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RESEALED ERYTHROCYTES: FORMULATION APPROACHES AND RECENT TRENDS

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ABSTRACT

Erythrocytes also known as red blood cells(RBCs) are biconcave discs with an average diameter of 7.5mm, thickness of 2.0m in periphery, 1m in the center, which are highly specialized oxygen carrier in the body, transported via circulatory system. They taken oxygen in the lungs orgills and release it while squeezing through the capillaries. 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". Resealed erythrocytes, as a drug delivery system has excellent capacity to enhance the therapeutic index and patient compliance. The process of production of erythrocytes within the body called erythropoiesis and produced in red bone marrow under hemopoeitic hormone called as erythropoietin. The present review details various features, drug loading methods and applications of resealed erythrocytes.

KEYWORDS: Resealed, Erythrocytes, RBC, Loading, Entrapment.

I. INTRODUCTION

Erythrocytes also known as red blood cells(RBCs) are biconcave discs with an average diameter of 7.5mm, thickness of 2.0m in periphery, 1m in the center, which are highly specialized oxygen carrier in the body, transported via circulatory system. They taken oxygen in the lungs orgills and release it while squeezing through the capillaries. The cytoplasm of red blood cells is rich in hemoglobin, iron that can bind oxygen and also responsible for its red blood color. The red bone marrow, where red blood cells develop and circulates in the body about 100-120days until the components are recycled by macrophages. The process of production of erythrocytes within the body called erythropoiesis and produced in red bone marrow under hemopoeitic hormone called as erythropoietin [1,2]

Resealed erythrocytes are prepared by the delivery of drugs and drug-loaded microspheres into the erythrocytes, have been extensively studied for their potential carrier capabilities. Such drug loaded carrier erythrocytes are prepared simply by collecting blood samples from the organism of interest, separating erythrocytes from plasma, entrapping drug in the erythrocytes, and resealing the resultant cellular carriers (called as resealed erythrocytes). Hence this process is based under osmotic condition. The drug loaded erythrocytes provided slow circulating depots and target to the diseases tissue organ[3,4].

1.1 Sources of Erythrocytes

Various type of erythrocytes used for drug delivery such as erythrocytes of mice, cattle, rats, rabbits, chickens, monkey, goat, sheep[5].

Isolation of Erythrocytes[6,7]

Blood withdrawn from cardiac/splenic puncture (in small animal) and through veins(in large animals) in a syringe containing a drop of anti-coagulant

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Collected into heparin zed tubes by veniputure

Centrifuged at 2500rpm for 5 min at 4^0c

Remove serum and buffy coats and washed the packed cells with three times with phosphate buffer saline(pH-7.4), 4^0c ina refrigerated centrifuge

Washed erythrocytes are diluted with PBS and stored at 4^0c.

1.2 Properties of Resealed Erythrocytes [8,9,10,11]

- It should be compatible to blood
- It should have low toxicity
- It should have Ability to carry broad spectrum of drug
- Drug should be release to target site
- The carrier system should have appreciable storage stability
- Circulate through the circulatory system
- Prospect of decreasing the side effectof drug
- Considerably uniform size and shape of carrier

1.3 Advantages [8,12,13]

- They are natural product of body, which are biodegradable in nature with no generation of toxic or harmful products
- Good entrapment efficiency is obtained
- No chance of triggered immune response
- After attaining plasma concentration , fluctuation in concentration decreases
- Alteration of pharmacokinetic and pharmacodynamic parameter of drugs can be done
- Ease of circulation and abilty to target RES organ
- Prolong systemic activity of drug with longer time in the body

1.4 Disadvantages [3,13]

- This method is time consuming
- There maybe chances of cell clumping and dose dumping
- Several drugs alter the physiology of RBCs
- In some cases, drugs carrier may cause toxicological problems

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II. METHODS OF DUG LOADING

2.1 Hypotonic method

Dilution method

According to this method, a volume of packed erythrocytes is diluted with 2-20 volumes of aqueous solution of drug. A hypertonic buffer was added to maintain the tonicity of solution. After that resultant mixture is centrifuged, the supernatant liquid is discarded and the pellet is washed with isotonic buffer solution. This dilution method is used for loading enzymes such as B-galactosidase, asparaginase, arginase, B- glucoside & bronchodilator such as salbutamol[13].

Dialysis method

In this method, a desired haematocrit is achieved by mixing washed erythrocyte suspension and phosphate buffer (pH 7.4) containing drug solution. After that the mixture is placed into dialysis bag and then both ends of the bag are tied with thread. An air bubble of 25% internal volume is left in the tube. During dialysis bubble serves to blend the content.

The tube is placed in a bottle containing 100ml of lysis buffer solution and placed on a mechanical rotator at 4^0c for 2hrs. Then dialysis tube is placed in 100ml resealing solution(isotonic PBS). Thus obtained resealed erythrocytes are then washed with cold phosphate buffer at 4^0c[13].

2.2 Osmotic lysis method

This method is also called as osmotic pulse method. In this method, erythrocytes are incubated in solution of a substance with high permeability, the solute will diffuse into the cells because of concentration gradient. Chemicals such as urea solution, polyethylene glycol and ammonium chloride have been used for isotonic haemolysis. Finally suspension was diluted with isotonic bufferd drug solution and cells were separated and resealed at 37^0c[14].

2.3 Preswelling method

This method was based on the principle of first swelling the erythrocytes without lysis by placing them in slightly hypotonic solution. This mixture is centrifuged at low speed. Then a small volumes of aqueous drug solution are added to lysis point. The supernatant liquid is removed. After that the mixture is centrifuged between the drug addition steps. The tonicity of cell mixture is restored at lysis point by adding a calculated amount of hypertonic buffer. Lastly the cell suspension is incubated at 37^0c to reanneal the resealed erythrocytes[15].

2.4 Electric cell fusion method

This method involves the initial loading of drug molecules into erythrocytes ghosts followed by adhesion of these cells to target cells. The fusion is promoted by the application of an electric pulse, which causes the release of an entrapped molecule. An example of this method is loading a cell-specific monoclonal antibody into an erythrocyte ghost. An antibody against a specific surface protein of target cells can be chemically cross-linked to drug-loaded cells that would direct these cells to desired cells[8].

2.5 Endocytosis method

Endocytosis involves the addition of one volume of washed packed erythrocytes to 9 volumes of buffer containing 2.5mm ATP, 2.5mm $MgCl_2$ and 1mm $Cacl_2$ followed by incubation for 2min at room temperature. The pores created by this method are resealed by using 154mm of Nacl & incubation at 37^0c for 2 min. the entrapment of material occurs by endocytosis. The vesicles membrane separates endocytosed material from cytoplasm thus protecting it from the erythrocytes and viceversa. It has been used for various drugs suchas primaquine, 8-aminoquinolones, vinblastine, chlorpromazine, tetracaine, vitamin etc[8,3].

2.6 Electro-encapsulation method

Lipid fusion method

This method involves lipid vesicles containing a drug can be directly fusedto human erythrocytes:it leads to exchange with a lipid-entrapped drug. Hence this method is used for entrapping ionositol monophosphate which helps to improve the oxygen carrying capacity of RBCs[3].

2.7 Chemical perturbation of the membrane

It is based on the increase in membrane permeability of erythrocytes when the cells are exposed to certain chemicals that the permeability of erythrocytic membrane increases upon exposure to polyene antibioticssuch as amphotericin B. this method is not very popular[8].

III. APPLICATIONS

3.1 In-vitro application

For in vitro, phagocytosis have been used to fascilitate the uptake of enzyme. The enzyme content with carrier RBCs could be visualized with the help of cytochemical technique. The most frequent in vitro application of RBC is that of micro-injection in which protein or nucleic acid was injected into eukaryotic cells by fusion process. Similarly in case of antibody, where antibody molecules are introduced using erythrocytic carrier system and it immediately diffuse throughout the cytoplasm[16].

3.2 In-vivo application

i. Slow drug release

Various agents encapsulated in erythrocytes are developed for slow release in circulation to allow effective treatment of parasitic diseases. Resealed erythrocytes serve as an ideal carrier for antineoplastic agent, antimicrobial agent, and vitamins and steroids[16].

ii. Carriers for enzyme

Enzyme can be injected into blood stream to replace a missing or deficient enzyme in metabolic disorders or to degrade toxic compounds accumulated in blood due to a disease like environmental, lysosomal storage disorders and kidney failure are the example which are treated by the administration of enzymes[14].

iii. Removal of RES iron overloaded

Desferoxamine-loaded erythrocytes have been used to treat excess iron accumulated because of multiple transfusion of thalassemic patients. Targeting this drug to the RES is very beneficial because the aged erythrocytes are destroyed in RES organ, which result in an accumulation of iron in these organs[1,17].

IV. EVALUATION PARAMETERS

- Shape and surface morphology
- Drug content
- Entrapment efficiency
- Turbulence fragility
- In-vitro stability
- Erythrocyte sedimentation rate
- In-vitro release and hemoglobin release

V. CONCLUSION

Resealed erythrocytes are a very efficient and novel method for drug loading and entrapment. It offers many advantages as drug targeting, better drug loading and drug delivery. The drugs can be directly delivered to the target sites. Drugs which have a potential for low absorption can be effectively delivered as resealed erythrocytes where the absorption limitation is a major hindrance for drug therapy. Resealed erythrocytes are non-toxic and can be used for many lipophilic and hydrophilic drugs. The use f this technology for drug targeting can be a major breakthrough for treatment of many diseases and it is also effective in terms of cost. The methods to produce resealed erythrocytes can be easily developed at the industrial level and used as a alternative for other drug carriers and transport mechanisms.

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