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The effect of trehalose administration on vascular inflammation in patients with coronary artery disease

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ABSTRACT

Background: In recent years, several trials investigated the role of anti-inflammatory agents in reducing cardiovascular events. Trehalose is a natural disaccharide able to reduce inflammation by enhancing macrophage autophagic activity. This action has been demonstrated to attenuate atherosclerotic plaque development in various pro-atherogenic animal models. However, at present, no data about the efficacy of this compound in human subjects have been published.

Methods: We performed a randomized, double-blind trial involving 15 patients with history of myocardial infarction and evidence of systemic inflammation (defined as C-reactive protein > 2 mg/L). The patients were randomly assigned, in 2:1 ratio, to receive either intravenous trehalose (15 g once weekly) or placebo for 12 weeks. The primary efficacy end-point was the change in arterial wall inflammation, assessed by quantifying ^{18}F-FDG PET/CT uptake in carotid arteries and ascending aorta.

Results: The MDS TBR change of the index vessel at 3-month follow-up was not significant in treatment and placebo groups. Furthermore, we could not demonstrate any significant difference between the trehalose group and control group in changes of cIMT from baseline to 3 months in the overall population. No significant changes in echocardiographic measurement were noted after trehalose treatment. Except for the change in urea level in placebo group (31.00 ± 6.59 vs. 25.60 ± 6.402 $P = 0.038$) no other changes were detected after treatment. Also, there was a significant difference between changes in alanine aminotransferase (ALT) trehalose and placebo groups.

Conclusion: This was the first study that specifically assessed the effects of intravenous trehalose on atherogenesis in human subjects. Trehalose treatment was characterized by an optimal safety profile, but no significant reduction in arterial wall inflammation could be observed. This might be a consequence of the small sample size of this trial. Larger studies are needed to better assess the efficacy of this compound in this clinical context.

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1. Introduction

Atherosclerotic cardiovascular disease (ASCVD) is among the most frequent causes of death and morbidity worldwide [41,39]. Its pathogenesis mostly relies on an interplay between an imbalanced lipid metabolism and a chronic inflammatory process of the arterial wall [41]. Lipid abnormalities represented the first element that attracted the attention of researchers studying atherosclerosis. The causal role of LDL-cholesterol in the formation of atheroma has been widely proven, and the introduction of lipid-lowering drugs allowed to greatly reduce the risk of atherogenesis-related complications. Despite this, even in case of optimal LDL-cholesterol reduction, cardiovascular events may still occur, and inflammation has been widely advocated as the major element explaining this residual risk [14,5,15]. Evidence supporting the importance of inflammation in the pathogenesis of atherosclerosis comes from the observation that markers of increased systemic inflammation, such as C-reactive protein (CRP), are consistently associated with the prevalence of underlying atherosclerosis and the risk of cardiovascular events, independently of cholesterol levels [35,4]. Therefore, in recent years, several trials investigating the possible role of anti-inflammatory agents in reducing cardiovascular events have been conducted, hypothesizing that these agents might lessen arterial wall inflammation and prevent its detrimental impact on atheroma growth and instability [27,42]. In the Canakinumab ANti-inflammatory Thrombosis Outcomes Study (CANTOS) [26], subcutaneous administration of canakinumab at the dose of 150 mg every 3 months led to a 15% reduction in cardiovascular events compared to placebo in patients with prior myocardial infarction and evidence of systemic inflammation (defined as a CRP level $>$ 2 mg/L); notably, however, a slightly higher incidence of fatal infections was observed in the treatment group. In contrast, in the Cardiovascular Inflammation Reduction Trial (CIRT) [25], low-dose methotrexate did not affect cardiovascular outcomes nor plasma levels of inflammatory markers in patients with a history of acute myocardial infarction (AMI) or multivessel coronary disease who additionally had either type 2 diabetes mellitus or the metabolic syndrome. More recently, in the COLchicine Cardiovascular Outcomes Trial (COLCOT) [37], low-dose colchicine (0.5 mg once daily) led to a 23% reduction in cardiovascular events compared to placebo in patients with a recent (within 30 days before enrollment) myocardial infarction.

In light of these differing – though promising – results, the search for a widely available and safe anti-inflammatory regimen that could effectively reduce atherogenesis in patients at risk of ASCVD continues. Inflammation is a complex mechanism, based on an intricate interplay between various cell types (such as neutrophils, macrophages, and lymphocytes) and soluble factors (such as inflammation-related serum proteins, antibodies, and cytokines); this theoretically allows multiple targets to be potentially addressed in pharmacological research [27]. In recent years, the role of the macrophage in arterial wall inflammation and atherosclerotic progression has gained growing interest [19,34,2,8]; more specifically, disruption of macrophage autophagy has been suggested as the main contributor to macrophage dysfunction and subsequent hyperactivation of inflammatory cascade [23,13,24].

Trehalose is a natural non-reducing disaccharide able to prevent protein denaturation; due to this property, it plays various protective roles against stress conditions such as heat, freeze, oxidation and dehydration [43]. The efficacy of its oral form is limited by the presence of trehalase enzyme in the intestinal wall, that causes its breakdown into glucose [7,31]; in its parenteral form, conversely, it has been safely used as a stabilizing excipient in a variety of research applications and in several FDA-approved therapeutic products [22]. Numerous studies have demonstrated trehalose's ability to induce macrophage autophagy and lysosomal biogenesis [20,6]. This capacity has led to some promising results for the treatment of neurodegenerative diseases [1,40,9,28,10,16]. Moreover, the potential of trehalose to induce and restore macrophage autophagy function in the atherosclerotic plaque has opened interesting perspectives on its use as an athero-protective agent. After some preliminary in vitro results [30], the demonstration of the potential anti-atherogenic benefit of trehalose has been substantiated in vivo in various pro-atherogenic animal models, in which trehalose administration was consistently associated with a significant attenuation of atherosclerotic plaque development compared to placebo, without significant differences in body weight or blood lipid levels [30,29,36].

Overall, these preclinical data – together with the established safety profile of the molecule – seem promising for the potential use of trehalose in the treatment of ASCVD via lipid-independent mechanisms in at-risk human patients, especially those who show a residual inflammatory activity despite achieving optimal LDL-cholesterol levels through lipid-lowering therapies. However, at present, no data about the clinical efficacy of this compound have been published.

The aim of this proof-of-concept study was thus to explore in humans, for the first time, the potential effect of intravenous trehalose in reducing arterial wall inflammation in patients with established ASCVD and increased systemic markers of inflammatory activity.

2. Methods

2.1. Study design

This study was a randomized, double-blind, placebo-controlled clinical trial with enrollment starting in 2019 and completion of the last study visit in 2021. As planned, we recruited 15 men between 18 and 80 years of age with history of myocardial infarction (MI) and percutaneous coronary intervention (PCI) $>$ 90 days before study inclusion (based on ST deviation, raised troponin and cardiac catheterization). Clinically stable subjects at the time of screening and able to tolerate the study procedure were required to have evidence of inflammation, defined as an hs_CRP $>$ 2 mg/l to be eligible for the study. Additional inclusion criteria were willingness to participate in the trials. Exclusion criteria included patients with impaired renal function (creatinine $>$ 3.0 mg/dL); diabetic patients; active hepatitis or severe hepatic dysfunction, active cancer, consumption of immunosuppressive drugs, active infectious or febrile disease and recipients of transplantation. Patients who met enrollment criteria were randomized (using computer-generated random numbers) in 2:1 ratio to either intravenous trehalose infusion weekly (15 g/week) or placebo groups (equal volume normal saline 0.9%) for a period of 12 weeks. All injections were conducted by a trained nurse in the presence of a specialist physician at a duration of 90 min. Safety was assessed at each injection visits and adverse effect questionnaire as well as assessment of symptoms and physical examination. Primary and secondary endpoints were assessed at baseline and end of study. All participants provided written informed consent and the study was approved by the institutional ethics committee (Ethical Code: IR.NIMAD.REC.1397.300). The trial is registered on ClinicalTrials.gov (NCT03700424) (Fig. 1).

The trial evaluated the potential efficacy of IV trehalose administration on arterial inflammation in patients with history of acute coronary symptoms (ACS).

2.2. Outcomes

The prespecified primary endpoint was arterial inflammation, as assessed by FDG-PET of the aorta. Other prespecified endpoints were carotid intima media thickness measured by ultrasound, cardiac function as measured by echocardiography, lipids, inflammatory and biochemical parameters.

2.3. Measurements

Baseline 18 F-FDG PET/CT examination and biochemical laboratory tests were performed in all patients. Patients were asked to be fasted for at least 4 h before PET/CT examination. Blood glucose levels were

checked to be below 160 mg/dL. All patients were examined 18 F-FDG PET/CT using Biograph Truepoint TrueV PET/CT scanner (SIEMENS Healthcare, Erlangen, Germany) in accordance with previous reports. One hour after the 18 F-FDG injection (5.2 MBq [0.14 mCi]/kg body weight), a three-dimensional PET/CT scan was started. CT was performed first to correct scattering and photon attenuation using a continuous spiral 6-slice technique with a voltage of 110 kV, a current of 70 mA, a pitch of 1 and a slice thickness of 5 mm. PET was performed immediately afterwards with an axial field of view of 21.6 cm and images were acquired from the cranial base to the mid-thigh obtained for 3 min/bed. Images were reconstructed with the three-dimensional ordered-subsets expectation maximization reconstruction algorithm (2 iterations, 21 subsets) with 3 mm FWHM of Gaussian filter with matrix of 168 × 168 after CT-based scattering correction and attenuation correction.

Arterial 18 F-FDG activity was determined by creating a volume-of-interest (VOI) containing the arterial wall and the lumen. SUV for each artery were calculated by measuring maximal SUV of drawn VOI within an arterial territory. The SUVs were normalized to venous 18 F-FDG activity by dividing them by the average venous ROI estimated from the inferior vena cava, which yielded an arterial target to background ratio (TBR).

The percent change in the MDS TBR of the index vessel calculated as (MDS TBR at 6 months – MDS TBR at baseline) / (MDS TBR at baseline) × 100 was defined as the primary endpoint.

2.4. Two-dimensional echocardiogram examination

Patients underwent echocardiography before treatment and 12 weeks follow-up. A 17-segment echocardiogram was performed to measure left ventricular end-systolic volume (LVESV), left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic diameter (LVESD), left ventricular end-diastolic diameter (LVEDD), interventricular septum thickness at end-diastole (IVSD) and LVEF by using standard methods of the American Society of Echocardiography, and were analyzed independently by two experienced observers who were unaware of patients' treatment assignments.

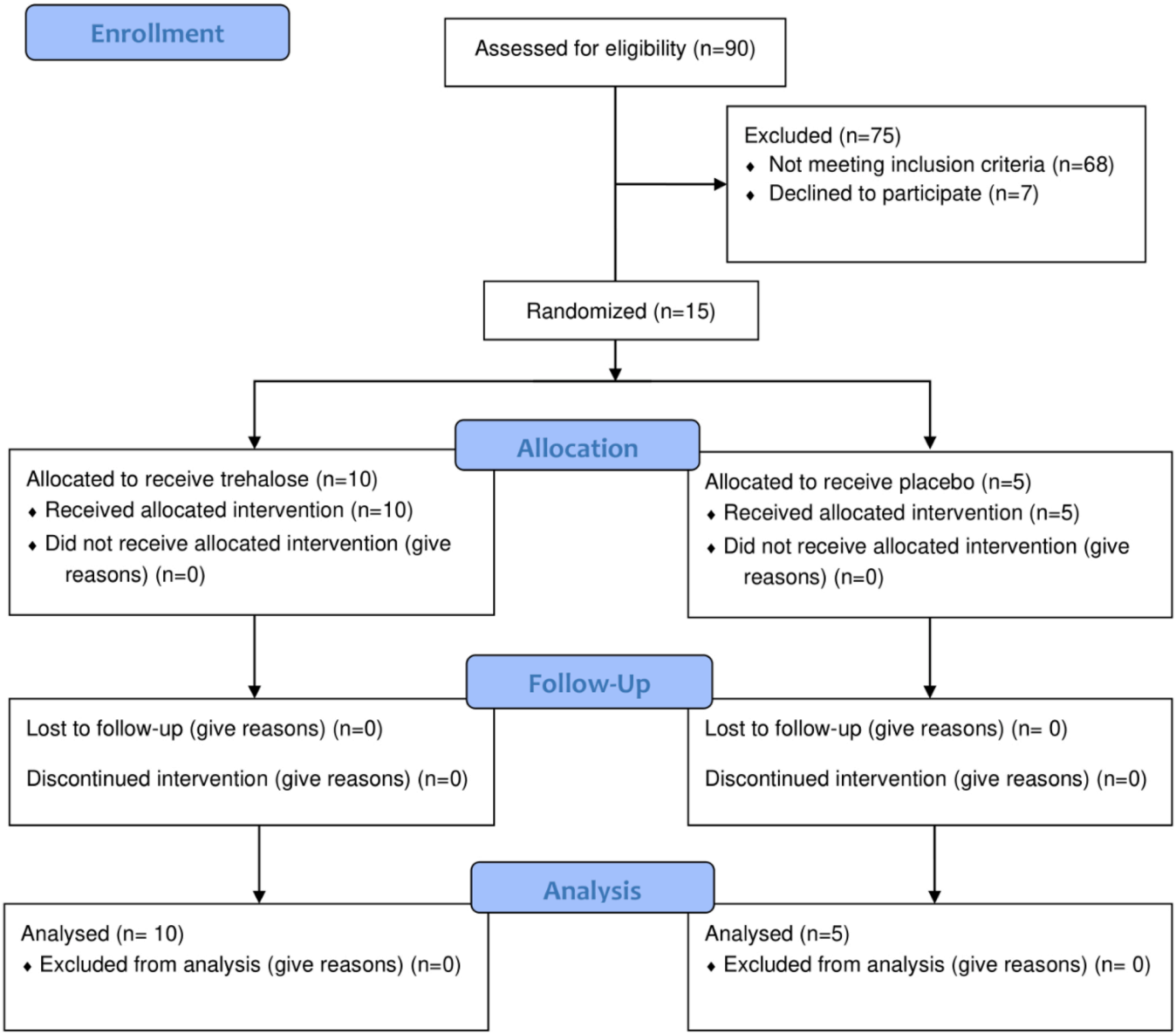

Fig. 1. Randomized controlled trial flowchart.

2.5. Intima media thickness

Patients underwent extracranial Doppler ultrasonography (ECD) with two cylindrical and linear probes. IMT of carotid arteries was measured by a linear probe at the frequency of 5–7 MHz in the distal part of the common carotid artery at the beginning and end of the study.

2.6. Laboratory assessments

Fasting blood samples were drawn from all participants to assess laboratory parameters including a lipid profile, liver enzymes (alkaline phosphatase (ALP, U/l), aspartate aminotransferase (AST, U/l), alanine aminotransferase (ALT, U/l), renal function tests (urea (mg/dl), creatinine (mg/dl)), bilirubin total and direct and hsCRP.

2.7. Statistical analysis

The data were analyzed using IBM SPSS software version 22.0 (SPSS Inc., Chicago, IL, USA). Categorical and continuous variables were described as frequencies (%), and mean ± standard deviation (SD), respectively. To compare the quantitative variables before and after intervention, paired-sample Student's t test was used. For multiple comparisons, the analysis of variance (ANOVA) was utilized. P-value less than 0.05 was considered statistically significant.

3. Results

3.1. Changes in arterial inflammation activity: index vessel analysis

As summarized in Table 1, baseline 18FDG PET/CT parameters were similar between the two groups. The MDS TBR change of the index vessel at 3-month follow-up was not significant in treatment and placebo groups ($p = 0.894$ vs $p = 0.677$ for right carotid), ($p = 0.146$ vs $p = 0.825$ for left carotid) and ($p = 0.310$ vs $p = 0.945$ for Aorta). Furthermore, the mean change in the MDS TBR of the index vessel (primary endpoint) was not significantly different between both groups (-0.015 ± 0.36 vs. 0.036 ± 0.17, respectively, $p = 0.765$ for right carotid), (0.122 ± 0.24 vs. 0.012 ± 0.11, respectively, $p = 0.360$ for left carotid) and (0.11 ± 0.33 vs. -0.01 ± 0.30, respectively, $p = 0.501$ for Aorta).

3.2. Changes in intima media thickness

We could not demonstrate any significant difference between the trehalose group and control group in changes of cIMT from baseline to 3 months in the overall population [-0.059 ± 0.11 mm vs. -0.080 ± 0.07 mm, $p = 0.722$] for right carotid and [-0.04 ± 0.13 mm vs. -0.03 ± 0.06 mm, $p = 0.881$] (Table 2).

Table 1
Changes in arterial inflammation activity: index vessel analysis.

MDS TBR of index artery	Trehalose	Placebo	p-value
Right carotid			
Baseline	0.92 ± 0.27	0.81 ± 0.19	0.466
Follow up	0.90 ± 0.28	0.85 ± 0.17	0.710
Change	-0.015 ± 0.36	0.036 ± 0.17	0.765
P-value compared with baseline	0.894	0.677	
Left carotid			
Baseline	0.78 ± 0.20	0.82 ± 0.15	0.692
Follow up	0.90 ± 0.22	0.83 ± 0.20	0.589
Change	0.122 ± 0.24	0.012 ± 0.11	0.360
P-value compared with baseline	0.146	0.825	
Aorta			
Baseline	1.20 ± 0.27	1.35 ± 0.26	0.345
Follow up	1.32 ± 0.24	1.34 ± 0.34	0.888
Change	0.11 ± 0.33	-0.01 ± 0.30	0.501
P-value compared with baseline	0.310	0.945	

MDS: most diseased segment; TBR: target to background ratio.

3.3. Changes in echocardiographic indices

Table 3 illustrates the echocardiographic measurement of the patients before and after treatment. No significant changes were noted after Trehalose treatment.

3.4. Changes in biochemical factors

Table 4 showed laboratory assessment before and after treatment. Except for the change in urea level in placebo group (31.00 ± 6.59 vs. 25.60 ± 6.402 $P = 0.038$), no other significant reduction was detected after treatment. Furthermore, there was a significant difference between changes in ALT trehalose and placebo groups.

4. Discussion

In this pilot-scale, proof-of-concept, randomized, placebo-controlled, double-blinded trial, the potential effect of intravenous trehalose administration in reducing arterial wall inflammation, evaluated by F-FDG PET/CT, was investigated in patients with established ASCVD and increased systemic markers of inflammatory activity. To the best of our knowledge, this was the first study that explored the potential effects of trehalose for the treatment of atherosclerosis in human subjects.

No significant differences could be found in the primary functional outcome measures of this trial. Trehalose administration did not significantly affect MDS-TBR in any of the three index vessels (i.e., the two carotid arteries and the ascending aorta), either in terms of change with respect to baseline or in terms of difference compared to placebo. A similar conclusion could be drawn for the secondary morphological outcome measures. Echocardiographic parameters were thoroughly comparable before and after trehalose administration in the treatment group, and no difference emerged also comparing the treatment group with the control group. The same held true also for the ultrasonographic evaluation of the carotid IMT. The laboratory findings confirmed in humans the apparent absence of a direct effect of trehalose on serum lipid levels. The few statistically significant results that emerged among the measured variables were most likely a statistical epiphenomenon of multiple testing procedures, as no clear pathophysiological rationale would apparently justify their occurrence.

Atherosclerotic plaque is mainly caused by disrupted macrophage autophagy which in turn leads to impaired efferocytosis, inflammasome formation and accumulation of foamy macrophages and apoptotic bodies [13,19,33]. As a result, inducing macrophage autophagy could be a viable method to slowing or even reversing plaque formation and mitigating pro-inflammatory responses [12,18,33]. Macrophage autophagy and lysosome development are found to be regulated by transcription factor EB (TFEB) [32,3]. Trehalose injection has been demonstrated to increase TFEB levels and nuclear translocation, which are likely to harness macrophage autophagy while decreasing macrophage apoptosis, protein aggregation, and IL-1 production [17,31].

Table 2
Changes in intima media thickness.

Intima media thickness (mm)	Trehalose	Placebo	p-value
Right carotid			
Baseline	0.75 ± 0.17	0.74 ± 0.05	0.902
Follow up	0.69 ± 0.08	0.66 ± 0.06	0.479
Change	-0.059 ± 0.11	-0.080 ± 0.07	0.722
P-value compared with baseline	0.138	0.364	
Left carotid			
Baseline	0.77 ± 0.19	0.78 ± 0.13	0.960
Follow up	0.73 ± 0.13	0.75 ± 0.08	0.824
Change	-0.04 ± 0.13	-0.03 ± 0.06	0.881
P-value compared with baseline	0.706	0.227	

Table 3
Changes in echocardiographic indices.

	Trehalose	Placebo	p-value
LVEF			
baseline	51 ± 8.43	49 ± 10.24	0.693
Follow up	52.5 ± 8.57	48 ± 10.95	0.395
change	1.50 ± 2.41	-1.00 ± 2.23	0.075
p-value compared with baseline	0.081	0.374	
LVEDV			
baseline	0.75 ± 0.17	0.74 ± 0.05	0.902
Follow up	0.69 ± 0.08	0.66 ± 0.06	0.479
change	-0.059 ± 0.11	-0.080 ± 0.07	0.722
p-value compared with baseline	0.138	0.364	
LVESV			
Baseline	0.77 ± 0.19	0.78 ± 0.13	0.960
Follow up	0.73 ± 0.13	0.75 ± 0.08	0.824
Change	-0.04 ± 0.13	-0.03 ± 0.06	0.881
P-value compared with baseline	0.706	0.227	
LVEDD			
Baseline	4.82 ± 0.63	5.32 ± 0.37	0.132
Follow up	4.70 ± 0.62	5.12 ± 0.32	0.195
Change	-0.11 ± 0.22	-0.20 ± 0.14	0.443
P-value compared with baseline	0.142	0.034	
LVESD			
Baseline	2.96 ± 0.58	3.4 ± 0.37	0.256
Follow up	2.95 ± 0.49	3.52 ± 0.32	0.074
Change	-0.01 ± 0.22	0.12 ± 0.14	0.548
P-value compared with baseline	0.932	0.523	
IVSD			
Baseline	0.91 ± 0.58	0.76 ± 0.37	0.313
Follow up	0.80 ± 0.49	1.84 ± 0.32	0.375
Change	-0.11 ± 0.19	1.08 ± 2.35	0.322
P-value compared with baseline	0.104	0.364	

Importantly, TFEB overexpression has recently been found to reduce inflammation and harness oxidative stress in human endothelial cells. Induction of TFEB in a transgenic rat was shown to diminish leukocyte infiltration and subsequent atherosclerotic plaque formation [17].

Given the promising results obtained in animal models and the purpose for which the present study was designed, the retrieved results need to be further discussed. The lack of a significant variation in the secondary morphological outcome measures is not fully surprising, as cardiac and vascular remodeling is a dynamic but slow process that usually manifests over a period of years rather than months [21,11]. On the contrary, the absence of a statistically significant difference in the primary functional outcome measures may raise some doubt about the actual presence of a clinically significant anti-atherogenic effect of trehalose when shifting from animal models to human subjects. However, several caveats should be considered before concluding for the ineffectiveness of this molecule as an athero-protective agent, which also correspond to the main limitations of this study.

First, this was a pilot-scale, proof-of-concept study, and was thus characterized by a limited sample size. Based on previously published data [38], a sample size of 10 patients in the treatment group would have been sufficient to achieve statistical significance only if the drug effect on MDS-TBR reduction had been greater than 20%. Such an effect size, however, approximately corresponds to the estimated percentage difference in MDS-TBR between patients with established ASCVD and healthy controls [38] and would have been greater than any previous result obtained through other pharmacological interventions, in which the MDS-TBR reduction mostly ranged between 5% and 15% [38]. Besides, F-FDG PET/CT failed to demonstrate any evidence of vascular wall inflammation and it was not cost-effective to evaluate all patients regarding vascular inflammation. Therefore, all participants were included only based on hs-CRP levels.

A third key point for the interpretation of the retrieved results lies in the dosing schedule chosen for the administration of trehalose. In high-cholesterol-fed rabbits -at present the model most similar to patients with ASCVD- trehalose was given at an intravenous dose of 350 mg/kg for 3 times a week, which would roughly correspond to a total weekly dose of 75 g in a patient of 70 kg. In the present trial, a dosing schedule of 15 g once a week was adopted; given the need for intravenous administration of the drug, this choice relieved the enrolled patients from the burden of a parenteral infusion on multiple days every week; however, it cannot be excluded that this dose might have reduced the anti-inflammatory and anti-atherogenic property of the drug.

In conclusion, the present study represents the first trial that evaluates the effects of intravenous trehalose on atherogenesis in human subjects. The benefit of trehalose treatment in the reduction of arterial wall inflammation, if present, is smaller than the one that could actually be detected based on the available sample size. On the other hand, the safety profile of the drug was confirmed to be excellent, as no drug-related adverse events occurred. Therefore, given the solid and favorable data obtained in animal models, the results from this pilot trial, rather than being considered as a conclusive answer about the ineffectiveness of intravenous trehalose in the treatment of ASCVD, should be more properly seen a key step for the finer design of a future phase II/III study conducted on a larger sample size and with higher dosing schedules. A demonstration of trehalose efficacy in reducing arterial inflammation, indeed, would open a promising way towards a widely available, safe and cost-effective therapy for patients with severe ASCVD, and in particular for those who have residual inflammatory risk despite optimal LDL-cholesterol levels with lipid-lowering therapy.

Table 4
Changes in biochemical factors.

	Trehalose			Placebo		
	Baseline	Follow up	change	baseline	Follow up	change
Urea	29.20 ± 9.95	33.20 ± 15.18	4.00 ± 13.00	31.00 ± 6.59	25.60 ± 6.02	-5.40 ± 3.84*
Cr	1.17 ± 0.24	1.30 ± 0.46	0.13 ± 0.27	1.10 ± 0.23	1.04 ± 0.20	-0.06 ± 0.05
TG	117.00 ± 72.56	161.20 ± 91.81	44.20 ± 75.74	122.80 ± 93.84	136.20 ± 104.94	13.40 ± 26.19
Chol	115.00 ± 40.00	129.50 ± 45.78	14.50 ± 40.16	109.60 ± 32.08	107.20 ± 17.54	-2.40 ± 19.55
HDL	34.40 ± 6.97	35.70 ± 8.90	1.30 ± 5.20	32.20 ± 4.43	34.00 ± 4.35	1.80 ± 3.49
LDL	64.60 ± 31.16	80.50 ± 36.53	15.90 ± 25.77	63.8 ± 26.09	60.00 ± 13.39	3.80 ± 16.3
AST	30.40 ± 8.87	25.40 ± 10.28	-5.00 ± 10.81	30.40 ± 4.50	26.00 ± 9.02	4.40 ± 6.50
ALT	25.50 ± 6.15	21.00 ± 10.39	-4.50 ± 10.21**	15.40 ± 5.77	24.00 ± 5.19	8.60 ± 7.95
ALP	222.70 ± 57.94	215.90 ± 61.59	-6.70 ± 42.06	204.00 ± 73.38	205.60 ± 74.29	1.60 ± 34.64
Bil T	0.55 ± 0.31	0.64 ± 0.59	0.08 ± 0.40	0.70 ± 0.60	0.86 ± 0.58	0.16 ± 0.30
Bil D	0.26 ± 0.25	0.24 ± 0.13	0.02 ± 0.18	0.28 ± 0.20	0.42 ± 0.27	0.14 ± 0.12
hs_CRP	9.15 ± 3.84	11.26 ± 6.82	2.10 ± 6.89	8.14 ± 4.71	12.40 ± 5.01	4.26 ± 6.78

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; Bil T, bilirubin total; Bil D, bilirubin direct; Cr, creatinine; Chol, cholesterol; HDL, high density lipoprotein; hs_CRP, high sensitive C reactive protein; LDL, low density lipoprotein; TG, triglycerides.

* significant difference compared to baseline.

** significant difference in trehalose group compared to placebo.

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CRediT authorship contribution statement

Tannaz Jamialahmadi: Conceptualization, Data collection, Writing – original draft, Approval of the final version. **Farshad Emami:** Data collection, Writing – review & editing, Approval of the final version. **Ramin Khameneh Bagheri:** Data collection, Writing – review & editing, Approval of the final version. **Hedieh Alimi:** Data collection, Writing – review & editing, Approval of the final version. **Fabio Bioletto:** Approval of the final version. **Simona Bo:** Approval of the final version. **Behzad Aminzadeh:** Data collection, Writing – review & editing, Approval of the final version. **Mohammad Ali Ansari:** Data collection, Writing – review & editing, Approval of the final version. **Faezeh Ehsani:** Data collection, Writing – review & editing, Approval of the final version. **Omid Rajabi:** Data collection, Writing – review & editing, Approval of the final version. **Shiva Ganjali:** Data collection, Writing – review & editing, Approval of the final version. **Maciej Banach:** Conceptualization, Writing – review & editing, Approval of the final version. **Amirhossein Sahebkar:** Conceptualization, Data collection, Writing – original draft, Approval of the final version.

Conflict of interest statement

None.

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