

A Novel BioTarget in Treatment of Heart Failure: Changes in Serum Galectin-3 Levels after Spironolactone Therapy

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Abstract

Objective: It has been aimed to investigate the Galectin-3 (GAL-3) levels and clinical responses after addition of spironolactone as a mineralocorticoid receptor antagonist (MRA) to the current treatment in patients with heart failure with low ejection fraction who received no aldosterone antagonist therapy previously.

Patients and methods: The study included 112 patients with Heart Failure (HF) who showed left ventricular Ejection Fraction (EF) of 35% or below, New York Heart Association (NYHA) Class II-IV symptoms and did not receive MRAs in their current treatment. Serum Gal-3 levels, serum BNP level, 6-minute walk test and class level of NYHA were examined before and 6 months after treatment of spironolactone in all patients.

Results: Mortality developed in 10 of 112 patients. Baseline and 6th month follow-up data obtained from 102 of 112 patients. Mean LVEF (%), BNP levels, Gal-3 levels, NYHA class of functional capacity and mean 6-minute walking test distance of the patients before treatment of spironolactone were $31.3 \pm 3.2\%$, 451.4 ± 50.3 pg/ml, 39 ± 21 ng/ml, 2,8 ± 0.59 and 305 ± 61 m respectively whereas, the same variables were found $32.1 \pm 2.8\%$ ($p=0.21$), 443.6 ± 49 pg/ml ($p=0.23$), 33 ± 22 ng/ml ($p<0.001$), 2.5 ± 0.47 ($p=0.037$) and 386 ± 87 m ($p<0.001$) respectively at 6th follow-up after treatment of spironolactone.

Conclusion: Spironolactone use is associated with regression of Gal-3 along with clinical improvement in HF symptoms. This may suggest that Gal-3 apart from being a biomarker of HF may also be a bio-target in HF management.

Keywords: Galectin 3; Myocardial and vascular fibrosis; Left ventricular ejection; MRAs

Introduction

Heart failure (HF) is the leading cause of mortality and morbidity in the western World [1]. Searching for therapeutics to cure HF has led studies to investigate the mechanisms particularly on neurohumoral activation [2]. During HF, increased levels of catecholamines resulting from activation of sympathetic nervous system lead to chronic stimulation of aldosterone levels causing adverse remodelling of the heart [3]. Aldosterone is a mineralocorticoid hormone that has been shown to play a pathophysiologic role in cardiovascular remodelling through cardiac hypertrophy, fibrosis and inflammation [4,5].

Galectin 3 (Gal-3) is a multifunctional protein that participate in various biological events such as angiogenesis and inflammation and an important member of the family of beta-galactoside-binding animal lectins mostly produced by macrophages. The current studies indicate Gal-3 as a novel prognostic marker for cardiac fibrosis associated with high risk for heart failure and mortality. Thus, elevated levels of Gal-3 are associated with cardiac fibrosis [6]. It has been demonstrated that GAL-3 expression is stimulated by aldosterone and stimulated GAL-3 plays a critical role in vascular remodelling and inflammation [7]. Use of mineralocorticoid receptor antagonists (MRAs) has provided 35% reductions in hospital admissions due to worsening heart failure by decreasing myocardial and vascular fibrosis [8]. Adding spironolactone to treatment in the patients who use angiotensin converting enzyme inhibitor provided a reduction in morbidity and mortality rates [9,10].

Data substantiating the hypothesis that decreased levels of GAL-3 after MRAs therapy may result beneficial outcomes remain unclear. To address this question, it has been aimed to investigate the GAL-3

levels and clinical responses after addition of spironolactone as a MRA to the current treatment in patients with heart failure with low ejection fraction who received no aldosterone antagonist therapy previously.

Methods

Study population

The study was carried out in the hospital of Çukurova University Faculty of Medicine between April 2013 and March 2014. Our study included 112 patients with heart failure who showed left ventricular ejection fraction (EF) of 35% or below, New York Heart Association (NYHA) Class II-IV symptoms and did not receive MRAs in their current treatment. The patients with creatinine clearance (CrCl) of 60 ml/minute or below (according to Cockcroft), advanced stages of liver failure (ALT and AST levels 3-fold higher than upper limit of normal interval), NYHA Class I symptoms, an ejection fraction above 35% and autoimmune and/or collagen tissue disorder were excluded from the study. The study aimed to evaluate serum Gal-3 levels after the use of spironolactone. Therefore, serum Gal-3 levels, serum BNP level, 6-minute walk test and class level of NYHA were examined before

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and 6 months after treatment of spironolactone in all patients. This study was performed according to criteria of Helsinki Declaration and approved by the Ethics Committee of Çukurova University Faculty of Medicine. Informed consent forms were obtained from all the patients.

Transthoracic echocardiography

All echocardiographic examinations were performed with a Vivid S5 cardiac ultrasound scanner (GE Vingmed Ultrasound, Horten, Norway) using a 2.5-3.5 MHz transducer. All patients were examined in the left lateral and supine position by precordial M-mode and 2-dimensional echocardiography. Blinded average interobserver and intraobserver reproducibility of measurement was evaluated and comparison revealed a Spearman correlation coefficient of 0.92 and 0.94, respectively. LV end-diastolic and end-systolic diameters, EF (%) and end-systolic left atrial (LA) diameters were measured from both M-mode in the parasternal long axis views and modified Simpson Method in the apical four chamber views.

Biochemical analysis

Prior to treatment of spironolactone, venous blood samples of all patients were obtained for tests of whole blood count, serum electrolyte levels, kidney and liver function tests, serum BNP and Gal-3 levels. Venous fasting blood sample was collected into EDTA-containing Vacuette tubes (Greiner Bio-One) and centrifuged at 3,000 g for 15 min at 4°C. Plasma samples were separated and stored at -70°C until usage within 3 months. The obtained serum was preserved at -80°C. Serum BNP and serum Gal-3 levels were tested in all patients when treatment of MRAs was initiated and at the end of sixth month. Assessment of Gal-3 level was performed using Human Galectin-3/LGALS3 ELISA Kit (Boster Biological Technology Co, Ltd, CA.). The concentrations of BNP were measured by a 2-site sandwich chemiluminescence immunoassay (Lot No: 22161145, reference No: 02816634) on the ADVIA® Centaur® platform (Siemens Healthcare Diagnostic, Ill., USA).

Six-minute walking test and determination of NYHA Classification

6-minute walking test was carried out to all patients included in the study prior to treatment of spironolactone and their functional classifications of NYHA were also evaluated. Six-minute walking test and functional classification of NYHA were repeated at the 6th month follow-up in all patients.

Statistical analysis

All analyses were performed using SPSS 20 statistical software package (IBM SPSS Statistics). Categorical analyses were expressed as numbers (n) and percentages (%) while continuous analysis were reported as mean, standard deviation, median, minimum and maximum as required. To evaluate the change in the measurements obtained before and after treatment, the paired sample t-test or Wilcoxon test was used depending on whether the statistical hypothesis was fulfilled. Additionally analysis of covariance is employed to determine the effect of other factors such as hypertension, diabetes, and the medications other than spironolactone (Beta blockers, ACEI/ARB) on GAL-3 levels after spironolactone use. The level of statistical significance for all tests was determined as 0.05.

Results

The study included 102 patients since mortality developed in 10 of 112 patients. Baseline and follow-up data at 6th month were obtained from all patients. The baseline characteristics of the patients

were presented in Table 1. The patients had mean age of 65 ± 13 and included 62 (60,8%) male subjects. The etiology of heart failure was found ischemic origin in 72 (70.6%) patients. Of the patients; 69 (67.6%) and 32 (31.3%) were respectively hypertensive and diabetic and mean creatinine/ eGFR values were 1.03 ± 0.36 mg/dl and 89.5 ± 28 , respectively. Mean heart beat rate was measured 76 ± 19 /min. The mean body mass index of the study population was 25 ± 1.2 . Of the patients, 90 (89%), 94 (92%) and 76 (75%) were receiving treatments of beta-blocker, ACEI or ARB and loop diuretic, respectively (Table 1). The treatment of spironolactone was initiated in all patients included in the study.

Mean LVEF % values of the patients before treatment of spironolactone was $31,3 \pm 3,2\%$ whereas that value was found $32,1 \pm 2,8\%$ ($p=0.21$) after treatment. BNP levels measured at baseline and at 6th follow-up after treatment of spironolactone were encountered respectively $451,4 \pm 50.3$ pg/ml and $443,6 \pm 49$ pg/ml ($p=0.23$) (Figure 1). After treatment of spironolactone was initiated to the patients; mean NYHA class of functional capacity regressed from $2,8 \pm 0.59$ to $2,5 \pm 0.47$ ($p=0.037$) while mean Gal-3 levels decreased from 39 ± 21 ng/ml to 33 ± 22 ng/ml ($p<0.001$) (Figure 2). Mean 6-minute walking test distance increased from 305 ± 61 m to 386 ± 87 m ($p<0.001$) (Table 2).

Diabetes, hypertension, use of beta blocker and use of ACEI/ARB are entered into co-variance model first individually then jointly to determine the possible effects on GAL-3 level after spironolactone therapy. In this model DM, HT, Beta blockers and ACEI/ARB entered in the model as cofactors together with GAL-3 levels after spironolactone use as a covariate. Diabetes, hypertension and use of beta blockers had no significant effect on serum GAL-3 levels after spironolactone therapy. From the joint co-variance model change in GAL-3 levels is significantly effected both by spironolactone and ACEI/ARB use (Table 3).

Discussion

In our study we have aimed to evaluate the rate of changing levels in Gal-3 level and some clinical endpoints of the patients by spironolactone therapy in treatment of HF with low EF. To the best of our knowledge, our study was the first prospective study to suggest that addition of spironolactone to the treatment of systolic heart failure patients with low EF decreases serum Gal-3 levels.

Activation of the sympathetic nervous system and renin-angiotensin-aldosterone system are the main neurohumoral compensatory mechanisms in HF, act to maintain cardiac output

Variable	Participants (n: 102)
Age (years)	65 ± 13
Gender, M/F, %	60.8 / 39.2
Etiology, ischemic/ non-ischemic, %	70.6 / 29.4
Hypertension, %	68
Diabetes mellitus, %	31
Heart rate/min	76 ± 19
Body mass index	25 ± 1.2
Creatinine, mg/dl	1.03 ± 0.36
eGFR, ml/min	89.5 ± 28
Beta-blocker, %	89
ACEI or ARB, %	92
Loopdiuretics, %	75
Digoxin, %	32

Table 1: Baseline characteristics of the study population

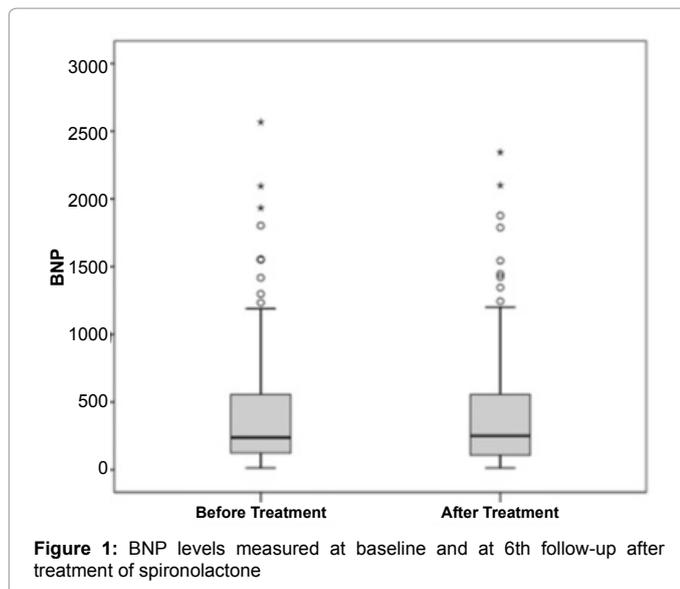


Figure 1: BNP levels measured at baseline and at 6th follow-up after treatment of spironolactone

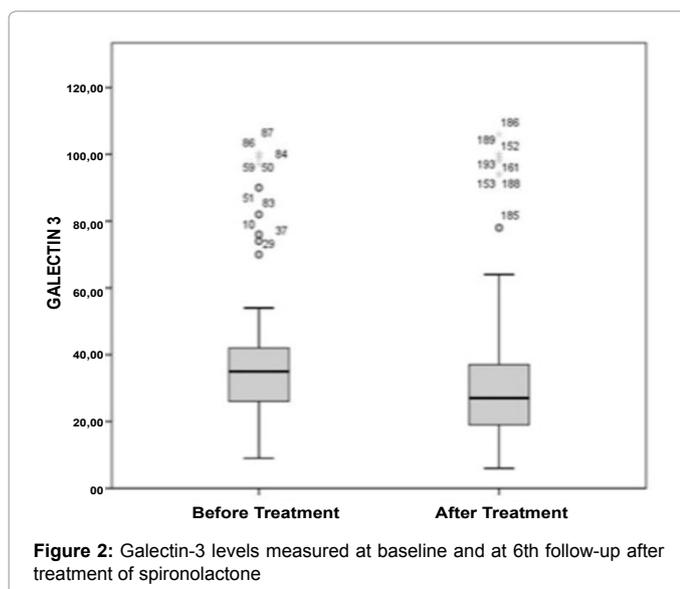


Figure 2: Galectin-3 levels measured at baseline and at 6th follow-up after treatment of spironolactone

by cardiac contractility, increasing water and salt retention, arterial vasoconstriction, and by activation of inflammatory cascades that contribute to cardiovascular repair and remodelling. Unregulated and prolonged neurohumoral activation in HF may, however, mediate a multitude of harmful effects [11,13].

Gal-3 is a novel biomarker that has been shown to mediate fibrosis in the heart failure [14]. The procollagens produced by myofibroblasts and fibroblasts activated due to immune response triggered secondary to acute and chronic myocardial damage present a collection in the extracellular matrix and subsequently cause myocardial fibrosis [15,16]. It has been demonstrated that myocardial Gal-3 level is upregulated in the hypertensive cardiomyopathies (CMP) prone to structural heart disease, myocarditis and CMPs induced by interferon-6, streptozosin-induced diabetic CMPs and angiotensin II-induced hypertensive animal models [15] Similarly, it has been detected that Gal-3 level significantly increases in the hypertrophic hearts with serious aortic stenosis, and in acute and chronic heart failure [16]. Gal-3 levels

have been evaluated by the HF-ACTION study in the patients with ambulatory chronic heart failure involving systolic dysfunction. By testing retrospectively in the stored plasma samples, high levels of GAL-3 concentrations were found associated with high NYHA class, high levels of NT-proBNP, low systolic blood pressure levels and low maximal oxygen consumption [17].

De Boer et al. have encountered that an early increase occurred in expression of Gal-3 in the hearts prone to heart failure and this increase causes fibroblast proliferation, collection of collagen and ventricular dysfunction [18]. It has been shown in the study of Calvier et al. conducted by in-vitro and in-vivo animal models that increased level of aldosterone increases expression of Gal-3 in the smooth muscle cells and over-expression of Gal-3 increases production of Type-1 collagens specifically [19].

In the light of these findings, one of the important aspects in treatment of heart failure should be the antagonization of the multiple inflammatory mediators including Gal-3. It has been encountered in the prospective 6-month follow-up examinations of our patients that a significant reduction has been encountered in the level of serum Gal-3 after addition of spironolactone therapy to standard treatment of HF and similarly a significant improvement was detected in the NYHA functional capacity and 6-minute walk test distance whereas no significant changing was determined in levels of LVEF and serum BNP. This result makes us think that regression in cardiac fibrosis in our relatively short 6-month follow-up process may have latter positive effects on cardiac filling pressures, LVEF and serum BNP levels. Another possible explanation for insignificant change of BNP levels is that, among the study population the majority of the patients were without congestion.

In a big cohort study, De Boer et al. have found that high levels of galectin-3 (normal values are 10-14 ng/ml, high values are 16-30 ng/ml depending on severity of heart failure and comorbidities) were associated with both all cause and cardiovascular mortality [20,21]. These findings have been confirmed with the detection of high rates of heart failure and mortality that developed in the cases with high levels of Gal-3 in the patient population of Framingham Heart Study [4].

Differently from the findings of our study, it has been encountered in the retrospective study of Fiuzat et al. carried out in the cases with systolic heart failure that MRAs therapy caused no significant changing in level of serum Gal-3 [22]. The authors of the study have stated for the potential rationales of this situation that administration of MRA may cause up-regulation in the level of GAL-3 and that this result may be probably associated with levels of Gal-3 obtained in the decompensated state of the patients.

As a study with supportive data for our results that conducted on the patients with heart failure with low systolic function, Tang et al. have found that GAL-3 levels were significantly lower in the patients who received b-blocker (13,4 ng/mL vs 14,9 ng/mL; P = 0.024) and spironolactone (13,1 ng/mL vs 14,3 ng/mL; P = 0.043) therapies than

Variable	Baseline	Follow-up (after 6 months)	P values
LVEF, %	31.3 ± 3.2	32.1 ± 2.8	NS
NYHA (level)	2.8 ± 0.59	2.5 ± 0.47	0.037
Gal-3 levels, ng/ml	39 ± 21	33 ± 22	<0.001
BNP levels, pg/ml	451.4 ± 50.3	443.6 ± 49	NS
6 MWD, m	305 ± 61	386 ± 87	<0.001

Table 2: The changes in clinical and laboratory parameters prior to and after treatment of spironolacton

Parameter	B	Std. Error	t	Sig.	95% Confidence interval	
					Lower bound	Upper bound
Intercept	-6.717	2.497	-2.690	.008	-11.677	-1.757
Beta Blocker therapy	-2.770	6.959	-0.398	.692	-16.593	11.053
ACEI/ARB therapy	-21.469	6.714	-3.198	.002	-34.805	-8.133
Hypertension	.575	2.975	.193	.847	-5.335	6.486
Diabetes	2.112	2.367	.892	.375	-2.589	6.813
GAL-3 levels before treatment	.986	.039	25.213	.000	.908	1.064
HT * DM interaction	.334	3.670	.091	.928	-6.957	7.625
ACEI/ARB * DM interaction	-13.798	6.932	-1.991	.050	-27.567	-.029

Table 3: Covariance analysis of Galectin 3 levels after spironolactone therapy

the patients who received no treatment [23]. It has been encountered in the randomized DEAL-HF study conducted on the patients with chronic heart failure that high concentrations of Gal-3 are associated with increased mortality [24].

MRAs, as the cornerstone in the treatment of standard heart failure, decelerates fibrosis process. The regression in the level of Gal-3, as an prognostic marker of myocardial fibrosis, due to spironolactone therapy, shows their positive effect on this process.

Limitations of the Study

The most important limitations of our study are the relatively small number of study population and being a single center study. To prevent the confounding effects on serum GAL-3 levels, the study continued with the same doses of ACEi/ARB and beta blocker before addition of spironolactone. Therefore dose-titration of these medications could not be made. Also, only one MRA (spironolactone) was used in our study and consequently response to other MRAs is not known. Since levels of Gal-3 are affected by renal failure, liver failure and collagen tissue disorders, the patients with these disorders were excluded from the study and this situation limited our study population. Finally, our data would be more accurate if the reduction of fibrotic adverse process in myocardium was confirmed by the examination of myocardial biopsy.

Conclusion

In a prospective analysis of a well treated cohort of ambulatory patients with systolic HF, spironolactone use is associated with regression of Gal-3 along with clinical improvement in HF symptoms. This may suggest that Gal-3 apart from being a biomarker of HF may also be a bio-target in HF management.

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