REENGINEERING ERYTHROCYTES A NOVEL DRUG DELIVERY SYSTEM

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Abstract

Drug delivery systems including chemical, physical and biological agents which will enhance the bioavailability, improve pharmacokinetics and reduce toxicities of the drugs. Carrier erythrocytes are one among the foremost promising biological drug delivery systems investigated in recent decades. Resealed erythrocytes are biodegradable biocompatible, possess long circulation half-life and can be loaded with sort of active drug substances. Resealed erythrocyte possesses several advantages over the other drug delivery system which makes it superior than other systems. Carrier erythrocytes are prepared by collecting blood sample from the organism of interest and isolate erythrocytes from plasma. By using various methods, the cells are broken and the drug is entrapped into the erythrocytes, finally they are resealed and therefore the resultant carriers are then called “resealed erythrocytes”. Resealed erythrocytes, as a drug delivery system has excellent capacity to reinforce the therapeutic index and patient compliance. It has got tremendous potential to achieve site specific drug delivery with minimum wastage of drugs and it also prolong the discharge of drug. So many drugs like aspirin, steroid, antineoplastic which having many side effects are reduce by resealed erythrocyte. The present review signifies various features, drug loading methods, evaluation, applications and clinical progress of resealed erythrocytes.

Introduction

Present pharmaceutical scenario is focused on advancement of drug delivery systems which maximize the drug targeting along with high therapeutic benefits for safe and effective management of diseases. To accomplish a necessary therapeutic concentration the drug has to be administered in large quantities, the major part of which is just wasted in normal tissues. Ideally, a “perfect” drug should exert its pharmacological activity only at the target site, using the lowest concentration possible and without negative effects on non-target compartments Various carriers has been used for the drug targeting among which cellular carrier offer a more prominent and potential advantages related to its biodegradability, non-pathogenicity, non-immunogenicity, biocompatibility, self degradability along with high drug loading efficiency. Erythrocytes, the most abundant cells in the human body, have potential carrier capabilities for the delivery of drugs. Erythrocytes are biocompatible, biodegradable, possess very long course half lives and can be loaded with a variety of chemically and biologically active compounds using various chemical and physical methods.

Erythrocytes

A healthy adult male and female has about 5.4 million RBC per L of blood and 4.8 million RBC per µL of blood respectively. Erythrocytes are biconcave discs with a diameter of 7-8 µm. They contain the O₂ carrying protein Hb, which is a pigment that gives whole blood red colour. Erythrocytes are highly specialized for their O₂-CO₂ transport function.1

The erythrocytes have flexible, elastic, biconcave and nucleated structure with mean diameter of 7.3µm and thickness of 2.2µm. The chemical constituents include water (63%), Haemoglobin (33.67%), methemoglobin (0.5%), glucose (0.8%), minerals (0.7%), non- haemoglobin protein (0.9%) and lipids (0.5%). The main aim of
these RBC’s is to transport gases for respiratory processes. The production rate of RBC is 2.5 million per second and life span of 100-120 days. [2]

Figure 1 : Erythrocytes [4]

Resealed Erythrocytes

Such drug-loaded carrier erythrocytes are prepared mainly by gathering blood samples from the organism of interest, separating erythrocytes from plasma, entrapping drug in the erythrocytes, and resealing the resultant cellular carriers. Consequently, these carriers are called resealed erythrocytes. [3]

Figure 2: Resealed Erythrocytes [5]

Advantages of Resealed Erythrocytes

- Biocompatibility.
- Biodegradability.
- Incorporation of wide variety of bioactive agents.
- Circulation throughout the circulatory system.
- Encapsulation of large amount of drug in the small volume of cells.
- Targeted specificity within reticuloendothelial system (RES).
- Protection against the premature degradation, inactivation and excretion of protein and enzymes.
- Prolonged systemic activity by long residence time in the body [6]

Disadvantages of Resealed Erythrocytes

- Possibility of leakage of cell and dose dumping.
- Some molecule alter physiology of cell.[7]

Isolation of Erythrocytes

- The blood is collected into heparinised tube by venipuncture.
- Blood is withdrawn from cardiac / splenic puncture (in small animal) and through veins (in large animals) in a syringe containing a drop of anti coagulant.
- The whole blood is centrifuged at 2500 rpm for 5 mins at 4±1°C in a refrigerated centrifuge.
The serums and Buffy coats are carefully removed and packed cell wash three times with phosphate buffer saline (\( \text{pH}=7.4 \)).

The washed erythrocytes are diluted with PBS and stored at 4°Celsius until used. \[8\]

**Table 1:** Various conditions and centrifugal force used for isolation of red blood cells.

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Species</th>
<th>Washing Buffer</th>
<th>Centrifugal Force (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Rabbit</td>
<td>10mmol KH(_2)PO(_4)/NaHPO(_4)</td>
<td>500-1000</td>
</tr>
<tr>
<td>2.</td>
<td>Dog</td>
<td>15mmol KH(_2)PO(_4)/NaHPO(_4)</td>
<td>500-1000</td>
</tr>
<tr>
<td>3.</td>
<td>Human</td>
<td>154mmol NaCl</td>
<td>&lt;500</td>
</tr>
<tr>
<td>4.</td>
<td>Mouse</td>
<td>10mmol KH(_2)PO(_4)/NaHPO(_4)</td>
<td>100-500</td>
</tr>
<tr>
<td>5.</td>
<td>Cow</td>
<td>10-15mmol KH(_2)PO(_4)/NaHPO(_4)</td>
<td>1000</td>
</tr>
<tr>
<td>6.</td>
<td>Horse</td>
<td>2mmol MgCl(_2), 10mmol glucose</td>
<td>1000</td>
</tr>
<tr>
<td>7.</td>
<td>Ship</td>
<td>10mmol KH(_2)PO(_4)/NaHPO(_4)</td>
<td>500-1000</td>
</tr>
<tr>
<td>8.</td>
<td>Pig</td>
<td>10mmol KH(_2)PO(_4)/NaHPO(_4)</td>
<td>500-1000</td>
</tr>
</tbody>
</table>

**Drug Entrapment Method**

- Chemical perturbation of the membrane
- Hypotonic hemolysis method
- Hypotonic dilution method
- Hypotonic preswelling
- Hypotonic dialysis
- Isotonic osmotic lysis
- Electro-insertion or electro encapsulation
- Entrapment by endocytosis
Various Evaluation Parameters and Their Determination Methods for Resealed Erythrocytes:

Table 2: Physical Evaluation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method /Instrument Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape and surface morphology</td>
<td>Transmission electron microscopy, scanning electron microscopy</td>
</tr>
<tr>
<td>Vesicle size and size distribution</td>
<td>Transmission electron microscopy, optical microscopy</td>
</tr>
<tr>
<td></td>
<td>Diffusion cell, dialysis</td>
</tr>
<tr>
<td>Drug release</td>
<td>Deproteinization of cell membrane followed</td>
</tr>
</tbody>
</table>

Table 3: Cellular Characterization

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method /Instrument Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmotic fragility</td>
<td>Stepwise incubation with isotonic to hypotonic saline solutions and determination of drug and hemoglobin assay</td>
</tr>
<tr>
<td>Osmotic shock</td>
<td>Dilution with distilled water and estimation of drug and hemoglobin</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate</td>
<td>ESR methods</td>
</tr>
<tr>
<td>% Cell recovery</td>
<td>Neubaur’s chamber, hematologicalanalyzer</td>
</tr>
<tr>
<td>% Hb content</td>
<td>Deproteinization of cell membrane followed by haemoglobin assay</td>
</tr>
</tbody>
</table>

Table 4: Biological Characterization

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method /Instrument Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterility</td>
<td>Sterility test</td>
</tr>
<tr>
<td>Pyrogenicity</td>
<td>Rabbit method, LAL test</td>
</tr>
<tr>
<td>Animal toxicity</td>
<td>Toxicity tests</td>
</tr>
</tbody>
</table>

Application of Resealed Erythrocytes

For Drug Targeting:
Resealed erythrocytes can be used for site-directed and target oriented drug delivery of loaded drugs.

- To The RES Organs
Resealed erythrocytes have been proposed for passive targeting to MPS/RES system where modified surface characteristics improved their selectivity and specificity towards target cells. The various approaches to modify the surface characteristics of erythrocytes include,
  - Surface modification with antibodies
  - Surface modification with glutaraldehyde
  - Surface modification with carbohydrates such as sialic acid
  - Surface modification with sulphydryl
  - Surface chemical cross-linking e.g. delivery of 125I-labeled carbonic anhydrase loaded in erythrocytes cross-linked with bisulfosuccinimidylsulberate and 3,3-dithiosulfosuccinimidyl propionate.
To the Liver
Enzyme Deficiency/Replacement Therapy
Enzymes can be infused into blood stream to supplant the missing or deficient enzymes in metabolic disorders. Exogenous enzymtherapy is complicated by the short half-life of enzymes in blood. Drug Loaded Erythrocytesstream, intolerance and occasionally toxicity against normaltissues. A strategy to eliminate or minimize the problems of immunologicalnature and toxicity, enzymes loaded erythrocytes havebeen employed .The enzymes used include β-glycosidase, β-glucoronidase, β-galactosidase .Use of glucocerebrosidesencapsulated erythrocytes in the disease caused by accumulation of the glucocerebrosides in the liver and spleen.

Treatment of Hepatic Tumors
Antineoplastic agents encapsulated in erythrocytes can be used for targeting to hepatic carcinoma. Various agents like bleomycin, methotrexate, andriamycin and asparagines have been successfully delivered by erythrocytes.

Removal of RES Iron Overload
RES cells are the primary and the major sites for iron accumulation has been entrapped in erythrocytes, for promising excretion of iron overload in the RES organs.

As Circulatory Bioreactors
Erythrocytes act as carriers for enzymes to serve as circulatory bioreactors. This immobilization of enzymes which decreases the level of circulating metabolite can be used as bioreactors.

Delivery of Antiviral Agents
Antiviral agents are entrapped in resealed erythrocytes for effective delivery and targeting. Resealed erythrocytes have been used to deliver deoxyxycytidine derivatives, recombinant herpes simplex virus Type 1 (HSV-1) glycoprotein B, azidothymidine derivatives, fludarabine phosphate and azathioprene and acyclovir.

Thrombotic Therapy
Anti-thrombotic agents loaded into the resealed erythrocytes have been proved effective as thrombolytic therapy. Several compounds have been reported for loading into the resealed erythrocytes includes aspirin and brinase and heparin.

In Oxygen Deficiency Therapy
An application of Inositol hexaphosphate-loaded erythrocytes for improved oxygen delivery is beneficial under the following conditions:
- High altitude conditions where the partial pressure of oxygen is low.
- Reduction in the number of alveoli, where interchange surface of the lungs is decreased.
- Increased resistance to oxygen diffusion in the lungs.
- Reduction in oxygen transport capacity.
- Mutation or chemical modification, which involves a decrease in oxygen affinity for haemoglobin.
- Increased radio sensitivity of radiation-sensitive tumours.
- Restoration of oxygen-delivery capacity of stored blood.
- Ischemia of myocardium, brain, or other tissues.

As Drug/Enzyme Carriers
Erythrocytes can be used as a carrier for delivery of bioactive compounds as circulatory depots. They can also be used as carriers for targeting drugs to liver, spleen and lymph nodes.

Erythrocytes as Carriers for Proteins and Macromolecules
Erythrocytes have been used for delivery of various proteins and macromolecules which includes insulin, recombinant human erythropoietin (rHuEpo), and mycotoxin and recombinant interleukin-2 (rIL-2).

Microinjection of macromolecules
Biological functions of macromolecules such as DNA, RNA, and proteins are used for various cell biological applications. Hence, various methods are used to entrap these macromolecules into cultured cells (e.g., microinjection). A relatively simple structure and a need of complex cellular components (e.g., nucleus) in erythrocytes make them good candidates for the entrapment of macromolecules. In microinjection, erythrocytes are used as micro syringes for injection to the host cells. The microinjection process involves culturing host eukaryotic cells in vitro. The cells are coated with fusogenic agent and then suspended with erythrocytes loaded with the compound of interest in an isotonic medium. Sendai virus (hemagglutinating virus of Japan, HVJ) or its glycoproteins or polyethylene glycol have been used as fusogenic agents. The fusogen causes fusion of cosuspended erythrocytes and eukaryotic cells. Thus, the contents of resealed erythrocytes and the compound of interest are transferred to host cell. This procedure has been used to micoroinject DNA fragments arginase, proteins, nucleic acids, ferritin, latex particles, bovine and human serum albumin, and enzyme thyminidinikase to various eukaryotic cells.
Clinical Progress

The clinical developments reported in this section have received approval by international regulatory agencies; many have also received the designation of ‘orphan drug’, and the most advanced are based on solid preclinical and/ or phase II/III clinical investigations.

Table 5 Therapeutic goals and drugs considered in this article related to the use of drug-loaded red blood cells as therapeutic agents[22]

<table>
<thead>
<tr>
<th>Conditions Treated</th>
<th>Drug</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia telangiectasia</td>
<td>Dexamethasone 21-phosphate</td>
<td>EryDel Italy &amp; USA</td>
</tr>
<tr>
<td></td>
<td></td>
<td><a href="http://www.erydel.com">http://www.erydel.com</a></td>
</tr>
<tr>
<td>Mitochondrial NeurogastrointestinalEncephalomyopathy (MNGIE)</td>
<td>Thymidine phosphorylase (TP)</td>
<td>St George’s, University of London UK</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The Clinical Trial Company UK</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orphan Technologies Ltd CH</td>
</tr>
<tr>
<td>Phenylketonuria</td>
<td>RTX-134</td>
<td>Rubius USA</td>
</tr>
<tr>
<td></td>
<td></td>
<td><a href="http://www.rubiustx.com">http://www.rubiustx.com</a></td>
</tr>
<tr>
<td>Acute lymphoblastic leukaemia/pancreatic cancer</td>
<td>Asparagines</td>
<td>ERYtech Pharma France &amp; USA</td>
</tr>
<tr>
<td></td>
<td></td>
<td><a href="http://www.erytech.com">http://www.erytech.com</a></td>
</tr>
</tbody>
</table>

Conclusion

The use of the erythrocyte as drug carrier is a fundamental advancement in the current exploitation of blood and may be applicable in medical fields for which no effective therapy is currently available. Some companies are leading the clinical applications with products currently in phase III trials and robust pipelines. The commercial medical applications of carrier erythrocytes are currently being tested by a newly formed company that is developing products for human use. The coming years represent a critical time in this field as commercial applications are explored. In near future, erythrocytes based delivery system with their ability to deliver controlled and site specific drug delivery will revolutionize disease management.

References

4. [https://images.app.goo.gl/8BckCm4K88dUg5w57](https://images.app.goo.gl/8BckCm4K88dUg5w57)
6. [https://images.app.goo.gl/5B6KYG9adpxKemXS7](https://images.app.goo.gl/5B6KYG9adpxKemXS7)