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Red blood cells: Supercarriers for drugs, biologicals, and nanoparticles and inspiration for advanced delivery systems☆,☆☆

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ABSTRACT

Red blood cells (RBCs) constitute a unique drug delivery system as a biologic or hybrid carrier capable of greatly enhancing pharmacokinetics, altering pharmacodynamics (for example, by changing margination within the intravascular space), and modulating immune responses to appended cargoes. Strategies for RBC drug delivery systems include internal and surface loading, and the latter can be performed both *ex vivo* and *in vivo*. A relatively new avenue for RBC drug delivery is their application as a carrier for nanoparticles. Efforts are also being made to incorporate features of RBCs in nanocarriers to mimic their most useful aspects, such as long circulation and stealth features. RBCs have also recently been explored as carriers for the delivery of antigens for modulation of immune response. Therefore, RBC-based drug delivery systems represent supercarriers for a diverse array of biomedical interventions, and this is reflected by several industrial and academic efforts that are poised to enter the clinical realm.

Contents

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1. Introduction: artificial, natural, and hybrid drug delivery systems

Drug delivery systems (DDSs) can be divided into three types: artificial, natural (biological) and hybrids. Artificial DDSs, including nanocarriers made of synthetic or natural components, offer advantages of rational design, diversity, and control of materials. Biological DDSs including cells and their fragments offer biocompatibility and utilization of natural mechanisms for transport, localization, and responsiveness to factors of microenvironment in the body. Hybrid systems offer the theoretical possibility of combining these advantages while mitigating shortcomings.

This article reviews a class of DDSs that largely falls within the second and third categories, namely, erythrocytes or red blood cells (RBCs). RBCs represent natural transport agents that can be used to improve vascular and systemic delivery of drugs and probes, either encapsulated in the cell's inner volume, or coupled to the surface of RBCs. This review focuses on the second approach for loading drugs to RBCs and explores the role of RBCs in other drug delivery strategies, including: (i) their effect on other DDSs; (ii) DDSs using RBC as a "supercarrier"; (iii) artificial DDSs imitating features of RBCs; and (iv) hybrid DDSs that combine artificial elements and RBC components.

2. RBC as carriers in drug delivery: brief overview

RBCs naturally deliver "cargoes" including oxygen throughout the body via prolonged circulation in the vascular system. RBCs circulate in humans for about 3 months and in mice for about 40 days. RBCs lack organelles and have a biconcave shape; hence their entire inner volume and extended surface can be used for carriage of diverse compounds. From a practical standpoint, RBCs represent the most abundant, biocompatible, affordable, and easy to handle biological carrier for DDSs [1–5].

These natural carriers can be employed for drug delivery, for example, via loading drugs into donor or autologous RBCs prior to transfusion to a patient. For more than forty years, RBCs have been explored for delivery of many drugs in models ranging from *in vitro*, to small animal models and primates, as well as in clinical studies in human patients. This section provides a background pertaining to key aspects of drug delivery by RBCs. Detailed reviews of this subject can be found in this volume (see Magnani) and elsewhere [2,4–7].

2.1. Pharmacokinetics, biodistribution, and targets of RBC-loaded drugs

RBC carriage offers unique features advantageous for delivery of many agents for improved management of some pathological conditions. First, carriage by RBCs cardinally changes the pharmacokinetics (PK) of drugs by prolonging their circulation time in the bloodstream, facilitating redistribution in blood from plasma to cellular elements and redistribution in tissues. This change in PK is especially desirable for drugs that: (i) require a long-lasting depot in blood; (ii) have intravascular therapeutic targets accessible to RBCs; or (iii) are supposed to act in the extravascular compartments accessible to RBCs. In many cases, these changes to PK effectively enhance drug bioavailability, allowing reduced dosages and therefore alleviating adverse effects.

Carriage by RBCs shifts the pathway for drug clearance from renal filtration to bile excretion, typical of products of hemoglobin degradation. This switch in clearance mechanism results from: (i) RBC size, which, by many orders of magnitude, exceeds the limit of glomerular filtration; and (ii) macrophages in the reticuloendothelial system (RES), which have direct access to blood cells (particularly, in the hepatic sinuses and splenic follicles) and normally eliminate senescent and damaged RBCs. As such, these host defense cells represent natural targets for drug delivery using RBC carriage.

With the exception of these compartments of the RES, "normal areas" for RBCs in the body are limited to areas connected by the vascular system. However, RBCs often exit this space in pathological sites including: (i) the CNS (for example, in stroke or traumatic brain injury); (ii) penumbra of the myocardial infarction zone in immediate post-ischemic period; (iii) alveolar and airway compartments in acute lung injury, pulmonary hypertension, and other conditions which lead to damaged endothelium; and (iv) other numerous cases of pathological vascular permeability and vessel damage. In theory, RBCs may provide "passive" drug delivery to these sites.

In the absence of these pathological conditions, the list of main targets accessible to RBCs includes components of blood, endothelium, and a few constituents of the RES. RBC-coupled drugs do not act upon targets inaccessible to their carrier, which limits non-specific, systemic, and other undesirable off-target effects. Furthermore, coupling to RBCs impedes a drug's freedom to interact with some targets even within the vascular compartment. For example, fibrinolytic plasminogen activators bound to RBC surfaces can act upon soluble therapeutic targets in blood, but not with endothelial receptors, which circumvents a main side effect of free plasminogen activators [8–10].

RBCs are attractive carriers for drugs that: (i) metabolize or capture agents circulating in blood; (ii) regulate blood clotting and thrombi transformations; (iii) require delivery to the endothelium; (iv) replace deficient lysosomal enzymes; and (v) regulate immune system (antigens, anti-inflammatory agents, stimulators, and inhibitors of phagocytes).

2.2. Loading drugs in RBC inner volume and to RBC surface

Drugs and probes can either be encapsulated into the RBC inner volume or coupled to the RBC surface (Fig. 1). Drugs encapsulated in RBC may interact with molecules diffusing from blood through the RBC membrane, but not with molecules outside the RBC membrane including components of blood and vascular walls. Thus, encapsulation in RBCs may reduce immune reaction to biotherapeutic agents or protect from pathways of inactivation.

In most protocols, loading drugs into the carrier RBC inner volume necessitates *ex vivo* manipulation. Hypotonic loading is achieved by incubating washed RBCs or blood with the cargo at hypotonic solutions which induce the formation of transient pores in the plasma membrane of swelling cells. This approach is currently used for several RBC carriers in clinical trials (Table 1) for the treatment of oncologic, inflammatory, and neurological diseases and commercial development is underway (Table 2). Newer developments include attempts to use cell-penetrating peptides to import therapeutic proteins in the carrier RBCs [11] and fusion of RBCs with drug-loaded liposomes [12].

Surface loading to RBCs can be achieved *ex vivo* by incubating either intact or modified RBCs with drugs or drug carriers. In such an approach, the drug of interest may react chemically with the RBC surface to form a covalent linkage or be non-specifically adsorbed. Most recently, biotechnological methods to culture genetically engineered RBCs *ex vivo* that bear recognition sequences have allowed for site-specific, covalent coupling of therapeutic proteins by way of transpeptidases [13]. Alternatively, drugs and carriers can be conjugated or fused with antibodies, antibody fragments, peptides, or other ligands that bind to RBC's surface. In the latter approach, RBC-targeted drugs can be used in two main protocols. First, this can be employed for *ex vivo* surface loading on donor or autologous RBCs, to avoid multi-step and inevitable damaging loading by encapsulation. Second, a simple intravascular injection of these RBC-targeted agents leads to rapid binding to RBC circulating in the bloodstream [14].

2.3. RBC surface loading: effects on cargo

RBC's surface can be used for the coupling of drugs, their carriers, and targeting agents. The prototype for the latter approach was designed in the early 1980s for targeting RBC-encapsulated drugs to the sites of vascular injury [15–18]. Antibody-coated RBCs have been studied *in vitro* and *in vivo* for targeting drugs to diverse cells—endothelium, smooth muscle cells, leukocytes, etc. [19–21]. More recently, RBCs painted