Evaluation of urolithin A efficacy in heart failure patients with reduced ejection fraction: A randomized, triple-blind, cross-over, placebo-controlled clinical trial

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Abstract

Background: Mitochondrial dysfunction and impaired mitophagy are integral to myocyte loss and the progression of heart failure. Urolithin A (UA), a microbiota-produced metabolite of ellagitannins and ellagic acid, is a known stimulator of mitophagy and mitochondrial biogenesis that has shown cardioprotective effects in experimental models.

Methods: A randomized, double-blind, placebo-controlled 2×2 cross-over trial was conducted in 10 patients with HF with reduced ejection fraction (HFrEF). The trial design involved two 4-week intervention periods of UA (500 mg BID) and placebo, separated by a 2-week washout phase. Patients underwent two-dimensional echocardiogram examination as well as blood sampling at the beginning and end of each period.

Results: All patients completed the study. The results failed to reveal any significant effect of UA supplementation on echocardiographic measures (LVEF, LVEDD, LVESV, and TAPSE). Nor were plasma concentrations of pro-BNP, glucose, and CRP ($p>0.05$) altered. Serum HDL-C levels were increased with UA compared with placebo (+6.46±2.33 mg/dL, $p=0.026$), whereas other lipid indices (LDL-C, triglycerides, total cholesterol, and VLDL-C) remained unchanged ($p>0.05$).

Conclusion: The results of the present study do not support any positive effect of UA supplementation in improving echocardiographic and biochemical indices of HFrEF. Further studies with higher doses of UA and longer supplementation duration are encouraged to be conducted.

Keywords: Mitochondrial dysfunction, Urolithin A, ellagic acid, Cardioprotective, Echocardiography, Heart Failure

Trial registration no. IRCT20210216050375N1

1. Introduction

Cardiovascular disease (CVD) remains one of the leading causes of premature death and increasing medical care expenses throughout the world (1-3). Cardiovascular diseases encompass a wide range of disorders affecting the heart, brain, and blood vessels, such as myocardial infarction, abnormal heart rhythms, angina, stroke, heart failure, cardiomyopathy, hypertensive heart disease, and so on (4, 5). Heart failure is a chronic and potentially fatal disorder caused by structural and functional deficiencies in the myocardium, which impairs the ability of heart to pump blood adequately enough to meet metabolic demands or accommodate systemic venous return all the time (6-8). In the early stages of heart failure, it may be asymptomatic, but gradually, with the progression of the disease, shortness of breath during activity and, in more severe cases, at rest, dyspnea from pulmonary congestion, orthopnea while lying down, weakness, premature fatigue, and lethargy from heart failure-induced circulation-related disorders in skeletal muscles, peripheral edema, and ascites from impaired venous return may occur (6, 7). Decreased left ventricular myocardial function is the most prevalent cause of heart failure; however, heart failure can also be caused by impairment of the pericardium, endocardium, myocardium, heart rate/rhythm, heart valves, or great vessels, either alone or in combination (9, 10). Treatment can be determined based on the type, stage, and class of heart failure. First-line drug therapy for patients with heart failure include angiotensin-converting enzyme (ACE) inhibitor (such as captopril, enalapril), beta blocker (such as atenolol and metoprolol), angiotensin receptor-neprilysin inhibitor (ARNI) (such as valsartan/sacubitril) and a sinoatrial node modulator (ivabradine). Surgery, and in more severe cases, heart transplants, are other treatments available for heart failure (11, 12). Despite the fact that these treatments can reduce the risk of death from the disease to some extent, the mortality

rate of this disease remains high. Therefore, the development of new medications with novel mechanisms for treating this group of patients is critical.

Cardiac cell mitochondria are vital for cardiovascular function because they provide energy in the form of ATP. Mitochondrial dysfunction is a critical factor in myocyte loss and the progression of heart failure. Mitophagy helps to stabilize mitochondrial structure and function, as well as cell survival and proliferation, by degrading damaged and dysfunctional mitochondria (13, 14).

Urolithins, also known as benzocoumarins, are secondary metabolites of ellagitannins and ellagic acid produced by the gut microbiota in the distal part of the gastrointestinal tract (15-17). A recent phase I clinical trial on the safety of urolithin A in elderly people confirmed previous findings and revealed that urolithin A is safe up to 2,500 mg/day with no major side effects or abnormal changes in laboratory test values (18). Furthermore, urolithin A dosages greater than 500 mg/day for 28 days improved mitochondrial biomarkers (19). Urolithin A not only activates the mitophagy process, it also stimulates mitochondrial biogenesis in skeletal muscle (19). Numerous studies investigated cardioprotective effects and anti-atherosclerotic activity of urolithin A using *in vitro* and *in vivo* experiments (20-22). Findings from urolithin A pretreatment in a mouse model of myocardial reperfusion injury and myocardial cells with hypoxia/reoxygenation injury showed that lactate dehydrogenase (LDH) leakage significantly reduced and cell viability increased in myocardial cells. It also decreased myocardial apoptosis and prevented reactive oxygen species (ROS) generation. Urolithin A treatment also improved heart function and reduced the size of myocardial infarction in ischemia-reperfusion mice (23).

In the present study, we designed a randomized, double-blind, placebo-controlled 4-week treatment period cross-over study with a 2-week washout phase to evaluate the efficacy of urolithin A in heart failure patients with reduced ejection fraction.

2. Method and material

Study Design

We designed a pilot randomized, double-blind, placebo-controlled 4-week treatment period cross over study with a 2-week washout phase in which each patient received urolithin or placebo (24). The washout period was considered effective and not to have influenced the results during the crossover design, as UA has a half-life of ~24 h with elimination following a single oral dose occurring after between 3 and 4 days (19). Mashhad University of Medical Science's Ethics Committee approved the study protocol and revisions. The research was carried out in accordance with the Helsinki Declaration standards. Before the start of the study, all patients gave written informed consent. The trial protocol was registered in the Iranian Registry of Clinical Trials (ID: IRCT20210216050375N1**)** (Ethics code: IR.MUMS.REC.1399.629.).

Study patients

Ambulatory male patients with heart failure who were 30-60 years old and receiving routine medical therapy were eligible. Patients were required to have a left ventricular (LV) ejection fraction (EF) of 40% or less and symptomatic heart failure [New York Heart Association (NYHA) class II–IV] despite maximum tolerated medical therapy. To reduce the heterogeneity in this small trial, only a single gender was selected. Given the higher prevalence of HFrEF in males, this gender was chosen for the study.

Exclusion criteria included having a systolic blood pressure greater than 180 mm Hg and a diastolic blood pressure greater than 100 mm Hg, not being able or willing to give informed consent, or having an estimated life expectancy of fewer than 12 months due to a cause other than heart failure,

a history of malignancy, hepatic failure (Aminotransferase >2 upper limit of normal), renal failure ((eGFR) < 20 ml/1.73 m2/min) and acute myocardial infarction (MI) in last two weeks.

Throughout the study, patients, investigators, outcome evaluators, and data analysts were not aware of therapy allocation. The appearance, texture, color, and taste of placebo (Avicel®microcrystalline cellulose (MCC)) and urolithin capsules were very comparable. For four weeks, Group A individuals were given oral urolithin (250 mg BID) in combination with conventional heart failure medication. Following a 2-week washout period, patients in Group A received oral placebo capsules and conventional heart failure medication for 4 weeks. Group B patients received the opposite procedure. During the washout phase, patients received only conventional heart failure medication. Patients had baseline examinations, including an echocardiogram (using a Samsung SE70) and laboratory assessments, both before and after enrolment (lipid profile, ProBNP, FBS, CRP).

Two-dimensional echocardiogram examination

Echocardiography was performed on each patient before and after each phase. A 17-segment echocardiogram was used to measure left ventricular end-systolic volume (LVESV), left ventricular end-diastolic diameter (LVEDD), and left ventricular ejection fraction (LVEF) and Tricuspid annular plane systolic excursion (TAPSE), was analyzed independently by two experienced observers who were unaware of the patients' treatment assignments.

Blood Sampling

After 12 hours of fasting, blood samples for laboratory assays were taken on the day of sampling. For each patient, blood samples were taken four times (before and after starting each period).

Routine Biochemical Analysis

Each subject had a complete fasting lipid profile that included total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), LDL-C, and VLDL. Serum lipid and FBS concentrations were determined enzymatically using commercial kits.

Statistical analysis

All measured values are represented as mean (SD). The standard 2×2 crossover design with baseline values was used to evaluate the treatment effect (drug versus placebo). In this design, subjects were randomly allocated to either the drug-placebo sequence or the placebo-drug sequence. In the drug-placebo sequence, the drug was given in the first period, followed by the placebo in the second period, whereas in the placebo-drug group, the placebo was given before the drug. Accordingly, the model can be used to describe the standard 2×2 crossover design as follows (24):

$$Yijk=\mu+Sik+Pj+Tj,k+Cj-1,k+Baselineijk+eijk$$

where,

Y_{ijk} is the response of the i^{th} subject in the k^{th} sequence at the j^{th} period,

subject $i = 1, 2, \cdots, n_k$ (i^{th} subject in k^{th} sequence)

period $j = 1, 2$ (First or second)

sequence $k = 1, 2$ (Drug-Placebo or Placebo-Drug)

Baselineijk is the baseline values of markers of the i^{th} subject in the k^{th} sequence at the j^{th} period.

Before testing the treatment effect, some factors such as carryover effect, period effect, and sequence effect were evaluated. Carryover effect refers to the residual impact of a treatment on subsequent periods, even after discontinuation. Period effect refers to the influence of time on study outcomes, meaning that the same treatment may have different effects at different times. Sequence effect refers to the order in which treatments are administered. To assess these effects in the data, a general linear model with analysis of variance constructed and executed using specific codes. The model included variables such as sequence (Drug → Placebo or Placebo → Drug), subject (patients), period (1 or 2), and treatment (Drug or Placebo).

3. Results

From 10 subjects who entered our study, all of them completed the study. The mean age of subjects was 38.3 ± 5.35. The prevalence of smoking was 10 % (Table 1).

Effect of Administration of Urolithin Versus Placebo on Biochemical Parameters

Results showed that neither the carryover effects nor the period effects were statistically significant for all variables. Therefore, the treatment effect can be reported for all variables using a crossover design. The treatment effect was only significant for HDL (p-value = 0.026). Accordingly, HDL levels in the treatment group were 6.46 units higher than in the placebo group (Table 2).

Effect of Administration of Urolithin Versus Placebo on Echocardiographic Indices

Results showed that neither the carryover effects nor the period effects were statistically significant for all variables. Therefore, the treatment effect can be reported for all variables using a crossover

design. The results failed to reveal any significant effect of urolithin treatment on LVEF, LVEDD, LVESV, and TAPSE values ($p > 0.05$) (Table 3).

Discussion

The results of the present study do not support any beneficial effects of UA supplementation in improving echocardiographic and biochemical indices of HFrEF. However, (to the best of our knowledge), this is the first clinical trial of UA in this setting. A single 'negative' trial should not be taken as conclusive proof that the intervention is ineffective, for a number of reasons:

Firstly, it is possible that the trial was under-powered to detect differences in key outcomes (despite the power being increased by the crossover design). Secondly the dose and duration of the study may have been suboptimal. Longer trials with a greater number of participants and higher doses of UA may show benefit. The lack of adverse effects (mild digestive discomfort such as nausea, diarrhea, or stomach cramps) in this trial would provide confidence in undertaking such an approach. Considering that the mechanism of action of UA is thought to result from promoting mitophagy (19), rather than by acting through a rapid signaling mechanism involving receptors and intracellular second messengers, it may be the case that a longer follow-up was warranted, than for a drug which might be expected to have an immediate mechanism of action.

The 'nutraceutical' approach to therapy relies upon identifying the active ingredients in natural products which bestow health benefits, and presenting them in a pharmaceutical formulation produced according to the principles of Good Manufacturing Practice (25). Once the product is marketed, it can undergo pharmacovigilance (or nutrivigilance) in the same way as conventional therapeutics in order to detect adverse effects (26). However, the most appropriate dose of UA to use in a nutraceutical formulation is hard to determine, owing to the fact that UA is usually produced by microbiota (15-17) in which case bio-ability may differ substantially from an orally-

ingested preparation. As such, we cannot be sure that the apparent lack of effectiveness of UA in this study was not a result of insufficient concentrations of UA reaching cardiac tissues.

Thirdly, it should be noted that participants in the trials were already taken guideline-directed therapies for heart failure. As such a ceiling effect may have made it difficult to observe further benefit being with the addition of another agent. It is possible that the 'nutraceutical' effect of UA supplementation may be optimal where patients are not already taking a range of existing therapies. Conversely, it may be the case that UA has greater potential for effectiveness in older, sicker patients (the participants in this trial had a mean age of 38, which is young for a heart-failure population). Either way, larger trials with greater diversity in population are warranted to exclude the possibility that this trial has overlooked s genuine beneficial effect of UA supplementation.

It is interesting to note that treatment with UA was associated with an increase in HDL, the mechanism of such an effect, and the implications are not clear. Whilst raised HDL-C is strongly associated with improved cardiovascular disease outcomes in a general population, interventions to increase HDL-C artificially have not consistently resulted in reduced cardiovascular events (27). Nevertheless, the HDL-raising properties of UA should be further investigated as a potential route to ameliorate the development of atherosclerotic disease processes.

Strengths and weaknesses of the study

This study was rigorously conducted as a 2×2 crossover design with triple blinding, and a crossover design such that patients acted as their own controls, thereby reducing variability in the data. A washout period was employed to prevent contamination between the two treatment periods. The endpoints used were objective and carefully measured. Together, the use of a placebo, objective outcome measures and blinding reduces the likelihood of erroneous results being

reported as a result of the nocebo/drucebo effect whereby a change in symptoms is reported as a result of patient expectation, rather than owing to a pharmacological effect of the intervention (28). Nutraceutical approaches to the treatment of debilitating conditions such as heart failure may be particularly susceptible to the drucebo effect owing to patient and physician's hopeful expectation of benefit of a 'natural' treatment.

The main weakness of the trial is that we cannot rule out the possibility of a Type II (false negative) error owing to insufficient statistical power or the choice of a suboptimal dose of UA or duration of treatment. It should also be noted that the trial was designed to evaluate imaging parameters and biomarkers, rather than hard clinical outcomes. 'Beneficial' changes in these surrogate endpoints would not have guaranteed reduction in hard clinical outcomes.

Conclusions

The results of the present study do not support any positive effect of UA supplementation in improving echocardiographic and biochemical indices of HFrEF at the dose and duration of follow-up employed in this study. However, the lack of any safety signals at this dose and duration of treatment should provide researchers with the confidence to undertake longer trials with larger doses of UA.

Graphical abstract: Urolithin A efficacy in symptoms of heart failure patients with reduced ejection fraction

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Table 1. Subject Characteristics

Variable	
Gender (male)	10 (100)

Age (years)	38.3±5.35
HTN	3 (30)
HLP	1 (10)
T2DM	0
Smoking	1 (10)
FBS (mg/dL)	92.00±15.87
Chol (mg/dL)	171.30±54.01
TG (mg/dL)	187.70±255.70
HDL (mg/dL)	45.90±11.17
LDL (mg/dL)	103.50±29.78
VLDL (mg/dL)	37.60±51.16
CRP (mg/dL)	9.34±14.13
ProBNP (pg/mL)	2042.30±3290.31

Data given as n (%) or mean (SD)
Abbreviation: HTN: hypertension, HLP: hyperlipidemia, T2DM: type 2 diabetes mellitus, FBS: Fasting blood Sugar, Chol: Cholesterol, TG: Triglyceride, HDL: High density lipoprotein, LDL: low density lipoprotein, VLDL: very low-density lipoprotein, CRP: C-reactive protein, ProBNP: pro b-type natriuretic peptide,

Table 2. Effect of Administration of urolithin Versus Placebo on biochemical Parameters, the Analysis of Variance Table for a Standard 2 × 2 Crossover Design controlling the pre values

Dependent variable	Effects	Mean difference (SE)	F value	P-value
FBS (mg/dL)	Sequence	-----	1.03	0.341
	Period	-----	0.90	0.374
	Treatment		0.38	0.555
	Drug vs. Placebo	4.22 (6.82)		
	Pre FBS	-----	2.78	0.140
Chol (mg/dL)	Sequence	-----	1.25	0.296
	Period	-----	0.44	0.529
	Treatment		0.23	0.650
	Drug vs. Placebo	7.59 (15.98)		
	Pre Chol	-----	18.14	0.004
TG (mg/dL)	Sequence	-----	1.82	0.215
	Period	-----	0.04	0.844
	Treatment		1.60	0.246
	Drug vs. Placebo	-29.67 (23.45)		
	Pre TG	-----	159.15	<0.001
HDL (mg/dL)	Sequence	-----	0.27	0.617
	Period	-----	0.00	0.974
	Treatment		7.86	0.026
	Drug vs. Placebo	6.46 (2.31)		
	Pre HDL	-----	0.08	0.789
LDL (mg/dL)	Sequence	-----	0.80	0.398
	Period	-----	0.05	0.829
	Treatment		3.28	0.113
	Drug vs. Placebo	10.31 (5.69)		
	Pre LDL	-----	1.15	0.320
VLDL (mg/dL)	Sequence	-----	1.89	0.206
	Period	-----	0.05	0.826
	Treatment		1.66	0.238
	Drug vs. Placebo	-6.06 (4.70)		
	Pre VLDL	-----	158.89	<0.001
CRP (mg/dL)	Sequence	-----	0.08	0.788
	Period	-----	2.28	0.175
	Treatment		0.01	0.937
	Drug vs. Placebo	-0.34 (4.16)		
	Pre CRP	-----	0.77	0.410
ProBNP (mg/dL)	Sequence	-----	0.51	0.494
	Period	-----	0.22	0.656
	Treatment		0.05	0.834
	Drug vs. Placebo	-71.74 (330.62)		
	Pre ProBNP	-----	48.83	<0.001

Abbreviation: FBS (Fasting blood Sugar), Cho1 (Cholesterol), TG (Triglyceride), HDL (High density lipoprotein), LDL (low density lipoprotein), VLDL (very low-density lipoprotein), CRP (C-reactive protein), ProBNP (pro b-type natriuretic peptide), ESV (End-systolic volume), EF (Ejection fraction), LVEDD (left ventricular end-diastolic diameter), TAPSE (Tricuspid Annular Plane Systolic Excursion). Sequence (the order of treatment administration in a crossover experiment), Period (time of a treatment administration). Pre means baseline.

Table 3. Effect of Administration of urolithin A versus placebo on echocardiographic indices. The Analysis of Variance Table for a Standard 2 × 2 Crossover Design controlling the pre values

Dependent variable	Effects	Mean difference (SE)	F value	P-value
ESV (ml)	Sequence	-----	0.04	0.843
	Period	-----	4.40	0.074
	Treatment		1.17	0.316
	Drug vs. Placebo	-18.10 (16.74)		
	Pre ESV	-----	63.45	<0.001
EF (%)	Sequence	-----	1.59	0.243
	Period	-----	0.00	0.966
	Treatment		0.05	0.834
	Drug vs. Placebo	-0.53 (2.43)		
	Pre EF	-----	27.32	0.001
LVEDD (mm)	Sequence	-----	0.01	0.919
	Period	-----	40.35	0.001
	Treatment		0.62	0.465
	Drug vs. Placebo	0.14 (0.18)		
	Pre LVEDD	-----	14.42	0.013
TAPSE (cm)	Sequence	-----	2.37	0.162
	Period	-----	0.02	0.900
	Treatment		3.82	0.092
	Drug vs. Placebo	0.25 (0.13)		
	Pre TAPSE	-----	5.52	0.051

Abbreviation: FBS (Fasting blood Sugar), Cho1 (Cholesterol), TG (Triglyceride), HDL (High density lipoprotein), LDL (low density lipoprotein), VLDL (very low-density lipoprotein), CRP (C-reactive protein), ProBNP (pro b-type natriuretic peptide), ESV (End-systolic volume), EF (Ejection fraction), LVEDD (left ventricular end-diastolic diameter), TAPSE (Tricuspid Annular Plane Systolic Excursion).

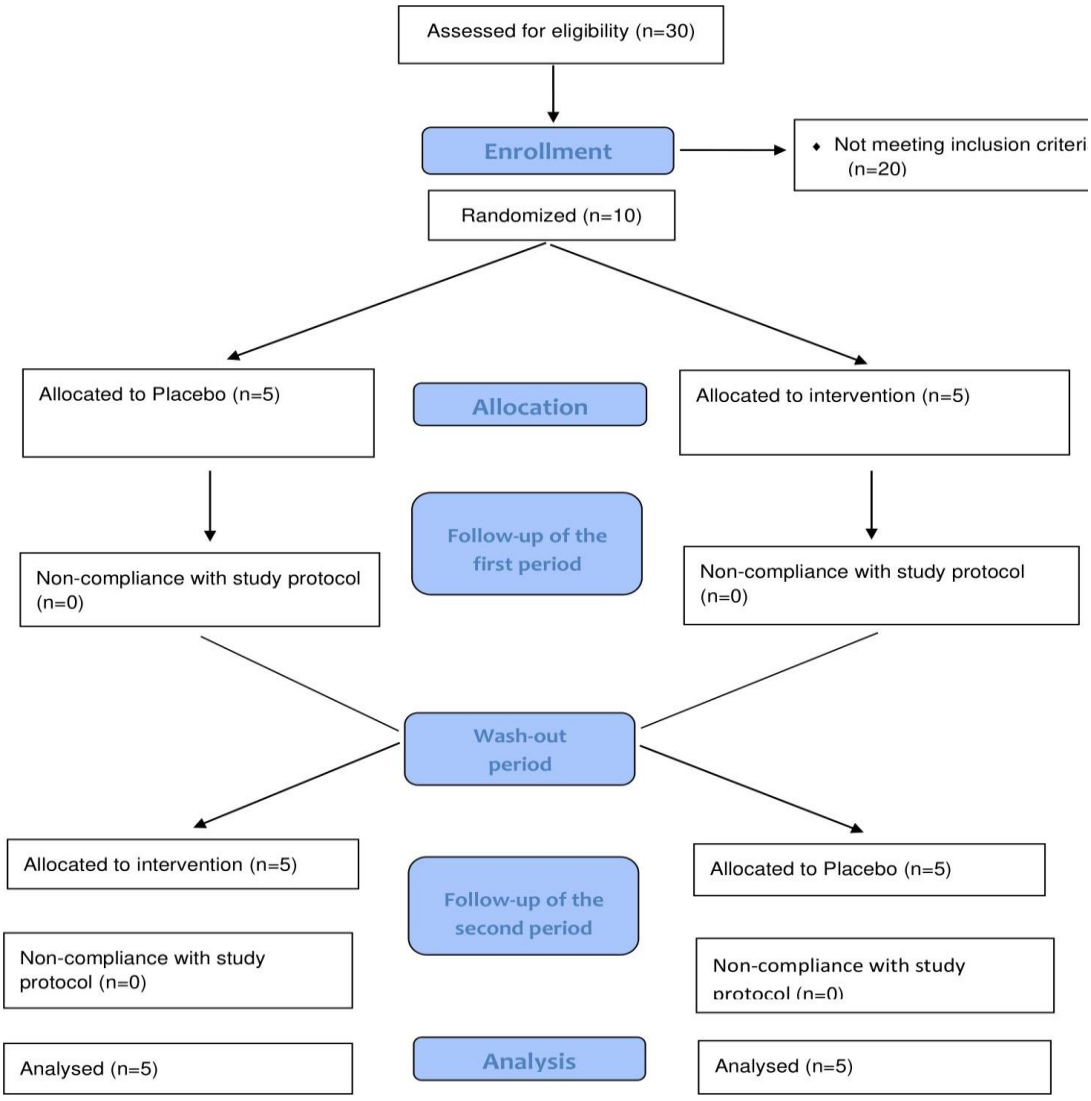

Fig. 1. Randomized controlled trials flowchart