Original article

Oral L-citrulline malate in patients with idiopathic pulmonary arterial hypertension and Eisenmenger Syndrome: A clinical trial

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ABSTRACT

Background and purpose: Citrulline is an amino acid which is produced by the urea cycle and also a precursor for NO, that is, a vasodilator for normal function of pulmonary vasculature. Thereby, enhancing L-citrulline malate in patients with idiopathic pulmonary arterial hypertension and those with congenital heart disease identified as Eisenmenger Syndrome results in reduction of pulmonary hypertension.
Methods and subjects: In this clinical trial before and after study, we assigned 25 patients with arterial pulmonary hypertension (idiopathic or Eisenmenger Syndrome) to receive L-citrulline malate 1 g three times daily for two weeks. The primary measurement was the change in exercise capacity, as considered as a result of the total distance walked in six minutes, from baseline to week 2. We also assessed mean pulmonary artery pressure, the change in the quality of life, and the change in pro-brain natriuretic peptide (BNP) level. The study was not powered to evaluate mortality.
Results: The mean walking distance in six minutes was significantly increased by about 44 m ($p = 0.005$) after receiving L-citrulline malate. Mean pulmonary artery pressure significantly reduced from 83.34 mmHg before receiving L-citrulline malate to 79.1 mmHg after that ($p = 0.01$). All dimensions of the quality of life had statistical differences after receiving L-citrulline malate except limit due to physical health, limit due to emotional health and social functioning ($p > 0.05$). Finally, pro-BNP difference was not statistically significant ($p = 0.9$).
Conclusion: L-Citrulline malate improves the distance walk in six minutes and also the quality of life of patients with idiopathic arterial pulmonary hypertension and Eisenmenger Syndrome and also reduced mean arterial pulmonary hypertension.

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Introduction

Pulmonary vascular tone is preserved as a result of the action of vasoprotective compounds such as nitric oxide (NO) [1]. The anion nitrite (NO_2^-) can produce NO endogenously in the body by the use of L-arginine and aNOS-independent mechanism [2,3]. Citrulline is an alpha amino acid which is generated by the urea cycle; through its metabolism endogenous NO can be formed. And

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it is metabolized to L-arginine in the vascular endothelium, kidney, and other cells. Oral administration of citrulline is more effective than L-arginine in producing an increase in the blood levels of L-arginine. As a result of not being a substrate for arginase, citrulline does not undergo intestinal or hepatic metabolism. So it does not induce its expression and activation. Therefore L-citrulline holds promise in the treatment of endothelial dysfunction, and perhaps of cardiovascular disease, in which L-arginine deficiency and bioavailability of NO are involved [4,5].

Cyclic guanosine monophosphate which is the ultimate product of NO results in vasodilatation of the pulmonary vasculature [6]. NO as a vasodilator is important for the normal function of the pulmonary vasculature. Loss of this mediator and the subsequent endothelial dysfunction is hypothesized to be a contributing reason for progress of pulmonary hypertension [1].

An important category of patients with pulmonary arterial hypertension (PAH) is idiopathic pulmonary arterial hypertension (IPAH) that has indefinite etiology and is defined by progressive destruction of small- and medium-sized pulmonary arteries and an increase in pulmonary arterial pressure and pulmonary vascular resistance. Occurrence of these pathologies finally leads to right heart failure and death [7]. And recent studies demonstrated that, early determination of PAH can be done by simple exercise echocardiography using the Master's two-step test [8].

Another category is congenital heart disease such as Eisenmenger Syndrome. In this category resistance of pulmonary vasculature is low and blood flow of the vasculature is high [9–11]. One of the therapeutic advances for IPAH is oral use of NO precursors, as a result of its vasodilatory features [12]. Efficacy of L-arginine is well-tested in the literature but there is a lack of data considering L-citrulline malate. Also based on other trials, combination therapy of two types of oral drugs, endothelin receptor antagonists and phosphodiesterase V inhibitors is an improvement in PAH therapy [13]. Therefore, this study will assess therapeutic efficiency of L-citrulline malate in two categories of patients with IPAH and Eisenmenger Syndrome. This randomized clinical trial employs the 6-min walk test as a marker of functional improvement of the patients, *pulmonary arterial pressure (PAP)*, the quality of life, and level of plasma pro-brain natriuretic peptide (BNP) in these patients.

Methods

Patient selection

Patients with PAH (idiopathic, congenital systemic-to-pulmonary shunts whether repaired or not) were included in this study.

Pulmonary hypertension has been characterized as an augmentation in mean PAP ≥ 25 mmHg at rest as evaluated by right heart catheterization (RHC) [14,15]. A 7.5 F, balloon-tipped, thermodilution catheter was positioned in the pulmonary artery for measurement of PAP, right atrial pressure, and pulmonary capillary wedge pressure for subjects. Standard formulas were used to calculate CI (cardiac index), systemic vascular resistance, and either pulmonary vascular resistance (PVR = [mean PAP − pulmonary capillary wedge pressure]/CO (cardiac output) or total pulmonary resistance (TPR = mean PAP/CO)) if the wedge position could not be reliably maintained.

Pulmonary pressures were also anticipated by Doppler echocardiography with the following diagnostic criteria: (1) significant tricuspid regurgitation, (2) enlarged or hypertrophied right ventricle without evidence of pulmonary stenosis, or (3) intraventricular septal flattening [16,17]. The ethics committee for human research of Shahid Beheshti University of Medical Sciences has reviewed and accepted the entire study protocol. A written well-versed

permission was attained from all patients. Our study protocol required all patients to continue their conventional therapy. The study protocol was registered in clinicaltrial.gov and the identifier number is NCT01683981.

Exclusion criteria of this study were: patients older than 70 years, patients with a walking distance in six minutes which is <100 meters (m), active pulmonary or extra pulmonary infection, serious coronaropathy and/or ventricular dysfunction, significant renal illness and/or hepatitis, detected immunosuppressive illnesses, carrier of known neoplasias, pregnancy, not having family support, psychosocial problems, drug or alcohol abuse, and negative response with established medical protocol.

Outcome measures

The primary measurement was the change in exercise capacity, as measured by means of the total distance walked in six minutes, from baseline to week 2. Other measurements were the changes in mean PAP, also the change in the quality of life, and the change in pro-BNP level. Quality of life measures were assessed according to the Short Form (SF)-36 Health Survey questionnaire. Adverse events were monitored throughout the study period.

Study protocol

Twenty-five patients were enrolled in this clinical trial, before and after study at the PAH clinic in the National Research Institute of Tuberculosis and Lung Disease (NRITLD) between August and October of 2012. This study recruited 20 patients (4 males and 16 females) with IPAH and a functional class II or III according to the diagnostic criteria defined by the European Society of Cardiology (ESC). This study also included 5 patients (3 males and 2 females) with Eisenmenger Syndrome with no complex congenital heart disease. All patients underwent an echocardiography as part of the initial diagnostic work up even if they had been diagnosed as having PAH. Furthermore, a six-minute walk test and a pro-BNP level were taken. L-Citrulline 1 g was administered as dry powder that should be diluted in 250 mg of water. Patients were followed for 2 weeks after being given L-citrulline malate 1 g by the 6-min walk test, pro-BNP level, and echocardiographic index such as PAP.

Statistical analysis

Continuous variables are presented as mean ± SD. For the categorical variables frequency and percentage were reported. The first and fourth end points, the six minute walking test and the level of pro-BNP were evaluated with the use of a Wilcoxon's rank-sum test. The secondary end point (mean PAP) was assessed with independent samples *t*-test. Quality of life was evaluated with chi-square test. Statistical significance was defined as a *p*-value lower than 0.05. All data were analyzed with SPSS version 17 for Windows (SPSS, Chicago, IL, USA).

Results

A total of 25 patients were assigned to receive L-citrulline malate at a dose of 1 g divided 3 times a day for two weeks.

Baseline characteristics

Baseline characteristics were not statistically different among all 25 patients. Seventeen patients (74%) were female and eight (26%) were male (Table 1). The mean age of the patients was 31.2 ± 9.27 years. The youngest participant was 18 years and the oldest was 51 years. Two patients were excluded from the study due to the adverse reactions of the prescribed drug.

Table 1
Baseline characteristics of the patients.

Female (%)	17 (74%)
Age (years) (mean ± SD)	31.2 ± 9.27
6 min walk (mean ± SD)	351.6 (116.4)
PAP (mean ± SD)	83.34 (22.4)
SF36 (mean ± SD)	
Physical functioning	61.5 (11.3)
Limit due to physical health	95.7 (14.4)
Limit due to emotional health	97.1 (9.5)
Energy-fatigue	54.8 (16.8)
Emotional well being	65.1 (7.8)
Social functioning	87.5 (0)
Pain	89.0 (15.9)
General health	60.0 (12.4)
Pro-BNP	339.7 (247.4)

PAP, pulmonary arterial pressure; SF36, Short Form 36 Health Survey; BNP, brain natriuretic peptide.

Table 2
Analysis of mean change of walking distance at 6 min, pulmonary arterial pressure and Short Form 36 from before to after receiving L-citrulline malate.

	Mean difference before receiving L-citrulline malate–after receiving L-citrulline malate (CI)	p-Value
Walking distance at 6 min	−44.1 (−73.2 to −15.1)	0.005
Pulmonary arterial pressure	4.2 (1.0–7.4)	0.01
SF36		
Physical functioning	−2.8 (−5.1 to −0.58)	0.02
Limit due to physical health	Not changed	–
Limit due to emotional health	Not changed	–
Energy-fatigue	−5.4 (−7.9 to −3.0)	<0.001
Emotional well being	−0.95 (−1.8 to −0.1)	0.02
Social functioning	Not changed	–
Pain	−2.2 (−4.0 to −0.4)	0.02
General health	−2.0 (−3.2 to −0.7)	0.004
Pro-BNP	−2.4 (−76.8 to −72.0)	0.9

SF36, Short Form 36 Health Survey; BNP, brain natriuretic peptide.

Exercise capacity

Distance walked in six minutes was increased after receiving L-citrulline malate compared to baseline ($p = 0.005$) (Table 2). No statistically significant difference between males and females was observed in the distance in six-minute walk ($p = 0.34$) (Fig. 1). Age was the other variable that was shown to be independent of the mean walked distance in six minutes before and after receiving L-citrulline ($p = 0.08$) (Fig. 2).

Pulmonary arterial pressure

Mean PAP before receiving L-citrulline malate (83.34 mmHg) (Table 1), as compared to its level after the treatment period (79.1 mmHg) (Table 1), was significantly lower ($p = 0.01$) (Table 2).

Quality of life

In all dimensions of the quality of life questionnaire, after receiving L-citrulline malate, the obtained score was higher or it was identical to its baseline level (Table 1). There was a significant difference between mean physical functioning ($p = 0.02$), energy-fatigue ($p < 0.001$), emotional well-being ($p = 0.02$), pain ($p = 0.02$), and general health ($p = 0.004$) (Table 2). There were no changes in dimensions such as limit due to physical health, limit due to emotional health, and social functioning.

Pro-BNP

Comparing pro-BNP before with after receiving L-citrulline malate, our results disclosed that it was higher after receiving L-citrulline malate and the difference was not statistically significant ($p = 0.9$) (Table 2).

Safety

Most adverse events were mild to moderate for all participants. The encountered adverse reactions of L-citrulline malate in the present study were edema in terminal limbs; urine frequency; increased cough; and heartburn. These were seen in two patients.
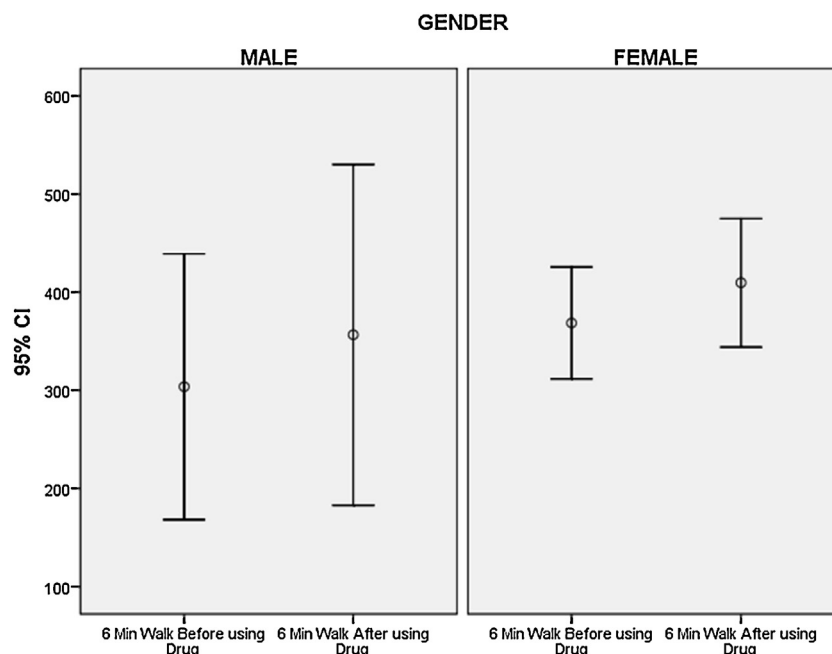

Fig. 1. Bar chart of walking distance at 6 min before and after using L-citrulline malate separated by gender.
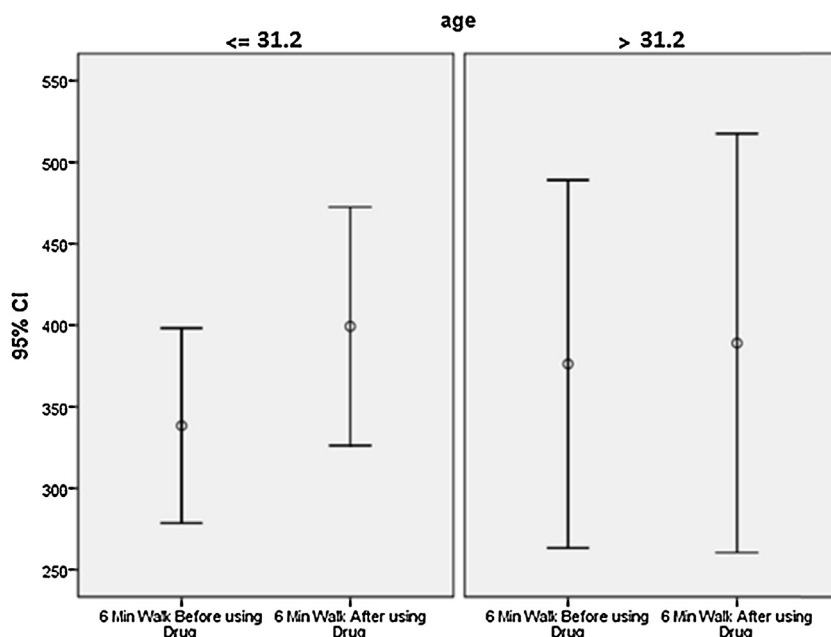
Fig. 2. Bar chart of walking distance at 6 min before and after using ʟ-citrulline malate separated by the mean age (upper and lower mean age).

Discussion

In this before–after clinical trial, ʟ-citrulline malate significantly improved exercise capacity, as assessed according to the six-minute walking test, in patients with PAH, whether it was idiopathic or it was due to congenital systemic-to-pulmonary shunts. This study was not designed to evaluate mortality. In the pulmonary vasculature, NO is formed from the metabolism of ʟ-arginine to ʟ-citrulline, two amino acids produced by the urea cycle. ʟ-Citrulline to ʟ-arginine salvaging in endothelial cells by the enzymes arginosuccinate synthetase and arginosuccinate lyase is proposed to be the principal mechanism for sustaining local ʟ-arginine availability for eNOS-catalyzed NO production [18]. Thus far, it has been shown that polymorphisms in the gene encoding carbamoyl-phosphate synthetase 1, the enzyme that produces ʟ-citrulline in the urea cycle, affects NO metabolite concentrations and its subsequent vasodilation [19]. Previous evidence suggests that in infants with persistent PAH of the newborn or after bypass surgery for congenital heart disease, plasma concentrations of arginine, citrulline, and NO metabolites are all low and this can be reversed by oral citrulline supplementation [20,21].

The distance walked in six minutes can be used as an independent predictor of death in patients with IPAH [22] and has been used as the primary measurement in most clinical trials concerning patients with PAH [23]. The treatment-correlated augmentation in six minute walking test of 44 m observed in this study is alike to the increases observed with the use of oral bosentan (44 m) [24] and is higher than the increase seen with the use of subcutaneous treprostinil (16 m) [25] and inhaled iloprost (36 m) [26] and is less than intravenous epoprostenol (47 m) [27] and with the use of oral sildenafil citrate (45–50 m) [28]. All patients in this study had PAH of WHO class II or III, which is not a measure of the severity of their condition. Other trials demonstrate that the sickest patients (those with PAH of WHO class III or IV) had the greatest improvement in the six-minute walking distance. Previous studies have shown that short-term administration of ʟ-arginine can improve endothelial dysfunction, which is related to increase exercise capacity in patients with congestive heart failure [29,30].

Thus, it is also possible that the increase in exercise capacity with ʟ-citrulline malate, as compared to ʟ-arginine, may be partly attributable to improvement in endothelium-dependent peripheral vasodilation in patients with PAH. Abnormalities in endogenous vasodilator substances such as NO have been proposed as being important in the development of PAH [31–33]. The biologic actions of ʟ-arginine, the precursor of NO, have been examined in a variety of cardiovascular diseases [29,34–36]. However, the therapeutic potential of orally administered ʟ-citrulline malate in patients with PAH remains unknown.

The consequence of the oral supplementation of ʟ-citrulline malate was a significant decrease in mean PAP and was similar to that observed with intravenous epoprostenol [27] and oral bosentan [24] in smaller studies. These findings suggest that ʟ-citrulline malate may cause pulmonary vasodilation at least partly via a NO-mediated mechanism.

ʟ-Citrulline malate also significantly improved the quality of life in these patients. Physical functioning, energy-fatigue, emotional well-being, pain, and general health were all improved in the present study. There were no changes in limits due to physical health, limits due to emotional health and social functioning. This study also demonstrated no reduction in pro-BNP plasma level of these patients, but one-week supplementation of ʟ-arginine tended to decrease plasma atrial natriuretic peptide and BNP, potential markers of right ventricular dysfunction [37,38]. With this dose of ʟ-citrulline malate, the main adverse events were mild to moderate and were not clinically significant. Intravenous epoprostenol has shown complex delivery systems, significant side effects, or both (e.g. catheter-related infections, sepsis, and pump malfunctions) [27], subcutaneous treprostinil (infusion-site pain) [25], inhaled iloprost (multiple daily inhalations) [26], and oral bosentan (abnormalities of hepatic function) [24].

This study has certain limitations. Lack of a control group might be named as the major pitfall of this study. Moreover, our patients were selected from those with minimal disease progression and hence our results cannot be extended to patients with severe forms of the disease. The short treatment period and not measuring laboratory data such as NO pathway, nitrite/nitrate, or cGMP and arginine metabolites in platelets were the other limitations of this trial. More randomized controlled trials with larger sample size with measurement of the NO pathway or arginine metabolites by blood samples are needed to confidently use our results in routine practice.

Conclusion

Short-term oral administration of L-citrulline malate modestly decreased PAH and improved exercise capacity and quality of life without serious adverse effects in patients with PAH (IPAH, Eisenmenger Syndrome). The study was not designed to attend to the important end point of mortality.

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