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Interference of cationic polymeric nanoparticles with clinical chemistry tests—Clinical relevance

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ABSTRACT

The development of medical nanosystems requires knowledge of their behavior in vivo. Clinical chemistry tests are widely used to estimate the systemic toxicity of nanoparticles. In this paper we have explored the impact of small positively charged nanoparticles–poly(amidoamine), phosphorous and carbosilane dendrimers – on biochemical parameters of standardized serum in vitro. All the dendrimers could shift the main biochemical parameters. Thus, in the case of patients having the normal, but 'boundary', values of biochemical parameters, nanoparticle-induced changes can be wrongly interpreted as evidence of some dysfunctions (hepatic, renal, etc.). Moreover, the effects of nanoparticles of metals, carbon nanotubes, quantum dots, fullerenes, dendrimers having been sized up to 4000 nm and the hundreds of reactive groups, can be significantly higher. Thus, preliminary evaluation of any nanomaterial in vitro is required in clinical chemistry tests before its application in vivo to draw the correct conclusions and benefit animals.

1. Introduction

The burst of interest in nanotechnology has led to the development of nano-based multifunctional systems for drug and gene delivery, imaging and diagnostics, giving a new aspect to medicine – personalized nanomedicine. In this approach, the practice of developing and administrating therapeutic agents are based on disease prevention, diagnosis and treatment, designed on an individual's genetic profile. Several companion diagnostics have been developed in association with the drugs (Petersen et al., 2014). Thousands of nanocomposites have been created and have to be tested as whether they can be non-toxic and effective personalized nanomedicines (Florence and Lee, 2011). Before clinical trials, nanoparticles are tested in vivo (Florence and Lee, 2011). To estimate their toxicity, clinical chemistry tests are widely used that are usually based on analysis of blood biochemical parameters (Okuda et al., 2006; Labieniec-Watala et al., 2014; Thiagarajan et al., 2013; Shcharbin et al., 2014) and have a high clinical significance which can be extremely helpful in emergency cases, i.e., stroke, infarction, accidents (Thomas, 1998). However, the nanoparticles differ completely from classic drugs, whose metabolism is well known and has become predictable. In particular, they interact with major biological structures: proteins, nucleic acids, membranes and cells (Maynard et al., 2011; Florence, 2012). Thus, the appearance of nanosystems as biomarkers can lead to their direct interaction with blood components, which could result in incorrect interpretation of clinical chemistry tests. To test whether this is the case, we made model studies using three kinds of polymeric nanoparticles: cationic poly(amidoamine), phosphorus dendrimers of the 4th generation and cationic carbosilane dendrimer of the 3rd generation. These dendrimers proved to be prominent gene and drug carriers (Tomalia, 2009; Mignani et al., 2013a,b, 2014; Sánchez-Nieves et al., 2013). The aim of our study was to estimate the effect of dendrimers on clinical

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chemistry tests of standardized serum when dendrimers were injected into serum in vitro (i.e., no in vivo application).

2. Methods

2.1. Clinical chemistry tests

For experiments, human standardized serum (Beckman-Coulter, USA) was used as a reference standard to clinical biochemical analyzer Olympus AU-400 (Japan) analyzed by a standard clinical protocol (Thomas, 1998). The reagents for the analyzer were obtained from Beckman-Coulter, USA or Sigma–Aldrich, USA. Enzyme activity and concentration of 11 blood proteins: (1) total protein, g/l (No. OSR6x32); (2) gamma glutamyl transferase (GGT), U/l (OSR6x20); (3) alkaline phosphatase (ALP), U/l (OSR6x03); (4) aspartate transaminase (AST), U/l (OSR6x09); (5) cholinesterase, μkat/l (OSR6x14); (6) alanine aminotransferase (ALT), U/l (OSR6x07); (7) α-amylase, U/l (OSR6x06); (8) albumin, g/l (OSR6x02); (9) lactate dehydrogenase (LDH), U/l (OSR6x26); (10) creatine kinase (CK), U/l (OSR6x79); (11) LDH isoenzyme test (2-hydroxybutyrate dehydrogenase (HBDH) activity), U/l (OSR6x29), were analyzed in the absence of dendrimers and their presence in 1, 10 and 50 μmol/l. The corresponding volumes of dendrimers were dialyzed, using MicroEqulibrium Dialyzer (Harvard Apparatus, USA) against PBS (150 mmol/l phosphate-buffered saline, containing 10 mmol/l Na-phosphate buffer, 140 mol/l NaCl, pH 7.4). Then 10 μl stock solution of a dendrimer in PBS was added to 500 μl of a freshly-prepared (by a standard protocol) serum and mixed gently for 15 min at 25 °C. The corresponding control samples were prepared and added to a serum in the same way. Every concentration had its own control sample. All the control samples had no significant difference (data not presented).

2.2. Dendrimers

Three different nanocomposites were used: commercially available cationic poly(amidoamine) dendrimer of the 4th generation (PAMAM) (obtained from Sigma–Aldrich, USA), and our own synthesized dendrimers: cationic phosphorus dendrimer of 4th generation (CPD), and cationic carbosilane dendrimer of the 3rd generation (CBS) No. BDEF009 (the detailed structures and description of dendrimers are presented in Supplementary data).

2.3. Statistics

Shapiro–Wilk test was used to check the normality of distribution, the data being expressed as mean ± SD of 7 independent experiments. Statistical significance was assessed using the one-way analysis of variance (ANOVA) with the post-hoc Newman–Keuls (N–Kt) and Duncan (Dt) multiple comparisons tests (0, 1, 10, 50 μmol/l), and also with the paired Student *t*-test (St) (Statistica 6.0, StatSoft Inc., USA). Origin 8.0 (Microcal Software Inc., USA) was used for curve fitting.

3. Results

The three dendrimers had no effect on activity of LDH, α-amylase, GGT, ALT. None of the dendrimers studied at 1 μmol/l concentration affected the ALP, AST, cholinesterase, CK or HBDH activities or the concentrations of total protein and albumin (data not presented). However, the activity of ALP, AST, cholinesterase, CK, HBDH, and the concentrations of total protein and albumin changed after the addition of dendrimers in concentrations of 10 and 50 μmol/l (Figs. 1–5).

3.1. Total protein and albumin

The dendrimers (50 μmol/l) induced false increases of the concentrations for both total protein and albumin in blood measured by their appropriate tests (Fig. 1). Also, CPD (10 μmol/l) induced false increases of the concentrations for both total protein and albumin tests (Fig. 1). There is a positive non-linear dependence between the number of cationic surface groups per dendrimer and the Δ (determined as Δ = (albumin value in presence of a dendrimer) – (the one in absence of it) (Fig. 2).

3.2. ALP

The reduction in ALP enzyme activity with PAMAM and phosphorous dendrimers was observed while CBS had no effect (Fig. 3A).

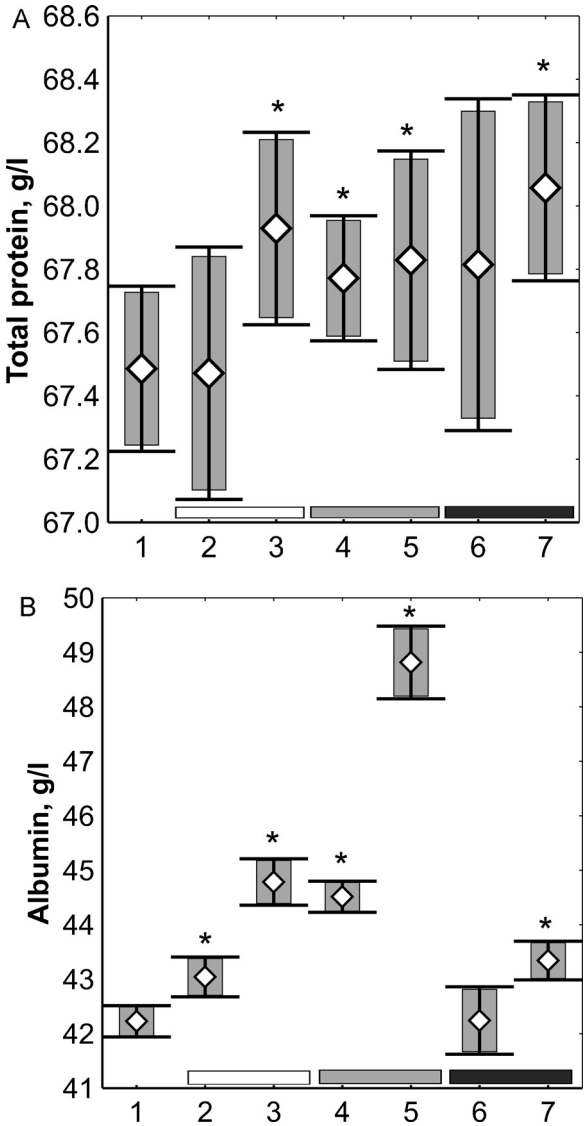

Fig. 1. Impact of dendrimers on total protein (A) and albumin (B) concentration tests. 1 – control, 2 and 3 – PAMAM 10 and 50 μmol/l, 4 and 5 – CPD 10 and 50 μmol/l, 6 and 7 – CBS 10 and 50 μmol/l. Box values – mean ± S.D., Whiskers values – mean ± 95% confidence intervals. $^{*}p < 0.05$ by N–Kt and Dt between control and a dendrimer-treated sample. The point at 1 μmol/l is not presented.

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