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J COMPONENT

ENHANCING THE ORAL BIOAVAILABILITY OF ANTIHYPERTENSIVE DRUGS

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Enhancing bioavailability of antihypertensive(Thiazide) drugs

1.On a survey of total adult population with hypertension, it was around 30% of people who have been taking medication to lower the blood pressure. As one in four adults suffer from hypertension in India.

2.As these condition have become more common and most of them suffer from the condition, it have became a long time illness with medication to be regularly taken.

3.Even though the medicine were regularly taken, there are numerous side effects and not the minimum dose of drugs met up in the body Which becomes a concern now.

*<u>**Usefulness of the Thiazide drug and their mechanism of action:**</u>

As they help to prevent high blood pressure and keeps the blood pressure at healthy level by:

1.reducing excessive amount of water and salts in the body and relaxing your blood vessels.

2.Making your heart beat with less force and blocking the nerve activity that restricts your blood vessel.

As the drugs main action centre is kidney which inhibits the sodium reabsorption thus elimination the salts from our body as it increase calcium reabsorption were also maintained.

As the main problem of the drug is poor bioavailability: minimum effective concentration is not met due to various reasons:

1.Poor Solubility of drugs: As it is slightly soluble in water and their aqueous solubility have to be improved or else it gets inactivated and gets eliminated from the stomach.

2.chemical,enzymatic and pH barrier in GI tract causes degradation of drugs as they were quite acidic in nature.

3.Inappropriate partition coefficient: it influences permeation of drugs through lipid membrane(low ionisation of drugs between two phases)

4.P-Glycoprotien meditated efflux: a drug transporter that determine the range of drugs that has to be uptake and efflux inside and out of the cell. There presence in liver, kidney causes absorption reduction and lowers their bioavailability.

So basic preliminary solution is to increase the drug’s dose viz anti-viral drugs but we get struck into a dilemma as increasing their dose may lead to Hypokalemia (results into low bp and heart disease)

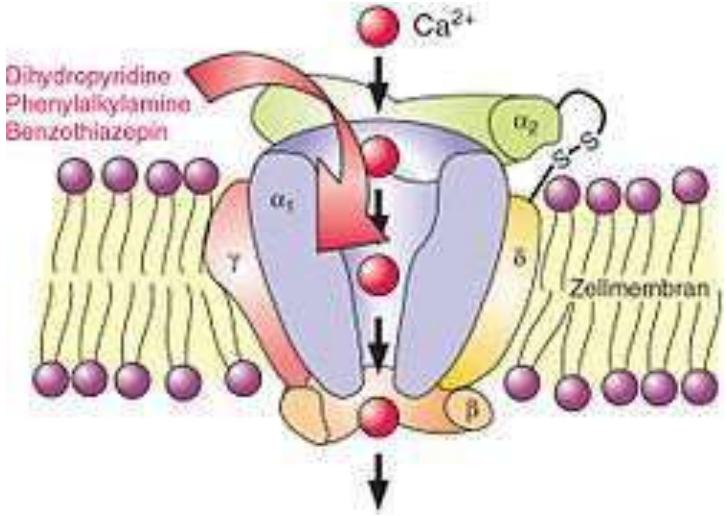
How do thiazide acts?

- They control hypertension by inhibiting reabsorption of Sodium and Calcium ions from Distal convoluted tubules in the kidney by blocking Na+Cl- symporter to the cells. It blocks the transportation of Na+ ions into the cells and bloodstream.
- Thus it allows excretion of salt and water as urine.
- This also increase Calcium reabsorption at the distal tubule and lowering the concentration of Sodium in epithelial cells creating an Na+/Ca2+ antiporter to maintain intracellular Na+ level facilitating Ca2+ to leave the epithelial cell into renal interstitium.
- As Calcium reabsorption also gets increased in response of sodium depletion.

- The thiazide diuretics increase sodium delivery to the distal segment of the distal tubule, this increases potassium loss (potentially causing *hypokalemia*) because the increase in distal tubular sodium concentration stimulates the aldosterone-sensitive sodium pump to increase sodium reabsorption in exchange for potassium and hydrogen ion, which are lost to the urine.
- Part of the loss of potassium and hydrogen ion by loop and thiazide diuretics results from activation of the renin-angiotensin-aldosterone system that occurs because of reduced blood volume and arterial pressure. Increased aldosterone stimulates sodium reabsorption and increases potassium and hydrogen ion excretion into the urine.

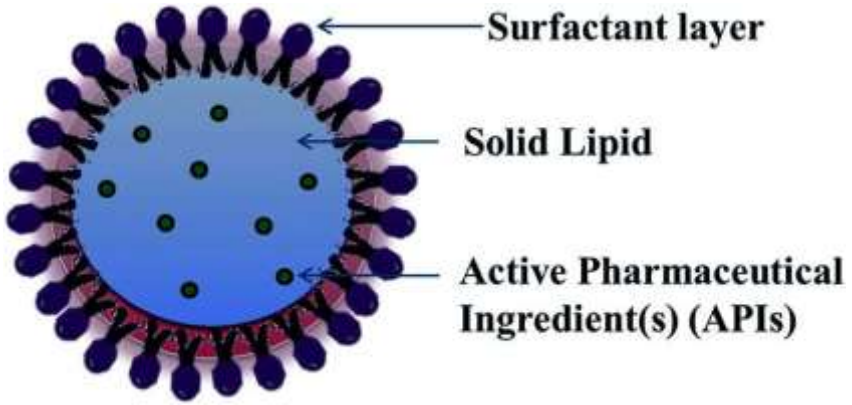
•Also the Calcium reabsorption plays a major role when sodium gets depleted and indirectly becomes one of the reason for Hypokalemia.

•To lower down the Calcium reabsorption, Calcium channel blocker comes in place with adding dose of amlodipine/alpha trinositol thus intracellular Ca2+ level gets reduced.

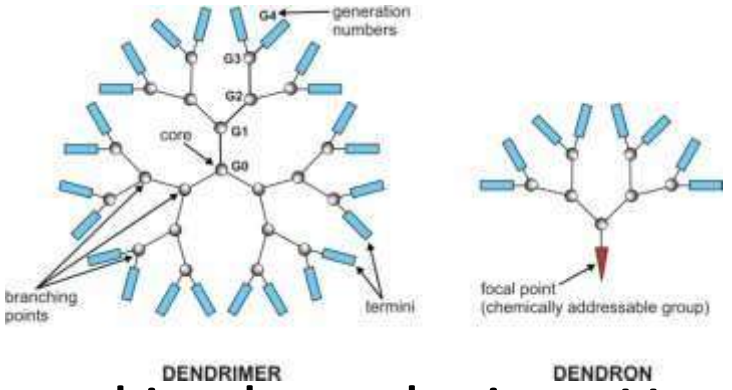


Nanotech based method were one such effective strategies to improve bioavailability, Solubility of antihypertensive drugs. They are:

1.Solid Lipid Nanoparticles: submicron colloidal carrier which is composed of physiological lipid, dispersed in water or in an aqueous surfactant solution. And the drug were embedded inside the lipid matrix. It is non-biotoxic and biodegradable.Here the drugs were encapsulated and holds the drug inside well so the drugs doesn't get eliminated inside the stomach and enhances lymphatic absorption(low 1st pass metabolism).



- **<u>2.Dendrimers</u>**: tiny synthetic polymers with several branching structure and drugs were loaded at the bases of the branch. It promotes aqueous solubility and drug enters into the blood stream.



- **<u>3.Nanosuspensions</u>**: Colloidal particle dispersed in the solution. Here the size of the drug were reduced to 1micro meter with help of a zirconium bead so that high dose of medication can be given and it has high dissolution rate.

REVIEW 2

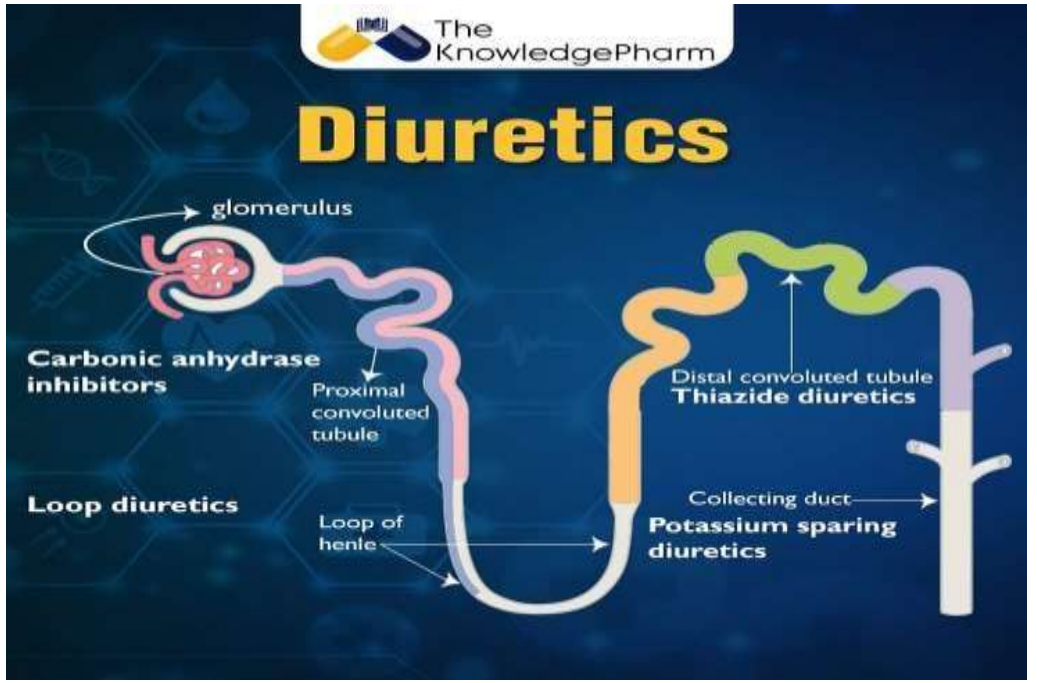
INTRODUCTION

- In our pervious review, we discussed about major factors that were affecting poor bioavailability of anti hypertensive drugs and pharmacokinetics and pharmacodynamics of thiazide drugs.
- Few nanoparticle based delivery system to improve solubility, effective drug distribution and preventing degradation.
- In the present review paper, we are going to discuss about some of the other nano-particle based drug distribution strategies that may improve effective and disadvantage of Solid lipid nanoparticles.
- Gene silencing of hypertension inducing receptor.

Rationale for using nanoparticles

- Oral route is the preferred way of drug administration. But the delivery of drug exhibits low aqueous solubility and permeability(BCS class IV) as the bioavailability of drug is very low and Ph of GIT varies for stomach(acidic) to intestine(basic). With the wide distribution of Ph it severely hamper the pharmacological activity of thiazide drug by oxidation and hydrolysis of protein drugs.
- Bioavailability gets decreases by various factors such as chemical degradation acidic Ph and enzymes like P450, protease cause significant degradation of antihypertensive drugs. Intestinal mucosal hinders drug permeation.

- Mucosal barrier consists of extrinsic barrier (microenvironment near the vicinity of mucus layer) and intrinsic barrier (epithelial cell monolayer). Intrinsic barrier is due to the presence of tight junction between adjacent cells.



MECHANISM OF DRUG DELIVERY

- Different mechanism by which any molecule can cross this barrier includes transcellular, paracellular, and transcytosis. Transcytosis being active transport pathway restricts large sized and charged molecules. When the mucosal barrier is permeated, molecules have to cross lamina propria where blood capillaries lie and molecule can get entry into the blood stream. Strategy to overcome intestinal barrier was to prepare mucoadhesive formulation which increases the contact time of the formulation with mucus thereby increasing drug concentration at the site of absorption

- Many mucoadhesives have the property of acting as permeation enhancer which can open tight junction and paracellular transport becomes possible. Another way to enhance GI permeability is transport through M cells. M cells have less quantity of protease enzyme and lacks mucus secretion. Lipophilic molecules have improved M cell transport.
- Lipid nanoparticles like SLN are transferred through intestinal barrier by clathrin-mediated transport. SLN is also transcytosed by caveolae-mediated endocytosis while NLC is transported by paracellular transport through tight junctions . Different nanoparticulate systems have been investigated to circumvent first-pass metabolism through lymphatic transport and includes nanoemulsion, liposome, SLN.

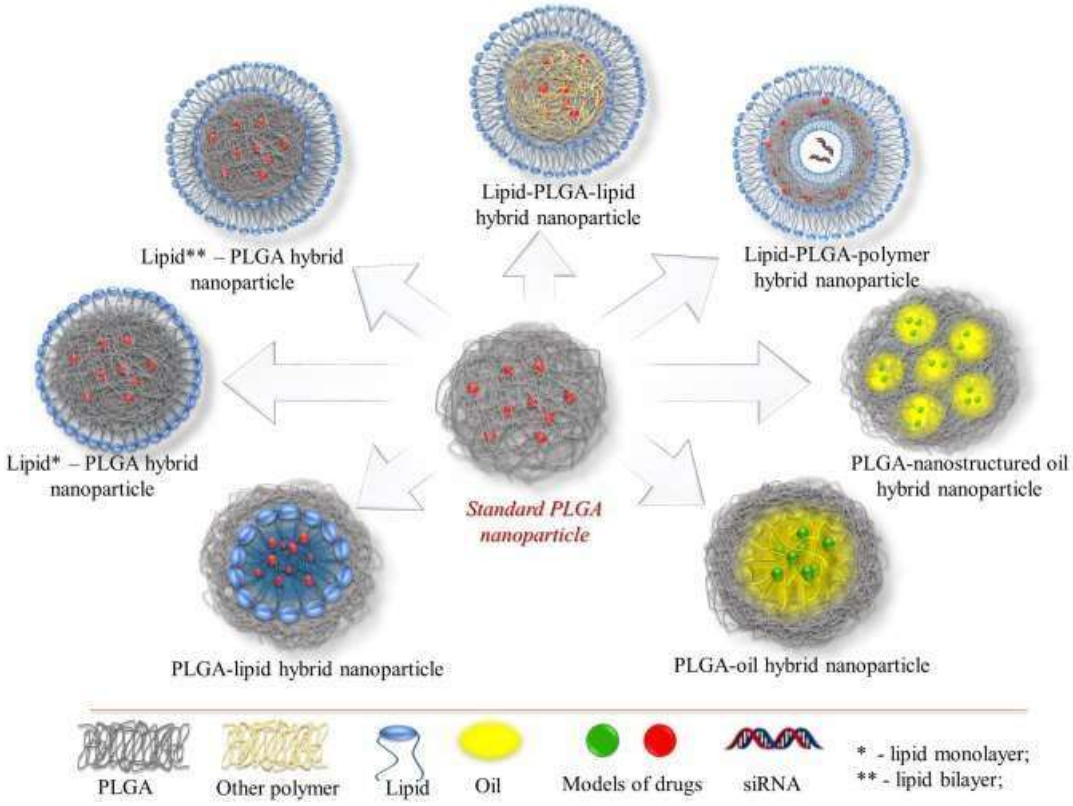
RATE OF REACTION

•Size range of 100–500 nm has been proposed to be ideal in the lymphatic uptake but rate of absorption is faster when size is below 100 nm. Negatively charged nanoparticles show higher lymphatic uptake than positively charged and neutral nanoparticles. Lipophilicity acts as an add-on for lymphatic uptake of drug. NLC of hydrophilic drugs acts as a better approach for enhancing the uptake of such drugs. Furthermore, efflux transporter like P-glycoprotein present on the intestinal wall causes efflux of several antihypertensive leading to poor oral bioavailability. Drug encapsulated in nanoparticle can avoid all these constraint and sustained action can also be achieved leading to dose reduction and frequency of dosing.

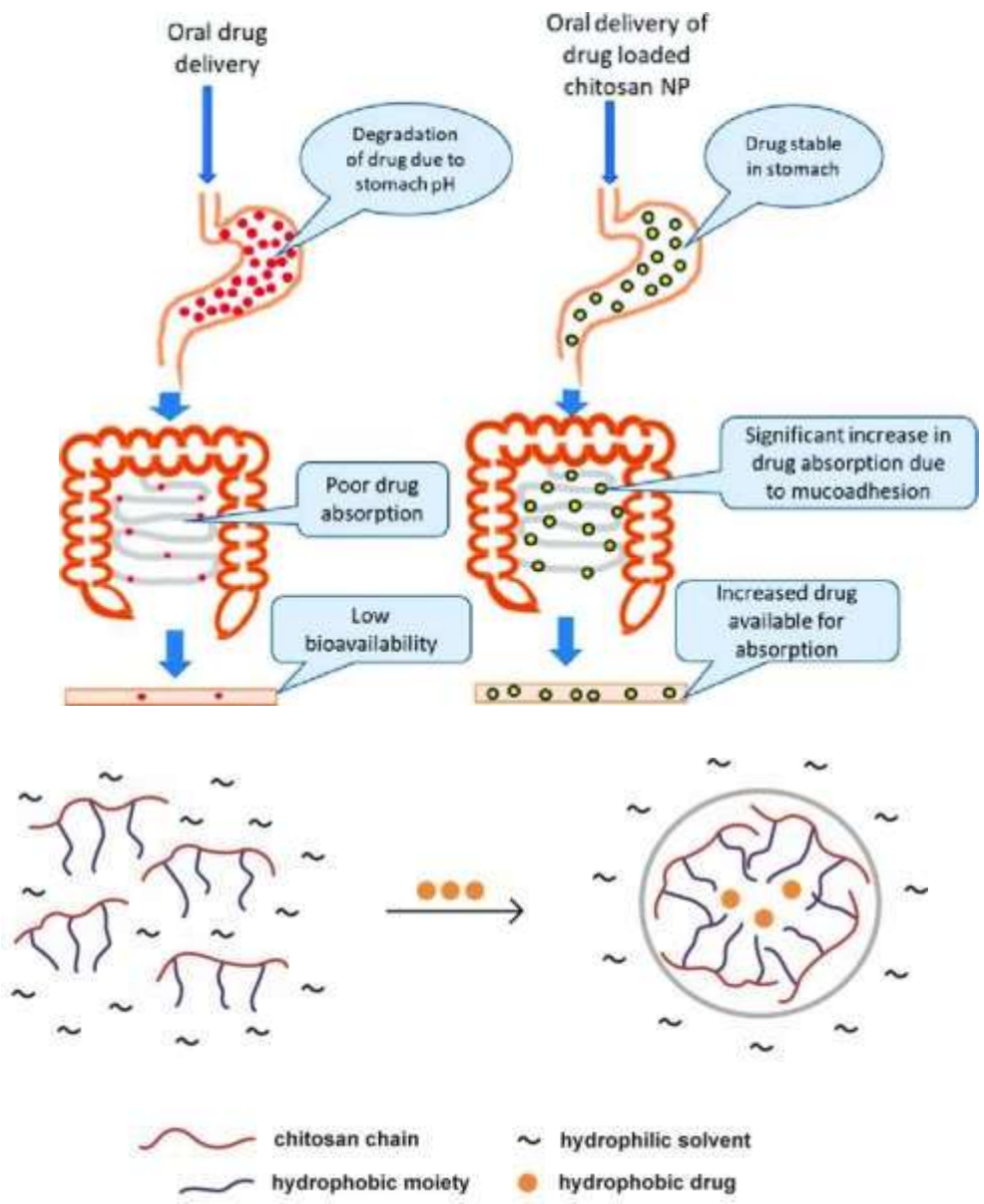
- As the thiazide drugs were Ph sensitive and to prevent acidic degradation they needed to be targeted in intestine/colon specific region. The Ph sensitive polymers can target the drug at specific region of GIT. Copolymers like EudragitS100/L100 can be used to target the drug at colon while it is suspectible to degradation at the upper part of GIT and enhance the bioavailabilty of the drug.
- Rate of drug release by the polymer is very fast. Drug release at the site of absorption creates concentration gradient which helps in permeation of drug from the site of absorption causing increase in drug Bioavailability. It was studied that the release of drug from the polymer was pH responsive and was evident to occur at the pH of the colon. There was avoidance of drug metabolism mediated by cytochrome P450 in the liver and gut wall. Thus it was concluded that the formulation has the capability to enhance oral bioavailability of the drug.

METHODS

PLGA [Poly(lactic-co-glycolic acid)] are made up of lactic acid and glycolic acid monomer which are endogenous and are degraded easily, so the toxicity associated with these NPs is minimal. PLGA is US FDA and EMA approved. They are available in different form depending upon the ratio of the monomer. They can entrap both hydrophilic and hydrophobic drug and can provide sustained release profile from days to years depending upon the ratio of the monomer. They can also be used to target specific tissue or organ after modifying their surface (Danhier et al., 2012). Shah et al. prepared PLGA NPs of felodipine.

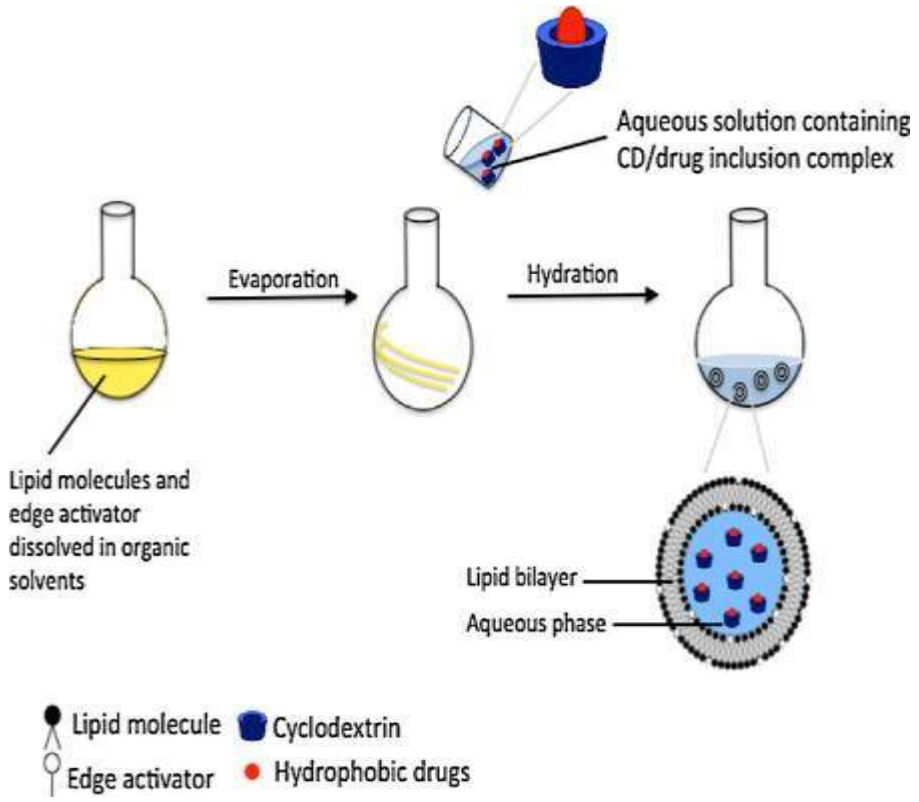


Chitosan is natural biodegradable, biocompatible, and nontoxic to human body. Chitosan is bioadhesive linear polysaccharide which is used as sustained release and site-specific delivery system for many drugs, including antihypertensive. Chitosan nanoparticles have enhanced the oral bioavailability of antihypertensive by preventing first-pass metabolism and degradation at acidic pH at upper GIT as chitosan are degraded by colonic microbes where pH is basic prepared ramipril-*β*-Cyclodextrin complexed nanoparticles of lecithin/chitosan. *In vivo* result showed 1.6 times decrease in systolic blood pressure of deoxycorticosterone acetate salt induced hypertensive rats. Chitosan nanoparticles emerged as a solution for oral administration of antihypertensive which are poorly soluble.



Lipotomes

This is another lipid-based novel dual-functioning nanocarrier developed by ElKasabgy for furosemide a poorly soluble drug. Lipotomes were prepared using lipid cetyl alcohol and surfactant Tween 80 by thin film hydration technique. Its dual function as claimed by the researchers is enhancement of drug solubility and bypassing first-pass metabolism of drug. Researcher compared enteric-coated lipotomes with enteric-coated lipid formulation without Tween 80 and marketed tablets. They found significant increase in the value of C_{max} of lipotomes (7.66 ± 3.52 ng/ml) than Tween 80 control preparation (3.62 ± 1.19 ng/ml), and marketed preparation (2.11 ± 0.81 ng/ml) showing efficacy of the lipotomes being absorbed efficiently. Also the relative bioavailability of lipotomes was 5.4 as compared to Tween control formula (relative bioavailability = 3.68). This result shows that the application of lipid excipient and surfactant Tween 80 individually plays vital role in enhancing clinical performance of the drug. The reason provided was that Tween 80 increases GI permeability, and lipid entrapped drug is circumvented by first-pass effect apart from its lymphatic uptake

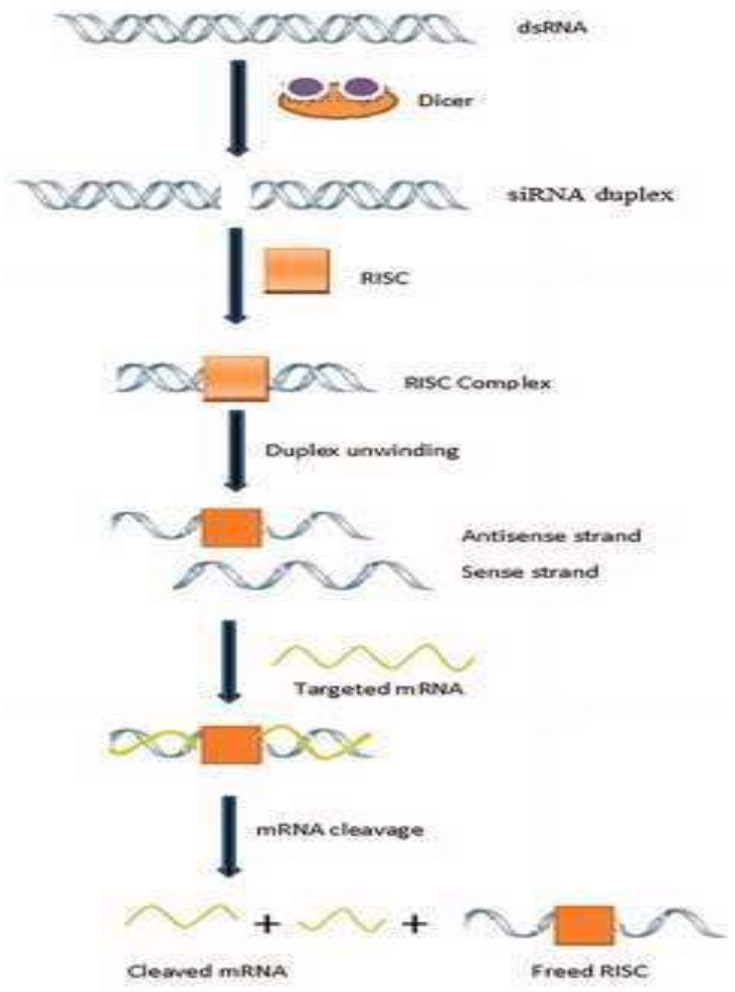


Limitation of using SLN:

- SLN has some limitation associated with it like expulsion of drug due to organization of solid lipid into more perfect crystal with time, which results in decrease in entrapment efficiency and loading capacity with time.
- This drawback associated with the SLN led to the development of NLC which is composed of liquid lipid apart from solid lipid. Liquid lipid is present within the solid lipid and does not undergo modification into stable structure; also solubility of drug in liquid lipid is higher than solid lipid, this results in enhancement of entrapment efficiency and loading capacity

Gene silencing to treat Hypertension:

- It is to make target mRNA non functional by it's cleavage. The mechanism of gene silencing is done by small interfering RNA produced by endonuclease "Dicer" leads to the formation of SiRNA duplex. RISC(RNA-induced silencing complex) gets incorporated into SiRNA duplex.
- SiRNA duplex undergo unwinding by RNA helicase resulting in antisense strand which remains with RISC (called activated RISC) while sense strand is degraded by exonuclease. This activated form of RISC binds with target mRNA and then RNAase activity is initiated by antisense strand of activated RISC.



- mRNA is cleaved into inactive fragments which become nonfunctional for protein synthesis. Thus gene silencing occurs, and receptor protein is not synthesized
- Gene silencing of particular receptor can help in regulating blood pressure. Small interfering RNA causes sequence-specific gene silencing thus receptor protein, which is the target of interest here is not synthesized.
- For example, AT1147siRNA was used by Vazquez et al. to silence AT1a, the subtype of angiotensin receptor, thus angiotensin II binding to this receptor is affected. siTRPC3 was another siRNA which decreased the expression of calcium-permeable transient receptor potential channel (TRPC). The protein level of which was decreased by using siRNA. These studies show promising approach using siRNA for treatment of hypertension

Conclusion:

- As these polymer and lipid based drug delivery have high beneficial in enhancing the bioavailability by eliminating first pass metabolism and problems like fast elimination of drug. Like these polymer based drug can be used for increasing dosage with level of increasing the ratio of monomer as well as sustained release of drug with long lasting action.

REVIEW 3

Introduction:

1. Angiotensin is a potent chemical formed in the blood causes muscles surrounding blood vessel to contract and also narrows cells.

2.The narrowing of blood vessel increase the blood pressure within the vessel and cause hypertension.

3.It also stimulates water and sodium reabsorption thereby increase in the blood volume and blood pressure.

4.Normal anti-hypersensitive drugs involves in blocking the action of Angiotensin converting enzyme 2(ACE2) binding with Angiotensin receptor 1(ATR1) on the muscle and surrounding blood vessel. So the blood vessel get enlarged and blood pressure get reduced.

Use of gene therapy medication for hypertension:

1.non-compliance by patients as single treatment may remain effective for many days and side effects were minimised.

2.It involves identification of effective target gene that is linked to hypertensive state and development of viral vector that can effectievely transduce transgene into cardiovascular relevant tissue and usage of promoter is also important.

3.But the limitation is on the choice of viral vector which should be able to transduce non-dividing cells with higher efficiency as tissue is terminally differentiated, should integrate with genome and long time expression of genome without any mutations. And it is quite of risk in using viral vector.

4.Eventhough wide variety of gene delivery vehicles are available but it involves in viral gene incorporation. So we use a novel strategy of incorporation naked DNA(antisense oligonucleotide) in the cationic liposomes will be a choice of method to deliver DNA across plasma membrane.

5.Cationic liposomes are safe, non-viral and can be successfully deliver DNA in vitro and vivo and have some limitations.

6.These liposome mediated delivery method also related to high cytotoxicity, low efficiency, low selectivity and transduction. These were some of the limitation that have to be rectified with various strategies.

Gene therapy for hypertension strategy

1.As we don’t know the exact pathophysiology of hypertension as it is not designated with particular tissue, as whole blood vessel gets involved and also cause multifactorial disorder.

2.Wide array of genes involved such as RAS, adrenergic receptors, endothelium etc. So targeting an antisense mRNA to any of the gene may reduce the synthesis of blood pressure increasing gene.

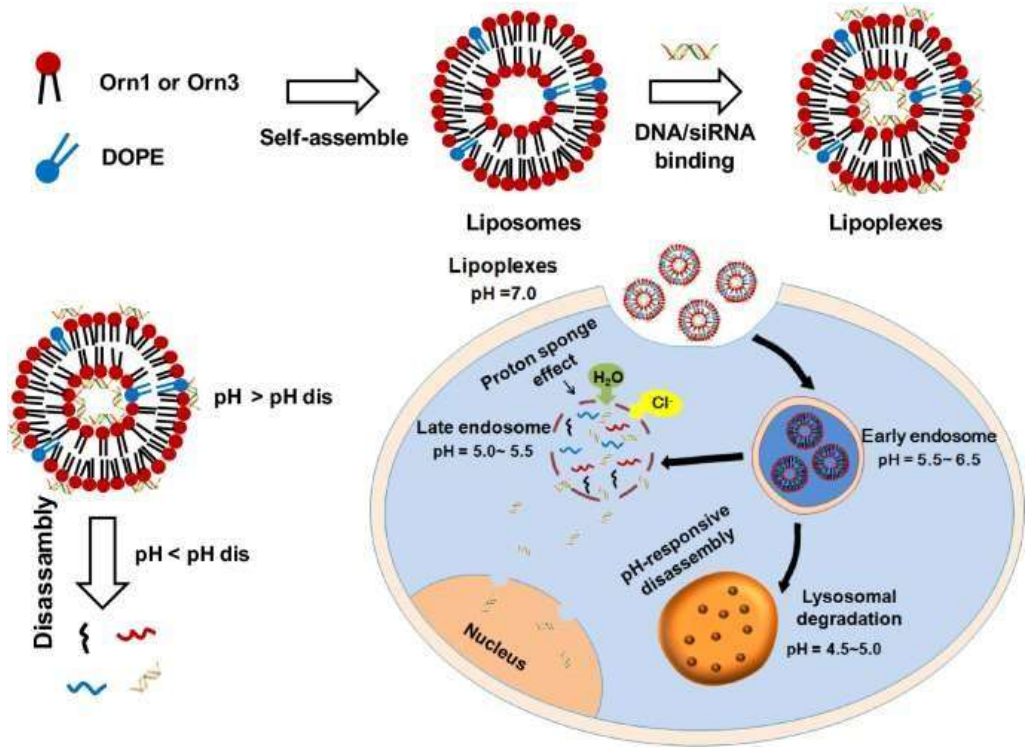
3.Gene therapy approach is based on gene silencing/ Knock down approach.

Gene ACE2 that increase high elevation of BP are targeted with anticipation of decreasing their transcription and translation resulting in RNA inhibition at genetic level.

It is done by designing an antisense mRNA that target to angiotensin gene with use of some primer designing equipments. So an antisense oligonucleotide(AS-ODN) of length 12-20bp is designed and hybridize specific mRNA that will prevent translation of specific protein.

on injecting antisense oligonucleotide by peripheral injection in rat models decrease in blood pressure upto 7days and also noticed decrease K+ and Ca2+ excretion.

Hypertension leads to the stage of hyperactive renin angiotensin system(RAS) leading to circulate angiotensin 2 by coordinated activites of kidney(renin), liver(angiotensin) and liver(ACE) and affects kidney, heart, arteries, brain and blood vessel.



Preparation of cationic liposomes DNA complex:

Here the DOTAP(1,2-Dioleoyloxy-3-(trimethylammonium)propane) and DOPE were used as main and helper lipids to deliver AGT1-AS-ODN(Angiotensin binding antisense oligonucleotide) to act as a novel angiotensin blocker with prolonged effect.

By optimizing the lipid/ODN ratio and with correct incubation procedure to produce antihypertensive effects with single dose upto 10 days. Distribution is up within blood vessel, kidney, liver and heart and most uptake takes place in the kidney.

1. DNA dilution were added in 0.4ml of opti-MEM.
2. Lipids were then added by gentle mixing.
3. Oligonucleotide and lipids were complexed in ringer's solution.

In vitro and clinical trial analysis of the DOTAP-ODN complex:

DOTAP complexed with short oligonucleotide increase the gene expression in blood vessel by 15 fold rather than using normal drug eventhough it is less efficient that viral vector.

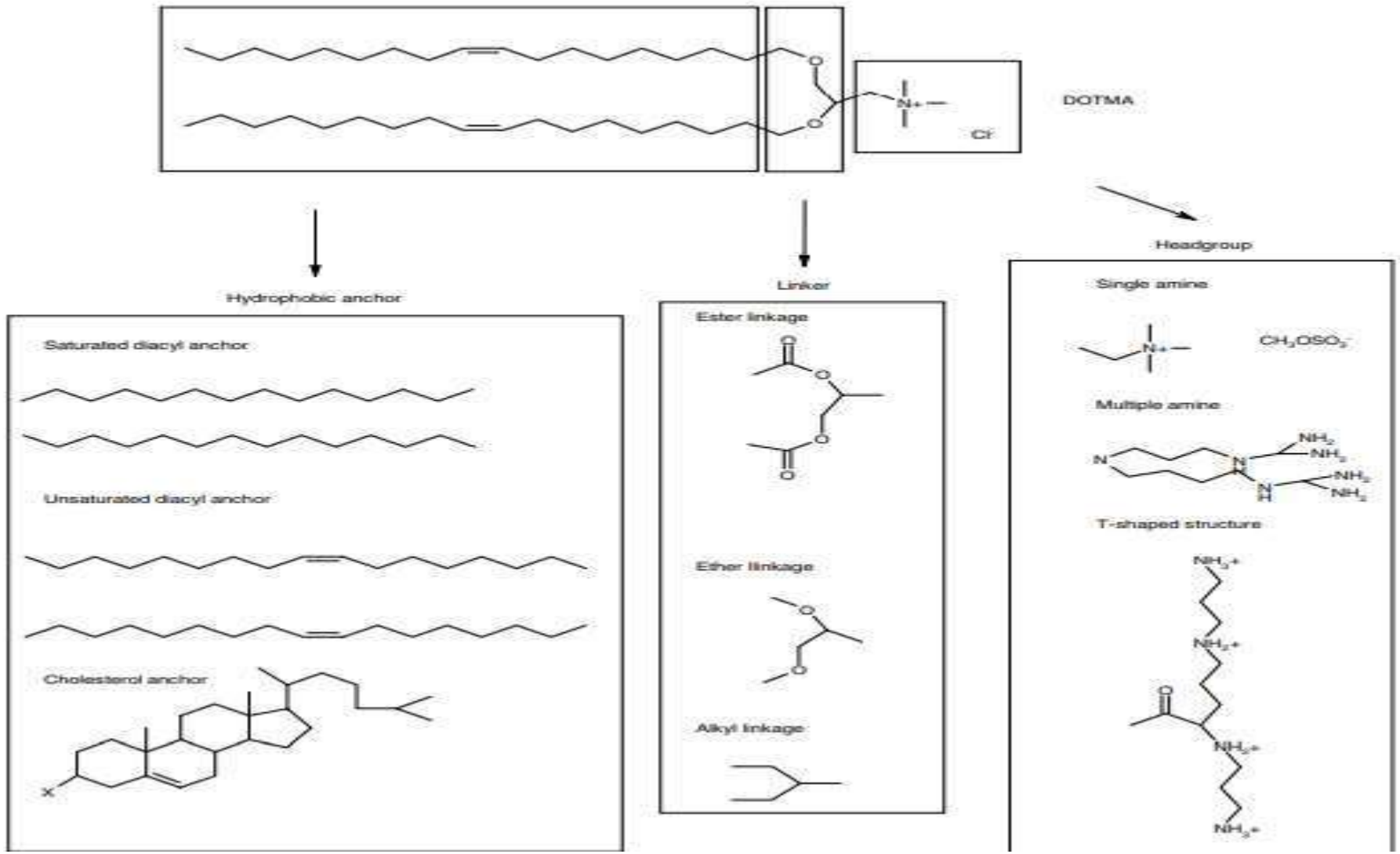
1.To determine optimal DNA: Liposome ratio:

cat promoter were complexed with DOTAP at increasing concentration from 6.25µg/ml to 60µg/ml, whereas the antisense oligonucleotide were kept constant concentration. Quantifying the stoichiometry of DOTAP and DNA interaction and their molar ratio were studied.

* High positive charged complex with condensed DNA results in homogenous size distribution(100-450nm)

*Negatively charged complex with more DNA will also have homogenous size distribution.

So equimolar ratio is the optimum concentration should be used for better transfection and to prevent DNA: liposomes aggregation results in heterogenous size distribution.


DOTMA
Cl
Hydrophobic anchor
Saturated diacyl anchor
Unsaturated diacyl anchor
Cholesterol anchor
X
Linker
Ester linkage
Ether linkage
Alkyl linkage
Headgroup
Single amine
$CH_3OSO_3^-$
Multiple amine
T-shaped structure

Also the size of linker groups such as alkyl chain length, ether linkages plays a major role in transfection efficiency and stability.

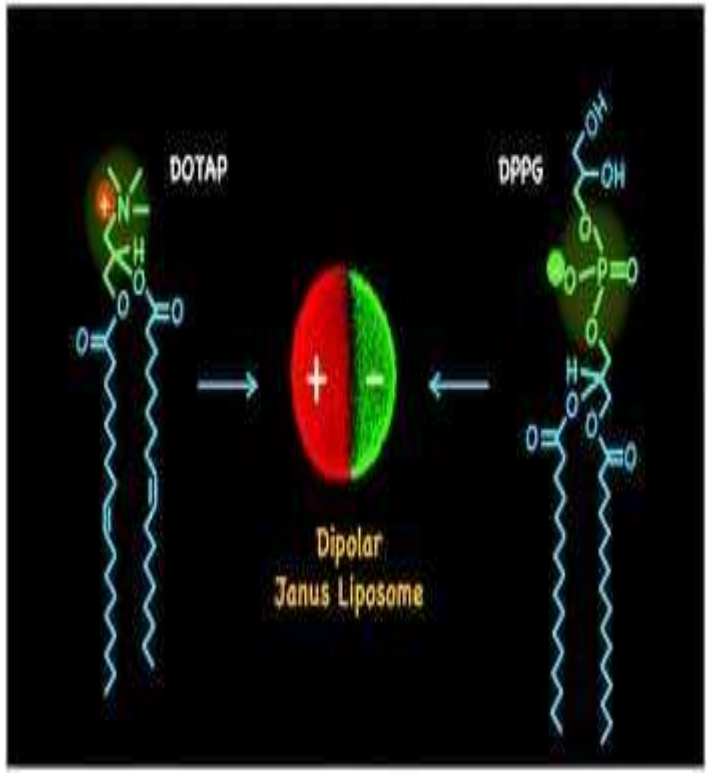
Increase in alkyl chain length results in decrease in transfection efficiency, so short length alkyl amine should be used in the liposomes.

Lipids with ether linkages such as DOTMA and DMRIE were more toxic than labile ester linkages such as DOTAP. So the nature of the linker group choosen on the basis of stability and biodegradability.

2.Effective transfection when using both DOTAP and DOPE rather than using DOTAP as cationic liposomes alone: because the usage of DOPE:

* undergo transition from bilayer to hexagonal configuration under acidic Ph which facilitate destabilisation of target membranes.

*helps in disassembling lipid based DNA formulation as the escape of DNA from endolytic vesicles happens and help bond weakening between lipids and DNA.



Ability to protect DNA against nuclease degradation is important regarding the biological activity of the complexes.

-negatively charged DNA cationic liposomes can't protect DNA efficiently and associates with nuclease degradation.

-positive charged cationic liposomes can protect DNA as they are condensed inside liposomes and thus obtain maximum resistance from different formulations.

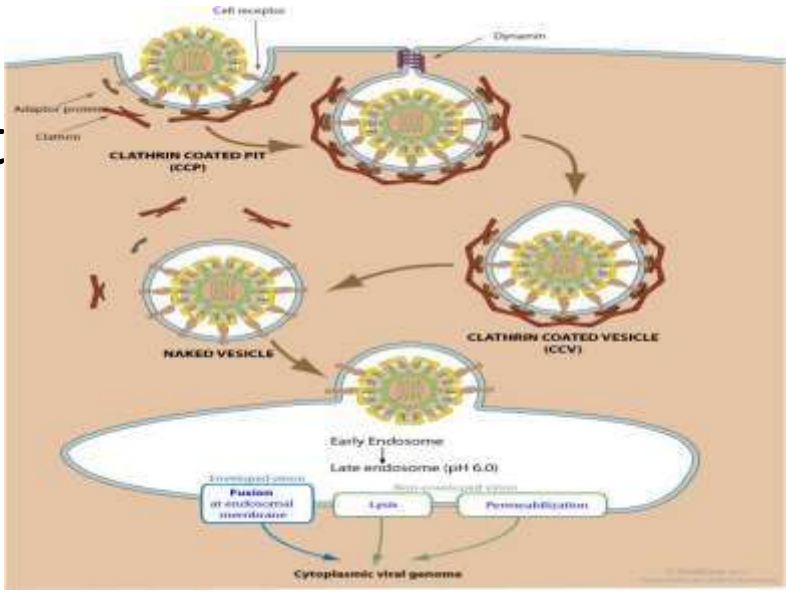
It will have high transfection, favours interaction and binding to cell surface as well as efficient protection of DNA against nucleases.

PEG modified cationic liposomes may increase the stability and storage of the DNA.

Particle size is a critical parameter for the cellular uptakes of lipoplex. As when the size of DNA-liposome complex size is more than 200nm there is a slow kinetics and facilitation and may cause endosomal escape.

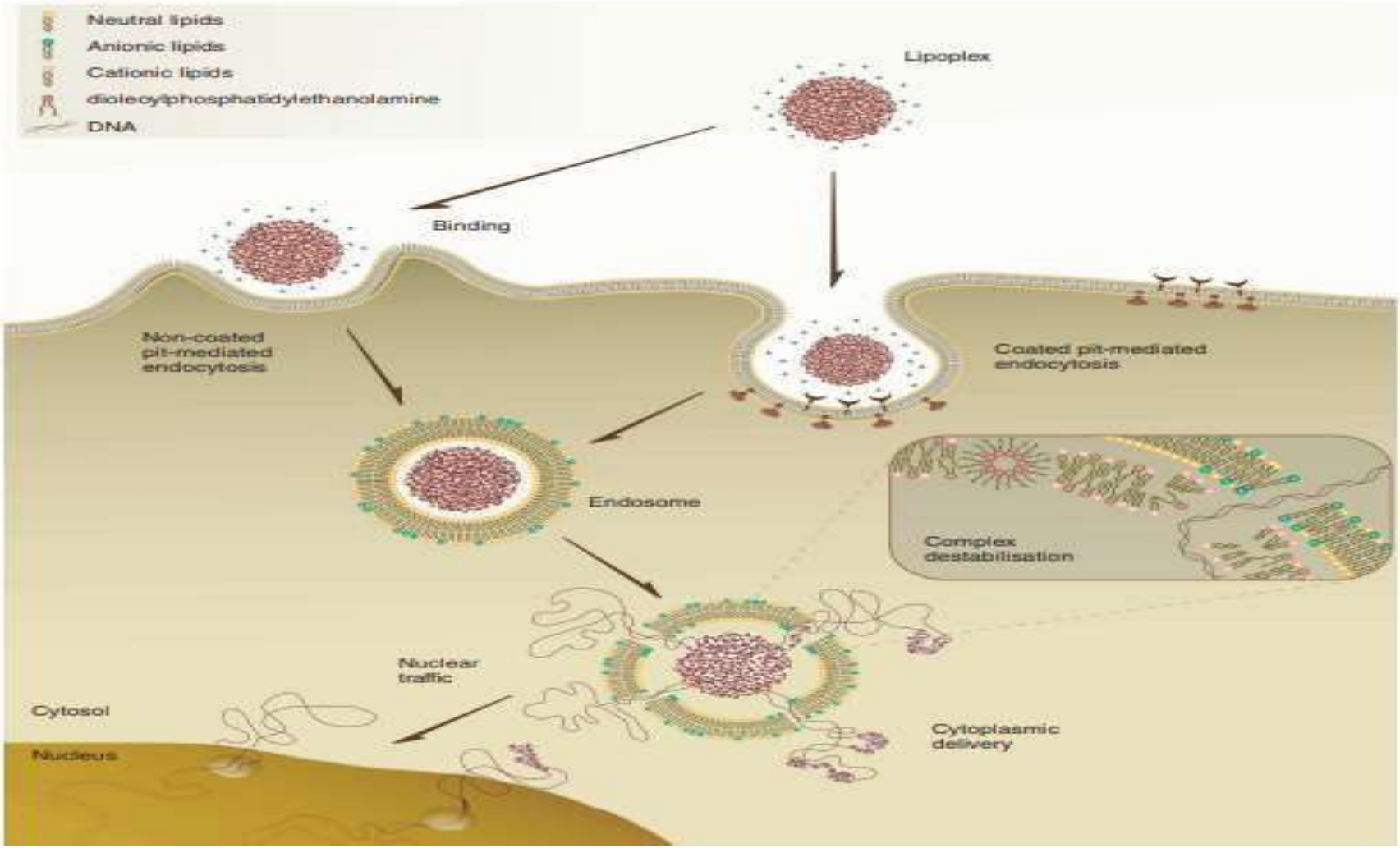
The DNA-liposome size ranging from 100-200nm were considered as optimum concentration and able to rapidly internalised via clathrin mediated endocytosis and reach lysosomal compartment readily.

To enhance cell internalisation by specifically target cationic lipid system to cells, through association of peptide ligands which direct the liposomes to bind towards receptor.



When cationic liposomes get interacts with the portions of blood vessel there is reduced in transfection efficiency and there is increase in disassociation of complex. It may also interact with macrophages, neutrophils and monocytes thus leading to disassembly and clearance of complex. So to overcome the problem, we use zwitter ionic lipids which may longer the circular time and lowers the toxicities.

Release behaviour of Cationic liposome and DNA complex:



A role for sulfated membrane-associated proteoglycans in the cell binding of cationic liposome–DNA complexes has been suggested . Sulfated proteoglycans are highly negatively charged components of cell membranes, and consist of a group of proteins covalently linked to one or more polysulfated glicosaminoglycan (GAGs) polysaccharides. These proteoglycans helps in the internalisation of the cells.

Clustering of proteoglycans bound to the particle triggers protein kinase C activity and the binding of actin to the cytoplasmic domain of proteoglycans, which then promotes the engulfment of the particle through a process similar to bacterial uptake.

Endosomal membrane destabilisation:

DOPE may be involved in helping the DNA dissociation from the lipoplexes due to the ability of its amine group to compete with cationic lipid for DNA phosphate groups, on lipoplex internalisation . It is possible that pore formation at the endosomal membrane may also be involved in the escape of the complexes or of free DNA into the cytoplasm.

In an attempt to improve the endosomal release of DNA, pH-sensitive fusogenic peptides have been associated to lipoplexes. This association is expected to result in a triggered destabilisation of the endosomal membrane on acidification of its lumen, in a manner similar to that used by certain types of enveloped viruses to infect their target cells.

Once in the cytoplasm, DNA has to reach the nucleus and surpass the nuclear membrane for transcription to occur.

it can be speculated that traces of cationic lipid still associated with DNA may play a role in the destabilisation of the nuclear membrane, thus facilitating DNA nuclear entry. In this context, studies by Zabner et al. have shown that microinjection of free plasmids into the nucleus of oocytes results in gene expression, whereas microinjection of lipoplexes do not, suggesting that lipid coating of DNA inhibits transcription.

Formulations:

1.GIT epithelial barrier are lipophilic membranes and strong barrier against genetic material and were not stable to the low pH in the stomach. The pH ranges from 1-2 in stomach and 8 in the large intestine. Our concern is the liposomes may not be stable in the broad pH range. Therefore Liposomes stability of function of pH by formulating liposomes in PBS solution at 6.4pH and then diluting them to pH in stomach intestine. Reproter genes may used to determine any gene alterations at stomach and intestine.

2.Digestive enzymes like bile salts, pepsin may breakdown cationic liposomes and genetic material during digestion. Invitro studies were happened by exposing liposomes at stimulated gastric fluid that contained pepsin for 30mins and then exposed at pancreatin for 30 mins.

On assessing the result with help of housekeeping gene using qPCR, some amount of cationic liposomes were digested and undigested liposomes showed 70% gene silencing effect. So to avoid liposomes digesting, the liposomal drug can be administered during the fasting stage, so that there will be no action of pepsin.

3.Presence of large number of multi drug resistant protein(p-GP) in GI tract effluxes the therapeutic DNA/RNA. To overcome, co-administration of p-GP inhibitor may reduce the efflux of genetic material.

The most viable mechanism of liposome is adsorptive endocytosis and the retentive property of the particles at the absorption site. Liposomes may be taken up by membranous cells on the surface of GI lumen and be transported to lymphocytes in the form of vesicles. The lymphatic absorption bypasses presystemic metabolism in the liver and provides a chance to target the lymphatic system.

Conclusion:

Eventhough on formulating the cationic liposomes for the effective uptake in lymphatic system, still bioavailability is the problem as we can enhance the bioavailability by another 10%. The normal bioavailability of AGT1 receptor blocking gene therapy drug is around 35-50% but it is quite enough for the hypternsive reduction for upto 7 days with taking prescription 3 times a week.

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