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Erythrocyte -Encapsulated (Red Blood Cell-Based) a Safe Methods for Delivery of Potent Drugs and Biologicals

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Abstract: Erythrocyte -encapsulated antibiotics have the potential to provide an effective therapy against intracellular pathogens. The advantages over the administration of free antibiotics include a lower systemic dose, decreased toxicity, a sustained delivery of the antibiotic at higher concentrations to the intracellular site of pathogen replication, and increased efficacy. In this study, the encapsulation of amikacin by human carrier erythrocytes prepared using a hypo-osmotic dialysis was investigated. The effects of the initial amikacin dialysis concentration and hypo-osmotic dialysis time on the encapsulation efficiency of amikacin were determined, and the osmotic fragility and hematologic parameters of amikacin- loaded carrier erythrocytes were measured. The efficiency of amikacin entrapment by carrier erythrocytes was dependent on the initial dialysis concentration of the drug. Statistically significant differences in the osmotic fragility profiles between control and carrier erythrocytes were observed, which were dependent on the hypo-osmotic dialysis time and on the dialysis concentration of amikacin. Mean hematologic parameters were evaluated and compared with unloaded, native erythrocytes; the mean corpuscular volume (MCV) of amikacin- loaded carrier erythrocytes was statistically significant smaller. Amikacin demonstrated a sustained release from loaded erythrocytes over a 48-h period, which suggests a potential use of the erythrocyte as a slow systemic-release system for antibiotics.
Keywords: Erythrocyte -encapsulated, carrier erythrocytes, mean corpuscular volume, hypo-osmotic

Introduction

Erythrocytes have been used as carriers for drugs, enzymes, and other macromolecules.[1], [2] and [3] Because of the increasing attention being devoted to the development of novel delivery systems, the erythrocyte as a delivery vehicle is a research field that has attracted a considerable amount of interest.[4], [5], [6] and [7]

Background

Erythrocytes have been widely used as carrier systems of different drugs, which induce changes in the pharmacokinetics and achieve a selective distribution to the reticulo-endothelial system (RES). Within the anti-infective agents field, this fact is specially advantageous, because some RES cells could act as a reservoir for facultative intracellular pathogens.

Translational Significance: Previous studies with animals showed important modifications in the kinetics of amikacin when it is incorporated in erythrocytes. Results of *in vitro* studies with human cells showed similar changes to those observed in rat, which suggests a potential similar sustained release effect when human amikacin carrier erythrocytes are injected. The advantages of these cellular carriers in comparison with other traditional carriers are their biocompatibility and lack of toxicity; their potential to transport selectively the drug to the monocyte–macrophage system [previously known as the reticulo-endothelial system (RES)] of the liver, spleen, and bone marrow; and their ability to behave as circulating bioreactors in a sustained fashion.[8], [9], [10], [11], [12], [13] and [14]

Several methods have been employed to encapsulate drugs and other molecules, such as enzymes and

peptides, within erythrocytes. The method of encapsulation and conditions employed has been shown to affect the *in vitro* and *in vivo* properties of the erythrocyte carrier. The hypo-osmotic dialysis procedure has shown to be preferable in terms of preserving the biochemical and physiologic characteristics of the erythrocyte. [3], [10], [11], [12], [15], [16], [17] and [18]

Within the field of antibiotics, apart from modifying the pharmacokinetic behaviour of the encapsulated drug, the carrier erythrocyte has the potential to target antibiotics specifically to the mononuclear phagocytic system, which can act as a reservoir for facultative intracellular pathogens,[19] such as *Mycobacterium*.[20] Pathogens located in the intracellular compartment, or phagosome, typically receive a lower concentration of antibiotic than the concentration which is administered systemically, and therefore for optimal efficacy relatively high concentrations are required, which often lead to adverse side effects.

Among different pathogens that reside within the monocyte–macrophage system, HIV must be emphasized because of the important consequences of the AIDS latency. Different antiviral agents have been encapsulated within carrier erythrocytes, which achieved optimistic results for the targeting of drugs to macrophages.[21], [22] and [23]

In previous studies, we optimized the conditions for the encapsulation of the aminoglycoside antibiotic amikacin in rat erythrocytes using a hypo-osmotic dialysis procedure.[24] We have demonstrated that rat loaded -carrier erythrocytes have hematologic and morphologic properties similar to the unloaded native erythrocytes, and that the loaded erythrocytes demonstrated a moderate change in osmotic fragility and exhibited a sustained *in vitro* release. Additional *in vivo* studies with rats revealed changes in the pharmacokinetics of the amikacin when it is administered within carrier erythrocytes. A greater accumulation of the antibiotic is observed in RES organs in comparison with the administration of free drug, which demonstrated the potential use of this system in the treatment of intracellular infections.[25]

Here, we report studies on the encapsulation of amikacin by human carrier erythrocytes; the encapsulation efficiency of amikacin using different hypo-osmotic dialysis times and different initial concentrations of antibiotic were investigated. The hematologic parameters and osmotic fragility of the loaded carrier erythrocytes were evaluated, and the *in vitro* release of amikacin from carrier erythrocytes was measured over time.

Conclusion

The results summarized in this review show some of the numerous potential biomedical applications of RBCs based drug delivery systems opening new perspectives to the possibility of using our cells for therapeutic purposes. Among these RBCs feature some unique advantages compared to other delivery systems making them not only natural, safe and abundant carriers but, being endowed with enzymes involved in bioconversion reactions, also active bioreactors. Recently, the role of erythrocytes as drug carriers have been documented in several reviews [9], [109], [110], [111], [112] and [113]. Herein we report, initially, some information regarding the preparation of drug-loaded RBCs and successively, numerous examples of RBCs as drug carriers, prevalently obtained through experiences in our laboratory. In particular, RBCs as carriers of nucleotide analogues, proteins, glucocorticoid analogues and antisense peptide nucleic acids are reviewed. Altogether, from the analysis of the data, the following conclusions can be drawn: RBCs are safe carriers that persist in circulation for months (unless specifically modified) and could release in circulation active pharmacological agents for an equivalent period. This delivery system permits to have in the blood a low and constant drug concentration that once specifically selected could be therapeutically useful without side effects.

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