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Influence of PAMAM dendrimers on human red blood cells

D.M. Domański, B. Klajnert, M. Bryszewska*

Department of General Biophysics, Institute of Biophysics, University of Łódź, 12/16 Banacha Street, Łódź 90-237, Poland

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Abstract

Polyamidoamine (PAMAM) dendrimers with different concentrations (1 nM–1 mM) (generations 2, 3, and 4) impact on human red blood cell morphology, and membrane integrity is studied. Erythrocyte shape changes from biconcave to echinocytic in dendrimers. Cell aggregation occurs. Polymers cause also concentration- and generation-dependent haemolysis.

Keywords: PAMAM dendrimers; Erythrocytes; Morphology; Haemolysis; Toxicity

1. Introduction

Dendrimers have attracted much interest since their synthesis in the mid-1980s [1] due to their unique nanoscopic architecture. There are attempts to use them in biomedical applications [2,3]. Functional groups presented on the surface have been utilized for the conjugation of drugs. In addition, a dendrimer interior has been shown to be capable of encapsulating various molecules. More information about dendrimer toxicity and biological properties is needed to continue studies on dendrimers in therapeutic applications.

The circulatory system seems to be the most convenient way of drug administration because an active compound within a relatively short time is able to reach distant tissues, which are unavailable directly. However, blood constituents can be the first and unwanted targets of drug action. Binding to plasma proteins, erythrocytes, leukocytes, platelets, and blood vessel walls may lead to serious problems, or at least dramatically lower the amount of drug available for therapy.

In this study, polyamidoamine (PAMAM) dendrimers impact on red blood cell morphology and haemolysis is checked. PAMAM dendrimers are synthesised from an ethylenediamine core by a successive addition of methyl acrylate and ethylenediamine. The number of terminal amino groups doubles after each cycle and the dendrimer is of higher generation. Here we have examined the haematotoxicity of three PAMAM dendrimer generations: second (G2), third (G3), and fourth (G4). The short characterisation of used dendrimers is presented in Table 1.

2. Experimental

Polyamidoamine dendrimers (generation 2, 3, and 4) were purchased from Aldrich (UK). Blood from healthy donors was obtained from the Central Blood Bank (Lodz). Blood was anticoagulated with 3% sodium citrate. Erythrocytes were separated from blood plasma and leukocytes by centrifugation (5000 × *g*, 5 min) at 4 °C and washed three times with phosphate-buffered saline (PBS; 150 mM NaCl; 1.9 mM NaH_2PO_4; 8.1 mM Na_2HPO_4, pH 7.4) and suspended in PBS. Erythrocytes were used immediately after isolation. Suspensions of red blood cells in PBS (haematocrit, 1%) were treated with different concentrations of dendrimers (1 nM–10 μM). Then cell samples were viewed under Nicon Eclipse 500 optical microscope using a magnification of 400 × and

Table 1
Characterisation of used dendrimers

Name, generation	Terminal groups	Number of terminal groups	Molecular weight [Da]	Diameter [nm]
PAMAM, G2	$-NH_2$	16	3256	2.7
PAMAM, G3	$-NH_2$	32	6909	3.6
PAMAM, G4	$-NH_2$	64	14,125	4.5

* Corresponding author. Tel.: +48-42-635-44-74; fax: +48-42-635-44-74.
E-mail address: marbrys@biol.uni.lodz.pl (M. Bryszewska).

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1000 ×. To study the effects of dendrimers on erythrocyte haemolysis, red blood cells were suspended in dendrimer solutions at a haematocrit of 1% and incubated for 1 h at 37 °C. The percent of haemolysis was measured spectrophotometrically from the absorbance at 415 nm [4]. For reference, red blood cells were treated with double-distilled water.

3. Results and discussion

The present work was aimed to investigate whether PAMAM dendrimers influence red blood cell morphology. In the circulation, erythrocytes have the shape of a biconcave disc—hence the name 'discocyte.' Discocytes in physiological conditions are highly deformable. It is essential for their survival in the microcirculation. Under the influence of intrinsic or extrinsic factors, discocytes can transform to echinocytes (crenated cells) or stomatocytes (cup-shaped cells) [5].

Changes in the red blood cell shape in response to interactions with dendrimers were studied by optical microscopy. Control cells were discocytes (Fig. 1A). Fig. 1B–D shows the shape changes of erythrocytes with increasing PAMAM G4 dendrimer concentration. The presence of 1 nM PAMAM G4 in a cell suspension induced echinocytic transformation. Cells displayed the characteristic irregular contour due to the folding of the periphery. At a higher dendrimer concentration (10 nM), cells elongated and spindle-shaped forms were observed. Drephanocyte-like forms in 100 nM dendrimer solution were recorded. Erythrocytes underwent similar shape transitions upon the addition of PAMAM G2 and G3 dendrimers; however, the extent of the process did not depend only on concentration but also on the dendrimer generation. For lower generations, similar changes in shape occurred at higher concentrations. In the presence of 1 μM PAMAM, G4 dendrimer glass was almost completely covered by attached cells, although plenty of echinocytes were still floating in suspension over the surface (Fig. 1E). Erythrocytes suspended in 10-μM solutions of dendrimer formed 40 × 80-μm clusters. The agglutinated red cells were difficult to disperse (Fig. 1F).

Changes in the erythrocyte shape were accompanied by dendrimer-induced haemolysis. PAMAM dendrimers

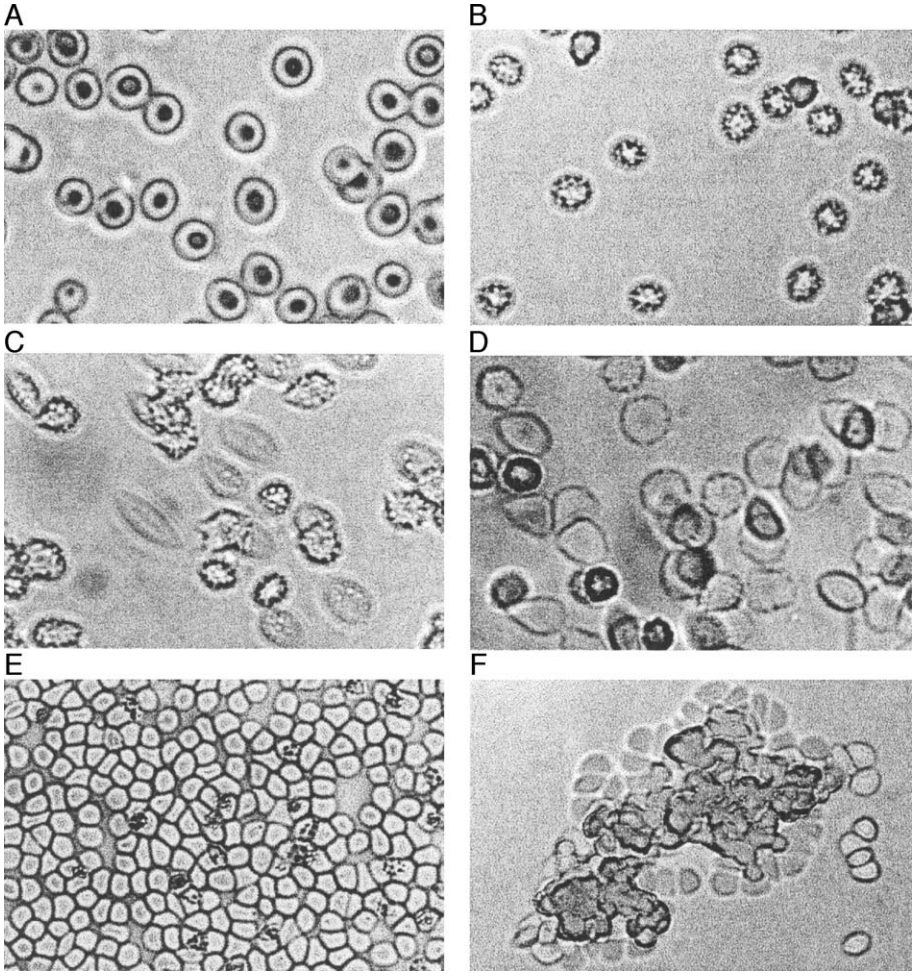

Fig. 1. Photographs of control erythrocytes (A): erythrocytes in PAMAM G4 dendrimer solution at a concentration of 1 nM (B), 10 nM (C), 100 nM (D), and 1 μM (E), and red blood cells clusters formed in the presence of 10 μM PAMAM G4 dendrimers (F) (magnification, 1000 ×).

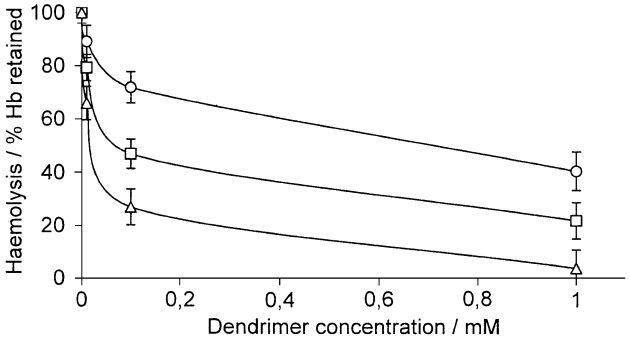

Fig. 2. Haemolysis induced by 1-h incubation at 37 °C with dendrimers PAMAM G2 (○), PAMAM G3 (□), and PAMAM G4 (△).

caused concentration- and generation-dependent haemolysis (Fig. 2). The higher dendrimer generation was used; a lower concentration was needed to release 50% of haemoglobin. For PAMAM G2, G3, and G4, it is equal to 735, 77, and 28 μM, respectively.

The haemolytic activity for amino-terminated dendrimers has earlier been reported by Malik et al. [6]. They also observed changes in the erythrocyte shape. In their case, the echinocytic transformation caused by dendrimers was more profound and spheroechinocytes were obtained.

The red blood cells' shape change and the haemolysis induced by dendrimers suggest that they interact with the erythrocyte membrane. The surface of a normal erythrocyte is negatively charged due to the presence of glycolipids and some glycated integral and peripheral proteins. Electrostatic repulsion among red blood cells prevents their self-aggregation and adhesion to the walls of blood vessels [7]. Cationic PAMAM dendrimers come close to the red blood cell surface as a result of electrostatic attraction. Thus, the formation of erythrocyte aggregates may be the consequence of their cross-linking by dendrimers.

When dendrimers are present on the red blood cell surface, there are two possible targets for their activity: lipids and membrane proteins. It has been shown that high-generation PAMAM dendrimers disrupt anionic lipid vesicles [8]. The dendrimer–vesicle interactions are dependent on the membrane composition (e.g., are weak for vesicles that contain a high fraction of PC and are strong for PE-containing vesicles). There are also studies postulating that the integrity of DMPC vesicles upon contact with PAMAM G7 dendrimers is maintained, although there are changes in ordering of the bilayer and rotational mobility of fatty acid chains [9]. The bilayer couple hypothesis explains echinocytic transformation as an effect of an asymmetric expansion of one monolayer [10]. The formation of echinocytes in our experiments can be due to partial dendrimer incorporation into a lipid bilayer, or due to pulling out of the outer monolayer by dendrimer molecules. PAMAM dendrimers also interact with proteins and change their conformation [11]. Further studies on enzyme activities and with isolated membrane proteins will help to reveal the mechanism of dendrimer–protein interactions.

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