THERAPEUTIC POTENTIAL OF "EXOSOMES DERIVED MULTIPLE ALLOGENEIC PROTEINS PARACRINE SIGNALING: EXOSOMES D-MAPPS" IS BASED ON THE EFFECTS OF EXOSOMES, IMMUNOSUPPRESSIVE AND TROPHIC FACTORS

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TERAPIJSKI POTENCIJAL "EGZOZOMALNIH MULTIPLIH ALOGENIH PROTEINA ZA PARAKRINU SIGNALIZACIJU, EGZOZOM D-MAPPS" JE ZASNOVAN NA EFEKTIMA EGZOZOMA, IMUNOSUPRESIVNIH I TROFIČKIH FAKTORA

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ABSTRACT

Due to their differentiation capacity and potent immunosuppressive and pro-angiogenic properties, mesenchymal stem cells (MSCs) have been considered as new therapeutic agents in regenerative medicine. Since most of MSC-mediated beneficent effects are a consequence of their paracrine action, we designed MSC-based product "Exosomes Derived Multiple Allogeneic Proteins Paracrine Signaling (Exosomes d-MAPPS), which activity is based on MSCs-derived growth factors and immunomodulatory cytokines capable to attenuate inflammation and to promote regeneration of injured tissues. Interleukin 1 receptor antagonist (IL-1Ra) and IL-27 were found in high concentrations in Exosomes d-MAPPS samples indicating strong anti-inflammatory and immunosuppressive potential of Exosomes d-MAPPS. Additionally, high concentrations of vascular endothelial growth factor receptor (VEGFR1) and chemokines (CXCL16, CCL21, CXCL14) were noticed at Exosomes d-MAPPS samples suggesting their potential to promote generation of new blood vessels and migration of CXCR6, CCR7 and CXCR4 expressing cells. Since all proteins which were found in high concentration in Exosomes d-MAPPS samples (IL-1Ra, CXCL16, CXCL14, CCL21, IL-27 and VEGFR1) are involved in modulation of lung, eye, and synovial inflammation, Exosomes d-MAPPS samples were prepared as inhalation and ophthalmic solutions in addition to injection formulations; their application in several patients suffering from chronic obstructive pulmonary disease, osteoarthritis, and dry eye syndrome resulted with significant improvement of biochemical and functional parameters. In conclusion, Exosomes d-MAPPS, due to the presence of important anti-inflammatory, immunomodulatory, and pro-angiogenic factors, represents potentially new therapeutic agent in regenerative medicine that should be further tested in large clinical studies.

Keywords: *mesenchymal stem cells, therapy, regeneration, immunosuppression, differentiation*

SAŽETAK

Mezenhimalne matične ćelije (MSCs), se zbog svojih imunomodulatornih i proangiogenskih karakteristika, primenjuju u regenerativnoj medicini. Kako MSCs parakrinim mehanizmom ostvaruju svoje imunomodulatorne i proangiogenske efekte, dizajnirali smo produkt „Egzozomalni multipli alogeni proteini za parakrinu signalizaciju (Egzozom d-MAPPS)", koji sadrži egzozome, faktore rasta i citokine koje produkuju MSCs i njima smanjuju inflamaciju i pospešuju regeneraciju oštećenog tkiva.

Antagonist receptora IL-1 (IL-1 Ra) i interleukin (IL)-27, su pronađeni u visokim koncentracijama u ovom produktu, što je ukazivalo na snažan antiinflamacijski i imunosupresivni potencijal Egzozom d-MAPPS. Uz to, u Egzozom d-MAPPS je zabeležena i visoka koncentracija receptora za vaskularni endotelijalni faktor rasta (engl. vascular endothelial growth factor receptor, VEGFR1), kao i hemokina (CXCL16, CCL21, CXCL14), što ukazuje na potencijal Egzozom d-MAPPS da indukuje neo-angiogenezu i pospeši migraciju ćelija koje ekprimiraju CXCR6, CCR7 i CXCR4.

Pošto su sve komponente Egzozom d-MAPPS (IL-1Ra, CXCL16, CXCL14, CCL21, IL-27 i VEGFR1) uključene u modulaciju zapaljenja pluca, oka i zglobova, Egzozom d-MAPPS smo davali, u vidu inhalacionih rastvora, kapi za oči, ili intraartikularnih injekcija, pacijentima obolelih od hronične opstruktivne bolesti pluća, osteoartritisa i sindroma suvog oka. Preliminarni rezultati su pokazali značajno poboljšanje kako funkcionalnih tako i biohemijskih parametara nakon primene Egzozom d-MAPPS.

Egzozom D-MAPPS, zbog egzozoma, antiinflamacijskih, imunomodulatornih i proangiogenskih faktora može da predstavlja nov terapijski agens u regenerativnoj medicini i njegov terapijski potencijal treba da se detaljnije ispita u kliničkim studijama sa velikim brojem pacijenata.

Ključne reči: *mezenhimalne matične ćelije, terapija, regeneracija, imunosupresija, diferencijacija*

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INTRODUCTION

Mesenchymal stem cells (MSCs) are adult, self-renewable stem cells which are, due to their differentiation capacity and immunomodulatory characteristics, used as new therapeutic agents in regenerative medicine (1-3). MSCs are fibroblast-like cells that express: CD105 (endoglin, also identified as SH2, a component of the receptor complex of transforming growth factor- beta (TGF-β) involved in proliferation, differentiation, and migration), CD73 (SH3/4, ectoenzyme that regulates the purinergic signaling through the hydrolysis of adenosine triphosphate (ATP)), CD44 (hyaluronan receptor involved in migration), CD90 (Thy-1, regulates differentiation of MSCs) [4]. Importantly, MSCs do not express CD14 (marker of monocytes), CD34 (marker of hematopoietic cells), CD45 (pan-leukocyte marker), CD79a and CD19 (marker of B lymphocytes) and lack expression of major histocompatibility complex (MHC) class II and co-stimulatory molecules, CD80 (B7-1), CD86 (B7-2), and CD40, suggesting a low immunogenicity *in vitro* and *in vivo* and their potential for safe allogeneic transplantation (5, 6).

MSCs have substantial differentiation potential. In addition to the cells of mesodermal origin (osteoblasts, chondroblasts, and adipocytes), MSCs are capable of generating neural cells, hepatocytes, alveolar epithelial cells, insulin-producing cells, cardiomyocytes, indicating their clinical application (7-14). Several lines of evidence suggested that MSCs have capacity to differentiate into functional cardiomyocytes (9), hepatocytes (10, 11), alveolar epithelial cells and lung precursor cells (13) contributing to the regeneration of injured myocardium, liver and lungs (12, 15-17). Additionally, in paracrine manner, through the production of immunomodulatory factors (indoleamine 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2), nitric oxide (NO), transforming growth factor beta (TGF-β), interleukin (IL)-10, interleukin 1 receptor antagonist (IL-1Ra) and growth related oncogene (GRO), MSCs are able to suppress detrimental autoimmune response and to attenuate autoimmune and chronic, inflammatory diseases (3, 18, 19).

In addition to their immunomodulatory characteristics [3, 18, 19], MSCs may promote angiogenesis, as well. Through the production of several pro-angiogenic factors (vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), TGF-β, platelet-derived growth factor (PDGF), angiopoietin-1, placental growth factor (PGF), IL-6, monocyte chemotactic protein-1 (MCP-1), epidermal growth factor (EGF)), MSCs induce generation of new blood vessels having beneficial effects in the therapy of degenerative and ischemic cardiovascular and neurodegenerative diseases (20).

It was recently revealed that immunomodulatory and pro-angiogenic paracrine effects of MSCs are, at least partially, mediated by MSC-derived exosomes: nano-sized extracellular vesicles that deliver proteins, lipids, DNA fragments, mRNA to the target cells: immune cells, endothelial cells (ECs), pericytes and other tissue-resident cells (21). MSC-derived exosomes, released into the extracellular milieu, can be either taken up by neighboring cells, (residing in the microenvironment of engrafted MSCs) or may be carried to distant sites via biological fluids where, in endocrine manner, modulate function of target cells (21, 22).

MSCs reside in perivascular niches of many diverse tissues and organs (bone marrow (BM), adipose tissue (AT), peripheral blood (PB), lungs, bone, heart, dental pulp (DP), amniotic fluid (AF), placenta (PL), chorion membrane (CM), chorion vili (CV), umbilical cord (UC), Wharton's jelly (WJ)) (23). Differences in extracellular milieu (influence of neighboring cells and their products, hypoxia) as well as intracellular conditions (expression of certain micro RNAs) significantly affect function and therapeutic potential of MSCs (23).

Several lines of evidence suggest that MSCs derived from placental tissues have superior cell biological properties such as improved proliferative capacity, life span and differentiation potential than MSCs derived from adult tissues. PL-MSCs have a higher expansion and engraftment capacity than BM-MSC [23]. Moreover, clonal subpopulations of PL-MSCs have been attributed with the potential to differentiate into tissues from all three germ layers. Accordingly, due to their capacity for neuronal differentiation, PL-MSCs have been proposed as one of the main candidates for stem cell therapy of multiple sclerosis, nerve injuries, and sensorineural hearing loss (23-26).

Ethical concerns related to the derivation of PL-MSCs should be disregarded by the fact that placental tissues are normally considered medical waste and can be recovered without harm to the donor or fetus (27). Bearing in mind the simplicity of the harvesting procedure for isolation of PL-MSCs and their huge therapeutic potential (28, 29), we recently developed: "Exosomes Derived Multiple Allogeneic Proteins Paracrine Signaling, Exosomes d-MAPPS", biological product which activity is based on placental derived biomaterials, growth factors, and immunomodulatory cytokines capable to attenuate inflammation and to promote regeneration of injured tissues. Herewith, we analyzed and discussed in detail concentrations of bio-active molecules in Exosomes d-MAPPS emphasizing its therapeutic potential in regenerative medicine.

MATERIAL AND METHODS

Exosomes d-MAPPS sample acquisition

Sterile Exosomes d-MAPPS is an engineered biologic product obtained from placental tissue, previously collected from healthy human donors. Blood samples were given by the donor prior to or at the time of collection and were tested by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) and were found negative using United States (U.S) Food and Drug Administration (FDA) licensed tests for detection of at minimum: Hepatitis B Virus, Hepatitis C Virus, Human Immunodeficiency Virus Types 1/2, Treponema Pallidum.

Placental tissue samples were obtained with patient consent as well as institutional ethical approval and kept at 4°C until processed.

Exosomes d-MAPPS sample was engineered as a sterile product, manufactured under current Good Manufacturing Practices (cGMP) regulated and reviewed by the FDA. Sterile Exosomes d-MAPPS sample incorporate Regenerative Processing Plant's (RPP) proprietary patented sterilization process to provide safe sterile product.

Exosomes d-MAPPS samples, used in this study, were manufactured under specific conditions in order to be applicable for bioavailability testing and for different therapeutic use.

Determination of cytokines, chemokines, growth factors and their receptors in Exosomes d-MAPPS samples

Concentrations of cytokines, chemokines, growth factors and their receptors in Exosomes d-MAPPS samples were determined as previously described (30). Briefly, about fifty milliliters of sample was concentrated to 1.0-ml protein with trichloroacetic acid. The acetone-washed protein pellet was resolubilized in urea, and proteins were processed with dithiothreitol and iodoacetamide and digested with trypsin. Tryptic peptides were quantified and 10 µg was loaded through pressure cell onto a biphasic column for online two-dimensional high-performance liquid chromatography (HPLC) separation (strong-cation exchange and reversed-phase) and concurrent analysis by nanospray using a hybrid mass spectrometer. Three salt cuts of 50, 100, and 500 mM ammonium acetate were performed per sample run, with each followed by a 120-min organic gradient to separate the peptides.

Resultant peptide fragmentation spectra were compared with proteome database concatenated with common contaminants and reversed sequences to control false discovery rates. Peptide spectrum matches (PSMs) were filtered and assigned matched-ion intensities (MITs) based on observed peptide fragment peaks. PSM MITs were summed on a per-peptide basis, and only those uniquely and specifically matching a particular protein were moved onto subsequent analysis. Briefly, peptide intensity distributions were log-transformed, normalized across biological replicates by LOESS, and standardized by median absolute deviation and mean centering across samples as suggested. Peptides were then filtered to maintain at least two hits in one replicate set, and missing values were imputed using a random distribution of low-level values. Peptide abundance trends for each protein were scaled to a specific, well-sampled reference peptide. Sample-to-sample variation was visualized by PCA, Pearson's correlation and hierarchically clustered using the Ward agglomeration method to generate a heat map of protein abundance trends normalized by z-score (30).

RESULTS

Exosomes d-MAPPS has strong anti-inflammatory and immunomodulatory potential

Since MSCs produce immunosuppressive and anti-inflammatory factors (3, 18, 19), we analyzed concentration of major MSC-derived immunomodulatory molecules in Exosomes d-MAPPS sample (Figure 1). For this purpose, levels of IDO, IL-1ra, IL-10, IL-4, IL-13, IL-18 binding protein (IL-18 Bpa), TGFβ1 and Latency associated peptide of TGFβ1 (LAP (TGFβ1), were measured (Figure 1A). Among

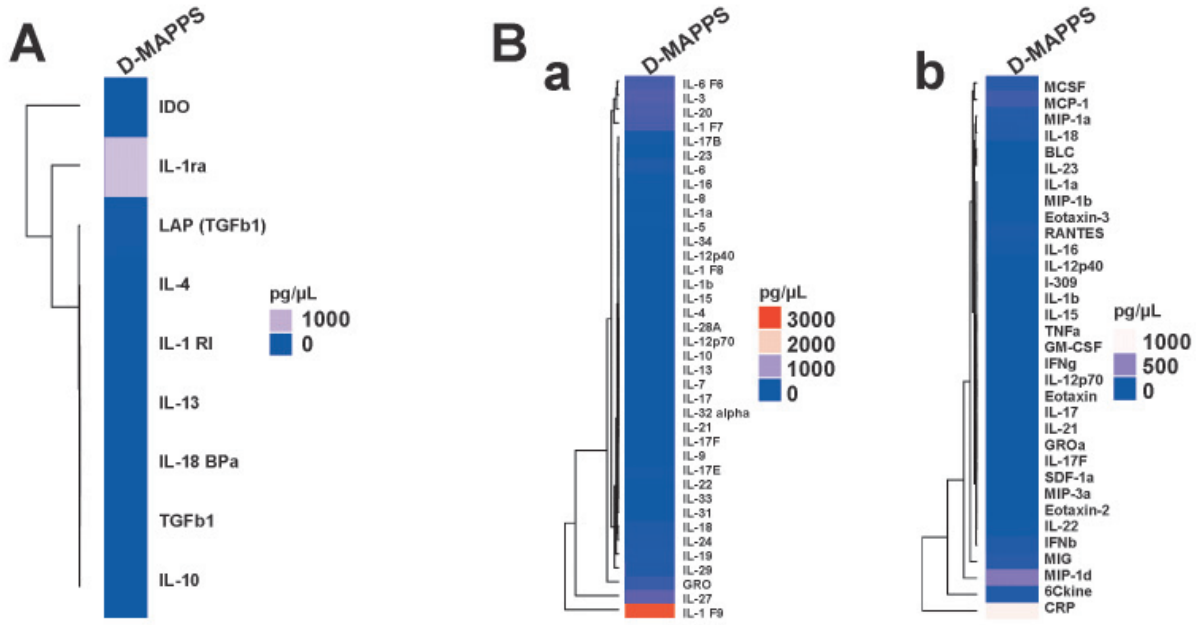

Figure 1: Inflammatory and immunomodulatory biomarkers in Exosomes d-MAPPS samples. (A) Different concentrations of 9 anti-inflammatory and immunomodulatory molecules are presented at heatmap. **(B)** Heatmap shows concentrations of 39 interleukins (a) and 33 inflammatory biomarkers (b) determined at Exosomes d-MAPPS sample.

measured immunoregulatory factors, IL-1Ra was found in high concentrations (1000 pg/μl). MSC-derived IL-1Ra is a naturally occurring cytokine which acts as an inhibitor of inflammatory cytokine IL-1. When IL-1Ra binds to the IL-1 receptor (IL-1R), binding of IL-1 is blocked and pro-inflammatory signal from IL-1 receptor is stopped. Accordingly, various pro-inflammatory events, initiated by IL-1:IL-1R binding, including the synthesis and releases of chemokines and enhanced influx of neutrophils, macrophages, and lymphocytes in inflamed tissues, are inhibited by IL-1Ra [4]. In line with these findings, high concentration of IL-1Ra, noticed in Exosomes d-MAPPS (Figure 1), indicates strong anti-inflammatory and immunomodulatory potential of this product.

In order to confirm strong anti-inflammatory properties of Exosomes d-MAPPS and to demonstrate that inflammatory mediators are not present in significant concentration in Exosomes d-MAPPS, levels of major inflammatory interleukins of innate and acquired immunity were evaluated (Figure 1B). As it is shown in Figure 1B, the main inflammatory cytokines of innate immunity (TNF-α, IL-1β, IL-12, IL-18) were not detected in Exosomes d-MAPPS sample. Similarly, Th1 (IFN-γ), Th2 (IL-4, IL-5, IL-10, IL-13) and Th17 (IL-17 and IL-22) cytokines were present in non-detectable concentrations indicating that neither one of T cell-dependent inflammatory pathways could not be elicited by Exosomes d-MAPPS. Among interleukins which might have dual (pro and anti-inflammatory) role (IL-6, IL-8, IL-27), only IL-27 was measured in Exosomes d-MAPPS sample (1000 pg/μl). IL-27 promotes regulatory and immunosuppressive effects of MSCs by enhancing MSC-dependent generation of IL-10 producing CD4+T cells within the population of activated helper T cells. Additionally, capacity of MSCs to induce apoptosis of inflammatory Th1 and Th17 cells in programmed death ligand 1 (PDL1) dependent manner is significantly enhanced by IL-27 (31). Accordingly, presence of IL-27 could be considered as an additional indicator of immunosuppressive properties and therapeutic potential of Exosomes d-MAPPS sample.

In addition to IL-27, IL-1F9 was noticed in high concentration in Exosomes d-MAPPS sample (3000 pg/μl; Figure 1Ba). Since currently there is no available information regarding the role of IL-1F9 in MSC-based immunomodulation, the role of this cytokine in Exosomes d-MAPPS-based effects should be explored and analyzed in further studies.

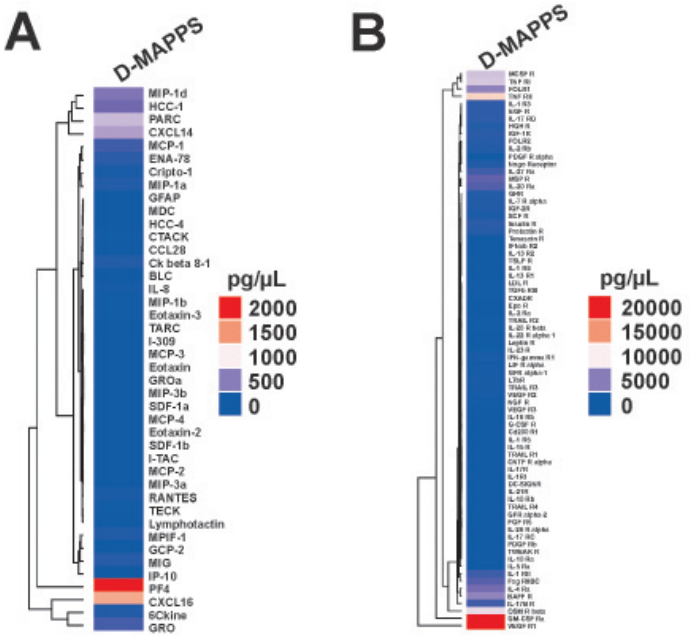

Figure 2: Chemokines and soluble receptors in Exosomes d-MAPPS sample. (A) Concentrations of 42 chemokines in Exosomes d-MAPPS samples are presented at heatmap. **(B)** Heatmap represents concentrations of 75 growth factor-related receptors and biomarkers in Exosomes d-MAPPS sample.

Exosomes d-MAPPS can promote migration of CXCR6, CCR7 and CXCR4 expressing cells

One of the main properties of MSCs is their homing capacity towards the site of the injury or inflammation where they, in juxtacrine and/or paracrine manner, suppress detrimental immune response and ongoing inflammation [3]. MSCs expressed chemokine-specific receptors (CXCR4, CX3CR1, CXCR6, CCR1, and CCR7) and are attracted by chemokines (CXCL12, CXCL14, CX3CL1, CXCL16, CCL3, CCL19, and CCL21) released from damaged tissues and inflammatory immune cells (32). Interestingly, MSCs are also able to produce chemokines which, in autocrine manner, enable migration of MSCs towards the site of injury or inflammation (32). In line with these observations, we measured high concentration of MSCs-derived chemokine CXCL16 in Exosomes d-MAPPS sample (1500 pg/μl) (Figure 2A). Since CXCR6, ligand for CXCL16, is highly expressed on MSCs and immune cells (memory/effector T cells, NK, NKT cells and plasma cells) (32), high concentration of this chemokine in Exosomes d-MAPPS sample strongly indicates that Exosomes d-MAPPS can be used as chemoattractant enabling migration of CXCR6 expressing cells into the inflamed or injured tissues.

Similarly, 6Ckine (CCL21) (ligand for CCR7 receptor) is measured at Exosomes d-MAPPS sample (500 pg/μl) (Figure 2A). Having in mind that CCL21:CCR7 axis is important for migration of MSCs in wounds, homing of naïve T cells in peripheral lymph nodes and for migration of antigen processing, activated DCs into peripheral lymph nodes and T cell-rich fields within injured lungs, synovia and eyes (33-38), high levels of CCL21 in Exosomes d-MAPPS, could be used for recruitment of CCR7 expressing MSCs and immune cells during Exosomes d-MAPPS-based modulation of skin/joint/eye/lung inflammatory diseases. In line with these findings, high concentration (2000pg/ml; Figure 2A) of platelet factor 4 (PF4), which is involved in tissue regeneration and wound repair (39), was noticed in Exosomes d-MAPPS sample confirming its potential therapeutic use in regenerative medicine.

CXCL14 was also detected in Exosomes d-MAPPS sample (500 pg/μl; Figure 2A). CXCL14 specifically binds to CXCR4 and, in a similar manner as CXCL12, is involved in CXCR4-dependant migration of MSCs into injured or inflamed tissues [40].

In addition to elevated levels of CXCL16, CCL21, PF4 and CXCL14, GRO-well known MSC-derived chemokine with strong immunosuppressive properties [41], has been detected in Exosomes d-MAPPS sample (500 pg/μl; Figure 2A). Human MSCs secrete GRO-γ which, accompanied with GRO-α, promote conversion of monocyte derived DCs (MDDCs) towards myeloid suppressive phenotype enabling generation of tolerogenic myeloid derived suppressor cells (MDSCs) (42). In line with these findings, presence of GRO in Exosomes d-MAPPS sample, strongly indicates its potential for *in vitro* generation of MDSCs and MDSCs-based cell therapy of autoimmune and chronic inflammatory diseases.

Exosomes d-MAPPS has the capacity to induce neovascularization in VEGF-dependent manner

Having in mind that generation of new blood vessels and re-vascularization are mainly responsible for MSC-dependent regeneration of ischemic tissues (20), we evaluated presence of angiogenesis-related growth factor receptors in Exosomes d-MAPPS sample in order to explore capacity of Exosomes d-MAPPS to induce neo-angiogenesis-based tissue regeneration. As it is shown in Figure 2B, high concentrations of VEGFR1 (20000 pg/μl) was determined in Exosomes d-MAPPS sample. VEGFR1 plays critical role in migration of MSCs and MSCs-based neo-angiogenesis [43]. VEGFR1 binds VEGF and is expressed by multiple bone marrow-derived cell types, including endothelial progenitor cells and MSCs. BM-derived endothelial progenitor cells and MSCs are mobilized into peripheral blood and recruited to the sites of ischemia in VEGFR1-dependent manner, where they participate in tissue repair and revascularization (42). Based on these results, it is highly expected that Exosomes d-MAPPS can modulate generation and maturation of BM-derived cells. In line with these observations are high concentrations of granulocyte-macrophage colony-stimulating factor receptor (GM-CSFR) which was also noticed in Exosomes d-MAPPS sample (20000 pg/μl; Figure 2B). Since signaling from GM-CSFR can promote an astonishing variety of cellular functions, including protection from apoptosis, progression through the cell cycle, early commitment to myelopoiesis, differentiation/maturation of committed progenitors, and multiple activation and motility functions in mature immune cells (44), Exosomes d-MAPPS can be used for controlled differentiation of BM-derived, GM-CSFR expressing cells.

DISCUSSION

Due to their differentiation capacity and potent immunosuppressive and pro-angiogenic properties, MSCs have been considered as new therapeutic agents in regenerative medicine (45, 46). Nevertheless, safety issues of MSCs-based therapy are still a matter of debate, especially in the long-term follow up (47). Several studies reported that transplanted MSCs, in response to the growth factors produced in the local microenvironment, differentiated into undesired tissues, mainly bone and cartilage (48, 49). Multiple areas of ossifications or calcifications were observed in infarcted myocardium after transplantation of MSCs (48, 49). Since most of MSC-mediated beneficent effects are consequence of their paracrine action, we designed Exosomes d-MAPPS, soluble product, which contains a broad number of MSC-derived immunomodulatory and pro-angiogenic factors (Figures 1-2). Among anti-inflammatory mediators IL-1Ra was presented in the highest concentrations in Exosomes d-MAPPS (Figure 1A). Recently, a well-characterized subpopulation of IL-1Ra expressing MSCs have been described (50). MSCs, in IL-1Ra dependent manner, were able to suppress inflammation and fibrosis in the lungs. Interestingly, therapeutic and anti-inflammatory effects of MSCs-overexpressing IL-1Ra were more effective than effects of recombinant IL-1Ra (50), indicating that MSC-derived IL-1Ra acts synergistically with other immunomodulatory cytokines and chemokines in suppression of immune response.

Among chemokines, CXCL16, CCL21 and CXCL14 were present in high concentrations in Exosomes d-MAPPS sample (Figure 2A). Each of these molecules is crucially involved in the pathogenesis of lung, synovial, and eye inflammation (51-57). Bronchial epithelium is an important source of CXCL16 (51) while its receptor CXCR6 is highly expressed on lung-infiltrated T cells (51). Moreover, an increased expression of CXCL16 was noticed in the lungs of bleomycin-treated mice while CXCR6 expression was markedly increased in the lung in patients with interstitial lung diseases (53), indicating the importance of CXCL16:CXCR6 axis in the pathogenesis of lung injury and inflammation. Similarly, CXCL16 plays an important role in recruitment of T cells in inflamed synovium of patients suffering from Rheumatoid arthritis, suggesting CXCL16 as a target molecule in biological therapy of Rheumatoid arthritis [54].

In similar manner as CXCL16, CCL21 significantly contributes to the recruitment of CCR7 expressing immune cells in inflamed synovia (55). Additionally, both CCL21 and CCR7 are significantly up-regulated in inflamed corneas [56]. CCL21 facilitate migration of inflammatory, antigen presenting dendritic cells (DCs) in CCR7 dependent manner from the cornea to draining lymph nodes, while local administration of anti-CCL21 may reduce recruitment of DCs resulting with the attenuation of corneal inflammation (56).

Smoking-induced expression of CXCL14 in the airway epithelium represents a novel potential molecular link between smoking-associated airway epithelial injury, chronic obstructive pulmonary disease (COPD) and lung cancer. Airway epithelium responds to cigarette smoking with altered CXCL14 gene expression, contributing to the dis-

ease-relevant phenotype that result with the development of COPD and lung cancer (57).

Since bone repair and regeneration depend on vasculogenesis and osteogenesis, both of these processes are essential for successful bone remodeling (58). Several lines of evidence suggest that pro-angiogenic VEGF-A and VEGFR1 play crucially important role in bone regeneration (59, 60). Despite the fact that VEGF-A: VEGFR1 axis could contribute to the regeneration of the bone, VEGFR1 mediated signaling contributes to the development of complications of ischemic retinopathies, including retinopathy of prematurity (ROP), age-related macular degeneration (AMD), and diabetic retinopathy (DR) (61). VEGFR1 expression was up-regulated during pathogenesis of choroidal neovascularization (CNV), a model of AMD. Accordingly, blockade of VEGFR1 suppresses pathological angiogenesis and vascular leakage in the eye (61).

IL-27, measured in high concentrations in Exosomes d-MAPPS sample (Figure 1B) has dual: immunomodulatory and angiostatic effect in the injured eye. It inhibits pathophysiological intraocular neovascularization by reducing VEGF production in macrophages (62) and at the same time attenuate ongoing inflammation by suppressing proliferation of IL-17 producing Th17 cells (63). In similar manner, PDF4, which was also present in high concentration in Exosomes d-MAPPS sample (Figure 2A), limits generation of Th17 cells and is crucially involved in suppression of Th17 immune response (64). In this way, we assume that Exosomes d-MAPPS, in IL-27 and PDF4-dependant manner, may attenuate potentially detrimental effects of VEGFR1 signaling in Th17 cell driven eye injury and inflammation.

Since all proteins which were found in high concentration in Exosomes d-MAPPS samples (IL-1Ra, CXCL16, CXCL14, CCL21, IL-27, PDF4 and VEGFR1) are involved in modulation of lung, eye and synovial inflammation, we analyzed effects of Exosomes d-MAPPS-based therapy in patients suffering from chronic inflammatory diseases (COPD, osteoarthritis and dry eye syndrome). Our preliminary results, obtained in several pilot trials, indicated that Exosomes d-MAPPS was well tolerated since none of undesired, side effects were observed in Exosomes d-MAPPS-treated patients. Importantly, in patients that received Exosomes d-MAPPS, we noticed significant attenuation of inflammation, accompanied with an improvement of biochemical and functional parameters of injured lungs, knees and eyes.

Having in mind that therapeutic effects of Exosomes d-MAPPS are attributed to the MSC-derived soluble factors which could be found within MSC-derived exosomes (65), we believe that, at least some of Exosomes d-MAPPS-mediated beneficent effects were related to the function of exosomes. Accumulating evidence has suggested that MSCs, via exosomes, suppress detrimental immune response, attenuate inflammation and promote tissue repair and regeneration (21). Compared with cells, exosomes have no risk of aneuploidy and are well tolerated by the immune system without the risk of rejection by immune cells after allogeneic transplantation (65). Additionally, due to their membrane-based structure, exosomes are able to cross the plasma membrane and to deliver their cargo into target cells throughout the body, indicating their potential to act in paracrine as well as endocrine manner (65). Accordingly, encouraging therapeutic effects of MSC-derived exosomes were observed in several animal models of organ specific and systemic inflammatory diseases, including acute and chronic injury of the eye and lungs (21, 66). It is well known that in early stage of corneal damage, injured epithelial cells produce IL-1 in order to elicit strong innate immune response (67). MSC-derived IL-1Ra, which was found in high concentration in Exosomes d-MAPPS sample (Figure 1A), binds to the IL-1R and prevents ongoing inflammation in the injured corneas. Similarly, it is well known that, in IL-1Ra dependent manner, MSCs are able to efficiently attenuate lung injury and inflammation (5). In line with these observations, we believe that IL-1Ra containing exosomes were responsible for the attenuated inflammation in Exosomes d-MAPPS-treated patients suffering from corneal injury and COPD.

In conclusion, Exosomes d-MAPPS, due to the presence of several important anti-inflammatory, immunomodulatory and pro-angiogenic factors, represents potentially a new therapeutic agent in regenerative medicine that should be further tested in large clinical studies.

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CONFLICT OF INTEREST

Dr. C. Randall Harrell and Dr. Crissy Fellabaum are employed at RPP.

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