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Review

Erythrocyte-based drug delivery in Transfusion Medicine: Wandering questions seeking answers

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ABSTRACT

Red blood cells (RBCs) represent the most commonly used and best-studied natural carriers in the history of drug delivery. Their abundance and long circulation half-life, their great immune-biocompatibility and biodegradability profiles, along with the availability of well established protocols for their safe collection, *ex vivo* processing and quality control make them advantageous as drug delivery systems (DDS). As a result, several drug-loading techniques (including encapsulation and surface conjugation) have been developed in order to construct RBC-based or RBC-inspired drug delivery vehicles for the effective treatment of infections, cancer, chronic and autoimmune diseases in both pre-clinical protocols and clinical trials. Despite the fact that the collected laboratory (*in vitro* and *in vivo*) and clinical data exhibit variable potential for translation into transfusion-associated prototypes and feasible protocols with significant clinical impact, little is known and done in the direction of drug delivery through RBC transfusion. Accordingly, several wandering questions for the application and utility of RBC-based drug delivery in transfusion medicine seek answers. By focusing on the most prominent of them, namely, "why not the stored/transfused RBCs", this review quotes some thoughtful considerations based on the current applications of RBCs as DDS, and on the potential application of RBC-based DDS in transfusion therapy.

Contents

1. Why red blood cells as DDS?

Several conventional drugs are characterized by poor stability and solubility, which undermine their therapeutic effectiveness, and risk of toxic adverse effects, including gastrointestinal reac-

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tions in the recipient [1]. The aim of drug delivery technology is the discovery of alternative systems that may be applied to overcome the limitations and toxicity of conventional administration and thus, to improve the pharmacological properties and therapeutic efficacy of the administrated drug, through better accessibility and specific targeting of cells and tissues [2]. Many different systems have been proposed over the last six decades, but only a few of them have found their way to clinical application.

Undoubtedly, the red blood cells (RBCs) represent one of the best-studied systems of natural carriers in the history of drug delivery; though RBC-based delivery systems have not yet overcome certain inherent problems that currently restrict their use mainly at the pre-clinical level. Actually, the physical association of drugs and nanocarriers with RBCs may change their pharmacokinetics, biodistribution, clearance and metabolism in undesirable ways, as extensively reported by other elegant reviews [3]. However, RBCs with features that perfectly match the necessary attributes for a candidate drug carrier or diagnostic tool remain advantageous over many other mammalian cells [4]. First, they are the most abundant circulating cells in the blood (about $4–5 \times 10^{13}$ cells), with a fairly long circulation life (almost 4 months) and the natural carriers of respiratory gases throughout the animal body for a lifetime. Moreover, their collection, *ex vivo* processing and quality control testing follow specific and well-established protocols [5]. In addition, their particular shape and geometry (high surface to volume ratio) along with their membrane structure and composition endow them with the remarkable deformability and durability needed to withstand the tremendous mechanical pressures prevailing in the capillaries [6,7]. Thus, the inherent ability of RBCs to preserve their own structural integrity may ideally serve the efficient delivery of cargos under the stresses of circulation.

Furthermore, the great immune-biocompatibility (especially in autologous administration schemes) and biodegradability of RBCs makes them suitable for drug carriers *per se* and their membrane a suitable coat for a wide variety of artificial nanocarriers [8]. By using a RBC camouflage, these small particles can evade the immune system and survive longer in the circulation. In the same way, in the case of drug encapsulation in whole RBCs, the presence of RBC-specific immunomodulatory markers "of self" [9], along with the lack of organelles and of highly concentrated toxic cytosolic products may provide extended life span in the internalized drugs, as well. At the same time, several well-characterized surface membrane proteins offer a variety of targeting sites for drug conjugation [10]. Finally, the well-known (although not complete) assessment of RBC elimination mechanisms *in vivo* allows their use for targeting the reticuloendothelial (RES) and complement systems [10].

The above mentioned answers to the query "why RBCs as DDS?" generate new and even more intriguing issues for consideration, including the probable usage of stored and transfused RBCs in drug delivery. This review focuses on well established applications of RBCs as drug delivery systems (DDS), on the potential use of RBC-based DDS in transfusion medicine and on the mutual benefits that this interplay might hold for both therapeutic strategies.

2. How the RBCs have been used in drug delivery?

In general, the DDS are classified into three broad categories: a) the artificial, b) natural, and c) hybrid systems. The RBC-based strategies belong to the second and third categories. Some of the strategies require encapsulation of the cargo (drug, nanoparticles *etc.*) into the cell, while others include conjugation of the cargo on the cell surface. In the most contemporary of them, parts of the erythrocyte (membrane, vesicles and surface markers) are used in combination with artificial nanocarriers. This section includes some of the most popular and "traditional" techniques that have been used in the field and a glimpse of the latest emerging RBC-inspired drug delivery strategies.

2.1. Encapsulation

Encapsulation of drugs into RBCs is the first type of RBC loading technique that was investigated 4–5 decades ago. It presupposes formation of transient pores on the RBC surface [11] through electrical pulsation, osmotic phenomena and chemical perturbation.

Hypotonic loading represents one of the oldest, and still most commonly used, strategies to produce modified RBCs. Despite having been applied for more than 30 years in drug delivery, this approach has evolved and is currently used in clinical trials for the treatment of neurological and oncologic disorders [3]. The process applied in the numerous DDS is the same: transient osmotic shock allows drug internalization first, and then membrane resealing occurs. The main hypotonic loading techniques that have been used were extensively reviewed by Ihler and Tsang [12]. Briefly, hypotonic dilution was the first and the simplest method tested for its efficacy in encapsulating drugs or chemicals in erythrocytes. RBC concentrates are diluted with an excess of aqueous drug solution and then hypertonic buffer is added in order to restore the tonicity. In hypotonic dialysis, a suspension of RBCs in isotonic buffer is incubated with large volumes of hypotonic buffer and then the tonicity is restored by addition of hypertonic buffer or by replacing the medium with an isotonic one. The drug can be encapsulated in the beginning of the procedure or after the incubation step. Hypotonic pre-swelling includes suspension of RBCs in a hypotonic solution, centrifugation and removal of the supernatant. The cellular sediment is then brought to lysis by addition of large volumes of aqueous drug solution and finally the tonicity is restored using a hypertonic buffer at 37 °C. The osmotic pulse is a continuous-flow method, in which the RBC suspension is pulsed through DMSO and then diluted with an isotonic drug solution. It has been used for the incorporation of inositol hexaphosphate, which binds to the 2,3-DPG site of hemoglobin, into RBCs to decrease their affinity for oxygen [13,14].

Chemical perturbations of the membrane and electroporation have been also used for encapsulating drugs into RBCs. In the first process, the membrane is chemically modified (and usually, irreversibly destructed) by polyenic antibiotics (*e.g.* amphotericin B), urea, ethylene, glycol, ammonium chloride or halothane, which increase its permeability [15]. Electroporation uses electric shock to achieve the same effect [16]. The main disadvantage of this method is that its use is limited to only enzymes, drugs and substrates which would not further and drastically alter the membrane permeability due to osmotic phenomena. Furthermore, the stability of the loaded cells is a key limitation for the long-term application of this technique at the clinical level.

The latest approaches in drug encapsulation include application of mechanical stress, fusion of RBCs with liposomes and treatment by cell penetrating peptides. Casagrande et al. recently developed a new mechanical stress-based method for drug encapsulation [17] that uses a construction composed of two reservoirs connected by a glass capillary. A compressor attached to each reservoir creates pressure to promote cell flow through the capillary. Evaluation of the pore diameter is achieved by measuring dextran uptake by the cells through flow cytometry. A risk for membrane integrity, which is expected to differ widely between RBC samples based to donor-related differences in the mechanical fragility of the membrane, is a handicap of this method. Another promising approach for drug encapsulation is the fusion of RBCs with drug-loaded liposomes. This method is reported to be superior to hypotonic dialysis in terms of loading efficiency, shape modifications, phosphatidylserine (PS) exposure and deformability [18]. Finally, a state of the art drug delivery technology involves membrane permeating peptides