



Role of platelet-derived microparticles in transfer of the chemokine receptor CXCR4 to CXCR4-negative cells

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Abstract

Background: Membrane-derived microparticles (PMPs) are produced from platelets during activation, storage, and apoptosis. PMP can transfer some adhesion molecules such as CXCR4 to CXCR4-negative cells. In this study, the ability of PMPs to deliver CXCR4 molecule to CXCR4-null targets (Daudi, K562 and U937 cell line) was evaluated and the different concentrations of PMPs were examined to transfer CXCR4.

Methods: In this experimental study, PMPs were prepared using serial centrifugations. After confirmation of PMP with flow cytometry, PMP concentration was evaluated using the Bradford method. CXCR4-negative cell lines (1×10^5 cells/ml) were cultured in RPMI1640 with 10% FBS and 1% antibiotic. PMPs in 7 different concentrations were added to cell culture plates and incubated for 1 hour at 37°C and 5% CO₂. The presence of CXCR4 on cells was analyzed by flowcytometry.

Results: In this study, characterization of PMPs and cell lines were done by flow cytometry. Then, the PMPs' ability to transfer CXCR4 to null cells (Daudi, K562 and U937 cell lines) was evaluated in 7 concentrations (10, 20, 50, 125, 250, 500, 1000 µg/mL); incubation lasted for 1 hour. The best result of transferring CXCR4 by PMP was done in the concentration of 250 µg/mL.

Conclusion: PMPs in different concentrations can transfer CXCR4 to target cells. Also, the increase of PMPs concentration up to 250 µg/mL can increase the CXCR4 presence on null cells.

Keywords: CXCR4, Microparticles, Platelet

Conflicts of Interest: None declared

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Introduction

PMPs are small circulating membrane fragments shed from the surface of eukaryotic cells (1). It has recently been shown that microparticles derived from monocytes may transfer CCR5 among cells (2). PMPs are small vesicles of circulating membranes that are released from the surface of eukaryotic cells (1). Recently, it has been shown that microparticles derived from monocytes have a role in transferring CCR5 among cells (2).

PMPs are also the most frequent microparticle in blood (3). Platelets shed microparticles during activation, storage, and apoptosis, which can transfer some adhesion

molecules and other materials such as arachidonic acid (1).

The presence of CXCR4 on megakaryocyte and platelet and their PMPs have been shown previously (4). It is also well documented that these cells may be infected by HIV-1 (5-10).

CXCR4 is the receptor of chemokine SDF-1 (stromal - derived factor 1) and widely expressed on hematopoietic cells, active T lymphocyte, megakaryocyte, platelets, and PMPs (11). Transferring CCR5 or CXCR4 receptors on CD4⁺ cells can cause CD4⁺ cells infection with human

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↑What is "already known" in this topic:

What is "already known" in this topic: PMPs transfer CXCR4 to null cells (Daudi, K562 and U937 cell lines).

→What this article adds:

The transfer of CXCR4 to CXCR4-negative cells by PMPs is dose-dependent.

immunodeficiency virus (HIV) strains. T lymphocyte tropic HIV strains (T- tropic HIV strain) are viruses that have the tropism to CD4+ T-cells and can infect these cells using CXCR4 coreceptor HIV is a lentivirus (a subgroup of retrovirus) (11, 12) that enters cells after binding to the CD4 protein and one of several chemokine receptors. Lymphotropic (X4) viruses utilize the CXCR4 chemokine receptor, and macrophage tropic (R5) viruses use the CCR5 chemokine receptor as a coreceptor for entry (6, 13-16).

In 2010, investigators tried to use different concentrations of MPs for drug release (17) and suggested that different concentrations of PMP may have an effect on chemokine transfer.

This study was conducted to demonstrate the existence of CXCR4 on PMPs and the ability of PMPs to transfer CXCR4 to CXCR4-null targets (Daudi, K562 and U937 cell lines). This study also determined the optimum concentrations of PMP for CXCR4 transfer.

All cell lines (Daudi, K562 and U937 cell lines) are CXCR4 negative according to previous studies. K562, Daudi, and U937 cell lines are originated from erythroid, lymphoid, and monocytic lineage, respectively.

Methods

PMP preparation

In this experimental study, platelets were obtained from platelet concentrate bags at a local blood transfusion center (Tehran Blood Transfusion Department; Vesal Branch). Five bags were transferred to the lab 3 days after donation.

All contents of the bags were poured in 50 mL falcon tubes. The tubes were centrifuged at 300g for 15 minutes to settle the remaining RBCs and WBCs. Then, the supernatant was collected and centrifuged at 1200 g for 15 minutes to settle platelets. In the final step, the supernatant was centrifuged at 16 000 g for 20 minutes. The pellets were washed 3 times by phosphate buffer saline (PBS) to remove proteins. PMPs were suspended in PBS.

PMP characterization

CD41 is a platelet specific marker; thus, 5 µL of FITC mouse antihuman CD41a (BD bioscience, Clone HIP8 (RUO)) was added to the isolated microparticles to characterize microparticles. After 30 minutes of incubation in dark at 4°C refrigerator condition, the microparticles were analyzed by Partec flow cytometry and analyzed with FloMax software. These particles were evaluated for size by zeta sizer at Faculty of Pharmacy of Tehran University of Medical Sciences by Jahromi et al; the same protocol was used in this study to obtain PMPs (18).

PMP concentration

To measure PMP concentration, bovine serum albumin (BSA) was prepared in 1000 µg/mL as a standard for Bradford method. To draw the standard curve, BSA was diluted in 6 concentrations; 200 µL of Bradford reagent

and 10 µL of the standards samples were mixed. Then, ODs were read in 595 nm by Nano drop spectrophotometer (Nano Photometer™ Pearl).

Coculture of cell lines and PMPs

Selected cell lines were purchased from Iran Cell Collection Bank (Pasteur Institute). These cell lines were cultured in RPMI 1640 media (Bio sera) with 10% FBS (GIBCO), 100 IU/mL penicillin, and 100 µg/mL streptomycin (GIBCO). To characterize the expression of CXCR4, the cell lines were stained with anti-CXCR4 antibody and analyzed by flow cytometry. Then, after optimization of the incubation time for coculture, cell lines were incubated (1×10^5 cell/mL) with the PMPs (10, 20, 50 125, 250, 500, 1000 µg/mL) for 1 hour in 5% CO₂ Incubator (37°C). All tests were repeated 3 times for all the cell lines before and after treatment with PMPs.

Flow cytometry

After the coculture time, cells were centrifuged at 300 g for 10 minutes, and the pellets were washed with PBS. Then, cells were prepared to analyze the surface CXCR4 presence. For this purpose, 5µL of purified mouse anti-human CD184, Clone 12G5 (RUO), was added to each tube. After 30 minutes of incubation, cells were washed 3 times by PBS to remove free antibodies (Abs). Next, 2 µL of goat antimouse IgG Fc (FITC) (Abcam, ab97264) was added. The cells were analyzed by flow cytometry 30 minutes after incubation in dark at 4°C refrigeration. The cell lines and PMPs were also evaluated for CXCR4 expression before co-incubation with cells.

Data were statistically analyzed by SPSS software version 22 using paired sample t test ($p < 0.05$).

Results

BSA was prepared in 6 different concentrations and the samples were read in 595 nm and the standard curve was drawn. The PMP concentrations (1000 µg/mL) were selected according to the standard curve. PMPs were prepared by PBS in 7 different concentrations of 10, 20, 50, 125, 250, 500, 1000 µg/mL. Flow cytometric analysis of PMPs showed that they were originated from platelets because they had CD41 (75%), which is a platelet specific antigen. PMPs also highly expressed CXCR4 (70%). Also, 3 cell lines were negative for CXCR4 expression (Table 1). Data are presented in Figure 1.

After 1-hour incubation with PMP, cell lines were isolated and stained by anti-CXCR4 Ab and analyzed. Data showed they were positive. The results for 3 cell lines (Daudi, K562, and U937) are presented in Table 2. Also, data showed that the amount of CXCR4 was increased as PMP concentrations elevated. The highest amount was related to PMPs with 1000 µg/mL.

Table 1. The percentages of CXCR4-expressing on PMPs and cell lines before co-incubation

Cells and PMPs	% CXCR4
K562	No expression
Daudi	No expression
U937	No expression
PMP	70%

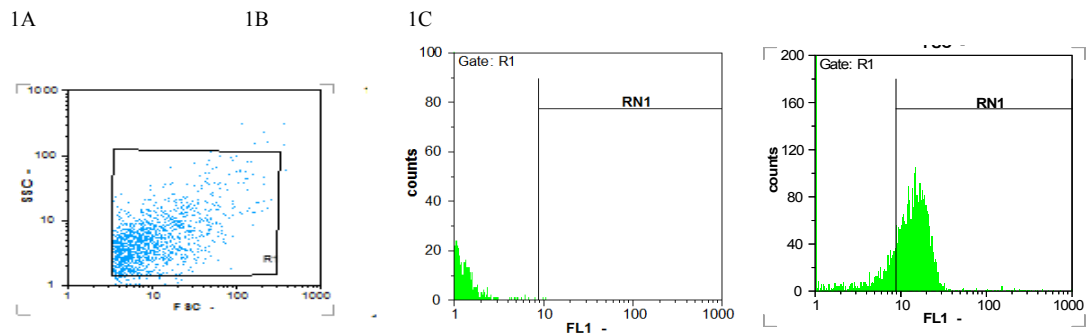


Fig. 1. Characterization of PMP by flow cytometry method. Figures 1 A and 1B show PMPs gate and PMPs Isotype control. Figure 1C shows CXCR4 expression on PMP (70%).

Table 2. The percentage of CXCR4-expressing on different cell lines (Daudi, U937, and K562) after incubation with different concentration of PMP

PMP concentration (µg/mL)	% CXCR4-expressing on different Cell line			p
	Daudi (Mean±SD)	U937 (Mean±SD)	K562 (Mean±SD)	
10	16±0.14	9±0.03	19.3±0.03	0.002
20	17±0.01	12±0.02	32.5±0.01	0.001
50	20±0.03	20±0.01	42.3±0.03	0.001
125	21±0.04	48±0.03	50.2±0.02	0.000
250	28±0.04	54±0.01	64.4±0.02	0.000
500	28.7±0.01	56±0.02	66.2±0.01	0.090
1000	29±0.02	57±0.03	68.4±0.01	0.200

Table 3. The percentages of fluorescence intensity of CXCR4 on K562, U937, and Daudi cell lines

PMP concentration (µg/ml)	% Fluorescence intensity of CXCR4 Mean± SD		
	U937cell line	Daudi cell line	K562 cell line
10	17.10±0.04	17.10±	19.1±0.02
20	14.30±0.07	14.30±0.04	24.10±0.03
50	16.6±0.08	30.25±0.03	30.25±0.09
125	21.10 ±0.01	33.1±0.05	33.1±0.04
250	21.98±0.05	21.98±0.06	35.6±0.05
500	22.55±0.04	32.21±0.04	36.3±0.08
1000	22.92±0.05	32.9±	36.66±0.04

Figures 2, 3, and 4 show flow cytometric analysis for CXCR4 on K562, Daudi, and U937. As this figure show, the amount of CXCR4 on K562 was increased with in-

creasing PMP concentration. The percentage of fluorescence intensity of CXCR4 was higher in the K562 cell line exposed with PMP compared to that of U937and

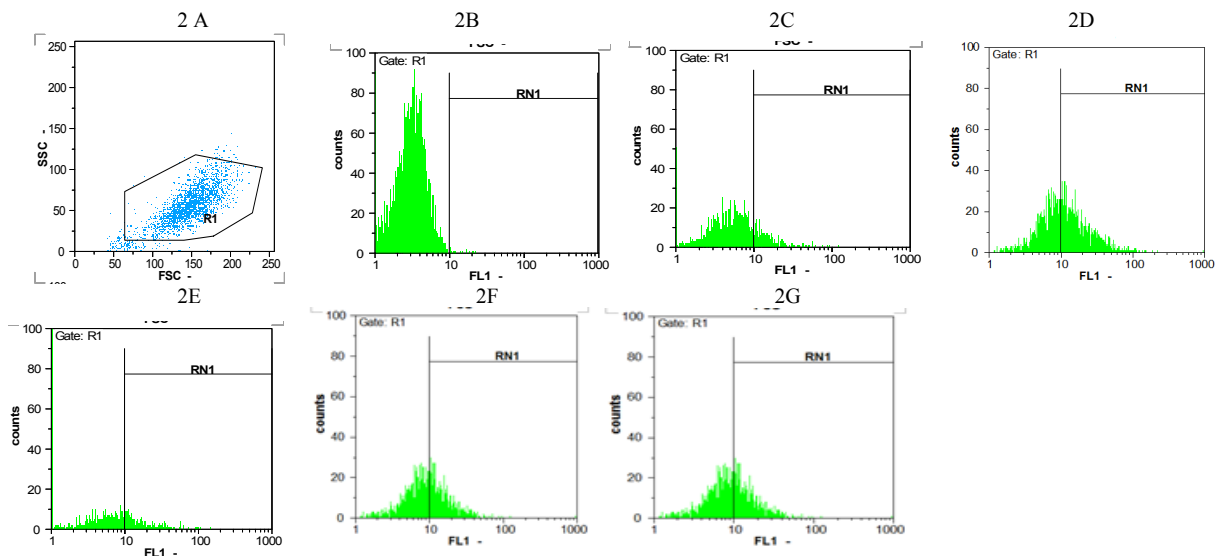


Fig. 2. The percentages of CXCR4-expressing on K562 cell line after incubation with different concentration of PMP. Figures 2 A and 2B show K562 cell line gate and Iso type control. Figure 2 C shows the result of 10 µg/ml concentration of PMP (19%), 2 D: 20 µg/ml (30%), 2E: 50 µg/ml (42%), 2F: 125 µg/ml (50%) and 2 G: 250 µg/ml (64%).

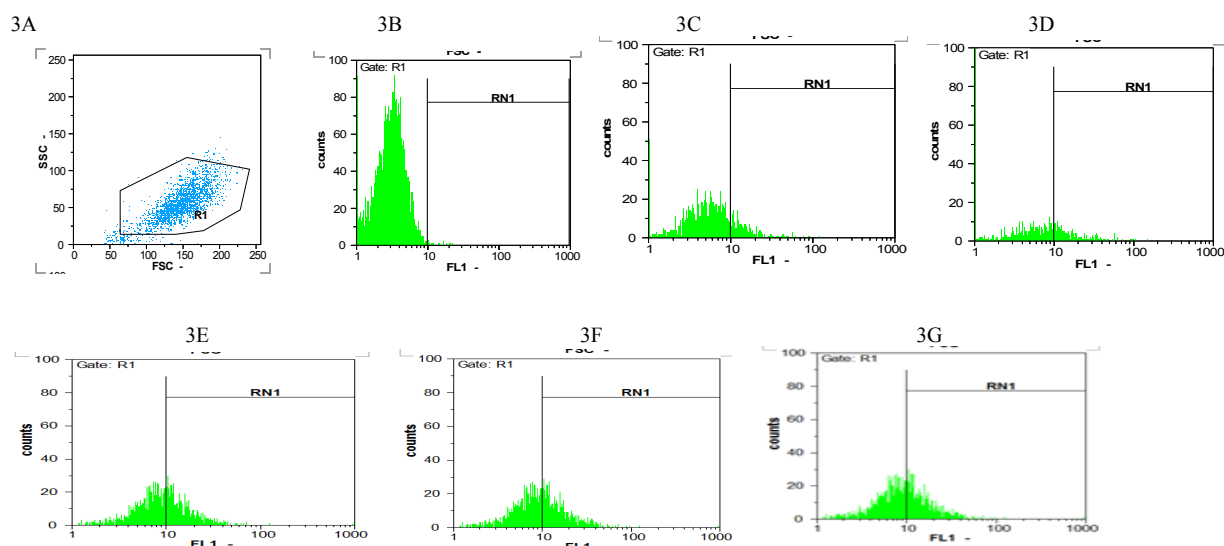


Fig. 3. The percentages of CXCR4-expressing on Daudi cell line after incubation with different concentration of PMP. Figures 3 A and 3B show Daudi cell line gate and Isotype control, 3C show the result of 10 $\mu\text{g/ml}$ concentration of PMP (9%), 3D: 20 $\mu\text{g/ml}$ (12%), 3E: 50 $\mu\text{g/ml}$ (20%), 3F: 125 $\mu\text{g/ml}$ (48%) and 3G: 250 $\mu\text{g/ml}$ (54%).

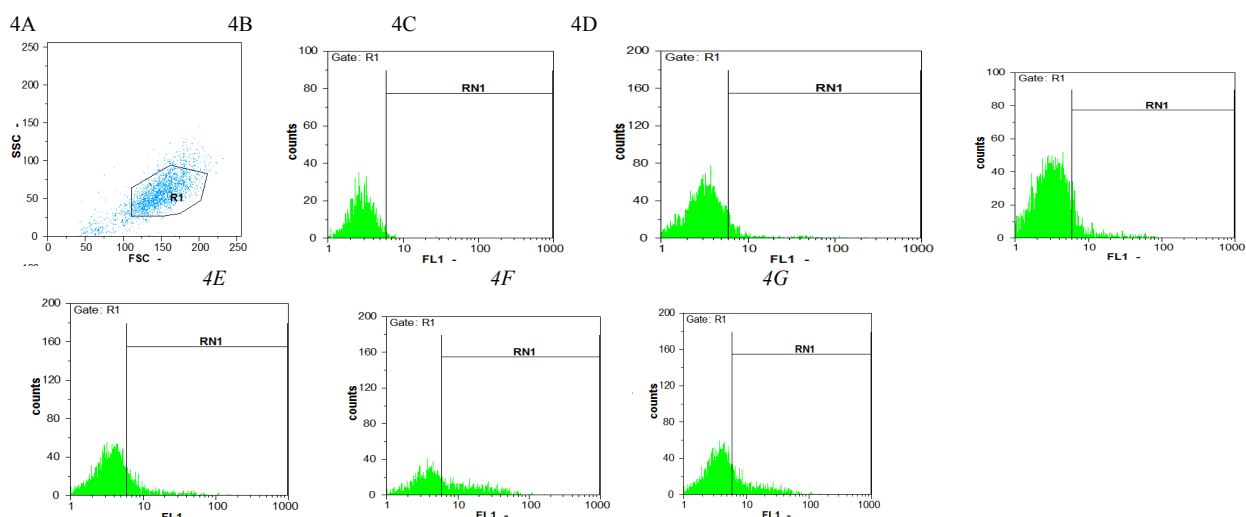


Fig. 4. The percentages of CXCR4-expressing on U937 cell line after incubation with different concentration of PMP. Figures 4 A and 4B show U937 cell line gate and Isotype control. Figure 4C shows result of 10 $\mu\text{g/ml}$ concentration of PMP (16%), 4D: 20 $\mu\text{g/ml}$ (17%), 4E: 50 $\mu\text{g/ml}$ (20%), 4F: 125 $\mu\text{g/ml}$ (21%) and 4G: 250 $\mu\text{g/ml}$ (28%).

Daudi cell lines. The fluorescence intensity was increased with the concentration of increasing PMP (Table 3). Jahromi et al previously showed that MP had the right size for PMP recognition according to this protocol (18).

Data were analyzed using paired sample t test by SPSS ($p < 0.05$) and showed that the increase of PMP concentration caused an increase in the CXCR4 presence on the studied cells. Also, 250 $\mu\text{g/ml}$ concentration provided the best result for transferring CXCR4 by PMP.

Discussion

Several studies have shown that PMPs can deliver their antigen to other cells. Some researchers reported that PMPs can attach hematopoietic stem cell and improve their engraftment (4, 19). Majka showed platelet-derived microvesicles can transfer platelet-specific immune reactive antigens on the surface of endothelial cells and CD34+ hematopoietic stem/progenitor cells (20). Also, a

study showed the internalization of human immunodeficiency viruses (HIV) by megakaryocytes and platelets (8).

Majka showed that the platelet-derived microparticles can transfer platelets antigens on the surface of endothelial cells and CD34+ hematopoietic stem/progenitor cells (20).

The possibility of infection of cells which do not express HIV-entry receptors was observed by several investigators; however, the molecular mechanisms leading to these infections are not understood (6).

Several investigators found that there is a possibility of infection of some cells that do not express HIV-1 receptors. Nevertheless, the molecular mechanisms leading to these infections are not completely understood (6).

In this study, it was found that PMP could transfer CXCR4 to null cells. The production methods for PMPs are different. Some researchers use freeze-thaw method to increase the number of PMPs (21) and some use thrombin

and other agonists to activate platelets and release PMP (22). We previously studied the effect of some methods such as freeze-thaw and speed of the centrifuge on the amount of PMPs (23). In freeze-thaw method and platelet activation, the surface composition may change. We collected PMPs from random platelet concentrates because this method had the least effect on the PMP surface composition (20). Also, we used the same protocol Jahromi used (18). It has been shown that megakaryocyte and platelets have CXCR4 on their surface (4).

In a study by Keryer-Bibens et al, incubation time was around 24 hours. However, in this study, PMPs were incubated with cells for an hour, which is more practical and the interaction among cells and PMPs was much lower compared to Keryer-Bibens method (24). PMPs or megakaryocyte-derived microparticles (M-MPs) can transfer CXCR4 to null cells and make them susceptible to infection by X4-HIV.

In the Keryer-Bibens et al study, normal erythroblasts, glioblastomas U87, MAGI, and hematopoietic cell lines UT-7, HEL and TF-1 were coincubated with PMPs and M-MPs. They activated platelets to obtain PMPs, but they did not mention the amount of PMPs (number or concentration) and the incubation period (6). Researchers showed that negative cells can be infected with X4-HIV after incubation with PMPs (6).

In this study, 7 different concentrations of PMPs were examined to minimize the dose dependency of this process. However, the increase in the concentration of PMP to transfer CXCR4 receptor to CXCR4-null cells in concentrations of 500 and 1000 µg / mL PMPs was not statistically significant, which may be due to the short incubation time or limited adsorption of cell lines for this receptor. More studies are needed to clarify that the adsorption of CXCR4 receptor to cell lines is dose-dependent or not. Previously Jahromi showed the dose dependency of PMPs for the production of IgG antibody from human peripheral blood B-lymphocytes (18).

Conclusion

In this study, the role of PMPs in transferring the chemokine receptor CXCR4 to CXCR4-negative cells (Daudi, K562 and U937 cell lines) was evaluated. In the present study, PMPs were used in 7 concentrations (10, 20, 50, 125, 250, 500 and 1000 µg/mL). The best result of transferring CXCR4 by PMP was seen in 250 µg/mL concentration that may be due to the dose dependency of this process.

Acknowledgments

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Conflict of Interests

The authors declare that they have no competing interests.

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