Effects of Levosimendan on TNF-alpha, BNP and MMP-1 in Patients with Heart Failure with Anemia

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Abstract

Background: Levosimendan is known with its two-sided effects of strengthening myofibril contraction without increasing myocardial oxygen demand. Anemia is a deteriorating situation that causes increase of drug dosing in patients with heart failure.

Objectives: In this study, we compared the effectiveness of levosimendan treatment in decompensated heart failure patients with or without anemia.

Methods: Twenty-three anemic patients having class 3 or 4 heart failure according to New York Heart Association (NYHA) and an ejection fraction of below 35% were included to the study. Another 23 patients with the same cardiac diagnosis but without anemia served as control group. Twenty-four hours levosimendan treatment was added to the traditional heart failure treatment of these patients. Samples were taken to measure serum tumor necrotizing factor alpha (TNF-alpha), aminoterminal pro-brain natriuretic peptide (NT-proBNP) and matrix metalloproteinase-1 (MMP-1) levels before and after the administration.

Results: There was no significant difference between serum TNF-alpha and MMP-1 levels before and after the treatment ($p>0.05$). Although NT-proBNP level decreased in both groups after the treatment this was not statistically significant ($p=0.531$ and $p=0.913$ for anemia and control groups respectively). Significant restoration of functional capacity was seen in both groups assessed according to NYHA ($p<0.001$ and $p=0.001$ for anemia and control groups respectively).

Conclusion: Levosimendan treatment shows similar effects in heart failure patients with anemia to that of patients without anemia. However, the early effect of this treatment on TNF-alpha, NT-proBNP and MMP-1 levels is not evident. It provides significant improvement in functional capacity without influence from anemia. (Arq Bras Cardiol 2012;99(1):659-664)

Keywords: Heart failure / drug therapy; anemia; tumor necrosis; factor-alpha; natriuretic peptide; brain; matrix metalloproteinase 1.

Introduction

Heart failure (HF) is an ongoing, progressive syndrome defined by impairment of cardiac functions and increase in neurohormonal activity. The level of circulating proinflammatory cytokines like tumor necrosis factor-alpha (TNF-alpha) increases in patients with HF. These cytokines also lead to remodeling of left ventricle and affect cardiovascular system by causing a contraction defect[1]. Plasma brain natriuetic peptide (BNP) levels increase in patients with left ventricle disorder. Plasma aminoterminal pro-brain natriuretic peptide (NT-proBNP) intensity changes reflect the response to HF treatment[2]. Matrix metalloproteinase (MMP) family is a wide enzyme group that has a role in regulating extra cellular structure. In the previous studies, they were proved to be strong markers of cardiac remodeling and HF progression[3,4] One of the organs in which blood circulation deteriorates due to low cardiac output is the hemopoetic system. Anemia due to affected hemopoetic system causes resistance to treatment and aggravation of symptoms[5].

Cardiac contraction is impaired in 15-20% of the patients with chronic HF, which leads eventually to acute HF. Advanced treatment of acute HF is based on the infusion of medications called positive inotropic drugs. Levosimendan is a new intravenous drug used for acute instability of chronic HF and supports the heart through two mechanisms like sensitizing myofibrils to calcium and opening vascular potassium channels susceptible to adenosine triphosphate (ATP)[6]. It was shown that this drug affected TNF-alpha, BNP and MMPs and decreased their serum levels[7-10]. However, the effectiveness of levosimendan was not investigated in anemic and non-anemic subgroups.

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The aim of this study is to investigate the effect of intravenous levosimendan administered in addition to traditional treatment on functional capacity and TNF-alpha, NT-proBNP and matrix metalloproteinase (MMP-1) levels in anemic and control groups having decompensated HF.

Material and methods

Study population

Patients who were admitted to Trakya University Cardiology Department with left ventricle ejection fraction (EF) under 35% in echocardiography examination, resistant to traditional HF treatment, and having class 3 and 4 heart failure according to NYHA were enrolled to this study. Twenty-three of these patients had additionally anemia (blood hemoglobin value <13 g/dl in men and <12 g/dl in women). Another 23 patients without anemia served as the control group. Exclusion criteria were as follows: Patients with; 1) unstable angina or myocardial infarct in the last two weeks, 2) obstructive cardiomyopathy or non-restored obstructive valve disease, 3) symptomatic primary lung disease, 4) systolic blood pressure <80 mmHg or > 200 mm Hg, 5) resting heart rate >115/min, 6) serum creatinine >2.5 mg/dl, 7) increased aspartate aminotransferase and alanine aminotransferase levels more than twice of normal, 8) serum potassium level <3.5 or >5.5 mmol/dl, and 9) acute and chronic infectious diseases, and 10) patients taking immunosuppressive drugs. Twenty-four hour intravenous levosimendan treatment was added to the traditional treatment composed of beta-blocker, angiotensin converting enzyme inhibitor, furosemide, and spiranolactone as infusion with 0.1 mcg/kg/min dose.

Echocardiography

Transthoracic echocardiography was performed to all patients (Vivid 3 Pro, GE Medical Systems, Milwaukee Wisconsin). The examiner was blinded regarding the study details. Left ventricle ejection fraction and left ventricle sizes were assessed by M-mode and regulated Simpson methods.

Laboratory analysis

Blood samples were taken from each patient before levosimendan administration and right after 24-hour infusion. Blood samples were centrifuged at 2500 rpm for 10 minutes and kept at -80 degrees Celsius. Samples kept in the freezer were processed using enzyme linked immunosorbent assay with Microplate Reader MPRA41 device to check TNF-alpha (Human TNF alpha ELISA, Daiclone, Besancon France), NT-pro BNP (BNP FRAGMENT EIA, Biomedica Wien), and MMP-1 (Human pro-MMP-1, R&D systems, Minneapolis) levels.

None of the patients required discontinuation of infusion or dose decrease during levosimendan infusion and all included patients completed the study.

Age, sex, coronary artery disease, history of diabetes mellitus, medications, HF class according to NYHA, blood pressure, heart rate, and information on electrocardiography were recorded.

Statistical analysis

In the statistical analysis, normal distribution was assessed by Kolmogorov-Smirnov one sample test. Mann Whitney U and Wilcoxon tests were used for numeric variables. Chi Square and Marginal homogeneity tests were applied for categorical variables. Statistically significant was accepted as $p<0.05$.

This study was approved by local ethics committee of Trakya University Hospital. Written consent was obtained from all patients.

Results

A total of 46 patients were enrolled in the study. Average hemoglobin level was 11.1±1 g/dl in anemic group and 13.5±1 g/dl in control group. Demographic features and laboratory values of both groups before the study are shown on Table 1. Blood levels and functional capacities of both groups before and after treatment are shown on Table 2. Average left ventricle ejection fraction was 26.8% ± 5.4 in anemic group and 24.4% ± 5.9 in control group ($p=0.175$, Table 1).

Average NT-proBNP, TNF-alpha, MMP-1 levels before treatment were 11.8±8.4 pmol/lt, 0.004±0.02 ng/ml, and 3.7 ± 2.3 ng/ml respectively in anemic group and 9.6±5.1 pmol/ml, 4.1±20 bg/ml, and 3.5±2.8 ng/ml respectively in control group ($p>0.05$, Table 1). Hypotension did not develop in both groups during levosimendan infusion. Ventricular tachycardia developed in 2 anemic patients (8%) and in 1 control (4%) patient during infusion ($p=0.255$). Average NT-proBNP, TNF-alpha, MMP-1 levels after treatment were 9.0±6.0 pmol/ml, 20.7±46.3 ng7ml, and 3.8±2.7 ng/ml respectively in anemic group and 9.3±7.4 pmol/ml, 14±25.7 ng/ml, and 3.2±2.8 ng/ml respectively in control group ($p>0.05$, Table 3).

No significant difference was seen in both groups compared to pre-treatment values. However NT-proBNP levels decreased in both groups ($p>0.05$, Table 2).

While 10 (43%) of 23 control patients had class 3 and 13 (57%) had class 4 HF at the beginning of treatment, 5(22%) had class 2, 11(48%) had class 3 and 7(30%) had class 4 HF at the end of treatment ($p=0.001$, Table 2). While 9 (39%) of 23 anemic patients had class 3 and 14 (61%) had class 4 HF at the beginning of treatment, 6 (27%) had class 2, 13 (56%) had class 3 and 4 (17%) had class 4 HF at the end of treatment ($p<0.001$, Table 2).

Discussion

In this study, we observed that levosimendan treatment did not have any effect on TNF-alpha and MMP-1 early period and had a statistically insignificant decreasing effect on NT-proBNP in patients with anemia and control group. Due to the presence of similar effects in anemic patients and control group, we concluded that effectiveness of levosimendan treatment did not decrease in anemia. Although no significant effect of this treatment on blood levels was seen in both groups, we observed its clinically improving effect on functional capacity.

Original Article

Table 1 – Basal characteristic features and laboratory levels of study patients

Parameter		Anemic group mean ± SD / n (%)	Control group mean ± SD / n (%)	P
Age (years)		64.5 ± 14.3	65.3 ± 11.8	0.886
Men / Women		20(86) / 3(14)	13(56) / 10(44)	0.024
Ischemic DCMP		14(60)	10(44)	0.023
Non-ischemic DCMP		9(40)	13(56)	0.542
Hypertension		13(56)	13(56)	0.862
Diabetes Mellitus		8(34)	7(30)	0.853
Systolic BP (mmHg)		98.2 ± 11.5	104.7 ± 8.4	0.056
Diastolic BP (mmHg)		64.1 ± 7.1	70 ± 8.5	0.017
Heart Rate (pulse/min)		95 ± 13	92 ± 13	0.347
NYHA class 3		9(39)	10(43)	0.765
NYHA class 4		14(61)	13(57)	0.362
TNF - alpha (ng/ml)		0.004 ± 0.02	4.1 ± 20	0.975
NT - proBNP (pmol/l)		11.8 ± 8.4	9.6 ± 5.1	0.531
MMP-1 (ng/ml)		3.7 ± 2.3	3.5 ± 2.8	0.652
Echocardiographic meaurements	LVESV (ml)	149.7 ± 68.3	146.3 ± 41.7	0.750
	LVEDV (ml)	202.3 ± 84	191.7 ± 46.8	0.775
	LVEF (%)	26.8 ± 5.4	24.4 ± 5.9	0.175
Drug use	Beta Blocker	19(82)	14(60)	0.189
	ACEI	13(56)	15(65)	0.763
	Furosemid	22(95)	18(78)	0.187
	Spiranolactone	16(69)	13(56)	0.542
	Digoxin	9(39)	12(52)	0.554

DCMP: dilated cardiomyopathy, BP: blood pressure, NYHA: New York Heart Association, TNF-alpha: tumor necrotizing factor alpha, NT-proBNP: aminoterminal probain natriuretic peptide, MMP: matrix metalloproteinase, LVEF: left ventricle ejection fraction, LVESV: left ventricle end-systolic volume, LFEDV: left ventricle end diastolic volume, ACEI: angiotensin converting enzyme inhibitor

Table 2 – Comparison of blood parameters and functional capacity in anemic and control groups before and after treatment

Parameter	Anemic group mean ± SD / n			Control group mean ± SD / n		
	Before treatment	After treatment	P	Before treatment	After treatment	P
TNF-alpha (ng/ml)	0.004 ± 0.02	20.7 ± 46.3	0.975	4.1 ± 20	14 ± 25.7	0.666
NT-proBNP (pmol / ml)	11.8 ± 8.4	9.0 ± 6.0	0.531	9.6 ± 5.1	9.3 ± 7.4	0.913
MMP-1 (ng/ml)	3.7 ± 2.3	3.8 ± 2.7	0.652	3.5 ± 2.8	3.2 ± 2.8	0.373
NYHA class 2	0	6	< 0.001	0	5	0.001
NYHA class 3	9	13		10	11	
NYHA class 4	14	4		13	7	

NYHA: New York Heart Association, TNF-alpha: tumor necrotizing factor alpha, NT-proBNP: aminoterminal probain natriuretic peptide, MMP: matrix metalloproteinase

Table 3 – Comparison of blood parameters and functional capacity in anemic and control groups after the study

Parameter	Anemic Group mean ± SD / n	Control Group mean ± SD / n	P
TNF-alpha (ng/ml)	20.7 ± 46.3	14 ± 25.7	0.547
NT-proBNP (pmol/ml)	9.0 ± 6.0	9.3 ± 7.4	0.890
MMP-1 (ng/ml)	3.8 ± 2.7	3.2 ± 2.8	0.507
NYHA class 2 (n)	6	5	0.850
NYHA class 3 (n)	13	11	
NYHA class 4 (n)	4	7	

NYHA: New York Heart Association, TNF-alpha: tumor necrotizing factor alpha, NT-proBNP: aminoterminal probrain natriuretic peptide, MMP: matrix metalloproteinase

In previous studies, efficacy of levosimendan treatment was shown by assessing hemodynamic, clinical and neurohumoral variables[11,12]. However, in these studies, anemic patients were not compared to non-anemic patients.

The aim of decompensated HF treatment is to make the patient clinically stable, to provide blood supply to organs, to provide a sufficient level of filling pressure and to make sure that patient returns to traditional treatment as soon as possible. Levosimendan supports cardiovascular function and impaired organ perfusion in decompensated heart failure patients with two-sided effects[13,14].

Anemia is an independent risk factor for mortality in patients with left ventricle insufficiency[15]. In a study conducted in anemic patients with HF, it was observed that medication need for the treatment of HF decreased along with the recovery of anemia[16]. This means that anemia causes increase in the required drug dose for HF and development of drug resistance. HF treatment in anemic patients remains uncertain[17]. Therefore knowing the efficacy of administered drugs in anemic patients in situations like decompensated HF that require urgent treatment could be practical for clinicians in daily practice.

In 1990, it was reported that level of circulating TNF-alpha, a proinflammatory cytokine, increased in patients with HF[18]. Since then, many studies were conducted stating that proinflammatory cytokines like TNF-alpha can play a very important role in regulating myocardial structure and functions in advanced phases of HF. It was found that cytokines contributed to changing cardiovascular functions via mechanisms like leading to a re-shaping of myocardium and decreasing myocardial beta adrenergic receptors[19]. It was shown that levosimendan treatment decreased TNF alpha levels[8,20,21]. This anti-inflammatory effect of levosimendan treatment was explained by various mechanisms. The most important of these are decrease of extra-cardiac cytokine production through restoration of peripheral tissue perfusion upon the effect on cardiac calcium metabolism[8]. No significant changes were seen between TNF-alpha levels of both groups before and after the treatment but a significant change was observed in NT-proBNP levels.

In another study conducted by Follath et al.[11], levosimendan treatment was compared with dobutamin treatment and placebo. In this study, no significant changes were seen between TNF-alpha levels of levosimendan and dobutamin groups before and after the treatment. However, the significance was higher in the placebo group.

Similar to the results of Parissis et al.[8], in our study, we did not observe any significant difference in TNF-alpha levels of both groups before and after treatment. In addition, we observed increase in TNF-alpha levels in both groups after the treatment.

Brain NP and NT-proBNP levels are associated with the severity of HF[22,23]. There is a number of evidence confirming that plasma BNP and/or NT-proBNP levels are independent predictors of total mortality, cardiovascular mortality and hospitalizations due to both in acute and chronic HF[24,25]. BNP and NT-proBNP oriented treatment is found to be superior than clinical decision oriented treatment[2]. It was shown in previous studies that levosimendan treatment caused decrease in BNP and NT-proBNP levels[7,9,20,21]. The decreasing effect of levosimendan treatment on BNP and NT-proBNP was found to be associated with its decreasing effect on end-diastolic cardiac wall tension[7]. In a study conducted by Adamopoulos et al.[20], effect of dobutamin and levosimendan treatment on NT-proBNP was observed. In this study, which included also a placebo group, no significant change was seen in NT-proBNP levels in dobutamin and placebo groups while significant decrease was seen in levosimendan group. In our study, we found insignificant decrease in NT-proBNP levels in both groups before and after the treatment.

Matrix metalloproteinase is an enzyme group which takes part in processes like tissue re-constitution with the ability to disintegrate basal membrane components. MMP activity was found to increase after myocardial infarct and decrease in left ventricle dilatation was seen with the decrease of MMP activity[26,27]. In the study by Tziakas et al.[9], significant regression observed in blood MMP levels with levosimendan treatment. In this study, effect on MMP was associated with left ventricle wall tension decreasing and peripheral vasoconstriction restoring effect of levosimendan treatment. MMP production decreases also due to its effect on proinflammatory cytokines. We observed decrease in MMP-1 level in control group after the treatment. However, it did not reach a significant level.

We observed significant improvement of functional capacity specified according to NYHA. However, this improvement was not parallel with blood parameters. One reason of this might be the lack of loading dose application during levosimendan administration. Because loading dose was applied regardless of patient characteristics in previous studies. 24 mcg/kg/min loading dose was applied in LIDO study, 6 mcg/kg/min loading dose was applied for 10 minutes in studies which observed TNF alpha and MMP levels[7-11,20]. Loading dose in levosimendan treatment is recommended in patients with the need of acute effect in situations like post-MI or after cardiac surgery. Since all the patients in our study were decompensated in chronic HF basis, we did not administrate loading dose.

We did not observe any significant difference when comparing the post-treatment levels of both groups. This indicated that levosimendan treatment does not show different effects in anemic patients and control group.

Conclusion

We concluded that levosimendan treatment applied both in anemic patients and control group with class 3 or 4 HF according to NYHA's classification and resistant to traditional HF treatment provided improvement in functional capacity and that efficacy was not different

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

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