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БИОФИЗИЧЕСКИЕ АСПЕКТЫ ВЗАИМОДЕЙСТВИЯ ФИБРИНОГЕНА И МЕМБРАНЫ ЭРИТРОЦИТОВ ПРИ ИХ АГРЕГАЦИИ: ОЦЕНКА С ОПТИЧЕСКИМИ МЕТОДАМИ

Аннотация: Механизмы индуцированной фибриногеном агрегации эритроцитов до конца не изучены. Одна из моделей предполагает адсорбцию молекул фибриногена на мембране эритроцитов, приводящую к сращиванию клеток в агрегаты. В данной работе мы представляем результаты оценки адсорбции

фибриногена на мембране эритроцитов с использованием оптических методов.

Ключевые слова: эритроциты, фибриноген, флуоресцентная микроскопия, цитометрия.

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BIOPHYSICAL ASPECTS OF THE INTERACTION BETWEEN FIBRINOGEN AND MEMBRANE OF ERYTHROCYTES AT THEIR AGGREGATION: ASSESSMENT WITH OPTICAL TECHNIQUES

Abstract. The mechanisms of fibrinogen-induced RBC aggregation are not studied completely. One of the models suggests thr adsorption of fibrinogen molecules on RBC membrane, leading to the bridging of cells in aggregates. In this work we present the results of the assessment of fibrinogen adsorption onto RBC membrane using optical techniques.

Keywords: erythrocytes, fibrinogen, fluorescence microscopy, cytometry.

INTRODUCTION

Fibrinogen is the major determinant of RBC aggregation (RBC-A) in blood plasma [1,2]. Despite the rheological significance, the mechanisms of fibrinogen-induced RBC-A are not completely studied [3,4]. One of the discussed models is the adsorption of fibrinogen macromolecules onto RBC membrane, leading to the bridging of cells in aggregate when RBC contact with each other [5]. There were reported several limitations of this “molecular bridging” model [6]. One of the most common problem is the question of adsorption of fibrinogen macromolecules onto RBC membrane. In the present report we present the results of the assessment of fibrinogen adsorption onto RBC membrane using optical techniques.

MATERIALS AND METHODS

Blood samples in a volume of 10 μl were taken from healthy male donor by finger-pricking method using a sterile lancet. RBC were washed three times in phosphate-buffered saline (pH 7.4). After that RBC were put in the solution of fibrinogen, labelled with fluorescent marker Alexa488, and incubated at 37°C during 1-2 hours for microscopy measurements and during 30 minutes for flow cytometry experiments.

RESULTS

The microscopic photographs of different cells at different concentrations of fibrinogen-Alexa488 conjugate (2.0, 2.5 and 3.0 mg/ml) are presented in fig. 1 (adopted from Supplementary Information from Semenov et al. [7]).

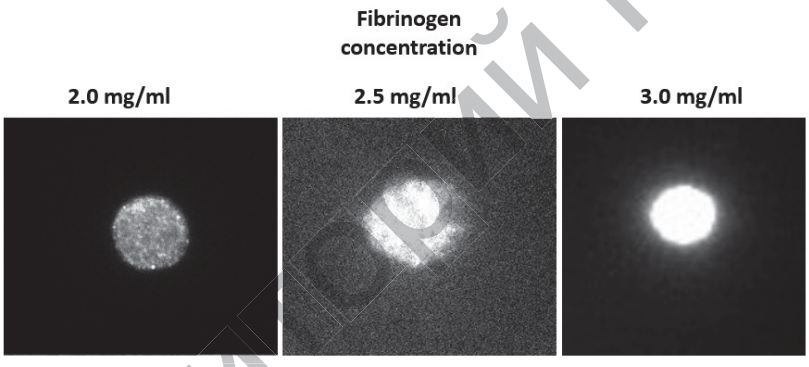

Figure 1. The microscopic images of RBC, incubated in the solution of fibrinogen, labelled with Alexa488 (Thermo Fisher, F13191) at different concentrations of conjugate. The images are adopted from Semenov at el. [7].

The results of the fluorescent microscopy measurements of fibrinogen-Alexa488 adsorption on RBC membrane were verified by flow cytometry. The results are presented in fig. 2 (adopted from [7]). We can see, that the number of counts for the "control Alexa488-labeled fibrinogen" (brown bars in fig. 2), which represents erythrocytes stained with the fibrinogen-Alexa488 conjugate, were found to be more than 2 times higher than the values, recorded for the "control (not stained)" sample (blue diagrams in fig. 2), which represents the autofluorescent signal from erythrocytes. This result suggests that fluorescently labeled fibrinogen macromolecules adsorb onto the

RBC membrane surface. To study the specificity of the fibrinogen adsorption on RBC membrane, prior to the staining with fibrinogen-Alexa488, we exposed the washed RBC to the effects of fibrinogen-binding receptors inhibitors. It can be seen, that the presence of fibrinogen-binding inhibitor in the solution before the staining led to the significant decrease in the fluorescent signal, detected in the FITC channel of the cytometer (**** $p < 10^{-4}$, Kruskal–Wallis H-test).

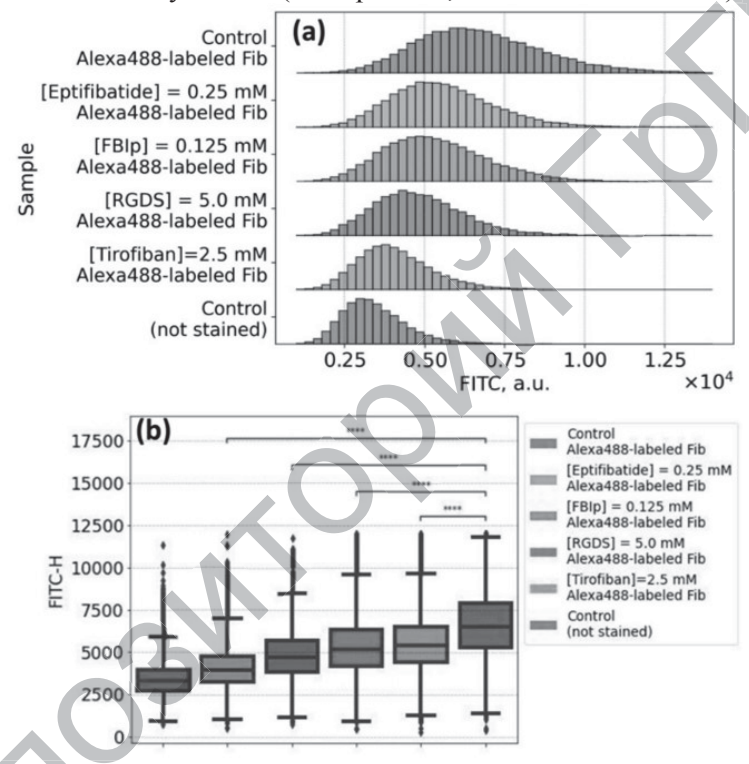

Figure 2. The results of the flow cytometry assay of the adsorption of fibrinogen, labelled with Alexa488, at concentration 3.0 mg/ml, on the membrane of RBC. The signal was detected in FITC-H channel of the cytometer (excitation 488 nm; emission 525 (40) nm): (a) signal distributions; (b) signal values. The results of the measurements of the effects of fibrinogen-binding inhibitors are also presented. **** $p < 10^{-4}$, Kruskal–Wallis H-test. Adopted from authors article [7].

CONCLUSIONS

Despite the localization of the negative charge on both fibrinogen molecules and RBC membrane, we can clearly observe the adsorption of fibrinogen on RBC. Generally there are two possible mechanisms of this process: non-specific interactions and specific interactions. Non-specific interactions, which include primary hydrophobic interactions and hydrogenic bonds, may arise and oppose the electrostatic repulsions between negative charges of fibrinogen and RBC membrane. However, our results on the effects of fibrinogen-binding inhibition (fig. 2), which is in agreement with results of other groups [8–10], allow to suggest that the specific character of the adsorption of fibrinogen prevails. The specificity of the interaction supposes the existence of the fibrinogen receptor on the surface of RBC membrane. Such receptor is able to recognize the specific fragments of fibrinogen macromolecule and form chemical bonds despite the electrostatic repulsion. Modern studies of the specific interactions between fibrinogen and RBC membrane characterize such receptor as a glycoprotein receptor class IIb/IIIa. These receptors play an important role in the aggregation of platelets at their activation by fibrinogen. Further studies on the structure and functioning of the fibrinogen-binding receptor on RBC membrane are very important because they potentially present novel opportunities to regulate the rate of fibrinogen adsorption on RBC membrane and therefore correct the hemorheological disorders caused by elevated fibrinogen-induced RBC aggregation.

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ЗАВИСИМОСТЬ МИКРОРЕОЛОГИЧЕСКИХ СВОЙСТВ КРОВИ ОТ ВОЗРАСТА ЭРИТРОЦИТОВ

Аннотация: Процесс агрегации эритроцитов сильно влияет на вязкость крови и гемореологию крови в целом. Целью данной работы было оценить изменение параметров агрегации эритроцитов при их старении in vivo. Лазерный пинцет использовался для захвата одиночных эритроцитов разных возрастов и измерения сил между этими клетками. Результаты показывают