Acta Biomed 2022; Vol. 93, N. 3: e2022066 DOI: 10.23750/abm.v93i3.12449

Original article

Plasma-derived exosomes implement miR-126-associated regulation of cytokines secretion in PBMCs of CHF patients in vitro

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Abstract. *Background and aim* The investigation of regulatory effects of intra-exosomal compounds, especially microRNAs, has promising therapeutic prospects in the treatment of numerous diseases, including cardiovascular disorders. In this study, we investigated the effect of healthy donors` plasma exosomes (HDPE) on the production of cytokines by PBMC cells of patients with congestive heart failure (CHF) and showed the integral role of miRNA-126 in CHF-mediated changes of mononuclear paracrine secretion. *Methods* Peripheral blood mononuclear cells (PBMCs) were isolated from a peripheral blood of fifteen patients with CHF (age, 66,8 ± 9,8 years; left ventricular ejection fraction, 44 ± 19%). The concentration of cytokines (IL-10, ICAM-1, VEGF-A, TNF-α and MCP-1) in culture medium and PBMCs was measured by ELISA. The level of miRNA-126 expression in PBMCs was performed by real-time PCR. *Results:* Dramatic increase of ICAM-1 level in activated PBMCs of CHF patients, as well as an increase of the IL-10, ICAM-1 and TNF-α levels in the culture medium was observed. It was accompanied by CHF-related miRNA-126 overexpression in PBMCs. HDPE treatment distinguished by a tendency to reduction in miRNA-126 expression by CHF PBMCs and correlated with upregulation of IL-10, ICAM-1, TNF-α and MCP-1 with normalization of cytokines secretion. *Conclusions*: The altered paracrine secretion of cytokines by CHF PBMCs and miRNA-126 overexpression in vitro was found. HDPE treatment modulated production and secretion of most of studied cytokines by CHF PBMCs in vitro. The experimental application of exosomes for the modulation of paracrine secretion and PBMCs cellular functions may be promising for CVD therapy, including endothelial dysfunction and CHF. (www.actabiomedica.it)

Key words: Plasma-derived exosomes, PBMC cells, congestive heart failure, miRNA-126, cytokines, paracrine secretion.

Introduction

Heart failure (HF) is an immense socio-economic burden worldwide and remains a fundamental cause of mortality and disability despite the significant development of therapy strategies and personalize healthcare. HF treatment costs varies from lower than 1,000 USD per patient in low-income countries to between 5,000-15,000 EUR in Europe, and around 17,000-30,000 USD in the United State (1).

Congestive heart failure (CHF) is a chronic progressive condition that decreases the efficiency of the heart muscle, through damage or overloading, and is accompanied by a gross lesion of its pumping function (2). Given the range of pathophysiologic factors contributing to the initiation and progression of CHF, endothelial dysfunction plays an important role: monocyte-endothelial cell interactions are thought to be critical for development of chronic cardiovascular disorders (3, 4). Numerous clinical and animal model studies have

consistently demonstrated an increase in serum levels of pro-inflammatory cytokines (such as TNF-α, IL-1, IL-6, galectin 3, MCP-1 and TNF-1,2 receptors) during CHF progression sustaining the assumption that inflammation may contribute to CHF (5, 6). At the same time, the development of CHF strongly correlates with the occurrence of mitochondrial dysfunction, a reduced maximal respiration, increased of ROS production and expression of pro-inflammatory cytokine genes in peripheral blood mononuclear cells (PBMCs) (7-9). A variety of critical inflammatory and anti-inflammatory markers has discerned that have been subsequently examined as potential targets for the CHF therapy. Among other factors, the class of microRNA (miRNA) molecules has distinctive interest both as cardiovascular-specific diagnostic markers of CHF progression and as potential modulators in the molecular therapy of endothelial dysfunction and heart failure conditions (10, 11). Aberrant expression of miRNA-126 was found in patients with heart failure, atrial fibrillation and coronary artery disease, suggesting an essential regulatory role of miRNA-126 in cardiovascular system (12, 13). Affecting to the genes transcriptional activity and the expression of cell adhesion molecules (ICAM-1, VCAM-1, E-selectin) and proinflammatory proteins (TNF-α, CXCL12, VEGF) through NF-ϐ dependent pathways, miR-126 is strongly involved in the regulation of monocyte-endothelial cell interactions and CVD pathogenesis (14, 15). The direct involvement of MIR-126 in the regulation of the functions of different populations of PBMCs in various chronic pathologies, including CVD, has been shown (16-18).

Therapeutic approaches, which decrease risk factors, lead to an improvement of the clinical status and have a positive effect on HRQoL of the patients are the most demanded. Many studies have confirmed that stem cell-derived and healthy donor` plasma exosomes content large spectrum of specific molecules (lipids, proteins, DNAs, miRNAs, including miR-126) with a cardioprotective function: they might promote cell proliferation and differentiation, survival, angiogenesis, leucocyte modulation, decrease apoptosis and inhibit fibrosis, etc. (19, 20). Some types of exosomes (derived from synovial fluid and synovial fibroblasts) can manage anti-inflammatory signaling, eventually suppressing proinflammatory cytokine production and osteoarthritis progression (21). However, based on the current literature data, the quantity of experimental and clinical studies related to plasma-derived exosomes in the area of cardio-vascular disorders have been poorly investigated. Therefore, the purpose of our study was to explore the miRNA-126-related effect of healthy donors' plasma exosomes on the paracrine secretion of peripheral blood mononuclear cells of patients with CHF in vitro. Overall, our data represent exosomes as potential therapeutic tools for the correction of PBMC paracrine functions in the treatment of CHF.

Materials and methods

Patient characteristics

The study was conducted on 15 patients with CHF who had suffered a myocardial infarction in the last 5 years and came to our hospital from January 2020 to July 2021 for treatment. The diagnosis of heart failure was performed by clinically experienced cardiologists following established guidelines and in common criteria were required: LVEF 40-49% by echocardiography, proB-type natriuretic peptide (NT-proBNP) (Biosite, USA) greater than 400 pg/L, and ferritin level lower than 100 μg/L in serum, more detail (Table 1). Exclusion criteria: patients with advanced liver disease, respiratory diseases, renal failure, malignant disease,

Table 1. Clinical characteristics of 15 patients

Characteristic	Data
Age, years	66,8 ± 9,8
Male gender, %	8 (54%)
Female gender, %	7 (46%)
NYHA Types	II-III
Left ventricular ejection fraction	45 ± 19 %
NT-proBNP, pg/ml	664 ± 238
Ferritin, μg/L	95 ± 42
Anemia, %	2 (13%)
COLD*, %	1 (7%)
Obesity, %:	
Class I	5 (33%)
Class II	2 (13%)

Data was expressed as MEAN ± SD and % unless otherwise stated
*COLD - chronic obstructive lung disease

septicemia, current steroid therapy, and other inflammatory diseases. Healthy age-matched control subjects (n=13) were included. All healthy subjects had a physical examination, blood analysis, and echocardiography.

Ethical declaration

The present study complied with the Declaration of Helsinki. The study protocol was approved by an Ethical Committee at Bogomolets National Medical University (C128, 23.12.2019 C). All patients and volunteers gave written informed consent to participate in the present study.

Experiment design

The design of our study and the main methodological steps are shown (Fig. 1). Briefly, in block diagram: biomaterial for the study was obtained from patients in a cardiac department and healthy donors. Exosomes were taken from the plasma of healthy donors. After 24 hours of cultivation with LPS (leukocyte activation), a suspension of exosomes was added to the CMC medium for further incubation during the day. Studies of cells cytokine secretion by ELISA were performed in the following biomaterial of subjects: 1) the culture medium of control PBMC; 2) lysate PBMC without exosomes; 3) culture medium on the background of incubation of PBMC with exosomes; 4) lysate PBMC after incubation with exosomes; 5) serum of venous blood. miRNA-126 concentration was detected by RT-PCR. Morphological verification of exosomes was performed by electron microscope.

Isolation and verification of exosomes

Exosomes were isolated from the plasma of control group individuals using the Total Exosome isolation kit (Invitrogen, USA) according to the manufacturer's protocol. The principle of exosome isolation lies in the fact, that by binding water molecules the total isolating reagent of the exosome displaces less soluble components from the solution, in particular vesicles. This allows them to be collected by centrifugation. After 30 min incubation with the reagent at 2-8° C, the suspension with exosomes was precipitated by centrifugation at 10,000×g for 5 min at room temperature. The resulting precipitate of exosomes was carefully resuspended in sterile PBS buffer.

Isolation and cultivation of PBMCs

According to the method of Antonakos et al., peripheral blood was collected into the heparinized tube, and PBMCs were separated using the Ficoll-Hypaque method as soon as possible (22). 6 ml of peripheral venous blood was collected from each patient. The cell suspension was washed three times with PBS (wash off platelets, etc.), and PBMC was counted with trypan blue-eliminated dead cells. Then, the cells were resus-

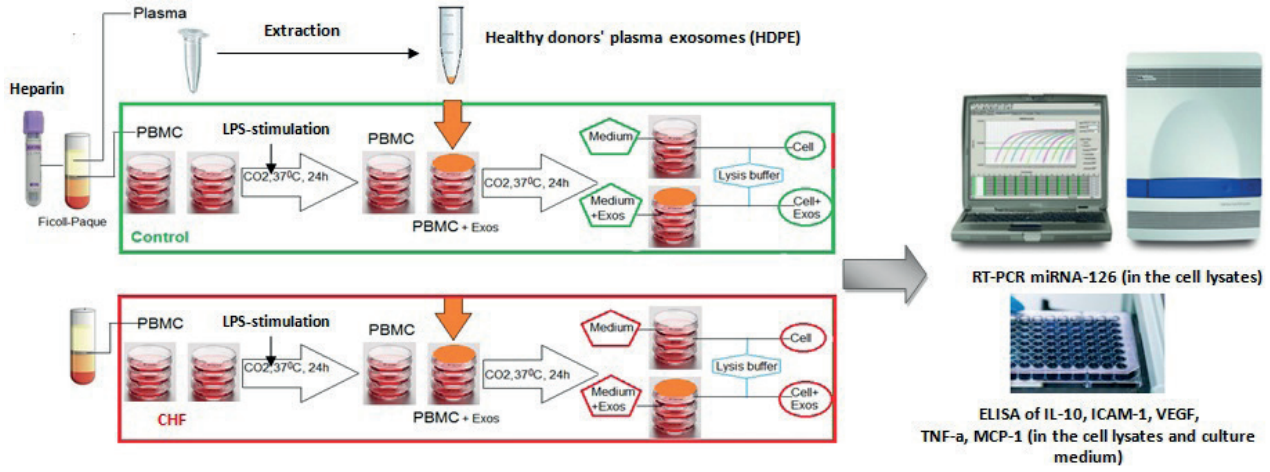

Figure 1. Scheme of the study design.

pended in RPMI-1640 evenly. Before passaging, we adjusted the PBMCs concentration to ensure the count of cells entering the experiment was the equal for each subject. Isolated PBMCs were seeded in a 6-well cell culture plate at a concentration of 106 cells per ml. 10 ng/ml LPS (Sigma-Aldrich, Germany) was added for stimulation (cell activation) and after cells were incubated for 24h. PBMCs were cultured in RPMI-1640 (Sigma-Aldrich, Germany) medium with 10% fetal bovine serum (Sigma-Aldrich, Germany), 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified carbon-dioxide incubator with 5% CO_2 at 37°C. After LPS-stimulation PBMCs were treated with a suspension of HDPE and incubated during 24h.

Sample preparation and ELISA

PBMCs cultivation medium (M) was collected after 24h incubation with or w/o HDPE and stored (-20°C), while PBMC cells were lysed in RIPA buffer (Abcam protocol) at +4°C. The protein concentration in the samples was determined on a semi-automatic biochemical analyzer BS-3000M (China), using a biochemical kit "Diagnosicum Zrt" (Hungary). The content of target interleukins in M and CL samples was determined by ELISA using ELISA KITs (Elabscience, USA) on the microplate reader RT-2100C (China). Each observation index was repeated 3 times.

Isolation of total RNA and real-time PCR analysis

The plasma level of miR-126 was quantified by RT-PCR. Total RNA was isolated by a PureLink miRNA Isolation Kit (Life Technologes, USA), followed by a reverse transcription using a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, USA) to synthesize the first strand of complementary DNA (cDNA). cDNAs were then subjected to RT-PCR amplification using a 7500 Fast RT-PCR System (Applied Biosystems; Thermo Fisher Scientific, USA). TaqMan®Universal PCR Master Mix (Applied Biosystems, USA) and primers TaqMan® MicroRNA Assays for miR-126 (Applied Biosystems, USA) were used for amplification. The relative expression level of miR-126 was determined by 2-ΔΔCT method. Each observation index was repeated 3 times.

Statistical analysis

The results of all experiments are expressed as mean ± SEM for at least seven rats per group. Each experiment was repeated three times. The hypothesis of normality distribution of data was tested by the Shapiro-Wilk test. Statistical differences between the groups were compared using the ANOVA test. Differences were considered to be significant when $p \leq 0.05$. All statistical analysis was performed using IBM SPSS Statistics, version 23.0 (SPSS Inc., USA).

Data availability

The data associated with the paper are not publicly available but are available from the corresponding author on reasonable request.

Results

In accordance with the design of the experiment, the research work was carried out with isolated blood mononuclear cells of CHF patients, who have undergone standard clinical diagnostics and confirmation of the CHF diagnosis at the university clinic (Table). To assess the dynamic of cytokines secretion by PBMC cells, isolated from normal and CHF donors, including with/without HDPE treatment in vitro, we performed immunoenzymatic detection of IL-10, ICAM-1, VEGF, TNF-α and MCP-1 proteins in PBMC lysates and in the culture medium.

In the culture medium was a significant increase of IL-10, ICAM-1 and TNF-α level (secreted by CHF patient PBMC cells) by 1.28, 4.4 and 1.31 times respectively, compared with the control group (Fig.2A,B,D). At the same time, the level of the VEGF protein in the culture medium did not change, and the level of MCP-1 decreased by 1.37 times in comparison with the PBMC of healthy donors (Fig.2C). HDPE treatment significantly reduced the ICAM-1 level in the culture medium by 1.4 times in the pathology group and increased its content in the control - by 1.64 times. Furthermore, HDPE treatment associated with an increase of the secreted angiogenic protein VEGF level in the PBMC culture medium in both groups (by 2.73 and 2 times in the control and

CHF groups, respectively) compared to "Exo-" groups. The concentrations of IL-10, TNF-α and MCP-1 proteins in CHF culture medium after incubation with HDPE were normalized to control values (Fig. 2A,D,E).

Changes in cytokine production by PBMC cells between two experimental groups were more pronounced than their secretion into the culture medium. Thus, we detected a significant rise of ICAM-1 protein level by 2.86 times and vice versa reduction of IL-10 and MCP-1 content by 2.72 and 2.95 times respectively, in the CHF patient PBMC cells compared with the healthy donors group (Fig.3A,B,E). Simultaneously, the level of the VEGF and TNF-α protein in the CHF PBMC cells, similar to the culture medium, did not change significantly compared to control (Fig.3C,D). Incubation with plasmatic exosomes significantly affected the intensity of cytokine mononuclear synthesis. HDPE treatment increased the content of ICAM-1 and TNF-α in PBMCs in the control group by 1.65 and 1.63 times and in the pathology group by 1.40- and 1.56 times, respectively (Fig.3B,D).

Intracellular level of MCP-1 protein under the same conditions increased by 1.85 times in the pathology group, while in the control group it changed insignificantly. Moreover, incubation with exosomes induced a decrease in VEGF content in PBMC cells in both the control group and in the pathology group by 2.10 and 1.52 times, respectively (Fig.3C). It is worth noting the effect of exosomes on the IL-10 production, which distinguishes it from the secretion trend of previous tested cytokines. The administration of a HDPE suspension led to a decrease in the IL-10 intracellular content in the control group by 1.31 times and to an increase in its level by 2.34 times in the CHF group (Fig.3A).

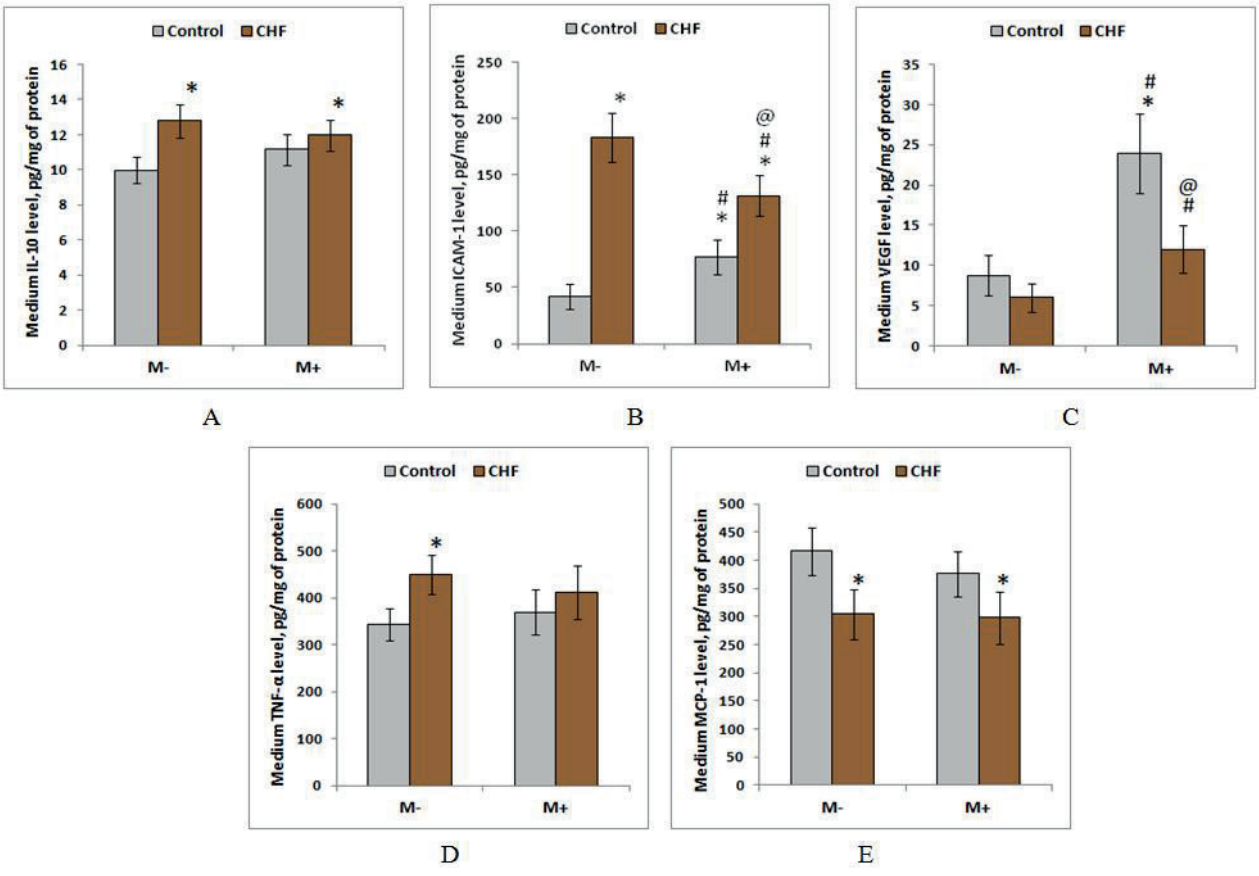

Figure 2. Concentration of IL-10 (A), ICAM-1 (B), VEGF (C), TNFα (D) and MCP-1 (E) proteins in the culture medium, secreted by LPS-stimulated PBMC cells of health volunteers (control, grey) and CHF patients (pathology, brown), incubated with or w/o plasma-derived exosomes. All data are presented as mean ± SEM of three independent experiments done in triplicate; $^{*}p<0.05$ vs. control «Exo-»; $^{\#}p<0.05$ vs. CHF « Exo-»; $^{@}p<0.05$ vs. control « Exo+» (n=25).

Importantly, that changes in the level of target cytokines in the culture medium and PBMC cells of CHF patients with/without exposure of HDPE were also accompanied by significant changes in the expression pattern of miR-126 under the given conditions. Thereby, a dramatic 4.2-fold increase in the expression of miR-126 in the peripheral blood mononuclear cells of CHF group compared with the healthy control group were detected (Fig.4). Notable, that after 24-h treatment with HDPE, the expression level of miR-126 practically did not change in the group of healthy donors, but decreased in the group of CHF patients by 1.6 times (Fig.4).

Discussion

The initiation and development of cardiovascular diseases closely correlate with the regulation of inflammation, which may provide novel strategies for the CVD treatment. In the last decades, investigations have focused on the association between the IL-10 cytokine family and the physiological and pathological progression of CVD, including CHF (23). As noted earlier, the level of the anti-inflammatory IL-10 protein in the activated mononuclear cells of patients with CHF dropped significantly in comparison with the PBMCs of healthy donors (Fig.3A). This strong correlated with an increase by the IL-10 content in the medium compared to the control (Fig.2A), which indicates the possible release of this anti-inflammatory factor by CHF mononuclear cells in response to generalized inflammation in the patient's body. LPS-stimulation, as an effective stimulator of the IL-10 synthesis by mononuclear cells (24), demonstrated a difference in the anti-inflammatory potential of PBMC by the IL-10 release into the medium between

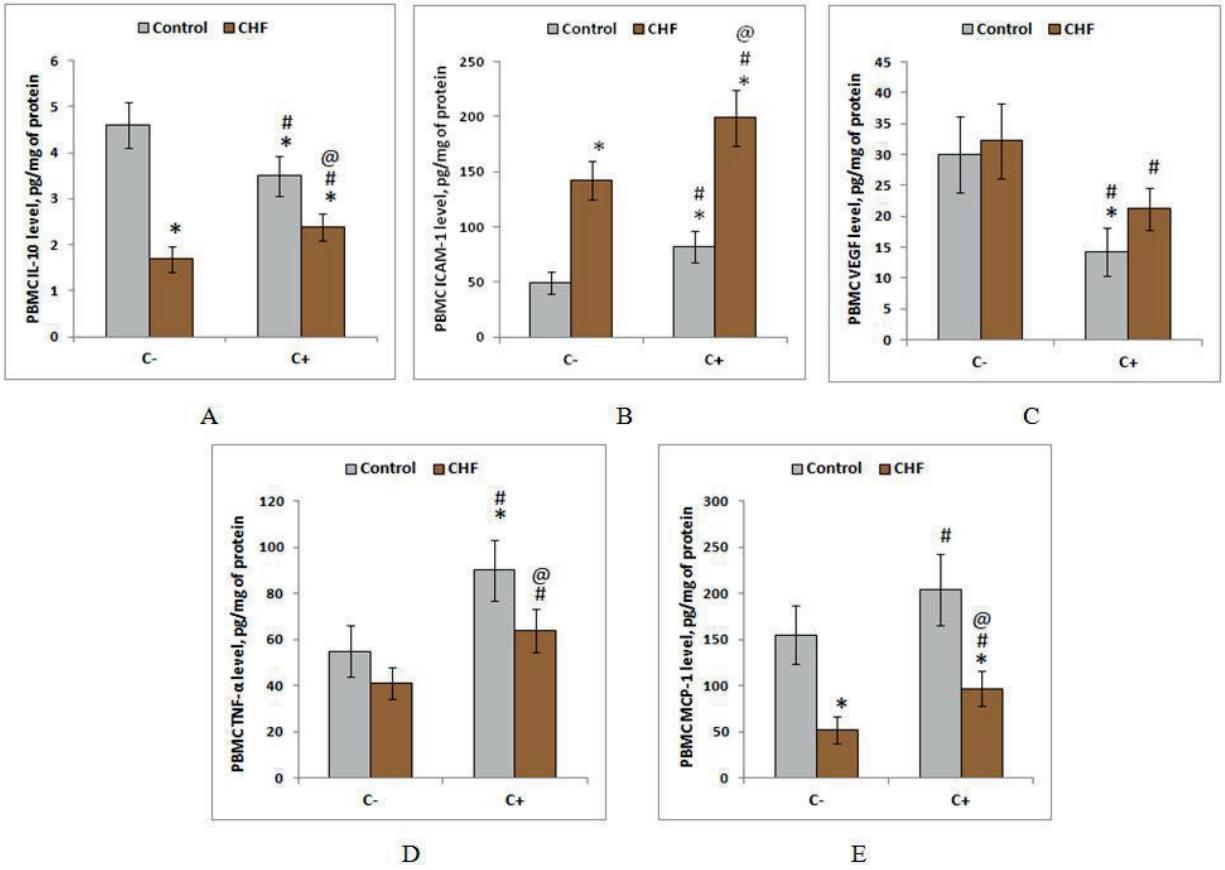

Figure 3. Levels of IL-10 (A), ICAM-1 (B), VEGF (C), TNFα (D) and MCP-1 (E) in LPS-stimulated PBMC cells of health volunteers (control, grey) and CHF patients (pathology, brown), incubated with or w/o plasma exosomes. All data are presented as mean ± SEM of three independent experiments done in triplicate; $^{*}p<0.05$ vs. control «Exo-»; $^{\#}p<0.05$ vs. CHF «Exo-»; $^{@}p<0.05$ vs. control «Exo+» (n=25).

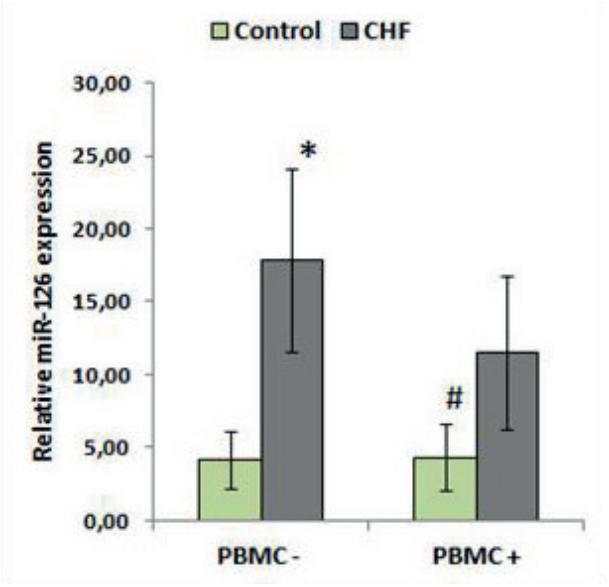

Figure 4. qRT-PCR analysis of miR-126 expression in LPS-stimulated PMBC cells of health volunteers (green) and CHF patients (grey), incubated w/o or with plasma exosomes («-» or «+»). All data are presented as mean ± SEM of three independent experiments done in triplicate; *p<0.05 vs. control «-»; #p<0.05 vs. CHF «-» (n=16).

the pathology and control groups. HDPE administration in the PBMC culture media contributed to a significant increase in the intracellular content of IL-10 in the CHF group (Fig.3A). This can be explained by the high anti-inflammatory potential of exosomes: a large number of scientific studies of the therapeutic properties of exosomes, including clinical trials, are being actively carried out today in relation to a variety of inflammatory diseases (25, 26). Moreover, HDPE stimulation slightly decreased IL-10 secretion by CHF mononuclear cells into the culture medium and equaled the control values.

As shown earlier, the secretion of pro-inflammatory cytokines by mononuclear cells and fluctuation in their serum concentrations plays an important role in the CHF pathogenesis (5, 6). It is well known, that intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1) enhance monocyte adhesion to the vascular endothelium and monocyte migration into the subendothelial regions of the vessels, stimulating the attraction of leukocytes to the inflammation focus (27). Our results, like another clinical serological studies (28, 29), have demonstrated a dramatic increase of ICAM concentration both in the LPS-stimulated PBMC cells and in the culture medium of CHF group to compare with control (Fig.2A, Fig.3A). A significant increase in the content of ICAM-1 in mononuclear cells and a decrease in the culture medium of CHF group were observed after incubation with an HDPE extract, which indicates a strong regulatory effect of exosomes on the production and release of ICAM-1 by mononuclear cells in the CHF condition. Considering that vascular endothelial cells are the main producer of ICAM-1 in vivo, the obtained results may be due to a special type of regulation of ICAM-1 expression in mononuclear cells: ICAM-1 is actively down-regulated in vivo until the monocyte leaves the circulation (30). The accumulation effect of intracellular ICAM-1 protein by CHF mononuclear cells after HDPE treatment correlates with data about monocytes- and endothelium-derived exosomes treatment of human monocyte-like MM6 cells in hyperglycemia conditions (31).

Despite the abundance of data about the evident elevation in the MCP-1 serum level in acute cardiovascular pathologies (32, 33), the question of MCP-1 serum concentration in chronic CVD remains open. MCP-1 is not only a key attractant for monocytes and macrophages and as such responsible for inflammation but might also be directly involved in the regulation of the local regeneration processes in the cardiovascular system (34). Moreover, the pattern of MCP-1 expression by mononuclear cells in cardiovascular disturbances, in contrast to infectious diseases (35), has been studied insufficiently. Our studies shown a significant reduce in the content of MCP-1 in PBMC cells of patients with CHF, as well as a slight decrease in the level of MCP-1, which secreted by cells into the culture medium (Fig.2E, Fig.3E). HDPE treatment increased the MCP-1 level in CHF mononuclear cells and also reduced its secretion into the culture medium, having reached the same with control value. HDPE-mediated up-regulation of intracellular ICAM-1 and MCP-1 in CHF PBMC cells and influence on cytokines releasing in culture medium may indicate modulative effect of plasmatic exosomes on the functions of activated mononuclear cells, such as adhesion potential, polarization, and transmigration (36, 37).

Current studies demonstrated that TNF-α is a key proinflammatory cytokine and an important part of the innate immune system which, upon stimulation of pattern recognition receptors, rises the expression of genes

required to control tissue inflammation and injury (6, 38). Since elevated serum concentration of TNF-α have been detected in patients with congestive heart failure (CHF) (39), TNF-α may potentially be involved in the maladaptive compensation seen in CHF. Our studies did not reveal significant changes in the level of TNF-α in activated CHF mononuclear cells vs. control, but demonstrated an increase secreted TNF-α level in the CHF group culture medium to compare with control (Fig.2D, Fig.3D). HDPE treatment increased the TNF-α level in CHF mononuclear cells and normalized its secretion into the CHF culture medium to control values. In general, the pattern of TNF-α expression in CHF PBMC cells and its secretion into the culture medium after HDPE treatment were similar to the MCP-1 concentrations. Thus, we cannot rule out a scenario, in which MCP-1 upregulation was mediated by elevation of intracellular level of the proinflammatory cytokine TNF-α (34).

VEGF plays an important role in cardiovascular system, because involve in the induction and regulation of both normal and pathologically altered angiogenesis and stimulates the proliferation of endothelial cells (40). Activated monocytes/macrophages may produce VEGF, bFGF and other factors which act on both the endothelium and the smooth muscle cells thereby inducing more VEGF production from other cells in the blood vessels (41). Our studies did not find any significant difference in the concentration of VEGF either in PBMC cells or in the culture medium between the CHF and control groups (Fig.2C, Fig.3C). At the same time, HDPE treatment led to a significant increase of VEGF level in both experimental groups, and contrariwise, to reduce of VEGF level in the PBMC lysates of both groups. Exosome-mediated elevation of secreted VEGF in the culture medium may indicate the activation of the PBMC cell potential to vascular tissue repair, wound healing and myocard tissue remodeling (42). Appositely, VEGF downregulation in CHF mononuclear cells after treatment with HDPE can be explained by a parallel rise of the IL-10 level in CHF condition, which may act antagonistically to the mononuclear angiogenic potential (43).

Describing in detail the HDPE regulation effects on the production and paracrine secretion of key functional cytokines by activated PMBCcells, it should be noted that these effects are due to biologically active components contained within exosomes (19-21). Considering that miRNA-126 is one of the most important substence of plasmatic exosomes, involved in the transcriptional regulation of many proangiogenic and proinflammatory genes (12-15), we decided to find out how the level of miRNA-126 expression in CHF PBMC cells changes during HDPE treatment. Our results demonstrated a dramatic increase in the level of miR-126 expression activated PBMC cells of the CHF group compared with control (Fig.4). An increase in the miR-126 expression in CHF condition correlated with an ICAM-1 up-regulation and interleukin-10 and MCP-1 down-regulation in activated mononuclear cells, as well as an increase in the paracrine secretion of IL-10, ICAM-1, TNF-α and a decrease of the MCP-1 level in the culture medium (Fig.2,3,4). HDPE treatment had a strong tendency to a decrease of miR-126 expression level in activated CHF mononuclear cells. This can be associated with the miR-126 exogenous pool, which entered PBMC cells during HDPE treatment (44). A decrease of the endogenous miR-126 expression in CHF condition after treatment with exosomes correlated with the IL-10, ICAM-1, TNF-α, MCP-1 up-regulation and VEGF down-regulation in the activated mononuclear cells, as well as with an increase in the paracrine secretion of VEGF and a decrease in ICAM-1 level in the culture medium. Thus, it can be assumed that an increase of the miR-126 endogenous expression by PBMC cells may be one of the effective adaptive mechanisms of mononuclear cells aimed at increasing the regenerative potential and intensifying cellular functions involved in angiogenesis, inflammation and tissue repair through a targeting transcriptional regulation of genes of a number of critical cytokines in CHF conditions (16-18).

Taking in account that experimental and clinical studies with the therapeutic use of easily attainable plasma-derived exosomes are negligible (45, 46), we tend to consider our primary studies as a promising basis for the establishment of new targeted approaches (including the miR-126-mediated mechanism) for the treatment and correction of impaired elements in the cardiovascular system both in endothelial dysfunction and CHF, as well as in CVD in general.

Conclusion

Our results demonstrate a significant changes in the concentration of target cytokines in LPS-stimulated PBMC cells of CHF patients in vitro: especially pronounced CHF-related alters both in the cells and culture medium were for the content of IL-10, ICAM-1 and MCP-1. HDPE treatment of activated CHF PBMCs had a modulating effect on the production and paracrine secretion of studied cytokines: exosomes had a normalizing effect on the level of VEGF-A, ICAM-1 and TNF-α. Important to note, that the concentrations of ICAM-1 turned out to be the most variable both in the CHF pathological group and in HDPE treatment. Concentration changes of the studied cytokines both in pathology and in HDPE treatment correlated with changes of the miR-126 expression in activated mononuclear cells, which makes miR-126 a potentially effective tool for regulating the processes of angiogenesis, inflammation and tissue regeneration in cardiovascular system through the modulation of monocyte functions. Our results are primary and for a deeper understanding of HDPE promising effects and their potential PBMCs-modulative and cardioprotective application in therapeutic strategies, studies need to be continued.

Funding: This work was supported by a research grant of the Ministry of Health of Ukraine (State registration 0119U101218).

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Received: 1 October 2021
Accepted: 27 October 2021
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