Red Blood Cell: Membrane and Metabolism Responses to Nanoparticles Exposure

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Abstract— Nanotechnology-based treatments have already demonstrated their potential for treatment of severe health problems like cancer, resistance to antibiotics or degenerative and traumatic disorders. However, research transition from experimental to clinical trials requires complete understanding of nanoparticles effects over human organism. Blood passage represents a common step in bio-distribution of nanoparticles and recently, effects over red blood cells (RBC) tend to represent a question of high interest among researchers. The special role played by RBC in overall homeostasis demands a detailed study of RBC’s membrane and metabolic processed affected by nanoparticles.

Keywords— nanotoxicology, metabolism, nanoparticles, red blood cell,

I. MEMBRANE INTERACTION AND TRANSPORT MECHANISM.

Erythrocyte membrane structure has been longly studied and the widely accepted model is that of a lipid bilayer having phospho- lipid molecules and glycolipids on the outer half and amino-phospholipids in inner half.[1] Transport of anions across the membrane is catalyzed by protein band 3, a 95,000-dalton integral membrane protein of RBC.[2] while other positive ions are transported through a non-selective ion channel, activated by prostaglandin E2.[3] Also, glucose follows a carrier-dependent mechanisms to pass through membrane[4]. Several studies reported interesting results on red blood cells (RBC)- membrane interaction with different nanoobjects. One such report combined various imagistic techniques for nanoparticles (NP) and red blood cells visualization. The purpose of method combination was to achieve the ability of particles’ detection for diameters of less than 0.2 µm and the ability to simultaneously visualize red blood cells and nanoparticles of interest. Laser scanning microscopy with digital restoration facility was used to detect fluorescent particles. For gold nanoparticles, conventional transmission electron microscopy was used. Energy filtering transmission electron microscopy was also involved in titanium dioxide nanoparticles’ analyzes. All obtained results suggest the ability of nanoparticles to transit RBC membrane by means of a distinct mechanism, different from endocytosis or phagocytosis.[5, 6] Consensus among authors exists in stating that, according to experimental results, attachment of NP to RBC membrane is a more facile process in the case of flexible particles as compared to rigid ones.[7, 8].

However, membrane interaction does not limit itself to attachment, as aforementioned. Internalization and rupture of cell membranes are also possible. Many studies have tried to decode the cellular mechanism involved in the transport of NPs across RBC membrane. Results have demonstrated that the contact between gold NPs with the red blood cell membrane is initially triggered by electrostatic interaction that exists between the negatively charged membrane and the positively charged NP surface. [6]

Following NP-RBC interaction various signs of toxicity have been reported for many types of NPs. Silica NPs have been reported to produce membrane ruptures and corpuscle formation in RBCs at 0.1wt% concentration. Also, agglutination of RBC has been detected, and, to a lesser extent, cell shape deformation and spherocytes presence among exposed RBC. [9]

The study of the RBC-nanoparticle interaction should also consider that adhesion of NP to RBC increased the circulation time. However, it is already proven that even so, NP clearance from circulation appears within a short time interval (hours from administration). Therefore the dynamics of NP-RBC clearance/NP-RBC adhesion needs to be carefully decoded for complete membrane –NP interaction understanding. Some studies suggest that, despite NP clearance, the RBC that were previously connected to them remain in the circulation. Researchers have demonstrated the attachment between NPs and RBCs is passively removed due to intercellular interactions and shear forces. As a consequence, NPs are removed from circulation, especially via spleen and liver pathways. Moreover, it has been stated that NP-RBC binding strength is directly and proportionally responsible for NP circulation lifetime. Any surface structure alteration, such as, polyethylene glycol(PEG)
can induce major changes in NP half-life (increase up tp 24 hours in case of PEG).[10]

II. HEMAGGLUTINATION

Recently, researchers directed their efforts towards testing nanoparticles’ capacity to induce hemagglutination. Although the typical cause of hemagglutination involves an antibody, coalescence of nanoparticles in solution with consequent hemagglutination is recently[4, 11] questioned. One such study evaluated partially quaternized poly(thio-1-(N,N-diethyl-aminomethyl) ethylene)s, a type of material proposed as a possible carrier for drugs lacking water solubility. The report concluded that the tested material is capable to promote hemolysis (through RBC bilayer rupture) and hemagglutination (by means of electrostatic interaction) [12]. Similar results were obtained when RBC were treated with nano-TiO2, with major signs of hemagglutination, sedimentation and hemolysis. Unlike them, micro- TiO2 did not induce RBC noxious effects. [13, 14] Alteration of primary nanoparticles structures have also been found to promote agglutination. One such chemical modification is the addition of a primary amine group on poly L-glutamic acid derivate. Such an addition has been reported to induce severe RBC agglutination. An interesting study, focused mainly on agglutination of red blood cells and platelets was performed for a newly synthesized material. High-density oxygen microwave plasma surface activation of polypropylene nonwoven fabric was used in the first step of material synthesis. Graft copolymerization with acrylic acid was performed next, with chitosan molecule conjugation in the last step. Hemagglutination and agglomeration of RBC was observed and the authors demonstrated that the effect can be caused by the acrylic acid –induced hydrophilic effect and by the chitosan conjugation. Also, RBC-polycations attraction was observed and demonstrated to be one of the hemagglutination-causing phenomena.[15, 16] Pro-agglutination effect was also observed for silica nanoparticles. The authors of the respective study conclude that should there be needed pro-agglutination enhanced effect for various applications it can be satisfactorily induced by interaction of silica nanoparticles with RBC. Such a technique would reduce the interaction caused by electro-negatively charged cells, by binding to the proteins of the RBC membranes. This interesting effect has also been demonstrated by other research groups, using microscopic techniques. [9]

III. CHANGES IN SEDIMENTATION RATE OF RBC

The role of electrical charge on RBC surface in zeta potential equilibrium and erythrocyte sedimentation rate determination has long been established. [17] Apart from hemagglutination, and hemolysis, studies on nano-TiO2 showed that RBC also present abnormal sedimentation after treatment. The effect is absent in the case of micro-TiO2. [13] Also, other synthetic particles revealed pro-agglutination effects when they are used in excessive RBC exposure. Demonstration of this effect is done by a research paper focused on carboxyl –functionalized polystyrene particles of 1100, 830, 450, 220, and 110nm, respectively. [18] However, effects are presented as inconstant in literature. Other types of nanoparticles, namely chitosan-functionalized nanoparticles, have demonstrated no erythrocyte sedimentation rate alterations following oral administration, making them useful for various nano-based therapies.[19] All data suggests that intensive testing is needed for toxicity evaluation, including agglomeration and erythrocyte sedimentation rate evaluation before any clinical applications to be proposed.[20]

IV. 2,3 DIPHOSPHOGLYCERATE (DPG) METABOLISM.

Synthetized from 1,3 Bisphosphoglycerate by Luebering-Rapaport shunt, 2,3 diphosphoglycerate represents (2,3 DPG) represents one of the most important player in oxygenation/deoxygenation of haemoglobin. It has significant affinity for deoxygenated haemoglobin. By binding to the beta subunits of haemoglobin and allosterically promoting the detaching of oxygen, the molecule is able to enhance tissue oxygenation.[21] Its synthesis has been demonstrated to be PH-dependent. Severe lactacidosis is associated with a reduction of 2,3 DPG caused by enzymatic inhibition (2,3 DPG mutase and severat glycolytic enzymes). [22] Although hemolysis has been investigated in relationship to many types of particles, little is known about oxygen transport function of RBC after nanoparticles exposure. However, several disorders are associated with 2,3 DPG impairment. Among them, diabetic neuropathy has attracted researchers’ interest. Recently, a nanoparticles treatment scheme has been proposed, employing 25Mg-PMC16nanoparticle (porphyrin adducts of cyclohexil fullerene-C60). Diabetic neuropathy animal models based on streptozotocin (STZ)-induction have demonstrated the ability of the treatment to compensatory increase the 2,3 DPG level resulting in improved tissue oxygenation and potential benefit in ameliorating diabetic neuropathy symptoms. Another study, dedicated to bio-polymers, such as branched polyethyleneimines studied the oxygen transporter function of RBC following exposure to synthetic material. Results revealed that, for some concentrations and at specific molecular weights impairment of tissue oxygenation can appear, as a result to 2,3 DPG decrease. These discoveries rise new questions to be addressed before any clinical-intend application of nanoparticles. [23]

V. ADENOSINE-TRIPHOSPHATE (ATP) METABOLISM.

Synthetized as a result of Embden-Myerhof Glycolytic
Pathway, ATP represents one of the critical molecules for RBC function. During osmosis, the ATP-dependent Na+-K+ pump promote the exit of Na+ from RBC along with the water molecules. In the absence of Na+-K+, ATPase in the membrane, Na+ would be retained in the RBC and swollen of the cells would appear, with their consequent elimination in the spleen.[24]

The mechanism of ATP release has also been, recently, associated to deformability processes by an interesting publication. The authors suggest that integrative studies (such as mechano-transduction) are required to depict cellular biochemistry and deformability. [25] Various nanotechnology-specific techniques, such as Fourier transformed light scattering have been used in ATP metabolism evaluation for RBC. The authors open new opportunities in evaluation of nano- RBC interaction and its effect over ATP equilibrium. Their recently published paper demonstrates that RBC scattering signals (either static or dynamic) are significantly modified by ATP-induced membrane metabolic remodeling.[26] Some types of nanoparticles have already been tested regarding their interference with ATP synthesis/degradation. Remarkably, in the case of silver nanoparticles, results showed no signs of toxic effects over the ATP/ADP ration and the polarity of the membrane. An increased activity of the Na+-K+ pump suggested a normal membrane polarization process following silver nanoparticles exposure, accompanied by a low calcium ion concentration.[27]

Other nanoparticles have been demonstrated to exert different effects. In a rainbow trout model, nano-sized copper have been suggested to be an ion-regulatory toxicant by interference to Na+-K+ ATP-ase.[28] Significant decrease in Na+-K+ pump in brain tissue following oral administration of iron oxide nanoparticles has been reported. Although the authors analyzed the tissue fragments and not blood, results could also suggest a similar effect in RBC membrane.[29] Nanotechnological treatment has also been proposed as a remedy for ATP metabolism impairment associated with severe disorders, such as diabetic neuropathy. For this life-affecting complication, Mg-PMC16 nanoparticles were administered to ameliorate RBC function.[30] Considering various possible membrane interactions in the event of nanoparticles exposure (see above), the ATP synthesis and metabolism remains an important issue for nano-toxicological experiments.

VI. EFFECTS OVER (NAD(P)+/NAD(P)H) SYSTEMS.

Formed as a result of glycolytic pathway NAD+ (and its reduced form: NADH) represent an essential cofactor in reconversion of methemoglobin following the enzymatic reaction catalyzed by methemoglobin reductase. The electron transfer, supported by the presence of NADH as a donor, helps the iron re-transition from ferric back to ferrous state.[31] Distinct from the aforementioned, the NADP+/NADPH system is associated with the hexose-monophosphate shunt which accounts for degradation of approximately 10% of glucose in RBC. NADPH serves as a crucial cofactor in antioxidant responses by contributing to reduced glutathione formation and consequently, conversion of peroxides to water by means of glutathione peroxidase.[32]

Little is known about the interference of nanoparticles with the two systems. Due to their capacity to act as electron donor-acceptor units, there is a high likelihood of interference with the complex surface charge of nanoparticles structures. However, one recent study focused on the effects of TiO2 on trout erythrocytes found no increased production of superoxide following NADH administration therefore infirming the theory of lipid peroxidation in RBC membrane. [33] Nano-Selenium has also been proposed for reducing oxidative RBC damage. However, the effects were not statistically significant in increasing NADPH synthesis and glutathione peroxidase activity aiming to protect RBC against free radical production. [34]

VII. SYNTHETIC RBC

The interest for RBC in nanomedicine applications has not been only directed towards the study of their interaction with nanoparticles. A new idea have emerged, that of designing synthetic RBC to suite numerous biological applications. For this purpose, different structures and techniques have been imagined, such as microencapsulation of RBC enzymes and hemoglobin,[35] using microcapsules’ membrane made of polylactic acid[36] or multi-enzyme systems.[37] Also, polyhaemoglobins (PolyHb) and perfluorochemicals are already in advanced phase III clinical testing while conjugated haemoglobins are presently approved for phase II clinical trial.[38]

An interesting paper focused on designing Hb nanocapsules for applications in transfusion medicine. The report presents as optimal PEG-PLA Hb nanocapsules, the ones which are prepared using a mixture of 4 different elements: polymerized Hb usage, utilization of increased molecular weight PLA, use of elevated concentrations of PEG-PLA and the crosslinking of the designed PEG-PLA Hb nanocapsules. Such a combination would offer the support for reaching maximal non-RBC systemic Hb for up to 3.66 gm/dl an interval to obtain a concentration of 1.67 gm/dl of 24.2 hours in rats and 41.5 hours in human.[39]

An important step forward has been represented by development of a new haemoglobin molecule presenting complete absence of vasoactive properties. For this purpose several methods have been proposed. Concepts started with utilization of an antioxidant oxygen carrier, such as
PolyHb-catalase-superoxide dismutase, continued with development of lipid vesicles-constructed artificial RBC and went up to more modern approaches. One of the most appreciated concept among researchers was that of designing artificial nano-RBC with polyethylene glycol–polycyclic acid copolymer membranes and haemoglobin and/or RBC enzymes content. The latter construct was demonstrated to have double circulation time as compared to previous approaches. [38]

Research towards synthesis of nano-sized RBC generated new experimental directions. A recent paper reports generation of a novel protein assay having RBC synthesis as fundament. The report describes a first technological step of RBC synthesis on nano-sized gold particles deposited on glassy carbon electrode surface followed by a second gold nanoparticles adsorption step and a final step of mammary cancer 15-3 antibody (anti-CA15-3) attraction on the surface. The immunodetection involving horseradish peroxidase- labeled CA 15-3 demonstrated high sensitivity and specificity in mammary cancer detection therefore opening new avenues towards detection of various other neoplasm locations.[40]

A more recent report proposes a novel synthetic RBC design by mixing polyaminic block copolymers into polyomersomes. The prototype has also been fluorescently labeled for near infrared tracking of biodistribution. Results show a good circulation time and tissue flow.[41]

VIII. CONCLUSION

Clinical applications of nanoparticles represent one of the most appealing target of nowadays research. Intensive toxicological experiments are under development and will be needed in the future. Due to its important role in blood and systemic homeostasis red blood cell should represent a milestone in any safety evaluation of all nanoparticles compounds. [42-44]

References


