DRUG DELIVERY TO LYSOSOMAL STORAGE DISEASES
VISWANADHA INSTITUTE OF PHARMACEUTICAL SCIENCES

CONTENTS

INTRODUCTION

- Lysosomes are sub cellular organelles found in all eukaryotic cells characterized by an acidic lumen.
- Lysosomes are rich in enzymes essential for the biochemical breakdown of molecules such as glycosaminoglycans, oligosaccharides, sphingolipids and other lipids.
- Lysosomes are nicknamed as "Suicide-bags" by cell biologists due to their role in autolysis.
- Lysosomes are the cell's waste disposal system

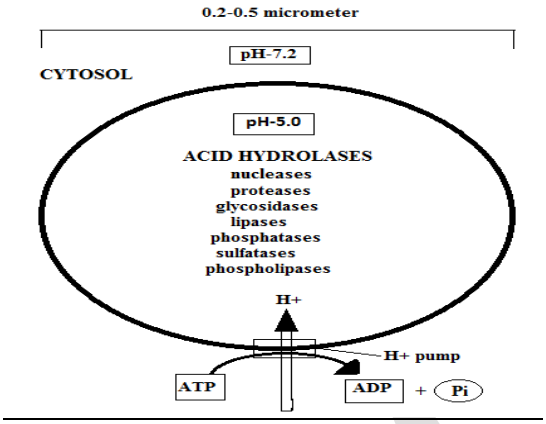
Functions:

- Helps in repair damage to the plasma membrane by serving as a membrane as a membrane patch, sealing the wound.
- Lysosomes are involved in three major cell functions
  1. **Phagocytosis**
  2. **Autophagy**
  3. **Endocytosis**

Lysosomal enzymes:

- Lysosomes have about 40 different types of hydrolytic enzymes.
- Some important enzymes found within lysosomes include
  . Lipase, which digests lipids
  . Carbohydrase,which digest carbohydrates (e.g sugars)
  . Proteases, cystin proteases (cathepsins) as well as aspartate protease.
  . Nucleases, which digest nucleic acids
  . Phosphoric acid monoesters.
- These enzymes have some properties in common:
  (a) They have in general an acid pH optimum,
  (b) They are generally resistant to autolysis and
  (c) They are glycoproteins.

Proton pump of lysosomes:



LYSOSOMAL STORAGE DISORDERS

- Lysosomal storage disorders (LSD) are a group of about 50 genetically inherited diseases caused by total or partial defects in an enzyme involved in the degradation of macromolecules within the lysosomes.

- This deficiency leads to a gradual accumulation of partially degraded molecules, causing progressive cellular impairment AND dysfunction function of organs.

- Most LSD's are characterized by their high morbidity and increased mortality, there are significant variations between different diseases, and among patients with the same disease.

- Tay-Sachs disease was the first lysosomal storage disorder (LSD) described, in 1881

- Gaucher disease was the second, in 1882

- The first link between an enzyme deficiency and a LSD (α-glucosidase and Pompe disease) was published in 1963 by Hers

- The successful treatment of a LSD, Gaucher disease with β-glucosidase, became available in the early 1990s
- It is now recognized that LSDs are not simply a consequence of pure storage, but result from perturbation of complex cell signaling mechanisms

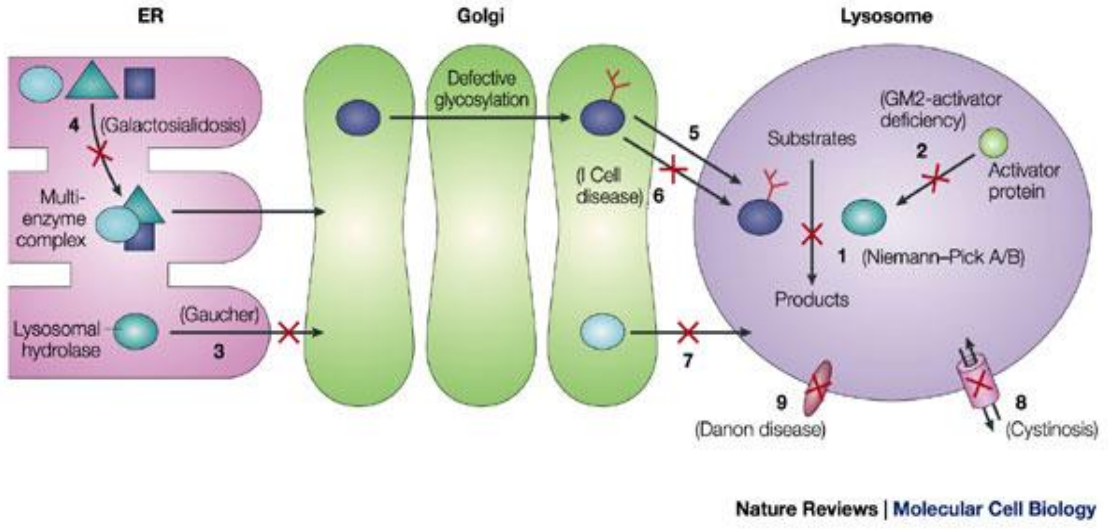
- These in turn give rise to secondary structural and biochemical changes, which have important implications for disease progression and therapy.

- Significant challenges remain, particularly targetting treatment to the central nervous and skeletal systems.

Biochemical and Cellular basis of lysosomal storage disorders

- Most mutations result in the delivery of a defective enzyme with a reduced catalytic activity to lysosomes

- Another (activator) protein required for optimal hydrolase activity is defective or absent
- A mutation that causes misfolding results in defective transport of a lysosomal hydrolase out of the endoplasmic reticulum
- Alternatively, defective transport of a lysosomal hydrolase out of the ER occurs because a multi-enzyme complex that is required for transport cannot form (Cathepsin A / sialidase / β-galactosidase)
- In the Golgi, defective glycosylation could result in an enzyme with reduced catalytic activity
- Alternatively, defective glycosylation with mannose-6-phosphate in the Golgi could produce an enzyme that cannot reach lysosomes
- Defects in other transport steps from the Golgi could also lead to an LSD
- Defects in integral lysosomal membrane proteins with transporter roles
- Defects in proteins that are involved in other vital regulatory events of lysosomal function (LAMP2, lysosomal associated membrane protein 2)



LSD Sub-Categories

- When a lysosomal enzyme (or another protein that directs it) is deficient or malfunctioning the substrate it targets accumulates, interfering with normal cellular activity.

Healthy Cell

LSD cell with accumulated substrate

- Sub-categories are based on the type of enzymatic defect and/or stored substrate product.
- For example, the mucopolysaccharidoses (MPS) are grouped together because each results from an enzyme deficiency that causes accumulation of particular glycosaminoglycan (GAG) substrates.

CAUSES OF LSD's:

LSD's occurs due to:

1. Defects in the lysosomal function
2. Defects in the hydrolytic enzymes
3. Defects in post translational processing of lysosomal enzymes.

SYMPTOMS OF LSD's:

Movement disorders, seizures, Dementia, deafness and blindness, Hepatomegaly, Splenomegaly, Pulmonary and cardiac problems, Bone disorders etc.

Some LSD's:

(a) METACHROMATIC LEUKODYSTROPHY

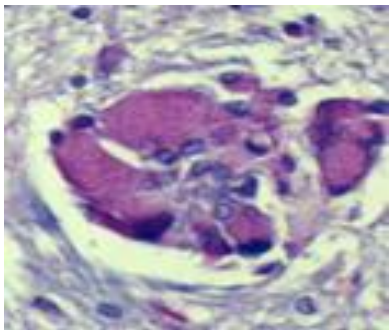
Metachromatic leukodystrophy (MLD) is an autosomal recessive **deficiency of arylsulfatase A** that results in **accumulation of the myelin lipid sulfatide** in oligodendrocytes and Schwann cells. In its most common variant, patients are normal up to **age of one or two years**, and then develop progressive peripheral neuropathy, psychomotor retardation, and blindness. Signs of white matter involvement (spasticity, brisk tendon reflexes, extensor plantar responses) are prominent. Less severe variants cause adult onset dementia, psychiatric disorders, and neuropathy.

Lysosomal storage of sulfatides kills oligodendrocytes and Schwann cells. Sulfatides discharged from dying cells are picked up by histiocytes. The white matter shows diffuse **loss of myelin** which spares the subcortical fibers. Peripheral nerves show a demyelinative neuropathy. Sulfatides are also asymptomatically stored in a variety of somatic cells such as the epithelium of the gallbladder and renal tubules. Sulfatide deposits stain pink with H&E. With acid cresyl violet, they take on a brown color (brown metachromasia), hence the term metachromatic leukodystrophy.

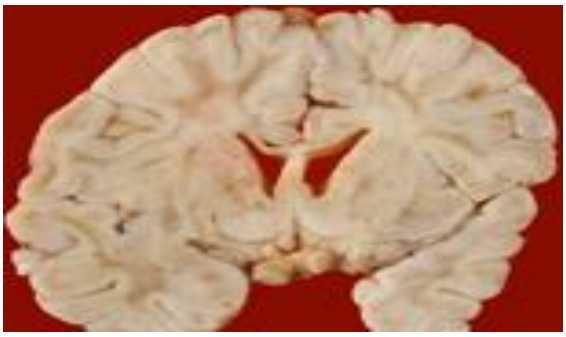
The biochemical defect of MLD **does not involve myelin synthesis, but rather the degradation and recycling of myelin lipids**. Myelin is probably formed normally initially. Subsequently, dysfunction and loss of myelin-producing cells cause loss of myelin. Myelination of some tracts begins in utero. The bulk of myelin is produced in the first two years of life. This is when severe MLD becomes clinically apparent. MLD and globoid cell leukodystrophy are LSDs.

(b) GLOBOID CELL LEUKODYSTROPHY (KRABBE'S DISEASE)

About one third of myelin lipid consists of galactocerebroside and its sulfated variant sulfatide. **Deficiency of galactocerebrosidase** (GALC) causes a severe infantile leukodystrophy, Globoid cell leukodystrophy (GCL) or Krabbe's disease. Children with the most common infantile form of GCL **appear normal at birth but, in a few months, develop irritability**, spasticity, progressive neurological regression, peripheral neuropathy and seizures and **usually die in one or two years, many in a few months**. Patients with late onset forms have a more protracted course eventually leading to severe disability and death.



Globoid cells



Krabbe's disease

In GCL, brain macrophages store galactocerebroside and are transformed into globoid cells. Most of the damage, however, is caused by **accumulation in the white matter of a related metabolite galactosylsphingosine (psychosine)**, which is toxic to oligodendrocytes. The combined effects of lipid imbalance and toxicity result in early and severe myelin degeneration. The white matter in GCL is devoid of myelin and axons (except for the subcortical fibers), firm because of gliosis, and contains globoid cells, which tend to accumulate around vessels. The cortex is normal and there is no galactocerebroside storage in neurons. There is neuronal loss in the thalamus, cerebellum and brainstem. Peripheral nerves show a demyelinative and axonal neuropathy with accumulation of galactocerebroside in Schwann cells and macrophages.

(c) GAUCHER DISEASE

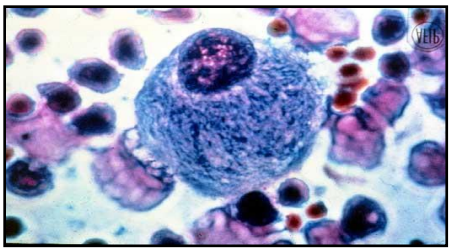
Gaucher disease (GD) is due to **deficiency of glucocerebrosidase** (glucosylceramidase) and is characterized by storage of glucocerebroside (glucosylceramide) in monocyte-macrophage cells. Three clinical phenotypes are recognized. The most common is type 1 which is especially prevalent in **Ashkenazi Jews**.

Type 1 GD presents **from childhood to early adulthood** and causes hepatosplenomegaly, bone disease (osteopenia, focal lytic or sclerotic lesions, osteonecrosis, pathologic fractures, chronic bone pain), anemia and thrombocytopenia due to hypersplenism, and pulmonary interstitial infiltrates. Spinal cord and root compression secondary to bone disease may also develop but there is no storage in the CNS.

Type 2 (acute neuronopathic) GD patients have hepatosplenomegaly similar to type 1, but **also develop neurological manifestations** (stridor, strabismus and other oculomotor abnormalities, swallowing difficulty, opisthotonus, spasticity) which **cause their death by 2 to 4 years** of age. There is no special ethnic prevalence for type 2 GD.

Type 3 (subacute neuronopathic) GD is frequent in **Northern Sweden** and has hematological and neurological manifestations similar to type 2 but milder and more slowly progressive. GD is the first LSD to be successfully managed by enzyme replacement.

GD is the prototype of storage histiocytosis. Lysosomal storage of glucocerebroside in cells of the monocyte-macrophage system leads to a characteristic cellular alteration of these cells. Gaucher cells (GC) have a large cytoplasmic mass with a striated appearance that has been likened to "wrinkled tissue paper" or "crumpled silk".



Gaucher cell 1882

GCs are present in the bone marrow, spleen, lymph nodes, hepatic sinusoids, and other organs and tissues in all forms of GD. An increased incidence of cancer including lymphoma, myeloma, and bone tumors has been reported in GD patients. There is no storage in neurons or glial cells. In type 2 and 3 GD, there are numerous GCs in perivascular CNS spaces and rare GCs in brain parenchyma. No part of the CNS is spared but the brainstem and deep nuclei are more severely affected than the cortex and account for most neurological deficits. Along with the presence of GCs, type 2 and 3 GD shows alsoneuronophagia, neuronal loss, and gliosis. No neuronal storage is seen. Neuronal degeneration and loss have been attributed to the neurotoxic action of glucosyl sphingosine, a by-product of glucocerebroside not normally present in the brain.

(d) MUCOPOLYSACCHARIDOSES (MPS)

Mucopolysaccharides (now called Glycosaminoglycans-GAGs) are synthesized in the Golgi apparatus and secreted and assembled in the extracellular space. They are produced by all cells, and are especially abundant in connective tissues. They are an important component of the matrix of connective tissue, cartilage and bone. For recycling, **GAGs are internalized and degraded in a stepwise fashion by lysosomal enzymes. Deficiency of these enzymes causes lysosomal storage of GAGs.** There are six clinical groups of MPS caused by deficiencies of ten GAG-cleaving enzymes.

Intracellular storage of GAGs in hepatocytes and other cells causes hepatomegaly, cellular dysfunction, and cell death. The most severe somatic changes in the MPS are due to accumulation of GAGs in matrix due to impaired recycling and to discharge of GAGs from dying mesenchymal cells. Because they are negatively charged, GAGs attract a lot of water that causes their molecules to swell to tremendous volumes. High GAG content of connective tissues affects collagen synthesis and causes increased collagen deposition.

The skin, connective tissues, and cartilage become swollen and distorted. The connective tissue and cutaneous changes cause facial deformity and macroglossia which gave rise to the insensitive term gargoylism. Cardiac valves and chordae tendineae become thickened and stiff. Endocardial and interstitial myocardial fibrosis develops. The intima of coronary arteries may be thickened to the point of occlusion and the aorta develops fibrous intimal plaques without lipid deposition. These changes cause a fatal cardiomyopathy and ischemic heart disease. GAG storage causes joint stiffening and swelling and complex skeletal deformities known as dysostosis multiplex. Storage in corneal fibroblasts causescorneal clouding.

GAG deposition in connective tissues of the brain and spinal cord causes thickening of the dura which along with distortion of vertebraeresults incompression myelopathy. Thickening of the arachnoid membrane impairs CSF flow, causing communicatinghydrocephalus. But the most devastating neurological effects of MPS are due to neuronal storage of gangliosides. The mechanism of this storage is poorly understood. It is probably due to inhibition of neuraminidase and other lysosomal enzymes induced by the storage of GAGs. Thus, in addition to the skeletal, cardiovascular and other lesions, many MPS also cause neuronal lipidosis. Gangliosides stored in

nerve cells take the form of concentric membranes (membranous cytoplasmic bodies) or stacks of membranes (zebra bodies).

(e) NIEMANN-PICK DISEASE TYPE C

Type A and B Niemann-Pick disease are **neurovisceral storage diseases caused by deficiency of sphingomyelinase**. Niemann-Pick type C (NPC) is an LSD with protean clinical manifestations including neonatal hydrops, neonatal hepatitis, storage histiocytosis and neuronal lipidosis. The material that is stored in lysosomes in NPC is not sphingomyelin but cholesterol. Patients with NPC can import LDL cholesterol into lysosomes and remove the cholesteryl ester generating free cholesterol, but they cannot move free cholesterol to its normal cellular destinations. Thus, cholesterol accumulates in lysosomes. The mutant gene is located on 18q and its product, the NPC1 protein, is a transmembrane protein which acts as "gatekeeper" in the transport of lysosomal cholesterol to its other cellular targets. The "filipin test", which is used for diagnosis of NPC, consists of feeding cultured fibroblasts with LDL cholesterol tagged with the fluorescent dye filipin. The fibroblasts show bright fluorescence due to accumulation of cholesterol. NPC is rare but its study has produced some important insights into intracellular cholesterol homeostasis and trafficking.

STRATEGIES FOR TREATMENT OF LSD'S or DRUG DELIVERY TO LSD's

- Traditional treatments for LSD's include
  1. **Bone marrow transplantation**
  2. **Enzyme Replacement Therapy**
  3. **Gene Therapy**
  4. **Small Molecule Therapy**
  5. **Substrate Reduction Therapy**
- Specific targeting to Lysosomes by replacing deficient Lysosomal enzyme.
- Purify the enzyme from human placental tissue. 3 different Glycosidases to remove terminal sugars on the enzyme's oligosaccharide chains expose mannose residues.
- The bloodstream mannose receptor on the surface of macrophages taken up by Endocytosis delivered to the natural target site

1. Bone marrow transplantation

- Replacement of the deficient enzyme activity by intravenous infusion of hematopoietic progenitor cells eligibility Transplantation occurs early (under three years).
- Transplantation occurs in the setting of appropriate neuropsychiatric follow-up, assessment, and support. A suitable donor is available.

2. Enzyme Replacement Therapy:

- Recombinant enzyme is administered to the patients intravenously in every week.

- This was effective and approved to treat patients with the non-neuropathic form of Gaucher disease, Fabry disease, Pompe disease, Mucopolysaccharidosis I (MPS I), MPS II and MPS VI.

- Patients treated with ERT show clinical benefits, this strategy is limited by the high cost and life-long dependence on 4–5 hours infusions.

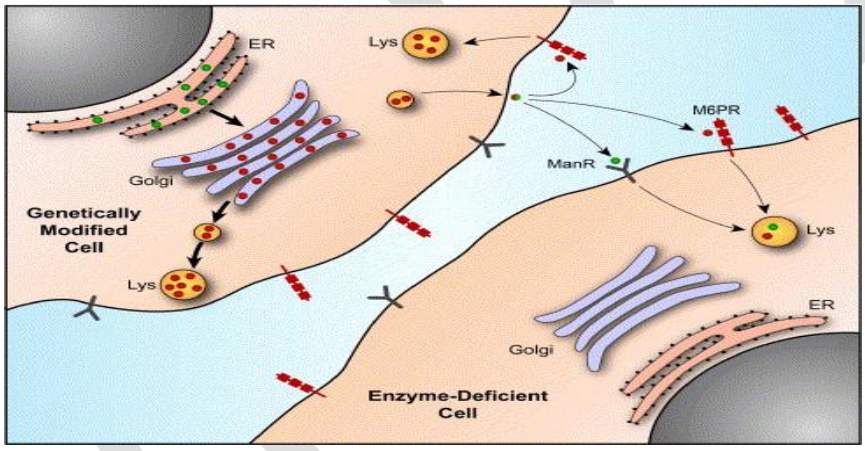
- ERT has limited ability to treat neurological and skeletal pathologies.

3. Gene Therapy:

Two different approaches to gene therapy:

1. Direct delivery of genes to specific organs, such as the central nervous system using viral vectors.
2. Genitically altering hematopoietic stem cells from patients to produce the missing enzyme and then returning the altered cells to the patient through bone marrow transplantation.

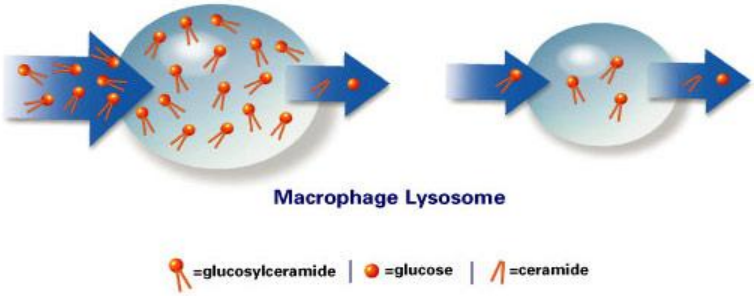
   Eg: In Gaucher disease, the glucocerebrosidase gene was transferred into autologous CD34+ cells through a retroviral vector.



4. Substrate reduction therapy:

- Reduction of the formation of the Lysosomal substance down to a rate at which the residual enzyme activity can catalized and incoming Lysosomal substance.

- **Disadvantage: SRT can be used only in the presence of residual enzyme activity**



Before and After SRT

5. Mechanisms for Lysosomal Delivery:

- Endocytosis provides a natural mechanism for delivery of exogenous enzyme to the storage material in the Lysosomes.

- Lysosomal enzymes are Glycoproteins to exploit Endocytosis mediated by the carbohydrate-recognizing receptors to enhance the uptake of circulating enzyme and to target the enzyme to different cells.

Two Mechanisms:

A. Clathrin-Dependent Endocytosis:

- Clathrin-dependent mechanisms including cell adhesion molecule (CAM) assisted RME pathway.
- Intracellular vesicles form invaginations in the membrane that are coated by the triskelion protein clathrin populating the cytoplasmic face of the membrane.
- Coated pits cover 1–2% of the plasma membrane surface area and allow for rapid intracellular vesicle budding, occurring as quickly as 1 minute.
- Clathrin-mediated endocytosis serves as main mechanism of internalization for macromolecules and plasma membrane constituents for most cell types.

B. Fluid Phase Endocytosis:

- FPE is a nonspecific adsorptive pinocytic mechanism which allows for the cellular incorporation of molecules contained in the extracellular fluid.
- Molecules absorbed via this pathway avoid direct binding with membrane constituents possess nonspecific charge and hydrophobicity membrane interactions.
- This process accompanies receptor-mediated absorption, internalizing receptor-ligand complexes and extracellular fluid

CARRIERS FOR LYSOSOMAL DRUG DELIVERY:

- The following methods used for effective Lysosomal drug delivery:

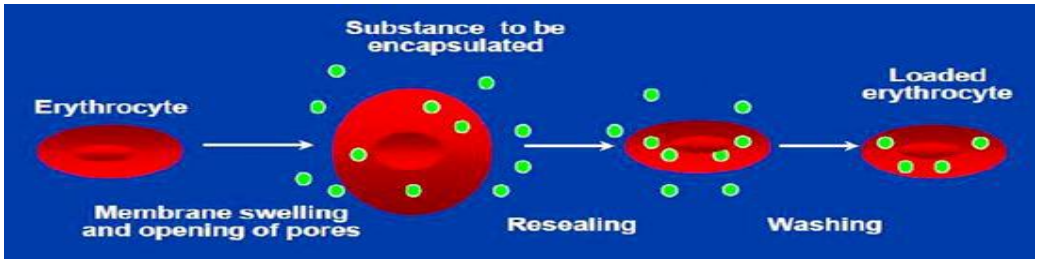
1. Resealed Erythrocytes
2. Liposomes
3. Microcapsules
4. Nanocarriers

1. Resealed Erythrocytes:

- The entrapment of enzyme in erythrocytes using Hypotonic haemolysis methods has enabled erythrocytes to be used as enzyme carriers in Enzyme Deficiency Therapy or Enzyme Replacement Therapy.

- These cells release enzymes into circulation upon haemolysis and act as a “circulating bioreactors” in which substrates enter into the cell, accumulate enzymes in RES upon haemolysis for future catalysis.

- Resealed Erythrocytes have been used for replacement of enzymes in lysosomes.



Mechanism of Resealing of erythrocyte:

Examples:

- Resealed erythrocytes have been proposed to deliver Lysosomal enzymes to lysosomes of the erythrophagocytic cells, replacement of the missing enzyme. Ex: β-glucoronidase, β-galactoronidase and β-glucosidase.
- Gaucher's disease was treated by encapsulating glucocerebrosidase in erythrocytes.

2. Liposomes

- Liposomes are pharmaceutical nanocarriers for replacement enzymes protect from inactivation in *in vivo* and enhance intracellular delivery, transport into lysosomes.

- Potential ability of liposome encapsulated immobilized enzymes to enter lysosomes of liver cells and is used for treatment of inherited diseases.

- Eg: Ability of liposomal β-galactosidase obtained from mixture of lecithin, cholesterol and sulfa tide to degrade GM1-gangliosidase in Lysosomes with pathological accumulation of this substrate was useful in treatment of globoid cell leukodystrophy.

- Liposomal β-glucocerebrosidase for gaucher disease was captured by kupfer cells in liver.

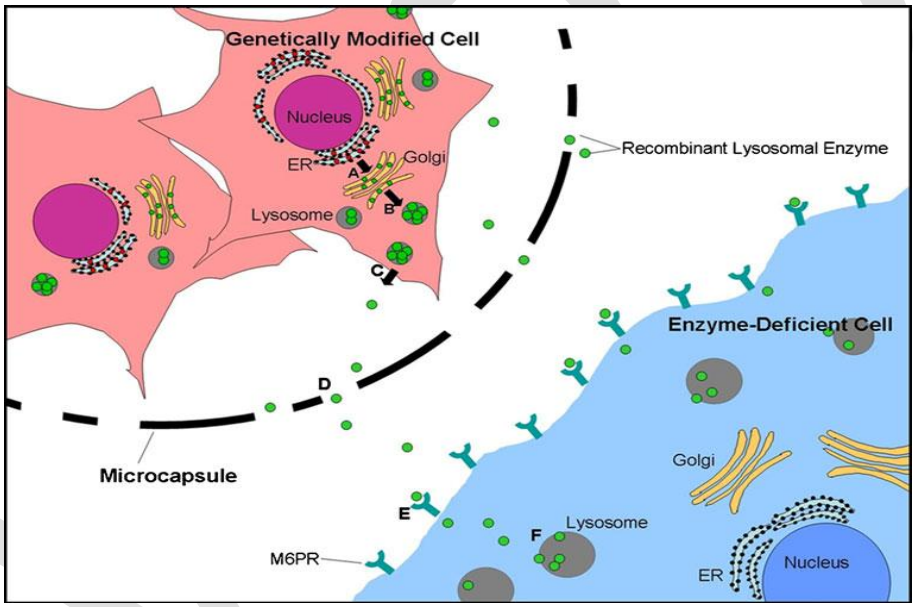
- Modification of Liposomes with mannose residues increased the capture because of presence of mannose-specific receptors on target cells.

- Liposomes are biodegradable lipid vesicles with concentric lipid bilayers alternating with aqueous compartments. Water soluble substances like enzyme can be entrapped in these aqueous compartment..
- Advantage is use of Liposomes is that they can be targetted to the diseased tissue.
- Liposomes are prepared by incorporating glycolipids such as cerebrosides into the matrix with the carbohydrate moiety of the glycolipids exposed on the surface of the Liposomes.
- Modification of the Liposomes by incorporating various glycolipids having non reducing terminal sugars facilitate the targeting of the Liposomes to different tissues carrying different terminal sugar specific receptors .
- Liposomes containing asialo GM1 ganglioside, when administered intravenously accumulated in the liver.

3. Microcapsules

- Clinical trials have been performed with gene therapy for different LSD's but has more side effects.

- An alternative- cell microencapsulation technology, a nonviral approach for the delivery of biologically active compounds, can overcome these hurdles and treat LSD.
- Cell microencapsulation is an approach in which cells are trapped in a semipermeable membrane, allowing the exchange of metabolites and nutrients between them and the external environment.
- The membrane prevents the access of the immune system to the cells, without the need for continued immunosuppression of the host.
- This technique allows the localized and controlled release, and long term duration of therapeutic products derived from the microencapsulated cell.
- Alginate, Agarose, chitosan and hyaluronic acid are polymers used for microencapsulation.
- Other materials used for cell encapsulation are diffusion chambers and hollow fibers, usually made with polyacrylonitrile vinyl chloride or polyethersulfone.
- Used in combination with some biomaterial remains in the interface between the cells and the fibers.
- LSD's are good candidates to be treated by somatic gene therapy based on cell microencapsulation.



The nascent lysosomal enzymes are glycosylated in the endoplasmic **reticulum of the genetically modified cells.** Traffic of lysosomal enzymes throughout the encapsulated cells.

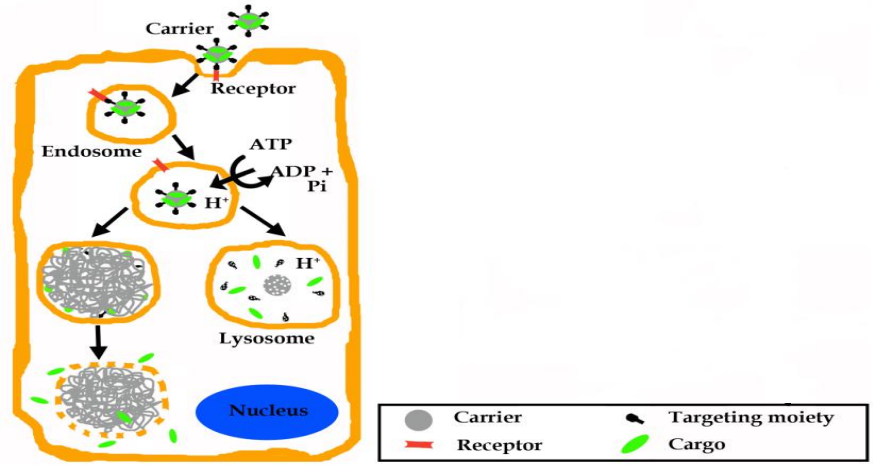
(a) The enzymes are phosphorylated at the residue of mannose-6-phpsphate in the Golgi apparatus
(b) Most enzymes are transported to mature lysosomes
(c) Some, however are secreted to extracellular environment and
(d) to outside of the microcapsule
(e) Phosphorylated enzymes bind to the mannose-6-phosphate receptors of the enzyme deficient cells
(f) where they are endocyted and subsequently targeted to the lysosomes

4. Nanocarriers:

- As opposed to gaining access to the cell by direct penetration into the cytosol, uptake within endocytic vesicles and subsequent selective permeabilization of these compartments for cytosolic release.

- Endocytosis - A group of processes by which cells engulf extracellular material with their plasma membrane, followed by pinching off the resulting vesicles into the cytosol.

- Uptake by endocytosis is regulated by numerous pathways (clathrin- and caveolar-mediated mechanisms, macropinocytosis) transport of the internalized materials to endosomes and lysosomes.



Delivery of PEG modified dextranase, which achieved prolonged by bypassing immuno recognition by lysosomal accumulation of dextran.

CELL THERAPY:

- Cell therapy utilized as a therapeutic generating system, cells from healthy subjects or cells from the patient are implanted in the body to provide sustained production of a molecule in the diseased patient.
- Cell itself can act as vehicle of delivery and sustained effects can be achieved.
- This is beneficial in certain proteins, hormones and small molecules (neurotrophic factors) - display short circulatory longevity and are easily subjected to proteases, shortening their therapeutic effects.
- These molecules produced at the required site, cell therapy provides crossing of physiological barriers.
- This is done in implantation of alginate microparticles carrying recombinant fibroblasts for sustained systemic release of -glucuronidase for mucopolysaccharidosis VII.
- Local implantation of the encapsulated cells into the brain lateral ventricles circumvents the blood-brain barrier result in delivery of the secreted enzyme.

STRATEGIES FOR BRAIN DELIVERY

- LSD's effectively treated by intravenous infusion of the missing enzyme by Enzyme Replacement Therapy(ERT).
- Patients with metabolic disease affects the central nervous system (e.g.type 2 or 3 Gaucher disease) partially respond to intravenous ERT because replacement enzymes prevented from entry of the brain by the BBB.
- The mannose and mannose-6-phosphate receptors involved in cellular uptake and transport to the lysosome.
- To introduce a replacement enzyme into the brain by direct injection has limited parenchymal diffusion rates in the brain.

FORMULATION ASPECTS

- An agent - lysosomal hydrolase enzyme, can be incorporated into a pharmaceutical composition.
- The composition is useful to diagnose, anaesthetise, or treat inhibit, prevent, a condition characterised by an insufficient level of lysosomal hydrolase activity.
- The pharmaceutical composition can be administered to a subject suffering from a lysosomal hydrolase deficiency or someone who is at risk of developing said deficiency.
- The pharmaceutical carrier is compatible, non-toxic substance suitable to deliver the polypeptides to the patient.
- Sterile water, alcohol, fats and waxes may be used as the carrier.
- Pharmaceutically acceptable adjuvants, buffering agents, dispersingagents and incorporated into the pharmaceuitical compositions.
- The carrier combined with the agent in any form suitable for administration by intraventricular injection or infusion,etc…
- Physiologocal saline, bacteriostatic water,phosphate buffer saline,dextrose solutions, glycerol solutions, water and oil emulsions made with oils of petroleum, animal, vegetable, or synthetic origin(peanut oil, soya bean oil).
- An artificial CSF can be used as a carrier.
- The carrier preferably be sterile and free of pyrogens.
- For ventricular administration, the composition must be sterile fluid and stable during manufacturing and storage.
- Prevention of the action of microorganisms can be achieved by antibacterial and antifungal agents,

Eg: parabens, chlorobutanol, phenol, ascorbic acid.

- Dosage of lysosomal hydrolase enzyme vary from individual to individual, depending on enzyme and its specific *in vivo* activity, the route of administration, the medical condition, age, weight or sex of the patient.

CURRENT AND FUTURE DEVELOPMENTS

- The cumulative incidence of the various LSD subtypes is relatively high, individually they are infrequent to rare clinical entities.

- The LSD's are viewed by drug regulatory agencies as 'orphan' disorders and exists appropriate legislation to promote venture capital investment to develop drugs for these indications.

- A major challenge in the development of treatment for these conditions lies within the majority of conditions there is severe neurologic involvement, and in certain cases the cellular insult may be present.

- The broadening spectrum of current and potential avenues of treatment for the LSD's raises the prospects of a brighter future for afflicted families.

CONCLUSION

- Lysosomal diseases are genetically inherited diseases.

- Though there is no effective cure or treatment for these diseases, Enzyme replacement, Substrate deprivation or dispersal, Enzyme enhancement, Bone marrow transplantation and Gene transfer methods are used to overcome these diseases.

- Targeting a drug or enzyme to lysosomes is mainly by endocytosis.

- Targeting nanomedicine complexes to the endolysosomal pathway have serious potential for improving drug delivery for the treatment of Lysosomal Storage Diseases.

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