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REVIEW ARTICLE

NOVEL DRUG DELIVERY SYSTEM

NOVEL DRUG DELIVERY CARRIER: RESEALED ERYTHROCYTES

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ABSTRACT

Nowadays, there are 30 and more main drug delivery products in the market. The total annual income for all of these is approximately US$43 billion with an annual growth of 15 % (based on global product revenue). The reasons for this increasing interest in drug delivery are due to the increasing need of safe drugs, capable of reaching the target and with minimal side effects. In fact the main problems associated with systemic drug administration are essentially related to the bio-distribution of pharmaceuticals throughout the body.

Resealed Erythrocytes are biocompatible, biodegradable, possess long circulation half-life and can be loaded with variety of active substances. Carrier erythrocytes are prepared by collecting blood sample from the organism of interest and separating erythrocytes from plasma. By using various methods the cells are broken and the drug is entrapped into the erythrocytes, finally they are resealed and the resultant carriers are then called "resealed erythrocytes". So many drugs like aspirin, steroid, cancer drug which having many side effects are reduce by resealed erythrocyte.

KEYWORD

Resealed Erythrocyte, Steroid , Cancer, Carrier , Biodegradability.

INTRODUCTION

To achieve a required 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. The delivery systems currently available enlist carriers that are either simple, soluble macromolecules (such as monoclonal antibodies, soluble synthetic polymers, polysaccharides and particulate biodegradable polymers) or more complex multicomponent structures (microcapsules, Microparticles, cells, cell ghosts, lipoproteins, liposomes, erythrocytes).[1]

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 circulation half lives and can be loaded with a variety of chemically and biologically active compounds using various chemical and physical methods.[2]

erythro = red and cytes = cell

erythrocyte is red cell. Erythrocyte is biconcave discs , anucleate Filled with hemoglobin (Hb) ,a protein that functions in gas transport. It contains the plasma protein spectrin.

Healthy adult male=4.5millions/µml

Healthy adult female=4.8million/ µml

Immature RBC are called "RETICULOCYTES."[3]

Properties of resealed erythrocyte of novel drug delivery carriers:

1) The drug should be released at target site in a controlled manner.
2) It should be appropriate size , shape and should permit the passage through capillaries. and Minimum leakage of drug should take place.
3) It should be biocompatible and should have minimum toxic effect.
4) It should possess the ability to carry a broad spectrum of drug.
5) It should possess specific physico-chemical properties by which desired target size could be recognized.
6) The degradation product of the carriers system , after release of the drug at the selected site should be biocompatible. It should be physico -chemically compatible with drug.
7) The carrier system should have an appreciable stability during storage.[4-9]

Advantage:

1) They are natural part of body , so they are biodegradable in nature.
2) The entrapment of drug does not require the chemical modification of drugs
3) The entrapment of drug also does not require the chemical modification of the substance to be entrapped.
4) They are non immunogenic in action and can be targeted to disease tissue/organ..
5) They prolong the systemic activity of drug.
6) Isolation of erythrocyte is easy and larger amount of drug can be encapsulated in small volume of cells
7) They can target the drug within reticulo-endothelial system.
8) They facilitate incorporation of protein and nucleic acid in eukaryotic cells by cell infusion with RBC.[10-14]

Disadvantage:-

1) They have a limited potential as carrier to non-phagocyte target tissue.
2) Possibility of clumping of cells and dose dumping may be there.[15-16]

Requirement for encapsulation:-

- Variety of biologically active substance (5000-60,000dalton)can be entrapped in erythrocytes.
- Non-polar molecule may be entrapped in erythrocytes in salts. Example : tetracycline HCl salt can be appreciably entrapped in bovine RBC
- Generally, molecule should be polar. and Non polar molecule should also been entrapped.
- Hydrophobic molecules can be entrapped in erythrocyte by absorbing over other molecules.
- Once encapsulated charged molecule are retained longer than uncharged molecule
- The size of molecule entrapped is a significant factor when the molecule is smaller than sucrose and larger than B-galactosidase.
- Two kind of polar in the dialyzed erythrocyte are exists , one set of pares exists at all times in the dialyzed cell and another set of pares appears and disappears constantly. the pare opening size is limited to 400-500A^{o}.[17-22]

Isolation of erythrocytes:-

The cellular content is about 40-50% of the blood volume and contains erythrocytes(red blood cells, RBC),Leukocytes(while blood cells, WBC) and thrombocytes (platelets).the primarily water (90 to 92%) and protein(7%).Blood is withdrawn from cardiac/splenic puncture(in case of small animal) and through veins (in case large animals) into a syringe containing drop of anticoagulant .The whole blood is centrifuged at 2500 rpm for 5min at 4± 4^{o}C in a refrigerated centrifuge. the serum and Buffy coats are carefully removed and packed cells washed 3 times with phosphate buffer saline(PBS pH 7.4).The washed Erythrocyte are diluted with PBS and stored at 4^{o}C until used.[23]

ENTRAPMENT METHOD

(1) Hypo-osmotic lysis method

Hypotonic lysis of cells in a solution containing the drug/enzyme to be entrapped followed by restoration of tonicity in reseal them. the ghost population so obtained are heterogeneous .three types of ghosts can be distinguished :type I ghosts which reseal immediately after haemolysis , type II ghosts which reseal after reversal of haemolysis by addition of alkali ions and type III ghosts which remain leaky under different experimental conditions.

Table 1

Various condition and centrifugal force used for isolation of erythrocytes

Sr.no.	Spcies	Washing Buffer	Centrifugal force(g)
1	Rabbit	10mmol KH_2PO_4/$NaHPO_4$	500-1000
2	Dog	15mmol KH_2PO_4/$NaHPO_4$	500-1000
3	Human	154mmol NaCl	<500
4	Mouse	10mmol KH_2PO_4/$NaHPO_4$	100-500
5	Cow	10-15mmol KH_2PO_4/$NaHPO_4$	1000
6	Horse	2mmol $MgCl_2$,10mmol glucose	1000
7	Sheep	10mmol KH_2PO_4/$NaHPO_4$,	500-1000
8	Pig	10mmol KH_2PO_4/$NaHPO_4$	500-1000

Erythrocyte have special capability that reversible shape change with or without accompanying volume change and for reversible deformation under distension (stress). they don't have internal membrane and no capacity to synthesize additional plasma membranes, the surface area is inevitably fixed,so Increase in volume initially leads to conversion of normal bioconcave , discocyte(normal erythrocyte) to spherocytes. Thus the cells becomes spheres as they accommodate additional volume with a fixed surface area. However , these swollen erythrocytes have little capacity to resist volume greater than 50-75% of the initial volume and when placed in solution less than about 150mOsm/Kg ,the membrane rupture , permitting escape of the cellular component.

These ruptured membranes can be resealed raising the salt concentration to its isotonic levels and upon incubation , the resealed erythrocytes assemble their normal bioconcave shape and recover impermeability to both macromolecules and ions . erythrocyte are resealed on addition of sufficient1.54 M KCl, which restores isotonicity. in experiments, where preservation of energy metabolism within the cells is desirable, 4mM magnesium salts , 10 Mm glucose and 2mM adenosine are included during resealing to attain as per above final concentrations.[24-28]

Loading by "red cell loader"

Magnani and coworkers, 1998 developed a novel method for the entrapment of non diffusible drugs into human erythrocytes. The equipment designed for this method was termed as "red cell loader" .

The method requires as little as 50ml of blood. By using a new apparatus, it is possible to entrap a variety of biological compound into erythrocytes in as little time as 2 hours at room temperature under blood banking condition ; the method is based on two sequential and controlled hypotonic dilutions of washed red blood cells followed by concentration with a haemofilter . Subsequent isotonic resealing of erythrocytes allow a 35-50% cell recovery and approximate 30% entrapment of added drug.[29-34]

(A) Hypotonic dilution

Hypotonic dilution was the first method investigated for the encapsulation of chemicals into erythrocytes and is the simplest and fastest . In this method, a volume of packed erythrocytes is diluted with 2–20 volumes of aqueous solution of a drug. The solution tonicity is then restored by adding a hypertonic buffer. The resultant mixture is then centrifuged, the supernatant is discarded, and the pellet is washed with isotonic buffer solution . The major drawbacks of this method include a low entrapment efficiency and a considerable loss of hemoglobin and other cell components. This reduces the circulation half life of the loaded cells. These cells are readily phagocytosed by RES macrophages and hence can be used for targeting RES organs. Hypotonic dilution is used for loading enzymes such as B-galactosidase and B-glucosidase , asparginase ,and arginase , as well as bronchodilators such as salbutamol.[35-39]

(B) Preswell dilutional haemolysis

. This Method was developed by Rechsteiner in 1975 and was modified by Jenner et al. for drug loading. The technique is based upon initial controlled swelling in a hypotonic buffered solution. This mixture is centrifuged at low g values. The supernatant is discarded and the cell fraction is brought to the lysis point by adding 100–120 ml portions of an aqueous solution of the drug to be encapsulated. The mixture is centrifuged between the drug-addition steps. The lysis point is detected by the disappearance of a distinct boundary between the cell fraction and the supernatant upon centrifugation. The tonicity of a cell

mixture is restored at the lysis point by adding a calculated amount of hypertonic buffer. Then, the cell suspension is incubated at 37°C to reanneal the resealed erythrocytes . Such cells have a circulation half life comparable to that of normal cells . This method is simpler and faster than other methods, causing minimum damage to cells.
Drugs encapsulated in erythrocytes using this method include propranolol , asparginase , cyclopohphamide, cortisol-21-phosphate ,w1-antitrypsin, methotrexate, insulin , metronidazole , levothyroxine , enalaprilate and isoniazide .[40-46]

(C) Hypotonic dialysis

This method was first reported by Klibansky Dale for loading enzymes and lipids. Several methods are based on the principle that semipermeable dialysis membrane maximizes the intracellular and extracellular volume ratio for macromolecules during lysis and resealing. In the 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 buffer. The medium is agitated slowly for 2 h. The tonicity of the dialysis tube is restored by directly adding a calculated amount of a hypertonic buffer to the surrounding medium or by replacing the surrounding medium by isotonic buffer . The drug to be loaded can be added by either dissolving the drug in isotonic cell suspending buffer inside a dialysis bag at the beginning of the experiment or by adding the drug to a dialysis bag after the stirring is complete .[47-49]
The use of standard hemodialysis equipment for loading a drug in erythrocytes was reported by Roper.In this method, the erythrocyte suspension and the drug to be loaded was placed in the blood compartment and the hypotonic buffer was placed in a receptor compartment. This led to the concept of “continuous flow dialysis,” which has been used by several other researchers . The loaded cells exhibit the same circulation half life as that of normal cells . Also, this method has high entrapment efficiency on the order of 30–50% ,cell recovery of 70–80%, high-loading capacity , and is amenable to automation with control of process variables . The drawbacks include a long processing time and the need for special equipment . This method has been used for loading enzymes such as B - galactosidase, glucoserebrosidase , asparginase , inositol, hexaphosphatase , as well as drugs such as gentamicin, adriamycin , pentamidine and furamycin , interlukin-2 , and human recombinant erythropoietin.[50-53]

(D) Isotonic osmotic lysis

This method, also known as the osmotic pulse method, involves isotonic hemolysis that is achieved by physical or chemical means. The isotonic solutions may or may not be isotonic. If erythrocytes are incubated in solutions of a substance with high membrane permeability, the solute will diffuse into the cells because of the concentration gradient. This process is followed by an influx of water to maintain osmotic equilibrium. Chemicals such as urea solution , polyethylene glycol , and ammonium chloride have been used for isotonic hemolysis. However, this method also is not immune to changes in membrane structure composition. In 1987, Franco et al. developed a method that involved suspending erythrocytes in an isotonic solution of dimethyl sulfoxide (DMSO) . The suspension was diluted with an isotonic-buffered drug solution. After the cells were separated, they were resealed at 37°C.[54-58]

(2) Chemical perturbation of the membrane

This method is based on the increase in membrane permeability of erythrocytes when the cells are exposed to certain chemicals. In 1973, Deuticke et al.

showed that the permeability of erythrocytic membrane increases upon exposure to polyene antibiotic such as amphotericin B . In 1980, this method was used successfully by Kitao and Hattori to entrap the antineoplastic drug daunomycin in human and mouse erythrocytes . Lin used halothane for the same purpose. However, these methods induce irreversible destructive changes in the cell membrane and hence are not very popular.[59-61]

(3) Electro-insertion or electroencapsulation

In 1973, Zimmermann tried an electrical pulse method to encapsulate bioactive molecules. Also known as electroporation, the method is based on the observation that electrical shock brings about irreversible changes in an erythrocyte membrane. In 1977, Tsong and Kinosita suggested the use of transient electrolysis to generate desirable membrane permeability for drug loading . The erythrocyte membrane is opened by a dielectric breakdown. Subsequently, the pores can be resealed by incubation at 37^{o}C in an isotonic medium.[62-63]

Characterization of resealed erythrocytes:-[64-69]

1. ***Drug content determination***

Packed loaded cells are deprotenized with acetonitrile after centrifugation at 3000 rpm far a fixed time interval. The clear supernatant liquid is assayed far drug content.

2. ***In-vitro drug release and Hb content***

The cell suspensions (5% hematocrit in PBS) are stored at 4^{o} C in ambered colored glass container.

Periodically clear supernatant are drawn using a hypodermic syringe equipped with 0.45 are filter, deproteined using methanol and were estimated far drug content.

The supernatant of each sample after centrifugation collected and assayed, % Hb release may be calculated using formula

% Hb release=A_{540} of sample-A_{540} of background
A540 of 100% Hb

3. ***Percentage cell recovery :***

May be determined by counting the no. of intact cells per cubic mm of packed erythrocytes before and after loading the drug.

4. ***Morphology :***

Phase contrast or electron microscope may be used far normal and erythrocytes.

5. ***Osmotic shock :***

Far 0.5 study, erythrocyte suspension (1 ml, 10%) were diluted with h20 (5 ml) & centrifuge at 3000 rpm far 15 minute. The supernatant was estimated far % Hb release spectrophotometrically.

6. ***Turbulence shock :***

It is the measure of simulating distribution of loaded cells during injection. In this drug loaded cells are passed through a 23 guage hypodermic at a flow rate of 10 ml/min which is comparable to the flow rate of blood. It is followed by collecting of an aliquot and centrifugation sample is estimated. Drug loaded erythrocytes appears to be less resistant to turbulence, probably indicating destruction of cells upon shaking.

7. ***Determination of entrapped magnetite :***

Atomic Absorption spectroscopic method is reported for determination of the concentration of a particular metal element in a sample.

The HCl is added to a fixed amount of magnetite bearing erythrocytes and content are heated at 60^{o}C for 2hours.then 20%w/v trichloro acetic acid is added and supernatant obtained after centrifugation is used to determine magnetite concentration using atomic absorption spectroscopy.

8. ***Erythrocyte sedimentation rate(ESR):***

It is an estimate of the suspension stability of RBC in plasma and is related to the number and size of the red cells and to relative concentration of plasma protein , especially fibrinogen and α ,β globulins.

This test is performed by determining the rate of sedimentation of

blood cells in a standard tube. normal blood ESR is 0 to 15 mm/hr. higher rate is indication of active but obscure disease processes.

9. Miscellaneous :-

Resealed erythrocyte can also be characterized by cell sizes ,mean cell volume ,energy metabolism ,lipid composition ,membrane fluidity , rheological properties ,density gradient separation

Route of administration :-

Intra peritoneal injection reported that survival of cells in circulation was equivalent to the cells administered by i.v. injection .They reported that 25% of resealed cell remained in circulation for 14 days they also proposed this method of injection as a method for extra vascular targeting of RBCs to peritoneal macrophages.Sub cuteneous route for slow release of entrapped agents .they reported that the loaded cell released encapsulated molecules at the injection site.[70-72]

Application:

(1) In-Vitro Application :

Carrier RBCs have proved to be useful for a variety of in vitro tests. For in vitro phagocytosis cells have been used to facilitate the uptake of enzymes by phagolysosomes. Enzymes content within carrier RBC could be visualized with the help of cytochemical technique. The biochemical defects such as the glucose- 6-phosphate dehydrogenase (G6PD) deficiency can be useful tool for discerning the mechanism that eventually causes these effects.

The most frequent in vitro application of RBC is that of micro-injection. A protein or nucleic acid was injected into eukaryotic cells by fusion process. Similarly, when antibody molecules are introduced using erythrocytic carrier system, they immediately diffuse throughout the cytoplasm. Antibody RBC auto-injected into living cells has been used to confirm the site of action of fragment of diphtheria toxin. Antibodies introduced using RBC mediated microinjection is recorded not to enter the nucleus, thus limiting the studies to the cytoplasmic level. Other in vitro tests include utilization of erythrocytes carrier to introduce ribosome inactivating proteins into cells by fusion technique.[72]

(2) In – Vivo Application :

i) Targeting of bioactive agents to RE System

Damaged erythrocytes are rapidly cleared from 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.

ii) Targeting to sites other than RES-rich Organs

Resealed erythrocytes have the ability to deliver a drug or enzyme to the macrophage-rich organs. Organ targeting other than RES have been tried recently with resealed erythrocytes. Some of the representative approaches are discussed in brief.

iii) Erythrocytes as Circulating Bioreactors

Erythrocytes have been realized as carriers for enzymes to serve as circulating bioreactors. Sometimes it is desirable to decrease the level of circulating metabolites that can enter erythrocytes. Erythrocytes have also been used as circulating bioreactors for the controlled delivery of antiviral drugs.

Delivery of Antiviral Agents:-

Several reports are available in the literature for the antiviral agents encapsulated in the resealed erythrocytes for effective delivery and targeting.

iv) 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.

v) 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 likewise, environmental, lysosomal storage disorders such as Gaucher's disease, hyperarginiaemia, hyperuricaemia, hyperphenyl- alaninaemia and kidney failure are only few examples of metabolic disorders that can be treated by administration of enzymes.[73-76]

Table 2
various application of resealed erythrocyte: [77-78]

Application	Drug/enzyme/macromolecule	References
Enzyme deficiency replacement therapy	β-galactosidase, β-fructo-furonodase, urease, glucose 6-phosphatedehydrogenase	Ihler et al 1973,1975
Thrombolytic activity	Brinase,aspirin,heparin	Eichler et al 1986
Iron overload	Desferroxamine	Green , 1985
Chemotherapy	Rubomycin , methotrexate , daunomycin , cytosine etc	Kitao and Hattori , 1980
Immunotherapy	Human recombinant interleukin-2	DeLoach et al 1991
Circulating bioreactor	Arginase,uriease,luciferase	Magnani et al 1992

Recent developments:-

Nanoerythrosomes:

Nanoerythrosomes are vesicles prepared by the extrusion of RBC ghosts , the average diameter of these vesicles being 100nm. The process gave small vesicles with the size of a liposomes . these spheroid particles were named 'nanoerythrosomes' and appear to be stable and maintain both the cytotoxic and antineoplastic activity of daunorubicin against mice leukemia P338D cells.(Jain and Jain,1996d ; Lejeune et al.,1994).[7]

Other:

Significant advances have been made with the use of erythrocyte for specific targeting to cells of the immune system.antiviral drugs can be pretreated to deliver drug directly to macrophages(Bischi et al , 1991).

Several laboratory techniques have developed for the encapsulation of allosteric effector of hemoglobin , inisitol hexaphosphate , which are effective at oxygen delivery, much more effective than normal erythrocytes. (Ropars et al ., 1992 ; woodson et al; 1987).[80]

CONCLUSION

During the past decade, numerous applications have been proposed for the use of resealed erythrocytes as carrier for drugs, enzyme replacement therapy etc.

Until other carrier systems come of age, resealed erythrocytes technology will

remain an active arena for the further research. The commercial medical applications of carrier erythrocytes are currently being tested in Europe by a recently formed company that is developing products for human use (Deloach and Way, 1994). 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 provide controlled and site specific drug delivery will revolutionize disease management.

The International Society for the use of Resealed Erythrocytes (ISURE) through its biannual meetings provides an excellent forum for exchange of information to the scientist in this exiting and rewarding field of research. For the present, it is concluded that erythrocyte carriers are "golden eggs in novel drug delivery systems" considering their tremendous potential.

Dissuction & Suggestions for future study:

In the near future, several therapeutic application will likely , occur in humans, using red blood cells as carriers for drug.some scientists have demonstrated that such engineered red blood cells are suitable for blood transfusion. Initial studies using even various cross linking agents are promosing and there is need for further studies using even lower concentration of cross linkers along with the encapsulation of a diffusible drug to fully evaluate the system. Further studies would concentrate on manipulation of the autologus properties of erythrocytes , improved understanding biology of the red cells and its membrane , development of pulsatile and feed back contol system, selective drug delivery to CNS and delivery of peptide and protein drugs.

Main suggestion for future study is that by carrier through we can transplant steroids and hormones to the targeting site. So we can decrease many side effect. In these field no one can deep think but by resealed erythrocyte we can improvise drug targeting area \ and reduces so many side effect. [81]

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