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Original article

Effects of α-lipoic acid therapy on sympathetic heart innervation in patients with previous experience of transient takotsubo cardiomyopathy

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ABSTRACT

Background: Takotsubo syndrome is a stress cardiomyopathy, characterized by reversible left ventricle (LV) apical ballooning in the absence of significant angiographic coronary artery stenosis. The frequent association with emotional stress suggests in this disease an autonomic nervous system involvement. We could think that a therapeutic treatment targeting heart sympathetic dysfunction could be of crucial importance.

Methods: From January 2010 to June 2012, 886 patients were consecutively evaluated at Cardarelli Hospital, Naples, Italy. Among these, 48 patients met takotsubo cardiomyopathy (TCM) criteria. Each patient was assessed with history and physical examination, 12-lead electrocardiogram, serum troponin, coronary arteriography, and left ventricular angiogram, perfusion myocardial scintigraphy with technetium 99m, with echocardiography and 123I-metaiodobenzylguanidine (MIBG) myocardial scintigraphy. At discharge, the surviving patients were randomly assigned to α-lipoic acid (ALA) treatment (600 mg once daily) or placebo. Following discharge, after the initial TCM event, patients returned to our outpatient clinic at Internal Medicine of the Second University Naples for the follow-up evaluation quarterly until 12 months. Routine analysis, myocardial damage serum markers, oxidative stress serum markers, pro-inflammatory cytokines, and sympathetic tone activity were evaluated in all patients.

Results: ALA administration improved MIBG defect size at 12 months compared to placebo.

Conclusions: Adrenergic cardiac innervation dysfunction in TCM patients persists after previous experience of transient stress-induced cardiac dysfunction. ALA treatment improves the adrenergic cardiac innervation. This study evaluates whether sympatho-vagal alterations are TCM event-related.

Introduction

Takotsubo syndrome is a stress cardiomyopathy (TCM), reported as a reversible form of acute heart failure, triggered by stressful events and associated with a distinctive left ventricular (LV) contraction pattern [1]. TCM mimics acute coronary syndrome

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and is accompanied by reversible LV apical ballooning, in the absence of significant angiographic coronary artery stenosis [1]. The left ventricle of TCM patients shows a particular shape resembling the Japanese ceramic fishing pot for trapping octopus, namely "takotsubo" [2]. Although the cause of this condition is still unresolved, in this disease the frequent emotional stress association suggests an autonomic nervous system involvement [3]. The excessive release of hormones, such as adrenaline, produced in high-stress situations, in TCM may cause heart muscle damage [4,5]. In fact, TCM events have been reported after accidental overdose of adrenaline [4] or associated with adrenaline-producing tumors, such as pheochromocytoma [5]. Thus, the sympathetic activity dysfunction appears to play a key role in the TCM pathophysiology. In most cases, in TCM patients, 123I-metaiodobenzylguanidine (MIBG) myocardial scintigraphy showed altered 123I MIBG distribution in several heart segments [6]. In more detail, the apical myocardium shows poor sympathetic innervations and reduction of MIBG tracer uptake. A possible explanation could be that the intense adrenaline discharge, acting on heart segments with different and abnormal innervation, produces a transient heart failure characterized by a particular LV shape. This distinctive "takotsubo" shape has a narrow upper portion and a lower enlarged portion that contracts poorly. Although TCM presentation has been described [7,8], there are still few data from large and prospective patient cohort studies following the initial TCM event. In the majority of cases, transient myocardial-stunning LV-related dysfunction involves the LV apical segments and there is a full recovery within a few weeks. With proper recognition and management, nearly all patients survive an acute takotsubo episode [8]. However, in approximately 5% of patients, a second (or third) stress-induced event may occur. Data suggest that the TCM recurrence rates in the first few years are likely in the range of 2–10% [2,9,10]. Because catecholamines may play a central role in TCM pathogenesis, we could speculate that recurrence rates should be lower in those patients maintained on comprehensive adrenergic blockade. However, recent data indicate that these drugs administered in traditional dosages did not absolutely prevent either the first or recurrent TCM episodes. In other words, 20–30% of these events occurred while beta-blockers were administered [11]. Despite appropriate aggressive treatment, about 5% of patients experienced either cardiac arrest (and survived) or died during hospitalization [8]. In light of these findings, since recurrent TCM is not entirely benign, a pharmacological treatment targeting the heart sympathetic dysfunction could be of crucial importance. To date, α-lipoic acid (ALA) administration has been shown to completely prevent heterogeneous MIBG distribution, decreasing MIBG uptake, and increasing norepinephrine content in diabetic cardiomyopathy [12]. However, the possible ALA treatment effect on TCM has not been examined to date. ALA treatment has been shown to restore sympatho-vagal alterations in diabetic cardiomyopathy [12,13], improving the adrenergic cardiac innervation. ALA treatment may affect the sympathetic tone balance by different action mechanisms as observed in stress-induced cardiomyopathy [12,13]. The sympathetic tone regulation may represent the therapeutic effect. We could speculate that, in TCM, ALA treatment may induce a similar effect regulating the complex interactions between vagal and adrenergic cardiac innervations. Because in TCM the sympathetic denervation appears to play a central role in disease pathogenesis [3–6], we may test in our study the ALA's therapeutic effect on sympathetic and vagal tone activity. On this basis, this study aimed to evaluate, in TCM patients, whether adrenergic cardiac innervation dysfunction and sympatho-vagal alterations persist after previous experience of transient stress-induced cardiac dysfunction. For these reasons in this study we investigated, in TCM patients, the ALA therapeutic effect compared to placebo.

Methods

Patients

From January 2010 to June 2012, 886 patients with suspected ST segment elevation myocardial infarction (STEMI) were referred to Cardarelli Hospital, Naples, Italy, for coronary angiography and consecutively evaluated at the Cardiac Intensive Care Unit (CICU) of Cardarelli Hospital (Fig. 1). Among these, 48 patients met the criteria for TCM: (I) acute onset of a cardiovascular event, usually associated with substernal chest pain, initially regarded as STEMI/evolving coronary syndrome; (II) cardiac biomarker modifications (creatine kinase-MB and troponin I); (III) systolic dysfunction, predominantly characterized by akinesia/hypokinesia of the mid-to-distal portion of the LV chamber, with hypercontractile basal LV; (VI) absence, by angiography, of significant atherosclerotic luminal narrowing in each of the 3 epicardial coronary arteries (0 to <25%); and (V) absence of pheochromocytoma, myocarditis, or hypertrophic cardiomyopathy [1]. Exclusion criteria were previous myocardial infarction, concomitant chronic diseases including diabetes mellitus, kidney, liver, and cerebrovascular diseases [1]. Informed consent was obtained from each patient and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. The Cardarelli Hospital Institutional Review Board approved the protocol and the informed consent was obtained from participants to the study.

Randomized trial

This study was performed in a double-blind using placebo manner. This study was performed in 48 patients with TCM. Each patient was assessed with history and physical examination, 12-lead electrocardiogram, serum troponin, coronary arteriography, and LV angiogram (an average of 6 h after admission to the hospital), perfusion myocardial scintigraphy with technetium 99m (99mTc), with echocardiography and MIBG myocardial scintigraphy. All patients were admitted to the CICU after coronary angiography. Currently recommended treatments for acute coronary syndromes, with therapy directed at relieving myocardial ischemia and preventing thrombotic complications, were provided to all patients [14]. At discharge, surviving patients with established TCM were managed and followed for 12 months after the event as outpatients. At discharge, the surviving patients were randomly assigned to ALA treatment (600 mg once daily) (ALA group) or placebo (placebo group). With regard to the full medical therapy, the use of concomitant treatments were uniform between the groups

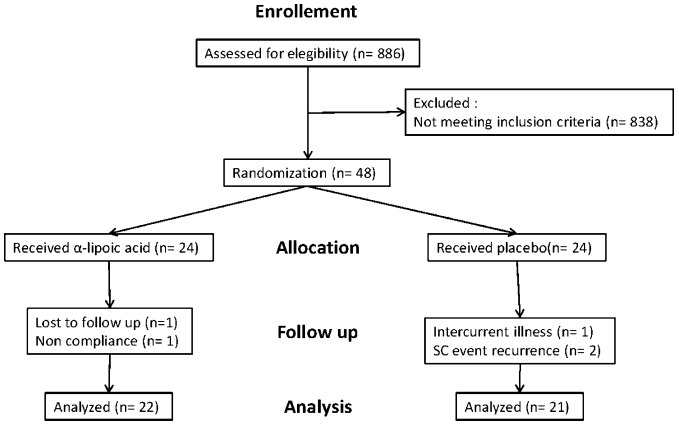

Fig. 1. The study flow chart. The study was conducted in a population of stress cardiomyopathy affected patients by following times: enrollment (886 patients), allocation (24 patients received α-lipoic acid, ALA, vs. 24 received placebo), follow-up and final analysis (22 ALA patients vs. 21 placebo patients). SC, stress cardiomyopathy.

and according to evidence-based international guidelines for acute coronary syndrome in all patients [14]. Following discharge, patients returned to our outpatient clinic at Internal Medicine of the Second University Naples for the follow-up evaluation quarterly until 12 months after the initial event of TCM.

Coronary angiography

Coronary angiograms at baseline were performed in at least 2 orthogonal views after intracoronary nitroglycerin. The analysis of all angiographic data was performed by operators who were unaware of the study groups (Infinix CS-I angiograph, Toshiba, Tokyo, Japan).

Echocardiography

LV function was evaluated in all patients by two-dimensional echocardiography at admission, 14 days, and 12 months after the acute event. Echocardiography (Philips IE 33, Eindhoven, The Netherlands) was performed by operators, who were unaware of the study groups, according to the international guidelines [15]. To assess left ventricle wall contractility, authors evaluated the echocardiographic score, that is wall motion score index. As suggested by echocardiographic guidelines [15], the left ventricle is divided into 5 walls: anterior, antero-septal, infero-septal, postero-lateral, and antero-lateral. Each wall is divided into a basal, mid, and apical segment and the apical, representing the 17th segment. Wall motion score index is a semi-quantitative analysis of regional systolic function. Each segment is analyzed individually and scored on the basis of its motion and systolic thickening. Ideally, the function of each segment should be confirmed in multiple views. This score is a 5-level score defined as follows: score 1 = normokinesis or hyperkinesis; score 2 = hypokinesis; score 3 = akinesis; score 4 = dyskinesis; score 5 = aneurysm. Wall motion score index is derived as a sum of all scores divided by the number of segments visualized [15].

Perfusion myocardial scintigraphy

At 5–7 days after CICU admission, patients received an intravenous injection of 27 mCi (1000 MBq) of technetium Tc-99m sestamibi. Single-photon emission computed tomography (SPECT) was performed within 6–8 h after the injection of the radioactive agent.

MIBG imaging

MIBG imaging was performed in all patients after CICU admission, with a median of 14 days after presentation of the symptoms, and at 12 months after the acute event. MIBG was performed as previously reported [16]. Briefly, the standard protocol for 123I-MIBG cardiac imaging requires that drugs that interfere with 123I-MIBG uptake should be withheld. Thyroid uptake of unbound 123I was blocked with 500 mg of potassium perchlorate given orally 30 min before 123I-MIBG injection. Between 148 MBq and 370 MBq of 123I-MIBG were injected intravenously at rest. Both planar and SPECT images were acquired 15 min after injection (early) and 4 h after injection (delayed). A dual head gamma camera (ECAM Siemens, Erlangen, Germany) equipped with a low-energy, high-resolution collimator was used. A 20% window was usually centered over the 159-keV photo peak of 123I for imaging. Anterior planar images of the chest were acquired using a 256×256 matrix. SPECT images were acquired using a 64×64 matrix over 180°, from the right anterior oblique position to the left posterior oblique position. Planar imaging allowed for global assessment of cardiac innervation, whereas SPECT allowed for regional evaluation. Quantitative evaluations were performed with a standard protocol previously described [16].

Laboratory analysis

Routine analysis

At admission, 14 days, and 12 months after the event, glycemia, lipid profile [total cholesterol (TC), triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol], transaminases, creatine kinase (CK), and creatinine were evaluated.

Myocardial damage

Serum levels of troponin I, CK, and CK-MB were evaluated at CICU admission.

Oxidative stress

At admission, 14 days, and 12 months after the event, nitrotyrosine plasma concentration was assayed by enzyme-linked immune sorbent assay (ELISA). Nitrotyrosine was determined as the modified amino acid as a product of free-radical (O^{2-}) interaction with nitric oxide (NO). The interaction of O^{2-} with NO is rapid and leads to inactivation of NO and production of the potent oxidant peroxynitrite. Detection of nitrotyrosine is strongly suggestive of increased generation of peroxynitrite [17].

Proinflammatory cytokines

Serum concentrations of tumor necrosis factor-α (TNF-α) were determined in duplicate using a highly sensitive and quantitative sandwich enzyme assay (Quantikine HS PharmPak, R&D, 395 Oyster Point Blvd, Suite 321 South San Francisco, CA 94080, USA). Plasma C-reactive protein (CRP) was determined using automated turbidimetry.

Sympathetic tone

We evaluated the sympathetic activity at admission, 14 days, and 12 months after the event, using laboratory analysis, by the evaluation of catecholamine plasma levels. Circulating catecholamines may reflect the systemic sympathetic activity in affected patients. Catecholamine plasma levels were measured by high-performance liquid chromatography. Brain natriuretic peptide was measured by enzyme immunoassay.

Study end point

The primary outcome was adrenergic cardiac innervation improvement, determined in ALA group by quantitative MIBG at 12 months. The secondary outcome was inflammation markers and oxidative stress reduction, and myocardial damage at 12 months after the initial event.

Statistical analysis

Data analysis

Data are shown as mean ± standard deviation. A general linear model (general form of ANOVA) was fitted to the data, with treatment group, sex, and age strata as the independent factors. When the dependent variable was measured at 12 months, the baseline value was also included as a covariate. Differences between groups were considered significant at $p \leq 0.05$. For the significance tests, we also report the estimated adjusted mean difference between the groups, i.e. antioxidant group-adjusted mean change – placebo group adjusted mean change. Bonferroni post-hoc test was used for statistical analysis. We report the analysis using data for participants who completed the trial, i.e. had measurements performed at baseline and 12 months. The analyses were performed using Sigma Plot 12 (Systat Software, Inc. SigmaPlot for Windows, Chicago, IL, USA).

Sample size calculation

The standard deviation of MIBG was assumed to be 0.0112, based on previously published data on the magnitude of the innervation deficit observed in the distal myocardial segments of TCM patients [18]. With 15 participants for each group, we estimated 80% power to detect a change of 0.015 between the mean MIBG of the placebo-treated and actively treated groups, when testing the null hypothesis using a two-tailed two-sample *t*-test at a 5% level of significance. A 20% loss due to early withdrawals and/or non-evaluable measurements was assumed and, combined with the effect of stratification on analysis, resulted in the requirement to recruit 22 patients per treatment group.

Results

Clinical characteristics

We enrolled 48 participants who met the TCM inclusion criteria (Fig. 1). The median age of TCM patients was 63.8 ± 5.8 years. TCM patients were all women and 47 of them were post-menopausal (Table 1). Clinical characteristics for the entire cohort are summarized in Table 1.

Presentation and hospital outcome

All patients had severe chest pain, dyspnea, or both during cardiovascular event and presented to the emergency department a median of 2 h (interquartile range, one to five) after symptom onset. All TCM patients had ST-segment elevation of at least 1 mm. In TCM patients, troponin I peak levels were only mildly elevated, with a median value of 0.25 ng/ml (interquartile range, 0.11–0.58 ng/ml; normal value, <0.06 ng/ml). In TCM, CK and CK-MB peak values were elevated with a median value of 191 UI/l (interquartile range, 95–304 UI/l; normal value, 33–194 UI/l) and 192 UI/l (interquartile range, 95–304 UI/l; normal value, 33–194 UI/l), 35 UI/l (interquartile range, 26.5–43 UI/l; normal value, 0–25 UI/l) and 37 UI/l (interquartile range, 28–44.5 UI/l; normal value, 0–25 UI/l), respectively in the ALA group compared to placebo. In TCM patients, the median left ventricular ejection fraction (LVEF) on the initial echocardiogram (admission) was 38 ± 9%. All TCM patients underwent emergency angiography on admission. TCM patients had normal coronary arteries or mild luminal irregularities, and no patient had angiographic epicardial spasm evidence (Table 1). All TCM patients had a similar contractile pattern, with preserved basal function, moderate-to-severe dysfunction in the mid-ventricle, and apical akinesis or dyskinesis (mean

Table 1
Patient characteristics.

	ALA group		Placebo group	
	Baseline	12 months	Baseline	12 months
Patients (*n*)	24	22	24	21
Mean age (years)	63.7 ± 6.5	63.2 ± 6.1	63.9 ± 5.2	63.1 ± 5.9
BMI (kg/m^2)	27 ± 1.62	26.9 ± 1.8	27.6 ± 2.2	27.3 ± 1.85
Waist-to-hip ratio	0.71 ± 0.02	0.7 ± 0.02	0.72 ± 0.03	0.71 ± 0.03
Systolic blood pressure (mmHg)	122 ± 9.9	121 ± 8.8	123 ± 11	122.5 ± 10.4
Diastolic blood pressure (mmHg)	81.3 ± 15.4	78.5 ± 10.7	82.2 ± 8.27	79.7 ± 5.7
Heart rate (bpm)	97.5 ± 7.86	78.9 ± 10.2	98.3 ± 5	78.16 ± 10.69
Blood glucose (mg/dl)	93.8 ± 7.24	96.6 ± 10	94.8 ± 9.12	96.7 ± 13.34
Total cholesterol (mg/dl)	208.4 ± 19.4	201.6 ± 20	206.3 ± 15.9	199.7 ± 19.09
Epinephrine (pg/ml) (IQR)	1013.5 (758–1240)	370.5 (336–475)*	999 (885–1142)	366.5 (226–535)*
Norepinephrine (pg/ml) (IQR)	2003.5 (1558–2665)	1047 (1009–1124)*	2012 (1778–2214)	1022 (1008–1137)*
Brain natriuretic peptide (pg/ml) (IQR)	995.5 (689–1240)	135 (119–153)*	995 (669–1236)	146 (124–158)*
C-reactive protein (mg/l)	3.8 ± 0.8	1.7 ± 0.64*	3.6 ± 0.8	2.7 ± 0.7*‡
Tumor necrosis-α (pg/ml)	5.5 ± 0.9	2.1 ± 0.95*	5.8 ± 0.8	3.98 ± 1*‡
Nitrotyrosine (μM)	0.5 ± 0.1	0.24 ± 0.14*	0.5 ± 0.08	0.46 ± 0.12‡
Smokers (% [*n*])	17 (4)	4 (1)	17 (4)	4 (1)
Previous disorder (% [n])				
Hypertension	50 (12)	45 (10)	44 (11)	43 (9)
Hyperlipidemia	44 (11)	50 (11)	54 (13)	61 (13)
TroponinI (ng/ml) (IQR)	0.25 (0.15–0.60)	/	0.25 (0.09–0.54)	/
CK (UI/l)	191.0 (95–304.5)	/	192 (97–306)	/
CKMB (UI/l)	35.0 (26.5–43.0)	/	37.0 (28–44.5)	/
Echocardiographic parameters				
Admission LVEF, %	38 ± 8	57.6 ± 5.88	37 ± 9	56 ± 6
Admission basal contraction, score	1.2 ± 0.3	1 ± 0.04	1.1 ± 0.2	1.02 ± 0.10
Admission midventricle contraction, score	3.4 ± 0.7	1.05 ± 0.17	3.0 ± 0.7	1.1 ± 0.28
Admission apical contraction, score	3.8 ± 0.8	1.1 ± 0.27	3.7 ± 0.8	1.2 ± 0.4
LVEF, % at 14 days	45 ± 10		44 ± 10	
14 days basal contraction, score	1.1 ± 0.1		1.0 ± 0.2	
14 days mid-ventricular at contraction, score	1.8 ± 0.6		1.6 ± 0.5	
14 days apical segments contraction, score	2.1 ± 0.7		1.8 ± 0.5	
Discharge medication				
Beta-blockers (% [*n*])	96 (23)	95 (21)	96 (23)	95 (20)
ACEi/ARB (% [*n*])	66 (16)	72 (16)	62 (15)	71 (15)
Aspirin (% [*n*])	92 (22)	95 (21)	96 (23)	96 (23)
Statin (% [*n*])	83 (20)	95 (21)	75 (18)	75 (18)

Representation of baseline characteristics of the patients assigned to either ALA or placebo treatment. Data are presented as mean ± SD, *n* (%) or median and interquartile range (IQR) according to their distribution.
ACEi, angiotensin-converting enzyme inhibitors; ALA, α-lipoic acid; ARB, angiotensin receptor blockers; BMI, body mass index; CK, creatine kinase; CKMB, creatine kinase MB type; LVEF, left ventricular ejection fraction. The * indicates a significant *p*-value ($p < 0.05$) in ALA and/or placebo group comparing follow-up vs. baseline. The ‡ indicates a significant *p*-value ($p < 0.05$) when comparing placebo group vs. ALA group. For troponin I, CK, and CKMB, peak values are reported. LVEF had improved to 45 ± 10% and the mid-ventricular and apical segments were only mildly hypokinetic, with echocardiographic scores of 1.1 ± 0.1 at the base, 1.8 ± 0.6 at the mid-ventricle, and 2.1 ± 0.7 at the apex. During echocardiographic examination at 14 days after admission, there were no significant differences in echocardiographic findings between the two groups of randomization.

echocardiographic scores, 1.1 ± 0.2, 3.2 ± 0.7, and 3.8 ± 0.8, respectively). By hospital day 14, LVEF had improved to 45 ± 10% and the mid-ventricular and apical segments were only mildly hypokinetic, with echocardiographic scores of 1.1 ± 0.1 at the base, 1.8 ± 0.6 at the mid-ventricle, and 2.1 ± 0.7 at the apex (Table 1). For this reason, LVEF did not significantly increase ($p > 0.05$) under ALA regimen at 12 months compared to placebo group. Myocardial perfusion using 99mTc (n 53) was assessed at the sub-acute phase (at 5–7 days after the event) in all patients. At rest, no significant (>5% of the total myocardial mass) impairment of myocardial perfusion was observed in TCM patients at the sub-acute phase (data not shown). In all TCM patients, the 123I-MIBG myocardial scintigraphy obtained 30 min after administering radiotracer showed LV myocardial uptake reduction, predominantly in the apical, anterior, and septo-apical segments and lateral–apical, and inferior segments (Fig. 2). At admission, catecholamines and B-type natriuretic peptide plasma levels were elevated in all TCM patients (Table 1). By hospital day 14, in TCM patients catecholamine plasma levels were one-third to one half of the peak values: norepinephrine, 988 (668–1240) pg/ml; epinephrine, 589 (335–741) pg/ml. Plasma B-type natriuretic peptide levels declined in all TCM patients: 225 (188–278) pg/ml. Moreover, at the onset of TCM, CRP, TNF-α, and nitrotyrosine plasma levels were elevated (Table 1). At day 14, they remained substantially elevated: CRP, 3.2 ± 0.9 mg/l; TNF-α, 5.2 ± 1.1 pg/ml; nitrotyrosine, 0.45 ± 0.1 μM.

Intervention study

At discharge (a median of 14 days after presentation), TCM patients were randomized to either ALA treatment or placebo (24 participants per group) (Fig. 1). There were no significant differences in any of the demographic or other baseline characteristics as well as full medical therapy of the study population (Table 1). There were also no differences between groups at baseline in systolic, and diastolic blood pressure, catecholamine circulating levels, and resting heart rate (Table 1). A total of 43 participants completed the 12-month intervention, 21 in the placebo and 22 in the ALA group (Fig. 1). The main reasons for study dropout are shown in Fig. 1. There was a similar course in systolic and diastolic blood pressure, body mass index, waste to hip ratio, and resting heart rate during the study in both treatment groups (Table 1). At baseline, there were no differences between the ALA and placebo groups in the CRP, TNF-α, and nitrotyrosine levels as well as catecholamine levels (Table 1). Compared with placebo, ALA supplementation resulted in significantly greater reductions in the CRP, TNF-α, and nitrotyrosine concentrations (Fig. 2). No differences between groups were observed in the catecholamine and B-type natriuretic peptide plasma levels and in the change between baseline and 12 months (Table 1). LVEF, global, and regional echocardiographic contractile patterns were similar in participants in both groups at baseline and in the change between baseline and 12 months (Table 1). No differences between groups were observed in the basal, mid-ventricular, and apical echocardiographic segmental scores (Table 1). The LVEF was increased slightly under the ALA regimen at 12 months, as compared to placebo group (Table 1). This difference did not reach statistical significance ($p = 0.59$). At baseline there were no differences between groups about global or any regional MIBG myocardial scintigraphy (Fig. 3). The ALA

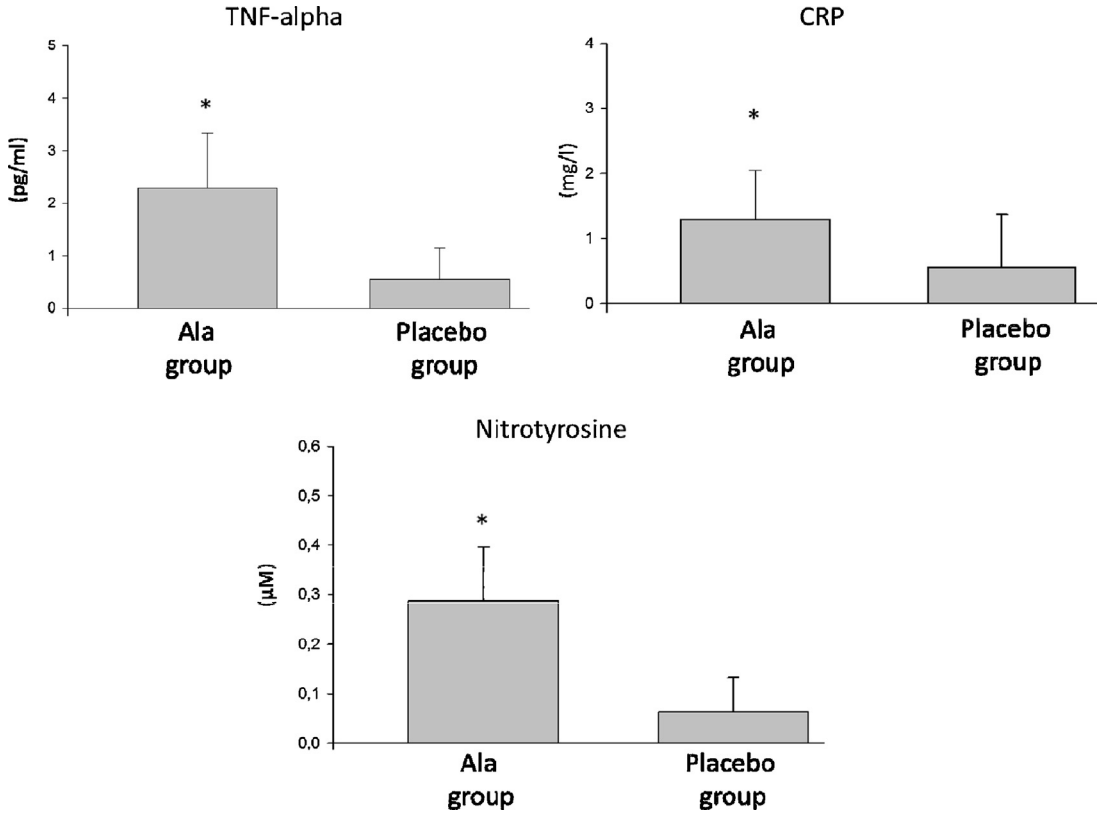

Fig. 2. Bar graphs representation of C-reactive protein (CRP), tumor necrosis factor α (TNF-alpha), and nitrotyrosine changes in patients who received α-lipoic acid (ALA patients) compared to placebo at follow-up. Data are expressed as mean and standard deviation (SD) and the * indicates a significant p-value ($p < 0.05$) in ALA group compared to placebo. C-reactive protein was significantly differently expressed at baseline and at 12-month follow-up in ALA group (3.8 ± 0.8 mg/l vs. 1.7 ± 0.64 mg/l, $p < 0.05$) and in placebo group, respectively (3.6 ± 0.8 mg/l vs. 2.7 ± 0.7 mg/l, $p < 0.05$). Tumor necrosis-α was significantly differently expressed at baseline and at 12-month follow-up in ALA group (5.5 ± 0.9 pg/ml vs. 2.1 ± 0.95 pg/ml, $p < 0.05$) and in placebo group, respectively (5.8 ± 0.8 pg/ml vs. 3.98 ± 1 pg/ml, $p < 0.05$). Nitrotyrosine was significantly differently expressed at baseline and at 12 months follow-up in ALA group (0.5 ± 0.1 μM vs. 0.24 ± 0.14 μM, $p < 0.05$), but not in the placebo group (0.5 ± 0.08 μM vs. 0.46 ± 0.12 μM, $p > 0.05$).

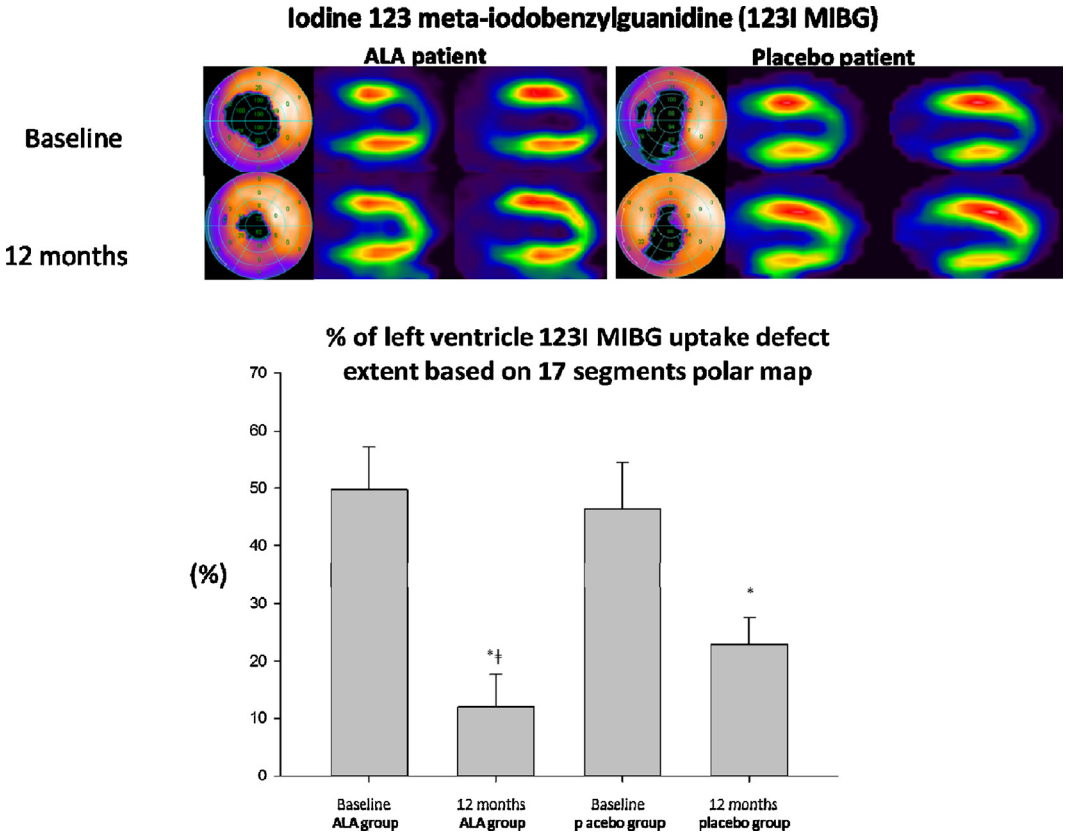

Fig. 3. Bar graphs are the representation of data expressed as mean and standard deviation (SD). The * indicates a significant *p*-value ($p < 0.05$) in α-lipoic acid (ALA) and/or placebo group comparing follow-up vs. baseline. The ‡ indicates a significant *p*-value ($p < 0.05$) when comparing placebo group vs. ALA group. Data are presented as mean + SD, data are distributed normally, and we have used ANOVA test for statistical comparisons. Upper part: representation of iodine 123 meta-iodobenzylguanidine (123I MIBG) distribution in left ventricle at baseline and 12-month follow-up in patients who received ALA (left) compared to placebo (right).

therapy was associated with a significant LV uptake increase as compared to placebo ($p < 0.01$) (Fig. 3). A significant change from baseline, a significant MIBG defect size reduction was observed in placebo-treated patients ($p < 0.05$) (Figs. 3 and 4). Analyses of the regional MIBG also showed that the ALA treatment induced considerably greater uptake increase predominantly in the apical, anterior, and apical-septal segments and apical-lateral and inferior segments (Figs. 3 and 5). After treatment, apical anterior and apical-septal segments uptake changes were related to significant TNF-α and nitrotyrosine reductions ($r = 0.38$, $p < 0.001$, $r = 0.29$, $p < 0.01$, respectively). There was a significant difference between ALA and placebo group after 12 months, because the 123-MIBG defect reduction was greater in the ALA group compared to the placebo group ($p < 0.05$) (Fig. 3). TCM recurred in 2 patients over a 12-month follow-up. Of note, the 2 patients with recurrent events were assigned to placebo treatment. At the time of TCM recurrence, there was no difference between the patients who did and did not have recurrence in the aspirin, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, beta-blockers, and statin drugs being used. The small number of patients and recurrences did not allow meaningful statistical analysis regarding factors predicting the TCM recurrence as well as the ALA treatment effect on the events.

Discussion

In the present study, for the first time to our knowledge, we demonstrated that 12-month ALA therapy improves sympathetic heart function in patients with previous TCM experience. The sympathetic activity dysfunction plays a key role in the pathophysiology of this reversible form of acute heart failure [1–5], as confirmed by our results about quantitative MIBG and catecholamine and B-type natriuretic peptide plasma level determination. Indeed, our data, according to the previous studies [18], provided evidence of the apical myocardial 123I-MIBG uptake impairment after 14 days from the acute event. Several mechanisms were proposed to justify the myocardial 123I-MIBG uptake reduction during acute phase. Distal denervation, following myocyte permanent damage, has been observed in patients with myocardial infarction [18]. In TCM patients, the normal coronary perfusion and the progressive 123I-MIBG uptake improvement both allow us to discard the hypothesis of myocardial sympathetic denervation from myocardial infarction. Alternatively, the 123I-MIBG uptake heterogeneity could be explained by the impairment of the uptake-1 function, which is an altered failing heart ATP-requiring system [19]. According to Owa et al. [20], we observed, in TCM placebo-treated patients, the non-complete uptake normalization of apical myocardial 123I-MIBG after 1-year follow-up. We can hypothesize that a persisting normal β-adrenoceptor function defected in the neurogenic stunned myocardium, in particular in the apical region, may be responsible for TCM recurrence. At this proposal, further larger studies including follow-up examinations in patients with transient mid ventricular ballooning syndrome could be interesting and particularly helpful. The mortality analysis revealed that TCM patients are three times more likely to die when compared to the general population [3]. After discharge, the incidence of sudden death (2.6%) was not negligible, and it merits further consideration. Currently, there are no data available to recommend proper preventive strategies besides an accurate patient follow-up, an aggressive cardiovascular risk factor

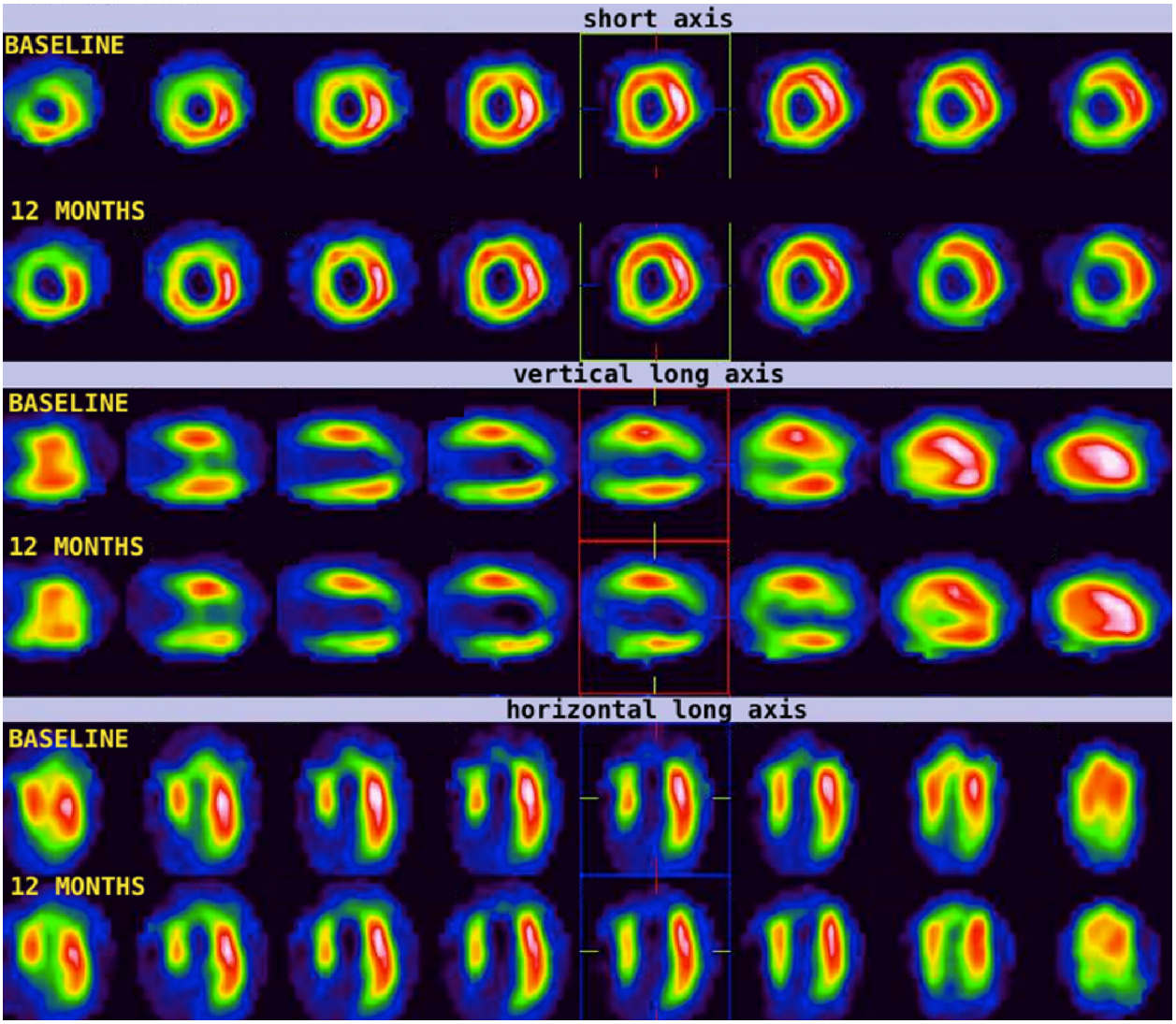

Fig. 4. Multiple slices from apex to base of short-axis, vertical long-axis and horizontal long-axis images, represented iodine 123 meta-iodobenzylguanidine (123I MIBG) distribution in left ventricle at baseline and 12-month follow-up in a placebo patient. These images represent the assessment of myocardial 123I MIBG distribution in a placebo patient with takotsubo cardiomyopathy at both subacute (baseline) and chronic phase (12 months). At baseline, 123I-mIBG uptake was severely reduced in the apex and in the apical and mid segments of antero-septal left ventricle (LV) wall, whereas myocardial perfusion at rest was normal, showing only moderate apical contractile dysfunction. 123I-mIBG scintigraphy was performed at 12-month follow-up after the acute symptomatology. At follow-up, images do not show an improvement of tracer uptake in LV apical segments. In placebo-treated patient, at 12-month follow-up, there is a non-complete normalization of apical myocardial 123I-MIBG uptake.

intervention, and an optimal co-morbidities management. In this context, the adrenergic cardiac innervation improvement may be beneficial in TCM patients. Indeed, there are two major findings in this study. First, we showed that ALA therapy is associated with a slight but significant left ventricle increased uptake as compared to the placebo group. In more detail, regional MIBG analyses showed that the ALA therapy induced a greater uptake increasing, predominantly in the apical, anterior, and septo-apical segments and lateral–apical and inferior segments. Second, we found that after treatment, apical anterior and septo-apical segment uptake changes were related to CRP, TNF-α, and nitrotyrosine blood level reductions. Our data were comparable to the observed ALA supplementation effects on diabetic cardiomyopathy [21]. Diabetic cardiomyopathy is associated with cardiovascular autonomic dysfunction, beginning at the apex of the ventricles, and progressing toward the base [21]. The oxidative stress role in diabetic cardiomyopathy pathophysiology is well known and an antioxidant ALA treatment has been shown to completely prevent heterogeneous MIBG distribution, decrease MIBG uptake, and increase norepinephrine content in diabetic cardiomyopathy [12,21]. Of note, the present study results indicate that ALA therapy determined a significant CRP, TNF-α, and nitrotyrosine plasma level reductions in TCM-treated patients compared to the placebo group. ALA acts by directly scavenging free radicals, increasing the activity of catalase and superoxide dismutase, and protecting peripheral nerves from lipid peroxidation [22,23]. In addition, ALA improves the antioxidant defense system through gene expression and inhibits nuclear factor κB [22,23]. In TCM patients, ALA appears to act through a reduction of oxidative stress and inflammation, as suggested by the strong evidence that changes in the anterior-apical and septo-apical segments uptake, after treatment, are related to TNF-α and nitrotyrosine reductions ($r = 0.38$, $p < 0.001$, $r = 0.29$, $p < 0.01$, respectively). In line with our evidence, the superoxide anion reduction, as well as the TNF-α decrements, completely abolished the increased catecholamine levels observed in heart failure [24]. Other heart failure markers, such as the urinary albumin to creatinine ratio, the urinary 24 h norepinephrine excretion, and high-sensitivity CRP levels, may

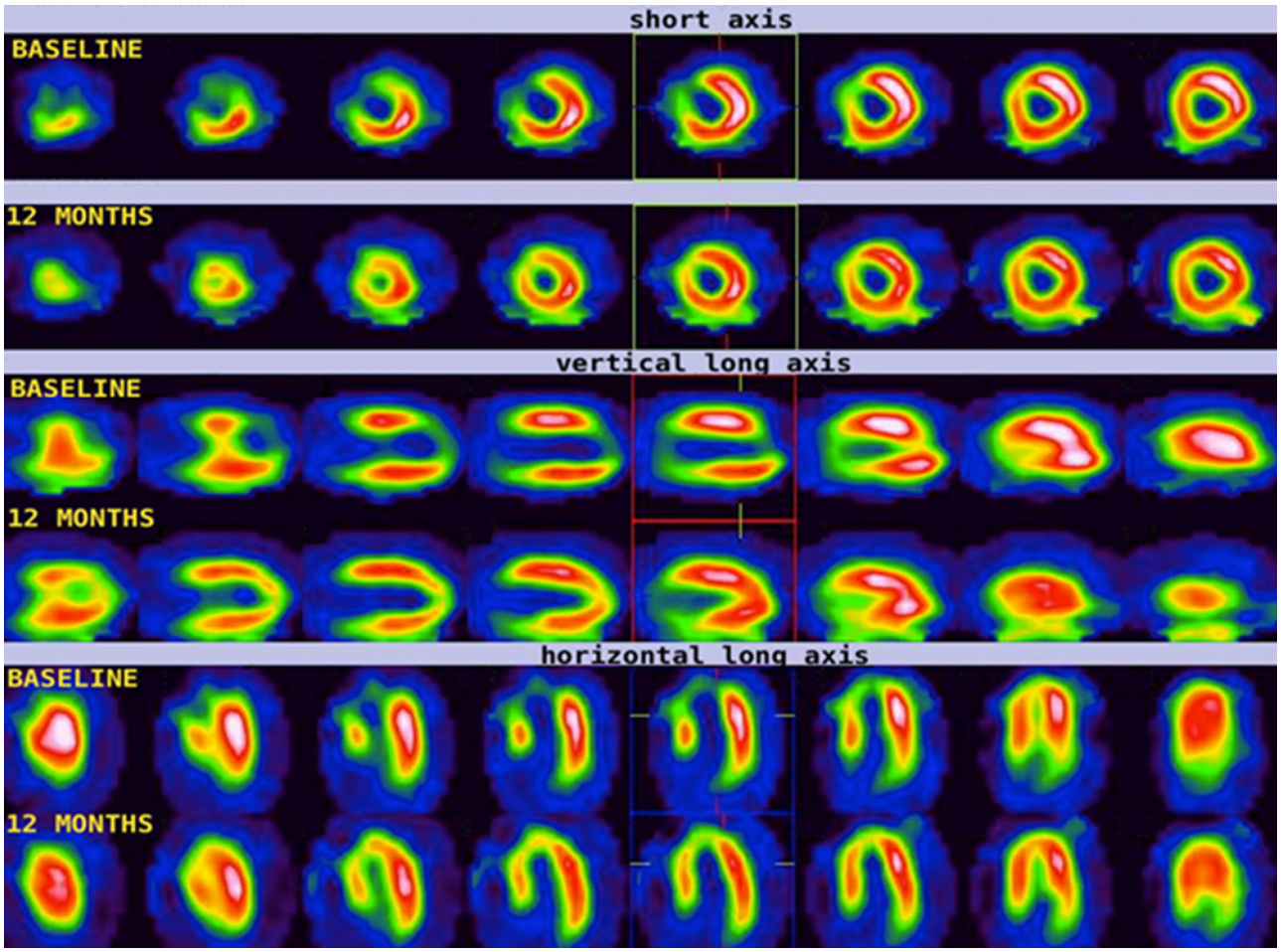

Fig. 5. Multiple slices from apex to base of short-axis, vertical long-axis and horizontal long-axis images represent iodine 123 meta-iodobenzylguanidine (123I MIBG) distribution in left ventricle at baseline and 12-month follow-up in a patient with takotsubo cardiomyopathy who received α-lipoic acid (ALA). These images represent, at baseline and at 12-month follow-up, the assessment of myocardial 123I MIBG distribution in ALA patient at both subacute (baseline) and chronic phase (12 months). In the subacute phase, 123I-MIBG uptake was severely reduced in the apex and in the apical and mid segments of antero-septal left ventricle (LV) wall, whereas myocardial perfusion at rest was normal, showing only moderate apical contractile dysfunction. 123I-MIBG scintigraphy was performed at 12-month follow-up after the acute symptomatology. At follow-up images, there is a reduction of 123-MIBG defect, with greater increases of the tracer uptake predominantly in the apical, anterior, and apical-septal LV segments.

reflect systemic and reversible inflammation compromising heart failure patients [25]. In fact, as authors have observed [25], the right treatment may reduce these inflammatory markers and partly mediate the reduction of albuminuria in heart failure. In our study, all these controlled molecular effects have been observed, described and correlated to the significant left ventricle MIBG uptake increase, predominantly in the apical, anterior, and septo-apical segments and lateral–apical and inferior LV segments. ALA therapy reducing oxidative stress and inflammation, and leading to the improvement of the adrenergic cardiac innervations, may control complex molecular pathways involved in LV coronary flow distribution in absence of coronary vessel obstructions. For these reasons, ALA oral therapy may be the target treatment to restore a defective LV segment vascularization in TCM-treated patients.

Study limitations

Study limitations include the limited power due to the relatively low number of patients, the relatively short duration of the study, and the limited assessment of changes in oxidative stress and inflammatory markers. In conclusion, the novelty of this study is that ALA treatment ameliorates the adrenergic cardiac innervation dysfunction in TCM patients. Identifying the direct and or indirect ALA action mechanism at a molecular level may provide an important novel insight for the improvement of therapeutic strategies to target pathogenic transient cardiomyopathy mechanisms such as takotsubo.

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