$)	10.04 ± 1.94	15.63 ± 3.82	0.125	7.06 ± 1.18	5.94 ± 1.13	0.336	0.205	0.029
Total nitrite	56.30 ± 3.35	63.97 ± 3.38	0.046	61.95 ± 5.23	47.59 ± 2.02	0.012	0.305	0.000
CRP	1.84 ± 0.26	1.90 ± 0.34	0.891	2.15 ± 0.30	1.86 ± .35	0.313	0.361	0.787

In young, relatively healthy cohort of women, a strong relation between biomarkers of endothelial activation, including VWF activity, high sensitive CRP, tissue plasminogen activator antigen, and total nitrite/nitrate concentration and migraine has been demonstrated.[21] Migraine is associated with reduction in the number and function of endothelial progenitor cells, serving as a marker for dysfunctional endothelium.[22]

Our results showed that daily consumption of 10 mg Enalapril results in significant FMD increase as compared to before treatment and control group. In line of our results, ACEI therapy has been associated with improved FMD and increased NO bioavailability in several other diseases with endothelial dysfunction.[5-11,23-25]

Although, NO has been implicated in migraine attacks, but the role of NO in migraine remains unclear. There are several studies that have shown decreased or unchanged NO metabolites in patients with migraine in interictal periods.[26,27] It has been shown that decreased NO bioavailability is associated with decreased cerebral blood flow.[28] In our study, there is a significant change in patients' nitrite concentration. Among the NO metabolites nitrite is a major oxidative metabolite, which was implicated to be an indicator of NO synthase (NOS) activity. It has been shown that up to 70-90% of plasma nitrite is derived from endothelial NOS (eNOS) activity in fasted humans and others.[29] Furthermore plasma nitrite levels progressively decrease with increasing cardiovascular risk load.[29]

It is well known that ACE inhibitors augment endothelium-dependent vasodilation through an increase in NO bioavailability, by an increase in NO production and a decrease in NO inactivation.

It has been shown that the ACEI therapy increased eNOS protein expression and cardiac NOS activity, however, inducible and neuronal NO synthase expression was not changed by the ACE inhibition.[25] So, it can be assumed that Enalapril might increase the eNOS expression in vessels of patients with migraine in our study. ACEIs suppress angiotensin II production and inhibit break down of bradykinin leading to increase in nitric oxide production.

Angiotensin II induces the production of reactive oxygen species and stimulates the expression of adhesion molecules and cytokines, leading to endothelial dysfunction, adhesion and invasion of leukocytes. So, ACEIs may also be contributed to increased NO bioactivity by limiting oxidative degradation of NO.[15]

Vascular inflammation is another downstream result of endothelial dysfunction. In our study, contrary to our hypothesis, Enalapril supplementation could not decrease CRP significantly. Similar results have been recently attained from ACEIs therapy in patients with several other endothelial dysfunction associated diseases.[30,31] These finding may suggest that beneficial effects of ACEIs cannot be explained by anti inflammatory action of these drugs.

Conclusions

In conclusion, our study revealed that Enalapril can possibly improve endothelial function. There are evidences confirming beneficial effects of ACE inhibitors and angiotensin receptor antagonist for migraine prophylaxis.[32,33] In this study the only recorded side effect of this drug was cough; so this drug is tolerable.

Endothelial dysfunction, which is more prevalent in patients with migraine, may be the underlying pathophysiology of stroke, myocardial infarction and other vasculopathies in these patients. An improved understanding of the role of the endothelial system in migraine may provide a basis for stroke prevention in these patients, including use of drugs that can prevent migraine, and restore the endothelial function simultaneously.

The limitation of our study was small sample size and non-cross over design.

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Conflict of Interests

Authors have no conflict of interests.

Authors' Contributions

SHJ carried out the design of the study, carried out the experiments, did the statistical analysis and participated in manuscript preparation. SAlS provided assistance in the design of the study, coordinated and carried out the experiments and participated in manuscript preparation and did the statistical analysis. KHG provided assistance in the design of study and carried out the experiments and participated in manuscript preparation. MS provided assistance in the design of the study and executed the FMDs and participated in manuscript preparation. SAhS collected the patients and performed the physical examinations and completing the questionnaires. All authors have read and approved the content of the manuscript.

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