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Note

Contribution of hydrophobicity, DNA and proteins to the cytotoxicity of cationic PAMAM dendrimers

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ABSTRACT

In most articles, cytotoxicity of cationic polyamidoamine (PAMAM) dendrimers toward red blood cells has been exclusively explained by their surface charge. We have focused on dendrimer hydrophobicity as a second possible factor that determines this cytotoxicity. Using PAMAM-NH_2 dendrimers from the 3rd to the 6th generations and PAMAM-NH_2-C_{12}(25%) dendrimer of the 4th generation bearing 25% acyl groups, these induced hemolysis that increased with their surface charge and hydrophobicity. Interaction of PAMAM-NH_2-C_{12}(25%) G4 dendrimer with blood proteins (γ-globulin, α-thrombin, human serum albumin) and calf thymus DNA (ctDNA) significantly reduced their cytotoxicity toward red blood cells.

Cytotoxicity of cationic polyamidoamine (PAMAM) dendrimers toward red blood cells has been exclusively explained by their surface charge (Malik et al., 2000; Domanski et al., 2004). Generally, cationic PAMAM dendrimers bearing —NH_2 termini are prevalent, and in the case of PAMAM dendrimers, generation-dependent hemolysis that are changes in red cell morphology arise. In contrast, anionic and neutral PAMAM dendrimers with —COONa and —OH groups, respectively, are neither hemolytic nor cytotoxic to a panel of cell lines *in vitro* (Malik et al., 2000; Domanski et al., 2004). These results were confirmed with many different kinds of dendrimers, such as PAMAM (Han et al., 2010; Ziemba et al., 2012a,b), polypropylenimine (PPI) (Mishra et al., 2010; Agashe et al., 2006; Ziemba et al., 2012a,b), carbosilane (Pedziwiatr-Werbicka et al., 2013), and viologen-phosphorus (Ciepluch et al., 2012). All available reports suggest that higher generations of dendrimers cause higher hemotoxicity, due to greater overall cationic charge on the surface (Jain et al., 2010). However, Santos et al. (2010) reported non-toxic and effective gene transfection into stem cells using 5th generation of the cationic PAMAM dendrimer randomly linked at the periphery to the hydrophobic chains.

We have focused on dendrimer hydrophobicity as a second possible factor that determines cytotoxicity. This aspect that seems important in relation to hemotoxicity is the influence of DNA and main plasma proteins on the level of hemolysis caused by dendrimers. The presence of serum albumin significantly reduces hemolysis caused by PAMAM dendrimers (Klajnert et al., 2010).

Many types of PAMAM dendrimers, γ-globulin, α-thrombin, calf thymus DNA (ctDNA), human serum albumin and other chemicals were obtained from Sigma–Aldrich (USA). Blood was obtained from healthy donors at the Republican Research and Practical Center of Hematology and Transfusion (Belarus). Red blood cells (RBC) were isolated as previously described (Ziemba et al., 2012a,b). Experiments were carried out in phosphate-buffered saline (PBS: 150 mM NaCl, 1.9 mM NaH_2PO_4, 8.1 mM Na_2HPO_4, pH 7.4). RBC suspension in PBS had a final hematocrit of 2%. The percentage of hemolysis was determined on the basis of hemoglobin (HGB) released into supernatants. RBCs were treated with distilled water to give a 100% hemolysis reference.

Hemolysis was induced by adding cationic PAMAM-NH_2 dendrimers of the 3rd (G3), the 4th (G4), the 5th (G5) and the 6th (G6) generations. Hemolysis increased in a generation-dependent way. The molar dependence of RBC hemolysis can be explained by an increase in surface charge of the dendrimers (Fig. 1A). However, when this data is expressed in the relationship of the % hemolysis and the number of dendrimers' surface charges in solution, an equal amount of cationic surface charges on the high generations of PAMAM dendrimers still proved more cytotoxic than

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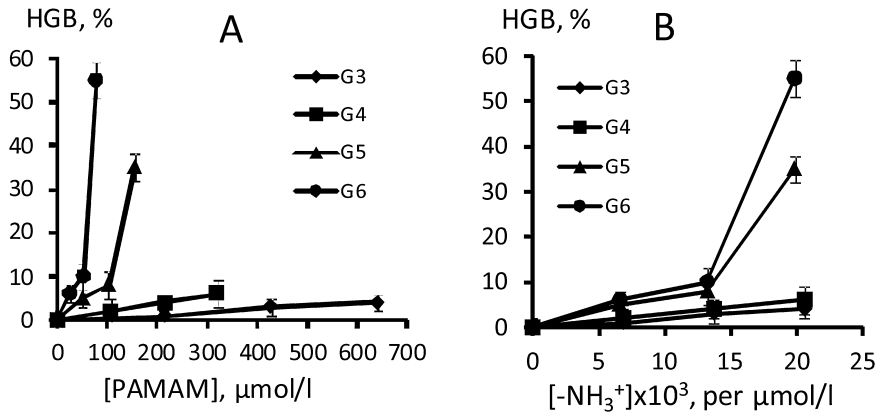

Fig. 1. Dependence of RBC hemolysis on molar: (A) concentrations of cationic PAMAM-NH_2 dendrimers, recalculated for the number of surface charges (B) 1% of RBS in PBS, pH 7.4, 20 °C, with a 2 h incubation.

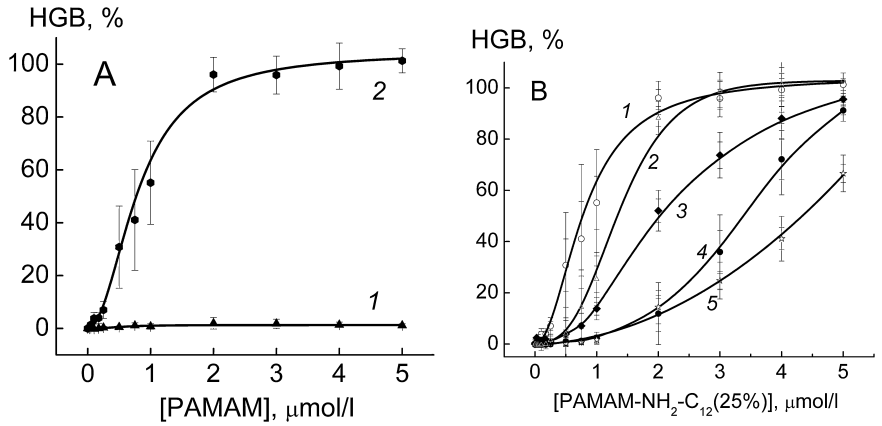

Fig. 2. (A) The dependence of RBC hemolysis on concentration of cationic PAMAM-NH_2 G4 (1) and PAMAM-NH_2-C_{12}(25%) (2) dendrimers. (B) Dependence of RBC hemolysis on the concentration of PAMAM-NH_2-C_{12}(25%) dendrimer in absence (1) and in presence of γ-globulin (2), α-thrombin (3), ctDNA (4) and human serum albumin (5). 1% of RBS in PBS, pH 7.4, 20 °C. 2 h incubation. [protein] = 15 μmol/l. [ctDNA] = 20 μg/ml.

lower ones (Fig. 1B). We hypothesize that this can be a consequence of increased hydrophobicity of high generations of PAMAM dendrimers that we have previously reported (Shakhbazau et al., 2010). To examine this hypothesis, we compared 2 dendrimers of the 4th generation: fully cationic PAMAM-NH_2 and partially cationic PAMAM-NH_2-C_{12}(25%) in which the 25% of NH_2 groups had been replaced by hydrophobic non-polar C_{12} groups (Fig. 2A). Replacement of 25% of NH_2 groups significantly increased hemotoxicity, which might be explained by its enhanced incorporation into erythrocyte membranes. Such an effect has been observed with many hydrophobic drugs (Machleidt et al., 1972). In the case of cationic dendrimers, their hemotoxicity was significantly reduced by the addition of human serum albumin (Klajnert et al., 2010). It was postulated that this effect was due to electrostatic interaction between the dendrimers and protein. It seems important to check whether the same phenomenon occurs in the presence of hydrophobic dendrimers. We therefore analyzed RBC hemolysis induced by PAMAM-NH_2-C_{12}(25%) dendrimer in the presence of blood proteins γ-globulin, α-thrombin, human serum albumin, and of calf thymus DNA (Fig. 2B). Both protein and ctDNA significantly reduced the impact of PAMAM-NH_2-C_{12}(25%) dendrimer on RBC, which can be explained by formation of complexes between dendrimers and proteins (Shcharbin et al., 2007; Giri et al., 2011) or ctDNA (Tang and Szoka, 1997; Al-Jamal et al., 2005; Fant et al., 2008). Formation of these complexes is based on both electrostatic and hydrophobic forces that neutralize electrostatic and hydrophobic interactions between the PAMAM-NH_2-C_{12}(25%) dendrimer and the erythrocyte membrane. This means that not only hemotoxicity of dendrimers alone, but the impact of complexes dendrimer–protein and dendrimer–DNA should be studied when dendrimers are going to be used in, *e.g.*, gene transfection. The results of Santos et al. (2010) are in agreement with ours results because (1) dendrimers are functionalized by natural fatty acids ($-(CH_2)_n-COOH$) in which non-polar acyl groups were masked by COOH groups, and (2) the cytotoxicity of complexes with pDNA has been analyzed.

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