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Review Article

Resealed Erythrocytes as a Potential Drug Carrier: A Review

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ABSTRACT

Drug delivery and drug targeting systems developed by various researchers are beleaguered to deliver the drug safely to the target sites without much interaction with other tissues of the body, so as to increase its bioavailability and reduce the adverse effects of that drug. In this approach, many drug carriers have been developed which are being widely used these days for increasing the efficacy and safety of the pharmaceutical products. Among the cellular carriers, the erythrocytes are the most potentiated drug carriers with very capitalizing properties like precise site specificity and sustained drug release. The circulation of erythrocytes into every corner of the human body is one such another advantage, thus making them useful for drugs which cannot be taken to their target site by any other carrier. Resealed erythrocytes are prepared by isolating erythrocytes from the plasma of the blood sample of an organism of interest, breakdown of cells using different methods and then entrapping the drug (which is to be administered into the body) into the erythrocytes by various techniques such as hypotonic haemolysis, hypotonic dilution, hypotonic pre-swelling method, dialysis method, isotonic osmocytosis, chemical perturbation of the membrane, electro-insertion method, entrapment by endocytosis, lipid fusion method etc., and then finally they are sealed back again, thus, accounting for their name – "Resealed Erythrocytes".

Keywords: Resealed Erythrocytes, Drug Carrier, Erythrocytes, reticulo-endothelial system

INTRODUCTION

A drug is administered into the body with a motive to get the required actions of that drug on a specific target. But we all are familiar with the fact that apart from its actual target, the drug also acts on some non-target sites, resulting in adverse drug reactions – which we call side effects (of that drug) in common terminologies. The various drug delivery systems available today aim at the target of making the drug exhibits its pharmacological action only on the specific required target site. These drug delivery systems include – monoclonal antibodies, soluble synthetic polymers, polysaccharides, particulate biodegradable polymers, microcapsules, microparticles, nanoparticles, lipoproteins, liposomes and cellular carriers such as – erythrocytes, leukocytes, platelets, hepatocytes and fibroblasts.

Erythrocytes (or the red blood cells) are probably the most common cells found in the human body. After long research, we found their intense application as a drug carrier in the drug delivery system. The resealed erythrocytes are biodegradable, biocompatible, non-immunogenic, non-pathogenic, self-degradable, reproducible, easy to prepare, possess prolonged circulation half-life and can be used to incorporate a wide variety of active drugs. All such properties make them a revolutionary drug carrier which can efficiently be used to increase the therapeutic effect of the drug as well as to prevent any possible toxic effects [1-4].

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Advantages of Resealed Erythrocytes

· This system of drug delivery can be used in situations where the drug needs to be delivered into the phagocytic cells

· The drugs which are meant to show their action in the vascular lumen are delivered by this drug carrier system [5].

· They make the inclusion of proteins and nucleic acids possible in eukaryotic cells.

· No chemical modifications of the drug are required for their entrapment them into erythrocytes.

· They help in prolonging the therapeutic pharmacological action of the drug due to their longer life span (of 120 days) than other carriers.

· Accomplishment of a stable concentration in plasma with possibly the drug release regulated by zero order kinetics.

· They have the capability to entrap a wide variety of drugs in them (due to their inert intracellular environment) and a large amount of drug can be incorporated in these small sized cells (due to their shape).

· They are biocompatible, biodegradable, non-immunogenic and non-pathogenic, thus are very non-toxic and safe for use [6-7].

Disadvantages of Resealed Erythrocytes

· The most serious problem encountered in the use of resealed erythrocytes is their degradation in vivo by the reticulo-endothelial system.

· They are not much useful to carry the drug to a non-phagocytic target.

· There are chances of clumping of the cells due to coagulation or other reasons.

· Possibility of dose dumping or leakage might be presented as their major drawback

· Several biochemicals present in the body may alter their physiology at different points of time [8-10].

Isolation of Erythrocytes

The erythrocytes of various mammals such as mice, cattle, pigs, dogs, sheep, goats, monkeys, chicken, rats and rabbits have been used till now for drug delivery purposes. In order to isolate erythrocytes from the blood, sample is collected in heparinized tubes, by cardiac/splenic puncture (in case of small animals) and venepuncture (in case of large animals), containing a drop of anticoagulant. Fresh blood gives higher encapsulation efficiency than older blood, therefore former is used more to get the best results. Fresh blood collected is immediately chilled to 4^{o} C and is stored for nearly about two days. After this, the erythrocytes are harvested and washed by the method of centrifugation (at an rpm of 2500 for 5 minutes in a refrigerated centrifuge). The washed cells are then subjected to buffer solutions (commonly phosphate buffer saline, PBS, is used having a pH of 7.4) to prepare their suspensions at various values of haematocrit that are desired. Then these suspensions are stored in acid-citrate-dextrose buffer solution at 4^{o} C for minimum 2 days before its use [13, 26].

METHODS OF DRUG LOADING IN ERYTHROCYTES

Hypotonic haemolysis: - The principle of this method is that erythrocytes readily undergo reversible swelling in a hypotonic solution. In this method, an increase in volume of the cell (due to osmosis that occurs because of being kept in a hypotonic solution), an initial change in the shape of the erythrocytes is observed from its original biconcave shape to spherical. This change in shape is accounted to the non-existence of a superfluous membrane in the erythrocytes, due to which their surface area is fixed. The increase in the volume of the cell is approximately 25-50%. These cells can withstand a tonicity upto 150 m osm/kg without losing their integrity. Above this value, the membrane ruptures and the cellular contents leak out. After this lysis, there is depletion in the cellular content and the remains left behind are known as erythrocyte ghost [11-12].

Hypotonic Dilution: - In this method, a fixed volume of packed erythrocytes is diluted with

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about 2-20 volumes of aqueous solution of the drug which is to be loaded into it. The tonicity of the solution is then re-established by the addition of a hypertonic buffer solution. The mixture/solution thus formed is then subjected to centrifugation. The supernatant obtained in the process is rejected and discarded while the pellet is washed with an isotonic buffer solution. However, the major drawbacks of this method are:

i. Low efficiency of entrapment

ii. Substantial loss of hemoglobin and other cellular components of erythrocytes, thus reducing its half life

Since these cells are voluntarily phagocytosed by the reticulo-endothelial systems' macrophages, they are used to target the organs which come under this system. This method is used to load enzymes such as B-galactosidase, B-glucosidase, asparginase, arginase and some bronchodilators such as salbutamol [4, 13-16].

Hypotonic pre-swelling: -This method is based on the initial controlled swelling of erythrocyte when it is kept in a hypotonic buffered solution. The mixture obtained by this is subjected to centrifugation at low values of rpm. The supernatant obtained after centrifugation is rejected and discarded while the cell fraction of the pellet is brought near a lysis point by means of addition of and aqueous solution of the drug (which is needed to be loaded into the erythrocyte). The mixture is centrifuged again between the steps of drug-addition. The lysis point of the cells is detected by the vanishing of the distinctive layer of boundary between the cell fraction the supernatant when subjected to centrifugation. Finally, the tonicity of the cell mixture is re-established at the last point by adding a specific measured amount of hypertonic buffer solution [6, 17].

Dialysis: -This method is particularly used to load enzymes and lipids. In this process, an isotonic buffered suspension of erythrocytes (with a hematocrit value of 70-80) is prepared and placed in a conventional dialysis tube immersed in 10-20 volumes of a hypotonic solution. Agitation is provided to it for 2 hours. The tonicity of the dialysis tube is re-established by the addition of a measured quantity of hypertonic buffer to the surrounding medium. The drug which is to be loaded into the erythrocytes can be added either at the beginning or at the end of agitation into the dialysis bag. This method has a high entrapping efficiency and is used to load enzymes such as B-galactosidase, glucoserebrosidase, asparginase, inositol, hexaphosphatase and drugs such as gentamicin, adriamycin, pentamidine and furamycin, inteleukin-2 and human recombinant erythropoietin [18-19].

Hypotonic osmocytosis: - This method is also known as "osmotic pulse method". In this method, isotonic hemolysis of the erythrocytes is done by physical or chemical methods. If the erythrocytes are subjected to incubation in a solution of a drug having high membrane permeability, an influx of drug into the erythrocytes would occur due to the concentration gradient followed by influx of water in order to main the osmotic pressure. This method is commonly used with chemicals such as urea solution, polyethylene glycol and ammonium chloride [20].

Chemical Pertubation of the membrane: - This method is based on the principle that specific chemicals when come in contact with the erythrocytes result in an increase in their membrane permeability. Such chemicals reported include – polyene antibiotic such as amphotericin B. This method is used to entrap antineoplastic drugs such as daunomycin. However, this method exposes the erythrocytes to certain irreversible and undesirable changes and hence is not commonly used [21-22].

Electro-insertion: -This method is also known as electro-encapsulation or electroporation. This method is based on the fact that electric

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current renders erythrocytes with irreversible changes in its membrane. The membrane of the erythrocyte breaks down due to the electric shock and thus becomes open and permeable. They are then suspended into a solution containing the drug which is to be incorporated into the erythrocyte. After this, the pores are resealed by subjecting them to incubation in an isotonic solution at 37^{o} C [23].

Entrapment by endocytosis: -The method of endocytosis entails the addition of one volume of erythrocytes to nine volumes of buffer solution containing 2.5 mM ATP, 2.5 mM $MgCl_2$ and 1 mM $CaCl_2$, followed by incubation for 2 minutes at room temperature. The pores created on the surface of the erythrocytes by this method are resealed by using 154 mM of NaCl followed by incubation for 2 minutes at a temperature of 37^{o} C. The entrapment of the drug to be loaded into erythrocytes occurs via the process of endocytosis. A vesicle is formed inside the erythrocytes after the process of endocytosis and the membrane of that vesicle separates the drug material from the cytoplasm of the erythrocyte [24].

Lipid fusion method: - A drug contained in a lipid vesicle can directly be fused to the erythrocyte. This principle is used in this method. After the fusion, exchange of drug takes place between the lipid vesicle and the erythrocyte. This method is used to entrap inositol monophosphate which results in a significant lowering in the oxygen carrying capacity of the hemoglobin present in cells. However, the efficacy of the drug entrapment is extremely low [25].

Mechanism of Drug Release From Resealed Erythrocytes

There are mainly three ways for a drug, loaded into the erythrocytes, to efflux out of them: -

- Phagocytosis
- Diffusion through the membrane of the cell
- Using a specific transport system [27-29].

APPLICATIONS OF RESEALED ERYTHROCYTES

In-Vitro Application: - For this purpose, use of phagocytosis cells was made to assist the uptake of enzymes by phagolysosomes. Enzyme content within carrier erythrocyte may be screened by using cytochemical technique. The deficiency of an enzyme glucose-6-phosphate dehydrogenase is one such biochemical defect which might prove vital in discriminating the mechanism which causes these effects. The concept of “microinjection” is the most pivotal in-vitro application of resealed erythrocytes:

- A protein or nucleic acid can be injected into eukaryotic cell by fusion process
- Antibody molecules can be injected into erythrocytic carrier systems which diffuse immediately into the cytoplasm.

However, the antibodies introduced into the erythrocytes are not recorded to enter into their nucleus. Thus, this restricts the further studies to the level of cytoplasm only.

In-Vivo Application: The various in-vivo applications of resealed erythrocytes are given as follows: -

Targeting of bioactive agents to RES (reticulo-endothelial system): - Damaged erythrocytes are rapidly cleared from the blood circulation by phagocytic Kupffer cells in liver and spleen. Resealed erythrocytes, by modifying their membranes, can therefore be used to target the liver and spleen. The various approaches to modify the surface characteristics of erythrocytes include surface modification with antibodies, gluteraldehyde, carbohydrates such as sialic acid and sulphydryl.

Targeting of sited other than RES organs: - Resealed erythrocytes have the ability to deliver a drug or enzyme to the macrophage-rich organs. Organ targeting other than RES have been performed by certain researchers.

Resealed Erythrocytes as circulating bioreactors: - Erythrocytes have been realized as carriers for enzymes to serve as circulating bioreactors. Sometimes it is desirable to

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decrease the level of circulating metabolites that can enter the erythrocytes. They have also been used as circulating bioreactors for the controlled delivery of antiviral drugs.

Resealed Erythrocytes as carriers for drugs: - Various bioactive agents encapsulated in erythrocytes are developed for the slow and sustained release in circulation to allow effective treatment of parasitic diseases. Resealed erythrocytes serve as an ideal carrier for antineoplastic agents, antimicrobial drug and vitamins and steroids.

Resealed Erythrocytes as carriers for enzymes: - Enzymes can be injected into the blood stream to replace a missing or deficient enzyme in metabolic disorders or to degrade toxic compounds accumulated in the blood due to a disease. Similarly, environmental, lysosomal storage disorders such as Gaucher's disease, hyperarginaemia, hyperuricaemia, hyperphenyl-alaninaemia and kidney failure are some of the examples of metabolic disorders whose treatment can be established by the administration of enzymes carried by resealed erythrocytes [30-31].

CONCLUSION

Insights of the review reveal that resealed erythrocytes have emerged as drug carriers which serve the characteristic of precise site specificity and sustained drug release. They offer vantage from other carriers having prolonged life, easily attainment of stable concentration etc. Thus, the use of resealed erythrocytes seems to be promising drug carriers with their utmost importance in treatment of many morbid conditions. However, this domain of drug delivery needs much further exploration so that the potential of resealed erythrocytes in future can be evaluated successfully.

↓ REFERENCES

1. Hamidi M. and Tajerzadeh H. Carrier erythrocytes: An overview, Drug Deliv., 2003; 10: 9-20.
2.Rossi L., Serafini S., Pierige F., Antonelli A., Cerasi A., Fraternale A., Chiarantini L. and Magnani M. Eryrthrocyte-based drug delivery Expert Opin. Drug Deliv, 2005; 2: 311-322.
3. Eichler H.G., Ramies H., Bauer K., Korn A., Bacher S. and Gasic S. Survival of gentamicin loaded carrier erythrocytes in healthy human volunteers Eur. J. Clin. Invest, 1986; 16(1): 39-42.
4. Adriaenssens K., Karcher D., Lowenthal A. and Terheggen H.G. Use of enzyme-based loaded erythrocytes in in-vitro correction of arginase-deficient erythrocytes in familial hyperargininemia, Clinical Chemistry, 1976; 22(3): 323-6.
5. Muzykantov V.R. Drug delivery by red blood cells: vascular carriers designed by Mother Nature, Expert Opin. Drug Deliv, 2010; 7(4):403-427.
6. Lewis D.A. and Alpar H.O. Therapeutic possibilities of drugs encapsulated in erythrocytes, Int. J. Pharm., 1984; 22: 137-146.
7. Zimmerman U. Cellular Drug-Carrier Systems and their possible targeting in targeted drugs, E.P. Goldberg (ed.), New York: John Wiley & Sons, 1983; pp. 153-200.
8. Mehrdad H., Adbolhossein Z., Mahshid F. and Soliman M.S. Applications of carrier erythrocytes in delivery of biopharmaceuticals, Journal of Controlled Release, 2007; 118(2): 145-160.
9. Alpar H.O. and Lewis D.A. Therapeutic efficacy of asparaginase encapsulated in intact erythrocytes , Biochem. Pharmacol, 1985; 34: 257-261.
10. Erchler H.G. Gasic S., Bauer K, Korn A. and Bacher S. In vivo clearance of antibody-sensitized human drug carrier erythrocytes , Clin. Pharmacol. Ther, 1986; 40: 300-303.
11. Ihler G.M. and Tsong H.C.W. Hypotonic haemolysis methods for entrapping of agents in resealed erythrocytes , Methods Enzymology, 1987; 149: 221-229.

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PHARMACY INFOPEDIA
ISSN: 2347-7881

12. Deloach J.R., Harris R.L. and Ihler G.M. An erythrocyte encapsulator-dialyzer used in preparing large quantities of erythrocyte ghosts and encapsulation of a pesticide in erythrocyte ghosts , Anal. Biochem, 1980; 102: 220-227.
13. Jaitely V., Kanaujia P., Venkatesan N., Jain S. and Vyas S.P. Resealed erythrocytes: drug potentials and biomedical applications , Indian Drugs, 1996; 33: 589-594.
14. Pitt E., Johnson C.M., Lewis D.A., Jenner D.A. and Offord R.E. Encapsulation of drugs in intact erythrocytes: an intravenous delivery system, Biochem. Pharmacol, 1983; 22: 3359-3368.
15. Deloach J.R. and Ihler G.M. A dialysis procedure for loading of erythrocytes with enzymes and lipids, Biochim. Biophys. Acta, 1977; 496: 136-145.
16. Talwar N. and Jain N.K. Erythrocytes as carriers of metronidazole: in-vitro characterization , Drug Dev. Ind. Pharm., 1992; 18: 1799-1812.
17.Jenner D.J., Lewis D.A., Pitt E. and Offord R.A. The effect of the travenous administration of corticosteroids encapsulated in intact erythrocytes on adjuvant arthritis in the rat, Brit. J. Pharmacol., 1981; 73: 212-213.
18. Dale G.L., Villacorte D.G. and Beutler E. High yield entrapment of protein into erythrocytes, Biochem. Med., 1977; 18: 220-225.
19. Klibansky C., PhD, thesis, Hebrew University, Jerusalem, Israel (1959).
20.Zanella A., Rossi F., Sabbioneda L., Russo V., Brovelli A., De Cal F., Fargion S., Fiorelli G. and Sirchia G. Desferrioxamine loading of red cells for transfusion, Adv. Biosci., 1987; 67: 17-27.
21. Deuticke B., Kim M. and Zolinev C. The influence of Amphotericin-B on the permeability of mammalian erythrocytes to non-electrolytes, aniona and cations , Biochim. Biophys. Acta, 1973; 318: 345-359.
22. Kitao T., Hattori K. and Takeshita M. Agglutination of leukemic cells and daunomycin entrapped erythrocytes with lectin in vitro and in vivo, Experimentia, 1978; 341: 94-95.
23. Kinoshita K. and Tsong T.Y. Hemolysis of human erythrocytes by a transient electric field, Proc. Natl. Acad. Sci.,1977; 74: 1923-1927.
24. Schrier S.L., Junga I. and Johnson M. Energized endocytosis in human erythrocyte ghosts, J. Clin. Invest, 1975; 56(1): 8-22.
25. Nicolau C and Gersonde K. Incorporation of inositol hexaphosphate into intact red blood cells I: Fusion of effector-containing lipid vesicles with erythrocytes, Naturwissenschaften, 1979; 66(11): 563-566.
26. Zimmermann U. JahresberichtderKernforschungsanlageJulichGmcH, Nuclear Research Center, Julich, 1973, pp. 55-58.
27. Jain S., Jain N.K. and Dixit V.K. Erythrocytes based delivery of isoniazid: preparation and in vitro characterization, Indian Drugs, 1995; 32: 471-476.
28. Hamidi M., Tajerzadeh H., Dehpour A.R., Rouini M.R. and Ejtemaee-Mehr S. In vitrocharacterization of human intact erythrocytes loaded by enalaprilat ,Drug Delivery, 2001; 8: 231-237.
29. Updike S.J. and Wakamiya R.T. Infusion of red blood cell-loaded asparaginase in monkey , J. Lab. Clin. Med., 1983; 101: 679-691.
30. Flynn G., McHale L. and McHale A.P. Methotrexate-loaded photosensitized erythrocytes: A photo-activatable carrier/delivery system for use in cancer therapy, Cancer Lett., 1994; 82(2): 225-229.
31.Chiarantini L., Rossi L., Fraternale A. and Magnani M. Modulated red blood cell survival by membrane protein clustering, Mol. Cell Biochem., 1995; 144(1): 53-59.