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Engineering erythrocytes for the modulation of drugs' and contrasting agents' pharmacokinetics and biodistribution☆

Luigia Rossi [a,b], Francesca Pierigè [a], Antonella Antonelli [a], Noemi Bigini [a], Claudia Gabucci [a], Enrico Peiretti [c], Mauro Magnani [a,b]

[a] Department of Biomolecular Sciences, University of Urbino, Urbino, PU, Italy
[b] EryDel SpA, Urbino, PU, Italy
[c] Department of Surgical Sciences, University of Cagliari, Cagliari, CA, Italy

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ABSTRACT

Pharmacokinetics, biodistribution, and biological activity are key parameters that determine the success or failure of therapeutics. Many developments intended to improve their *in vivo* performance, aim at modulating concentration, biodistribution, and targeting to tissues, cells or subcellular compartments. Erythrocyte-based drug delivery systems are especially efficient in maintaining active drugs in circulation, in releasing them for several weeks or in targeting drugs to selected cells. Erythrocytes can also be easily processed to entrap the desired pharmaceutical ingredients before re-infusion into the same or matched donors. These carriers are totally biocompatible, have a large capacity and could accommodate traditional chemical entities (glucocorticoids, immunossuppresants, *etc.*), biologics (proteins) and/or contrasting agents (dyes, nanoparticles). Carrier erythrocytes have been evaluated in thousands of infusions in humans proving treatment safety and efficacy, hence gaining interest in the management of complex pathologies (particularly in chronic treatments and when side-effects become serious issues) and in new diagnostic approaches.

Contents

1. Introduction

In order to achieve a therapeutic success, the plasma concentration of the majority of drugs, including both small chemical entities and biologics (*i.e.* recombinant proteins, antibodies and their derivatives, and nucleic acid-based therapeutics), should be within the therapeutic window and should result in a proper tissue distribution. Mainly owing to this reason, our understanding should no longer be limited to the mechanisms governing drug absorption, distribution, metabolism and excretion, but should also consider the compartmentalization of these processes and the interaction of the active substance with its

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delivery system and with specific and non-specific targets. In fact, *in vivo* fate of a drug is determined by its physicochemical properties, by the interaction of the same with the selected drug delivery system and in addition by the physiological and/or pathological features of the body receiving the treatment.

Administration of several active substances by conventional formulations and dosage forms may lead to their rapid clearance resulting in short plasma half-life, which is frequently not effective and/or does not exhibit sufficient *in vivo* pharmacological activities.

Limited plasma half-life of most therapeutic agents is commonly due to their susceptibility to degradation by liver and kidney enzymes, fast renal clearance by glomerular filtration (connected to hydrophilic properties as well as small size of the drug), recognition and subsequent processing by the reticuloendothelial system (RES) and/or to their potential immunogenicity [1]. These problems commonly lead to poor bioavailability and reduced *in vivo* activity which further limits the clinical applications and the therapeutic index of several drugs [2,3]. Solutions to these problems cannot be found in either frequent administrations or high dose to achieve the required therapeutic efficacy, since these are associated with significant side effects, reduced quality of life and compliance, besides the inconvenient economic burden, which decreases the benefit to risk ratio [4]. Instead, in order to ensure safety and efficacy, drugs should preferentially be delivered selectively to their target site and at an optimal concentration. In fact, lack of selectivity in biodistribution sometimes leads to unwanted side effects, particularly for drugs that have severe cytotoxicity and/or induce drug resistance [5].

1.1. Intravascular contrasting agents

As well as for therapeutics, clinical success of *in vivo* diagnostics depends on various parameters: pharmacokinetic, short and long-term tolerability in the body, safety and functionality in the desired organ, cell targeting. The most common imaging modalities include Magnetic Resonance Imaging (MRI) [6], Computed Tomography (CT) [7], Single Photon Emission Computed Tomography (SPECT) [8], Positron Emission Tomography (PET) [9], Ultrasound (US) [10] and Optical Imaging (OI) (bioluminescence and fluorescence) [11], as well as combinations of the same. Contrast agents are used to enhance visibility of specific tissues by increasing the signal-to-noise ratio referred to surrounding tissues, and therefore to provide clear discrimination between normal and pathological regions in areas of interest [12,13]; however, the optimum performance of contrast agents has not yet been achieved since there is always a competition between their desired distributions in specific organs and their highly active clearance mechanisms [14]. In fact, the amount and distribution pattern in different organs and tissues, and the rate of recognition and removal from the body [15] should be considered important criteria that determine how long a contrast agent takes to reach the signal peak and the retention time of the signal after its intravenous injection [16].

Knowing these parameters is crucial to enhance the expected functionality and to mediate the fate of a contrast agent in the body [17].

1.2. The emergence of cell-based delivery systems

A possible and interesting solution to overcome restrictions related to the use of drugs and contrast agents lies in the exploitation of cell-mediated drug delivery systems (DDS) that is an emerging and promising strategy to address the above challenges. Numerous are the DDS based on circulating cells like recently described in detail by Su Y. et al. 2015 [18]. Among them, erythrocytes stand out to be the most appealing in order to improve the pharmacokinetics, biodistribution and pharmacodynamics of therapeutics or diagnostics.

Inherent biochemical and biophysical properties of red blood cells (RBCs or erythrocytes) make them an ideal drug delivery platform: RBC-based therapies have a significant advantage over alternative technologies in terms of half-life, stability, versatility, safety and ease of manufacture. Thanks to their remarkable long life-span in circulation they act as potential reservoirs for a slow, controlled and sustained release of valuable payloads, performing both as passive carriers (*e.g.* proteins or diagnostics) or as active bioreactors due to the enzymatic systems that their possess (*e.g.* prodrugs) [19]. Prolonged retention time in blood can provide a chance to extend the duration of pharmacological activity both to increase the therapeutic action in circulation or to lengthen the period of imaging analysis mediated by contrast agents [5].

On the contrary, by exploiting the natural fate of RBCs, it is possible to quicken the removal of drug-loaded RBCs from circulation with the aim of rapidly and selectively targeting drugs to macrophages, leading to a change in the pharmacokinetics of the molecule of interest. The use of drug-loaded RBCs was first reported in 1973 for enzyme replacement therapy of inborn errors of metabolism [20] and since then, significant progress has been made as shown by the abundant literature produced in the last decades on red blood cells as drug delivery system and the most important results have been collected in several and influential reviews [21–25]. This strategy is based on the possibility to transiently open pores on the RBC membrane that can be easily crossed by non-diffusible drugs that will remain confined within the RBCs once pores close.

Among the available loading procedures, hypotonic dialysis allows a high percentage of cell recovery (range 50–80% according to experimental conditions, [26]) which, in addition, show a normal glycolytic activity, ATP and 2,3-BPG content [27]. Moreover, the recovered RBCs show morphological features quite similar to those observed in native cells with the presence of some microcytic and hypochromic cells [28]; the observed decrease in MCH and MCHC values [26] might affect the total capability of these cells to transport oxygen that could be estimated to range from 75 to 85% the original capacity. However, this should not have an impact on blood functions since the amount of loaded cells usually re-infused in patients represents a very small fraction of the total RBCs in circulation and thus the reduction of function would not be clinically relevant. Moreover, regarding survival of the red blood cells processed by hypotonic dialysis, erythrocyte mean half-life has been reported to be 28 days, that is within the normal range of 19–29 days [29,30].

Loading procedures have been used *in vitro* and *in vivo* for the encapsulation of many substances (*e.g.* anti-inflammatory, antimicrobial agents, enzymes, polypeptides, antibodies, therapeutic proteins, antitumor drugs, oligonucleotides, contrasting agents, *etc.*) in order to modulate their pharmacokinetics. In this review, we focus our attention on a strategy to overcome the problems of actual therapeutics and diagnostics concerning *in vivo* half-life extension, modulation of bioavailability and biodistribution profile in order to maximize the therapeutic efficacy and to minimize the amount of administered drug as well as the frequency and cost of therapeutic interventions. An additional review in this same issue of ADDS by Vladimir Muzykantov will describe additional strategies able to address the payload to selected sites within the vascular system [31].

2. Therapeutics: main limitations to their use and RBC-based strategy to overcome them

2.1. Enzymes as therapeutic agents

Recombinant proteins hold increasing potential as therapeutic agents and are already successfully used as very efficient drugs in the treatment of many pathophysiological conditions. Namely, recombinant proteins are used to treat endocrine disorders, to combat various cancers, to alleviate autoimmune diseases, as active pharmaceutical ingredients in many vaccines [32] and as replacement therapy for the treatment of enzyme deficiency diseases or as a degradation system of toxic metabolites or compounds secondary to some kind of poisoning. The main drawbacks of biopharmaceutical proteins are their suboptimal