Review Article

Review-Resealed Erythrocyte As A Drug Carrier

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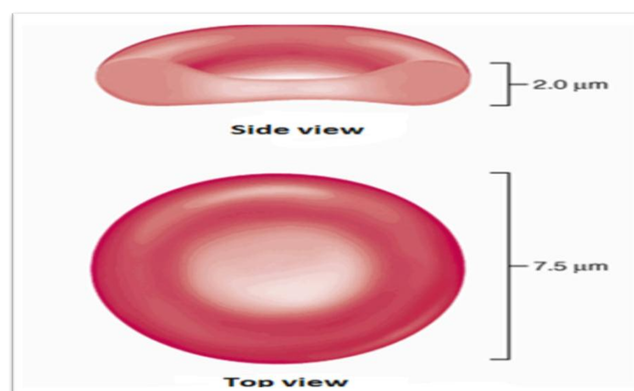
ABSTRACT
Resealed Erythrocyte is the drug delivery system which makes the most of the drug targeting along with high therapeutic benefits for safe and effective management of diseases. Erythrocytes can be used as carriers by targeting particular tissue/organ, for continuous or prolonged release of drugs. It offer a greater potential advantages related to its biodegradability, non-pathogen city, no immunogenicity, biocompatibility, self degradability along with high drug loading efficiency. 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". In this review, isolation of carrier erythrocytes, methods of drug loading, Characterization parameters, Stability and its applications were presented.

Keywords: Resealed Erythrocytes, Non-Pathogenicity, Antineoplastics, Spectrophotometrically.

INTRODUCTION
Present pharmaceutical picture is intended at development of drug delivery systems which make the most of the drug targeting along with high therapeutic benefits for safe and effective management of diseases. Targeting of an active bio molecule from effective drug delivery where pharmacological agent directed specifically to its target site. Drug targeting can be approaches by appropriate carrier or by either chemical modification. Various carriers has been used for the drug targeting among which cellular carrier offer a greater potential advantages related to its biodegradability, non-pathogenicity, nonimmunogenicity, biocompatibility, selfdegradability along with high drug loading efficiency . Erythrocyte, platelets and leukocytes have been proposed as cellular carrier systems.

Erythrocytes
Red blood cells are also known as erythrocytes they most regular type of blood cells by means of the blood flow through the circulatory system. They delivering oxygen to the body tissue RBCs take up oxygen in the lungs or gills and release it while squeezing through the body's capillaries. Cytoplasm of these cells contains high amount of hemoglobin, which contains iron responsible for red color of blood. Red blood cells are flexible biconcave disks that lack a cell nucleus and most organelles. About 2.4 million new erythrocytes are produced per second in our body.

Morphology and physiology of erythrocytes



Erythrocytes are the large amount abundant cells in the human body (_5.4 million cells/mm3 bloods in a healthy male and _4.8 million cells/mm3 bloods in a healthy female). The Dutch Scientist Lee Van Hock described in human blood samples in 1674. In the 19th century, Hope Seyler identified hemoglobin and its role in oxygen delivery to various organs. Erythrocytes squeeze easily through narrow capillaries due to its flexible and biconcave shape generally about 3 mm wide.

Mature erythrocytes are fairly simple in structure. They do not have a nucleus and other organelles. Their plasma membrane contains hemoglobin, a protein which contains heme that is responsible for O2–CO2 binding inside the erythrocytes. Erythrocytes plays important role in the transport of O2 from the lungs to tissues and the CO2 produced in tissues back to lungs. Erythrocytes do not consume any of the oxygen they are carrying as it does not contain mitochondria an energy is generated an aerobically. Erythrocytes live only about 120 days because of wear and tear. Worn-out erythrocytes are removed from circulation parts of the body.

Advantages of Resealed Erythrocytes

1. No chance of triggered immune response.
2. Biodegradability with harmful products or no generation of toxic products.
3. The considerable uniform size and shape of the carrier.
4. Relatively inert intracellular environment.
5. Prevention of degradation of the loaded drug from inactivation by endogenous chemicals.
6. The wide variety of chemicals and enzymes can be entrapped.
7. The modification of pharmacokinetic and pharmacodynamic parameters of drug can be done.
8. Attainment of steady-state plasma concentration which decreases fluctuations in concentration of drug.
9. Protection of the organism against toxic effects of drugs (e.g., antineoplastics)
10. Ease of circulation and ability to target RES organ
11. Prolong systemic activity of the drug while residing for a longer time in the body.

Isolation of erythrocytes

- ✓ Blood is collected into heparin zed tubesby venipunture.
- ✓ Blood is withdrawn from cardiac puncture (in small animal) and throughveins (in large animals) in a syring econtaining a drop of anti-coagulant.
- ✓ The whole blood is centrifuged at 2500rpm for 5 min. at 4 ±10C in a refrigerated centrifuge.
- ✓ The serum and buffy coats are carefullyremoved and packed cells washed threetimes with phosphate buffer saline pH=7.4).
- ✓ The washed erythrocytes are diluted with phosphate buffer solution and stored at 4oC until used.
- ✓ Various types of mammalian erythrocytes have been used for drug delivery, including erythrocytes of mice, cattle, pigs, dogs, sheep, goats, monkeys, chicken, rats, and rabbits. The encapsulation efficiency of the erythrocytes isolated from fresh blood is more than that of the aged blood. Fresh whole blood is the blood that is collected and immediately chilled to 4 C and stored for less than two days. The erythrocytes are then harvested and washed by centrifugation. The washed erythrocytes are suspended in buffer solutions at various hematocrit values as desired and are often stored in acid–citrate–dextrose buffer at 40C for as long as 48 h before use. Entrapment of dextran (molecular weight10–250 kDa) and loading of drugs in erythrocytes was reported separately.

METHODS OF DRUG LOADING

Hypotonic heamolysis

Erythrocytes have an outstanding capability for reversible shape changes with or without accompanying volume change and for reversible deformation under stress. An increase in volume leads to an first change in the shape from biconcave to spherical. This change is attributable to the absence of unnecessary membrane; hence, the surface area of the cell is preset. The cells assume a spherical shape to accommodate additional volume while observance the surface area constant. The volume gain is 25–50%. This method is based on the ability of erythrocytes to under reversible inflammation in a hypotonic solution. The cells can keep up their integrity up to a tonicity of 150 mos m/kg, above which the membrane ruptures, releasing the cellular contents.

On this position (just before Cell lysis), some passing pores of 200–500 Å are generated on the membrane. After cell lysis, cellular contents are exhausted the remains is called an erythrocyte ghost. Upon incubation; the cells resume their exclusive biconcave shape and recover unique impermeability.

Use of red cell loader

New method was developed for entrapment of nondiffusible drugs into erythrocytes. They developed a portion of equipment called a “red cell loader. With as slight as 50 ml of a blood sample, dissimilar biologically active compounds were entrapped into erythrocytes within a period of 2 h at room temperature under blood banking conditions. The process is based on in order hypotonic dilutions of washed erythrocytes followed by concentration with a hemofilter and an isotonic resealing of the cells. There was 35–50% cell recovery with 30% drug loading. The processed erythrocytes had usual survival in vivo. The

same cells could be used for targeting by improving their recognition by tissue macrophages.

Hypotonic dilution

Hypotonic dilution was the primary 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 unnecessary, and the pellet is washed with isotonic buffer solution. The main drawbacks of this method include low entrapment efficiency and a substantial loss of hemoglobin and other cell components. This reduces the circulation half-life of the loaded cells. These cells are generally phagocytosed by reticule endothelial system macrophages and hence can be used for targeting RES organs. Hypotonic dilution is used for loading enzymes such as galactosidase and glucosidase, asparginase, and arginase, as well as bronchodilators such as salbutamol.

Hypotonic preswelling

This method was developed by Rechsteiner in 1975 and was developed by Jenner et al. for drug loading. The technique is based upon initial controlled inflammation in a hypotonic buffered solution. This mixture is centrifuged at low *g*values. The supernatant is not needed and the cell fraction is brought to the lysis point by adding 100–120 Ltrs portions of an aqueous solution of the drug to be encapsulated. The mixture is centrifuged among the drug-addition steps. The lysis point is detected by the disappearance of a distinct limit between the cell portion and the supernatant upon centrifugation. The tonicity of a cell mixture is restored at the lysis point by adding a calculated quantity of hypertonic buffer. They cell have a circulation half-life comparable to that of normal cells. This method is simpler and faster than other methods, causing lowest amount injure to cells. Drugs encapsulated in erythrocytes using this technique include propranolol, asparginase, cyclopohphamide, 1-antitrypsin, methotrexate, insulin, metronidazole, levothyroxine, enalaprilat, and isoniazid.

Hypotonic dialysis

This method was first reported by Klibansky in1959 and was used in 1977 by Deloach and Ihler,and Dale for loading enzymes and lipids. Several methods are based on the principle that semipermeable dialysis membrane maximizes the intracellular: extracellular volume ratio for macromolecules during lysis and resealing. This process isotonic buffered suspension of erythrocytes with a hematocrit rate 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 quantity 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. The use of standard hemodialysis apparatus for loading a drug in erythrocytes was reported by Roper et al. In this method, the erythrocyte suspension and the drug to be loaded were located in the blood compartment and the hypotonic buffer was located in a receptor section. They 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 consist of a long processing time and the need for special equipment. This method has been used for loading enzymes such as galactosidase, glucoserebrosidase, asparginase, inositol hexaphosphatase, as well as drugs such as gentamicin adriamycin, pentamidine and furamycin, interlukin-2, desferroxamine, and human recombinant erythropoietin.

Isotonic osmoticlysis

This process, 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 isoionic. If erythrocytes are incubated in solutions of a matter with high membrane permeability, the solute will disperse into the cells because of the concentration gradient. This method 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 process also is not immune to changes in membrane structure composition. In 1987, Francoet al. developed a technique 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 divided, they were resealed at 370 C.

Chemical perturbation of the membrane
This process is based on the raise in membrane permeability of erythrocytes when the cells are exposed to certain chemicals. Amphotericin B. such as permeability of erythrocytic membrane increases upon exposure to polyene antibiotic In 1980, this technique was used successfully to entrap the antineoplastic drug daunomycin in human and mouse erythrocytes. However these process induce irreversible critical changes in the cell membrane and hence are not very popular.

Electro-insertion or electro encapsulation
In 1973, Zimmermann tried an electrical pulse technique to encapsulate bioactive molecules. Also known as electroporation, the process 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 make desirable membrane permeability for drug loading. The erythrocyte membrane is opened by a dielectric breakdown. Then, the pores can be resealed by incubation at 37 0C in an isotonic medium.
The process involves suspending erythrocytesin an isotonic buffer in an electrical release chamber. A capacitor in an external circuit is charged to a specific voltage and then discharged within a specific time interval through cell suspension to make a square-wave potential. The optimum intensity of an electric field is between 1–10 kW/cm and optimal discharge time is between 20–160 . Inverse relationship exists between the discharge time and electric-field intensity. The compound to be entrapped is added to the medium in which the cells are balanced from the commencement of the experiment. The characteristic pore diameter formed in the membrane depends upon the intensity of electric field, the discharge time, and the ionic strength of suspending medium. This procedure can be prevented by adding large molecules (e.g., tetrasaccharide stachyose and bovine serum albumin) and ribonucleose. One advantage of this technique is a more uniform distribution of loaded cells in comparison with osmotic process. The main drawbacks are the need for special instrumentation and the sophistication of the method. Entrapment efficiency of this process is 35%, and the life span of the resealed cells in circulation is similar with that of normal cells. Different compounds such as sucrose, urease, methotrexate, isoniazid, human glycophorin, DNA fragments, and latex particles of diameter 0.2 m can be entrapped within erythrocytes by this process. Mangal and Kaur achieved continuous release of a drug entrapped in erythrocytes with the use of electroporation.

Entrapment by endocytosis
This process was reported by Schrier et al. in 1975. Endocytosis involves the addition of one volume of washed filled erythrocytes to nine volumes of buffer containing 2.5 mM ATP, 2.5 mM MgCl2, and 1mM CaCl2, followed by incubation for 2 min at room temperature. The pores created by this technique are resealed by using 154 mm of NaCl and incubation at 37oC for 2 min. The setup of material occurs by endocytosis. The vesicle membrane separates endocytosed substance from cytoplasm thus defensive it from the erythrocytes and vice-versa. The various candidates entrapped by this method include primaquine and related 8–amino– quinolines, vinblastine, chlorpromazine and related phenothiazines, hydrocortisone, propranolol, tetracaine, and vitamin A.

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

Loading by lipid fusion
This method was used for entrapping inositol monophosphate to improve the oxygen carrying capacity of cells. However, the entrapment efficiency of this process is very low (1%). Lipid vesicles containing a drug can be frankly fused to human erythrocytes, which lead to an replace with a lipid entrapped drug.

CHARACTERIZATION PARAMETERS	ANALYTICAL METHODS / INSTRUMENTATION
1. PHYSICAL CHARACTERIZATION ✓ Shape and surface morphology ✓ Vesicle size and size distribution ✓ Drug release ✓ %Encapsulation ✓ Electrical surface potential and surface pH	Transmission electron microscopy (TEM), Scanning electron microscopy, Phase-contrast optical microscopy ✓ TEM, Optical microscopy ✓ Diffusion cell / dialysis ✓ Deproteinization (using methanol or acetonitrile) of membrane and assay for released drug or radio-labeled markers ✓ Zeta potential measurements and sensitive probes.
2. CELL RELATED CHARACTERIZATION ✓ % Haemoglobin content / volume ✓ Mean corpuscular haemoglobin ✓ % Cell recovery ✓ Osmotic fragility ✓ Osmotic shock ✓ Turbulent shock ✓ Erythrocyte sedimentation rate	✓ Deproteinization (using methanol or acetonitrile) of cell membrane and assay for Hb; Laser light scattering for cell vol ✓ Laser light scattering ✓ Hematological analyzer; Neubauer's chamber ✓ Stepwise incubation with isotonic to hypotonic saline solutions and estimation of drug and Hb. ✓ Dilution with distilled water and estimation of drug and Hb. ✓ Passing cell suspension through a 23 gauge needle, hypodermic needle (10 ml / min) and estimation of residual drug and Hb. ✓ ESR apparatus
3.BIOLOGICAL CHARACTERIZATION ✓ Sterility ✓ Pyrogenicity ✓ Animal toxicity	✓ Aerobic or anaerobic cultures ✓ Rabbit fever response test of LAL test ✓ Toxicity tests

Characterization of Resealed Erythrocytes

These characterizations are important to ensure their in-vivo performance and therapeutic benefits.

I. Drug Content Determination

Method: 0.5ml packed loaded erythrocytes are deproteinized with acetonitrile (2 ml) and then centrifuged at 2500 rpm for 10 minutes. Now the clear supernatant liquid is analyzed for drug content.

II. *In-vitro* drug release and Hb content: Both these properties are monitored periodically from drug-loaded cells

Method: The cell suspension (5% Haematocrit in Phosphate buffer saline) is stored at 4oC in amber colored glass containers. Periodically the clear supernatant are withdrawn using a hypodermic syringe equipped with 0.45μ filter, deproteinized with methanol and then estimated for drug content. The supernatant of each sample after centrifugation is collected and assayed. Hence, % Hb (Haemoglobin) release is calculated.

% Hb release= A540 of sample - A540 of background A540=Absorbance at 540 nm, A540 of 100% Hb Mean Corpuscular Hb = Hb (g/100 ml) × 10 Erythrocyte count (millions/cu mm)

III. Percent cell recovery

Percent cell recovery determine by counting the number of whole cells per cubic mm of packed erythrocytes before and after loading the drug.

IV. Morphology

Following types of microscopy are used for the morphological study of normal and drug loaded erythrocytes:

i. Phase contrast microscopy
ii. Electron microscopy
  a. Scanning electron microscopy
  b. Transmission electron microscopy

V. Osmotic fragility

This method is based on resistance of cells to haemolysis in decreasing concentration of hypotonic saline.

It is a reliable parameter for:

- ✓ *In-vitro* evaluation of carrier erythrocytes with respect to shelf life
- ✓ *In-vivo* survival of erythrocytes
- ✓ Study of effect of the encapsulated substances
- ✓ For stimulating and mimicking the bio-environmental conditions that are encountered on in-vivo administration.

Method: Normal and drug - loaded erythrocytes are incubated separately in stepwise decreasing % of NaCl solution (0.9%) at 37oC±2oC for 10 minutes , followed by centrifugation at 2000 rpm for 10 minutes. Then the supernatant liquid is examined for drug and haemoglobin content.

VI. Osmotic shock

This is used to describe a sudden exposure of drug loaded erythrocytes to an environment, which is far from isotonic so as to calculate the capability of resealed erythrocytes to withstand the stress and

keep up their integrity as well as appearance.
Method: Erythrocyte suspension (10% haematocrit,1 ml) was diluted with distilled water(5 ml) and centrifuged at 300 rpm for 15 minutes. Supernatant was estimated for % Hb release spectrophotometrically.

VII. Turbulence shock

This parameter indicates the effects of shear and pressure, by which resealed erythrocyte formulations are injected, on the integrity of the loaded cells. Drug loaded erythrocytes appear to be less resistant to turbulence because resealing of erythrocytes make them sensitive towards turbulence/ Mechanical agitation and hence estimation of turbulence shock study provides their expected performance in-vivo.
Method: Loaded erythrocytes(10% haematocrit,5 ml) are passed through 23-gauge hypodermic needle at a flow rate of 10 ml/minute(which is comparable to the flow rate of blood).After every pass, aliquot of suspension is withdrawn and then centrifuge at 2000 rpm for 10-15 minutes. Now the Hb content is estimated spectrophotometrically.

VIII. Determination of entrapped Magnetite

Resealed erythrocytes are entrapped with magnetite to make them Magnoresponsive.
Method: Magnetite bearing erythrocytes and Hydrochloric acid are heated at 60ºC for 2 hour. Now 20% w/v trichloroacetic acid is added. Centrifugation is done and supernatant is examined for Magnetite concentration using atomic absorption spectroscopy.

IX. Erythrocyte Sedimentation Rate (ESR)

ESR is the estimation of suspension stability of RBC in plasma and is related to:

- ✓ Number and size of red cells.
- ✓ The relative concentration of plasma proteins (especially fibrogen ,alpha and beta globulins)

This test is performed by determining the ESR of blood cells in a standard tube of ESR apparatus. Higher rate of ESR is indication of active but obscure disease processes. The normal blood ESR is found to be 0 to 15 mm/hour.

X. The Zeta Sedimentation Ratio

It is based on a measure of the closeness with which RBC's will approach each other after standardized cycles of dispersion and compaction.

XI. Miscellaneous

Lipid composition, Membrane fluidity, rheological properties, density gradient separation, energy metabolism, Biological characterization(sterility test using aerobic and anaerobic cultures, Pyrogenicity using rabbit fever response or LAL test, animal toxicity study)

Release Characteristics of Loaded Drugs

These are important to study so as to control efflux rate before constant release rate over prolonged period can be achieved. There are mainly three ways for a drug to efflux out from erythrocyte carriers.

I. Phagocytosis

RBC's are normally removed from circulation by process of phagocytosis following heat treatment or antibody cross linking. The drug could be released from macrophages after phagocytosis if linkage is susceptible to lysosomal enzymes.

II. Diffusion through the cell membrane

The rate of diffusion depends upon the rate at which a particular molecule penetrates through a lipid bilayer. It is greatest for a molecule with high lipid solubility. Hence considerable control over the rate of drug release is possible by introducing or eliminating polar or charged substituent.

III. Using a specific transport system

Many substances enter cells by specific membrane protein system because the carriers are proteins and specific as that of enzymes. Moderate modification in compound can often alter the rate of exit. The release of drugs from erythrocytes rapidly follows sustained release profile and rate of exit is proportional to instantaneous intracellular drug concentration i.e. first order kinetics. However, erythrocytes' carriers have the potential of releasing encapsulated substance following zero order kinetics. By incorporating polymers, release pattern may be modified. If the drug is encapsulated in a random population of erythrocytes, then constant fraction of cells will be removed each day and constant amount of drug will be made available each day.

Storage of Erythrocytes

The storage of resealed erythrocytes places a major challenge in their practical utility as drug delivery system and is important pre-requisite for the erythrocytes as a drug carrier. The Encapsulated preparation should be stored in such a way that there should be no loss of integrity. There are following methods for the storage of loaded erythrocytes:

I. Suspending in Hank's balanced salt solution at 4oC for two weeks.
II. After encapsulation suspending the cells in oxygenated Hank's balanced salt solution containing 1% soft bloom gelatin. The cells can be recovered by liquefying the gel by placing the tubes in water bath at 37oC and centrifugation under clinical conditions.
III. or Cryopreservation of erythrocytes at liquid nitrogen temperature.

APPLICATIONS

a. Slow Drug Release

Erythrocytes have been used as storehouse for the sustained delivery of various drugs like anticancer, antiparasitic, veterinary, anti amoebic,vitamins, steroids, antibiotics and cardiovascular drugs.

b. Drug Targeting

Ideally, drug delivery should be site-specific and target-oriented to show maximal therapeutic index with least undesirable effects. Resealed erythrocytes can act as drug carriers and targeting tools also. Surface-tailored erythrocytes are used to target organs of mononuclear phagocytic system/ reticuloendothelial system because the changes in the membrane are renowned by macrophages.

c. Targeting RES organs

Damaged erythrocytes are quickly blank from circulation by phagocytic Kupffer cells in liver and spleen. Resealed erythrocytes by developed 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
- ✓ Surface modification with gluteraldehyde
- ✓ 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 *bis* (sulfosuccinimidyl) suberate and 3, 3-dithio (sulfosuccinmidyl propionate).

d. Targeting the liver, Enzyme deficiency/ replacement therapy

Many metabolic disorders related to deficient or missing enzymes can be treated by injecting these enzymes. However, the problems of exogenous enzyme therapy include a shorter circulation half life of enzymes, allergic reactions, and toxic manifestations. These problems can be effectively overcome by administer the enzymes as resealed erythrocytes. The enzymes used include -glucosidase, -glucoronidase, -galactosidase. The disease caused by an accumulation of glucocerebrosidase in the liver and spleen can be treated by glucocerebrosidase- loaded erythrocytes.

e. Treatment of Hepatic Tumors

Hepatic tumors are one of the most prevalent types of cancer. Antineoplastic drugs such as methotrexate, bleomycin, asparginase, and Adriamycin have been successfully delivered by erythrocytes. Agents such as daunorubicin diffuse rapidly from the cells upon loading and hence pose a problem. This problem can be overcome by covalently linking daunorubicin to the erythrocytic membrane using glutaraldehyde or cis-aconitic acid as a spacer. The resealed erythrocytes loaded with carboplatin show localization in liver.

f. Treatment of parasitic diseases

The ability of resealed erythrocytes to selectively mount up within reticule endothelial system organs make them useful tool during the delivery of antiparasitic agents. Parasitic diseases that engage harboring parasites in the RES organs can be successfully restricted by this method. Results were positive in studies concerning animal models for erythrocytes loaded with antimalarial, antileishmanial, and antiamoebic drugs.

g. Removal of reticule endothelial system iron overload

Desferrioxamine (iron-chelating agent) loaded erythrocytes have been used to be concerned for excess iron accumulated because of various transfusions to thalassemic patients. Targeting this drug to the RES is very useful because the aged erythrocytes are spoiled in RES organs, which results in an accumulation of iron in these organs.

h. Removal of Toxic Agents

Cannon et al. reported inhibition of cyanide intoxication with murine carrier erythrocytes containing bovine rhodanase and sodium thiosulphate. Antagonization of organophosphorus intoxication by resealed erythrocytes containing a recombinant phosphodiestrase also has been reported.

i. Targeting organs other than those of RES

The various approaches include:

- ✓ Entrapment of paramagnetic particles along with the drug
- ✓ Entrapment of photosensitive material
- ✓ The use of ultrasound waves

- ✓ Antibody attachment to erythrocyte membrane to get specificity of action.

j. Delivered antiviral agents
Nucleosides are quickly transported across the membrane whereas nucleotides are not and thus exhibiting prolonged release profiles. The release of nucleotides requires exchange of these moieties to purine or pyrimidine bases.

k. Enzyme Therapy
Enzymes are widely used in clinical practice as replacement therapies to treat diseases associated with their deficiency (e.g., Gaucher’s disease, galactosuria), degradation of toxic compounds secondary to some kind of poisoning (cyanide, organophosphorus), and as drugs. The problems involved in the injection of enzymes into the body have been cited. One method to conquer these problems is the use of enzyme-loaded erythrocytes. These cells then release enzymes into circulation upon hemolysis and act as a “circulating bioreactors” in which substrates enter into the cell, interact with enzymes, and generate products or accrue enzymes in RES upon hemolysis for future catalysis.
The first report of successful clinical trials of the resealed erythrocytes loaded with enzymes for replacement therapy is that of – glucosere brosidase for the treatment of Gaucher’s disease. The disease is characterized by inborn deficiency of lysosomal- glucosere brosidase in cells of RES thereby leading to accumulation of glucosere brosides in macrophages of the RES. This leads to an growth of aminolevulinic acid in tissues, blood, and urine. This state leads to delicate porphyria and CNS related difficulty.

l. **Improvement in oxygen delivery to tissues**
Haemoglobin is the protein responsible for the oxygen-carrying capacity of erythrocytes. Under normal conditions, 95% of haemoglobin is saturated with oxygen in the lungs, whereas under physiological conditions, in peripheral blood stream, only 25% of oxygenated haemoglobin becomes deoxygenated. Thus, the major fraction of oxygen bound to haemoglobin is recirculated with venous blood to the lungs.

B. *In-vitro* Applications
Most important in-vitro application is that of Microinjection of macromolecules. DNA, RNA, and proteins are exploited for different cell biological applications. Hence, various methods are used to entrap these macromolecules into cultured cells (e.g., microinjection).

C. In microinjection, erythrocytes are used in micro- syringes for injection in the host cells. The microinjection method involves culturing host eukaryotic cells *in vitro*. The cells are coated with fusogenic agent and then suspended with erythrocytes full with the compound of interest in an isotonic medium. Sendai virus its glycoprotein's or polyethylene glycol has been used as fusogenic agents. The fusogen causes fusion of co-suspended erythrocytes and eukaryotic cells.

Novel Approaches (Recent Developments)
- ✓ **Erythrosome**

This process is achieved by modify a reverse-phase evaporation technique. These vesicles have been proposed as useful encapsulation systems for macromolecular drugs. These are particularly engineered vesicular systems that are chemically cross-linked to human erythrocytes’ support upon which a lipid bilayer is coated.
- ✓ **Nanoerythrosomes**

These are prepared by extrusion of erythrocyte ghosts to create small vesicles with an average diameter of 100 nm. Daunorubicin was covalently conjugated to nanoerythrosomes using gluteraldehyde spacer

CONCLUSION
This composite was more lively than free daunorubicin alone, both in vitro and in vivo. Moreover, the complex appears to be stable and preserve both cytotoxic and antineoplastic activity of daunorubicin against leukemia. Erythrocytes are loaded with diversity of biologically active compounds by various methods. Resealed erythrocyte method of drug loading is safe and useful for long period of time. Resealed erythrocytes carriers are, golden egg in novel drug delivery system and which is having several application as a carrier for drugs, enzymes and peptides.

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