Review Article

Resealed Erythrocytes As Drug Carriers -An Over View

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INTRODUCTION

The desirable properties of the carriers used in the drug targeting are to protect the drug from premature bioinactivation and direct the drug to the target site. The property desired increases the dose effectives of drug by reducing the dosage and frequency of administration.

This desirable property is achieved by using biological carriers (antibodies, liposomes, macromolecules, erythrocytes). Biological carriers can be used to achieve sustained and controlled systemic drug delivery. Such carriers can also be introduced directly in to the blood stream unlike synthetic carriers which have to be administered orally or parenterally.

Amongst all the different types of carriers' erythrocytes due to their unique nature and property are used in providing an ideal drug delivery system.

Erythrocytes as drug carriers

The developing RBC has the capacity to synthesize haemoglobin. The adult RBC however loses their capacity and serves only to carry haemoglobin. The use of cells as drug delivery system requires that the drug which are normally unable to permeate the membrane, should be made to traverse the membrane without causing any irreversible changes in membrane structure and permeability. Further the cells should be able to release the drug in controlled manner upon reaching the desired target.

RBCs have solid content of about 35% (rest 65% being water). Apart from this the erythrocytes have phosphate content which is in organic nature. The osmotic pressure of the interior of erythrocytes is equal to that of plasma and termed as **isotonic.** (equal to the osmotic pressure of 0.9% NaCl). If medium is **hypotonic** water diffuses into the cells and they get swelled and eventually loose their hemoglobin content and may burst. If medium is **hypertonic** (osmotic pressure more than 0.9% NaCl) they will shrink and become irregular in shape.

Some of the hemoglobin is lost and other cellular constituents are retained in the cells. On resealing they lose some of properties of the normal erythrocytes and referred as **"resealed erythrocytes".** Such erythrocytes which contain no or little haemoglobin are called **ghosts.** 3 types of ghosts can be distinguished: type-1 ghosts which reseal immediately after haemolysis; type-2 ghosts which reseal after reversal of haemolysis by addition of alkali ions; type-3 ghosts which remain leaky under different experimental conditions. RBC's are biocompatible provided that compatible cells are used in patients; there is no possibility of triggered immunological response.

Since resealed erythrocytes are being considered as novel carriers, it would be logical to review the **properties of the novel drug carriers**, which are

- It should be of appropriate size and shape to permit the passage through the capillaries.
- It should possess specific physico – chemical properties by which a desired target site could be recognized.
- Should be biocompatible and have minimum toxic side effects.
- Degradation products should be biocompatible.
- Minimum leaching\ leakage of drugs should takes place before target is reached.
- Possess ability to carry a broad spectrum of drugs with different properties.
- Physico- chemically compatible with drugs.
- The carrier system should have an appreciable stability during storage.

Advantages of resealed erythrocytes

- They are the natural products of the body which are biodegradable in nature.
- Isolation of erythrocytes is easy.

- Entrapment of drugs does not require the chemical modification of the substance to be entrapped.
- Non – immunogenic in action and can be targeted to diseased tissue or organ.
- Prolongs the systemic activity of drugs while residing for a longer time in the body.
- They can target the drugs with in RES.
- They facilitate incorporation of protein and nucleic acids in eukaryotic calls by infusion with RBC.

Limitations

- They have a limited potential as a carries to non phagocytic target tissue.
- Possibility of clumping of cells and dose dumping may be there.

Isolation of RBC

- Blood is withdrawn from cardiac or spleenic puncture (in case of small animals) and through veins in larger animals in to syringe containing a drop of anti coagulant (EDTA or heparin can be used as anticoagulant).
- The whole blood is centrifuged at 2500 RPM for 5 min. at 4 ± 1^{O} C using a refrigerated centrifuge.
- The serum and Buffy coats are carefully removed and packed cells are washed three times with phosphate buffer saline (PBS) ph 7.4.
- The washed erythrocytes are diluted with PBS and stored at 4^{o}C until used.
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Haematocrit Value and Erythrocyte Sedimentation Rate

The Haematocrit is the % volume occupied by the cells and is determined by simple centrifugation of blood. When blood in presence of some anticoagulant is centrifuged the cells settle down to the bottom of tube, while plasma rises up to the top. Normally the cell constitute about 45% (male) and 41% (female) of total volume. This is referred as normal **Haematocrit Value or Volume of packed red cells (VPRC)**

The erythrocytes are also characterized by **erythrocyte sedimentation rate (ESR).** When blood is mixed with an anticoagulant and kept for sometime, the erythrocytes form aggregates and settle down under the force of gravity alone, the rate at which sedimentation occurs is known as **erythrocyte sedimentation rate.**

Novel approach to target using RBC

For targeting only the erythrocytes membrane is used. This is obtained by splitting the cell in hypotonic solution and after introducing the drug into the cells allowing them to reseal into spheres. Such erythrocytes do not remain in the circulation for a long time as they are quickly sequestered and rapidly phagocytosed by RE cells in the liver and spleen. Such RBC'S can be used to target drugs to liver and spleen only, i. e. to those tissues which contain phagocytic cells and not other tissues in the body.

Resealed erythrocytes which modified surface characteristics by

- Heat treatment
- Glutaraldehyde treatment
- Sulfhydral reacting agents
- Antibodies

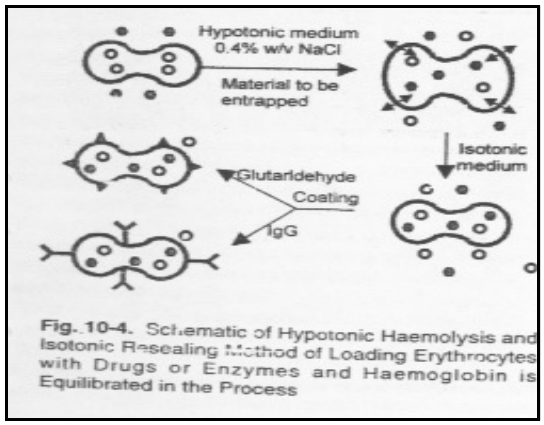
These are quickly removed from circulation by phagocytic cells located in liver and spleen. Since the damaged cells (RBC'S) are phagocytosed such modifications may be very helpful in targeting drugs to RES.

- Glutaraldehyde fixation of erythrocytes renders them resistant to both osmotic shock and turbulence induced lyses. This alters the cell (RBC) that is taken up by liver and spleen.
- At low concentration glutaraldehyde treated cells are removed by spleen and at high concentration by liver, since spleen is capable of selecting the cells with slight damage only. Liver lacks this discriminating power and also since it is highly vascular, highly damaged RBC'S are removed from liver.
- The external sulfhydral groups of RBC'S are oxidized to differing degrees and the circulating half life is reduced from 27 days to 8 min.
- One more method is modifying the circulatory and tissue uptake parameters of RBC'S is enzymaticallty modify the cell surface carbohydrates.
- Coating RBC'S with IgG increases half life of RBC.

Entrapment method

1. **Hypo – osmotic method**
   - dilution method
   - Dialysis method
   - Presswell method
   - Isotonic osmotic lyses
2. **Electrical break down method**
3. **Endocytosis method**
4. **Membrane perturbation method**
5. **Normal transport method**

1) Hypo–osmotic lyses method



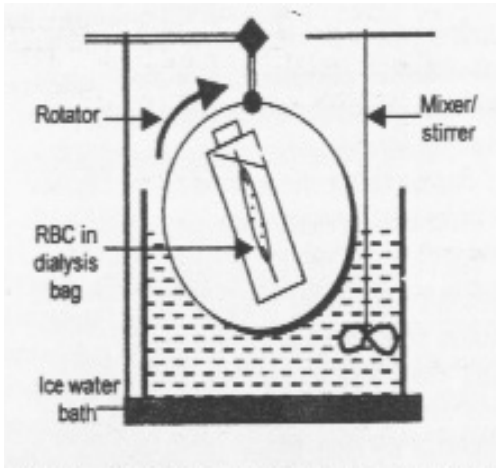
Schematic representation of hypo – osmotic lyses procedure

- In this process, the intracellular and extra cellular solutes of erythrocytes are exchanged by osmotic lyses and resealing. The drug present will be encapsulated with in the erythrocytes membrane by this process.

A) Dilution method

- The RBC'S are exposed to hypotonic solution (corresponding to 0.4 % Nacl), the erythrocytic membrane ruptures permitting escape of cellular contents and equilibrium is achieved with in one minute.
- The cells up to 1.6 times its original volume.
- The swelling results in the appearance of pores of 200 – 500 A^o in size.
- The length of time for which these pores remain open is not fixed. However at 0^oC the opening permits long enough to allow partial resealing of membrane.
- Increasing the ionic strength to isotonicity and incubating the cells at 37^o C causes the pores to close and restore the osmotic properties of the RBC'S.
- This method was used to entrap b – glucosidase and b – galactosidase.
- This method is simplest and fastest yet the capsulation efficacy is very low i. e. 1 – 8 %.
- Efficient for encapsulation of low molecular weight drugs.
- Most of the cytoplasmic constituents are lost during the osmotic lyses.

B) Dialysis method



Erythrocyte Dialyzer

- A desired Haematocrit is achieved by mixing washed erythrocyte suspension and phosphate buffer (pH 7.4) containing drug solution.
- This mixture is placed into dialysis bag and then both ends of the bag are tied with thread. An air bubble of nearly 25 % of the internal volume is left in the tube. During dialysis bubble serves to blend the content.
- The tube is placed in a bottle containing 200 ml of lysis buffer solution and placed on a mechanical rotator at 4^o C for 2 hrs.
- The dialysis tube is then placed in 200 ml of resealing solution (isotonic PBS pH 7.4) at room temperature 25 – 30 ^{o} C for resealing.
- The loaded erythrocytes thus obtained are then washed with cold PBC at 4^oC. the cells are finally resuspended in PBC.

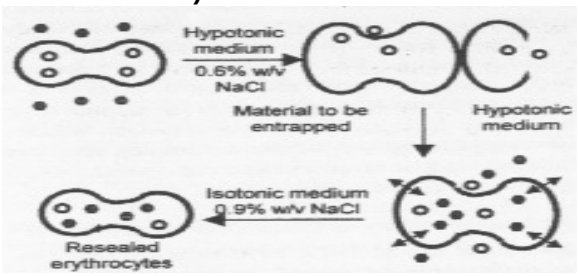
Advantages

- Good entrapment efficiency is obtained
- The volume of extra cellular solution that equilibrates with the intracellular space of erythrocyte during lyses is considerably reduced.

Disadvantages

- Time consuming method.
- The size distribution of loaded ghosts is not found to be homogeneous as revealed by studies with hydro dynamically focusing particle analyzer.

C) Press well dilution method



Press well Dilution Method

- It is based on the principle of first swelling the erythrocytes without the lyses by placing them in slightly hypotonic solution.
- The swollen cells are recovered by centrifugation at low speed.
- Then, relatively small volumes of aqueous drug solution are added to the point of lyses i. e. when there is minimum loss of constituents.
- The slow swelling of cells results in good retention of the cytoplasmic constituents and hence good survival in vivo.

Advantages

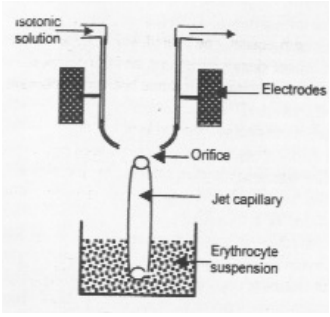
- Simple and quicker then dialysis method.
- Under optimum conditions resealed erythrocytes can survive in – vivo as long as native RBC'S.

D) Isotonic osmotic lyses technique

If erythrocytes are incubated in solution of a substance with high transerythrocytic membrane permeability the solute will diffuse into the cells due to inwardly direct chemical potential gradient. This will be followed by water uptake until osmotic equilibrium is restored.

- A transient permeability in erythrocyte wall could be produced using propylene glycol which also allows the drugs/ agents to diffuse in.
- The lysed erythrocytes are resealed under isotonic condition by dilution glycol free medium.

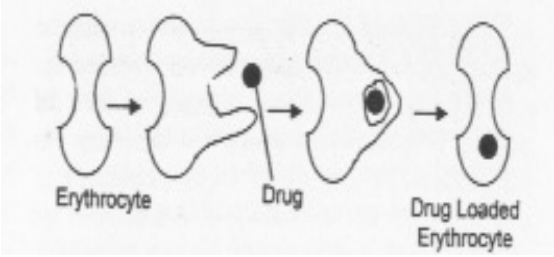
E) Electrical breakdown method



- Electrical breakdown of a cell membrane is observed when the membrane is polarized very rapidly (in nano to micro seconds) using voltage of about 2kV/ cm for 20µ sec which lead to the formation of pores and entrapment of drugs.
- Electrical breakdown probably takes place in the lipoid regions or at the lipid protein junction in the membrane.
- Pores formed are stable and it is possible to control pore size.
- Subsequently the pores can be resealed by incubation at 37° C in Osmotically balanced medium.

Disadvantage of this method is that it is very expensive

F) Endocytosis method



- Intracellular vesicles could be inducing in erythrocytes containing small molecules drugs or virus from external medium.
- This method is efficient for loading larger particles such as virus (up to 1000 nm diameter), enzymes and small molecules.
- In this method the vesicle membrane separates the endocytoced substance from the cytoplasm containing the drug which are sensitive to inactivation by cytoplasmic enzymes and also protect the erythrocyte membrane.
- The contents of vesicles, however may release into erythrocytes cytoplasm depending upon the nature of material.

Ex. Entrapment of glucose, insulin and b – glucouronidase by a chlorpromazine induced endocytosis has been reported.

G) Membrane perturbation method

- Antibiotics such as Amphotericin – B damage micro–organisms by increasing the permeability of their membrane to metabolites and ions.
- This property could be exploited for loading of drugs in to erythrocytes.
- Amphotericin – B was used to load erythrocytes with antileukamic drugs.
- Amphotericin – B interacts with the cholesterol of the plasma membrane of

eukaryotic cells causing change in permeability of the membrane.

Normal transport mechanism

- It is possible to load erythrocytes with drugs without disrupting the erythrocytic membrane in any way by incubating the drug and erythrocytes for varying period of time.
- After infusion the drug would in general, exit from the cell following the kinetics comparable to those observed for entry.

Lipid fusion method

Lipid vesicles containing ionsitol hexaphosphate with human erythrocytes the incorporated ionositol hexaphosphate in erythrocytes provided a significant lowering of the O_2 affinity for haemoglobin in intact erythrocytes.

IN VITRO CHARACTERIZATION

Resealed erythrocytes after loading are characterized for following parameters:

- Drug content
- In vitro drug and haemoglobin release
- Osmotic fragility
- Osmotic shock
- Turbulence shock
- Morphology

Drug Content

- Loaded erythrocytes are first depolarized with acetonitrile.
- Centrifugation at 2500 rpm for 10 mins.
- Clear supernatant is analysed for drug content.
- In case of magnetite loaded erythrocytes, a horse shoe magnet is placed adjacent to the base of centrifuge tube in order to retain the entrapped magnetite. The magnetic conc. is determined by atomic absorption spectroscopy.

In vitro drug and haemoglobin release

Normal and loaded erythrocytes are incubated at 37 ± 2^0 C in phosphate buffer saline in metabolic rotating wheel incubator path.

- Periodically samples are withdrawn with the help of hypodermic syringe fitted with a 0.8µ spectropore membrane filter.
- Samples are depolarized with acetonitrile and estimated for amount of drug released.

Osmotic shock

- Osmotic shock describes a sudden exposure of drug to an environment, which is far from isotonic to evaluate the ability of resealed erythrocytes to withstand the stress and maintain their integrity as well as appearance.
- Incubating erythrocytes (1 ml) with dis. Water (5 ml) for 15 min, followed by centrifugation at 3000 rpm for 15 min. may cause release of haemoglobin to varying degrees, which could be estimated spectrophotometrically.

REFERENCES

1. Green R and Widder KJ. Methods in Enzymology .Academic Press, San Diego.1987;149.
2. Ropars C, Chassaigne M and Nicoulau C. Advances in the BioSciences. Pergamon Press, Oxford, 1987; 67.
3. Lewis DA and. Alpar HO. Therapeutic Possibilities of Drugs Encapsulated in Erythrocytes. Int J Pharm. 1984;22:137–146
4. Zimmermann U. Cellular Drug-Carrier Systems and Their Possible Targeting In Targeted Drugs, EP Goldberg, Ed. John Wiley & Sons, New York, 1983; 153-200.
5. Jain S and NK Jain..Engineered Erythrocytes as a Drug Delivery.