World Journal of Pharmaceutical research

Volume 2, Issue 6, 2125-2141. Review Article ISSN 2277 – 7105

PROSPECTIVE PLAN ON NANOVESICULAR ORAL DELIVERY OF MACROMOLECULES

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Article Received on
26 August 2013,

Revised on 21 Sept. 2013,
Accepted on 24 October 2013

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ABSTRACT

For have handy reference on the development of nanovesiclular oral delivery system of macromolecules (NvODSM). Biopharmaceuticals of macromolecule origin (viz. proteins, peptides, monoclonal antibodies, and vaccines) explored to deliver associated proclaimed benefits. Interest evoke for presenting them as oral delivery system while synergising their effect through amalgamating nanotechnology. This abreast increases efficacy, specificity, tolerability and therapeutic index, and minimises toxicities. Amongst several nano-systems nanovesicles provide improved performance, stability, and patient compliance. They are receiving attentions and have potential for marketability. Their development and design is progressed to make best formula through method of optimisation. Which considered being a state of art involves multidisciplinary activity and creates troubles. Available literature unable to provide information on combating issues relating their development while some remains unhandy. In this context, information collected and presented as handy note which will be a helping hand and will offer an outstanding knowledge. This article features on combating issues relating development of NvODSM and has applicability in product evolution, with excellent feature.

KEY WORDS: Delivery, macromolecules, nanovesicles, nanotechnology, oral, strategy.

INTRODUCTION

Nanotechnology possesses enormous potential to produce novel pharmaceuticals. This achieves in devising nanotherapeutics and nanodevices and are under extensive study for improving performance. [1, 2] These attempts is to present them as disinfectants; biosensor or

bio-tracer based diagnostic agent for detecting toxins, pathogens, volatile compounds, and organic components of body fluids; and for monitoring diseases. [1, 2] Besides these they will revolutionise offering of device for drug targeting or their site-specific controlled delivery, and presenting of differential device-activity in dissimilar physiological environments, under direction of an external operator or physician. [2-6]

Semi-biological nanodevices offer versatile therapeutic services demonstrating unitary biochemical activities. Nanodevice amalgamating imaging and therapeutic function can provide prognostic information concurrent with therapeutic intervention. [1]

Amongst several nanodevices, designed for oral delivery of nanotherapeutic macromolecules, nanovesicles are gaining popularity. [7] These allow their paracellular and transcellular transcytosis, and site specific delivery or targeting with worthy biopharmaceutical and pharmacokinetic profile. [8-11] They are preferred for poorly soluble drugs and exhibit dynamic structure, comparing nanoparticles. [12-14] But have poor kinetic stability comparing nanoparticles. [2, 7] Consensus on improving specificity, efficacy, tolerability and therapeutic index and prognostic on nullifying toxicities are available. [2-5, 12, 15-19]

Nanovesicles are vesicular systems that encapsulates drug in a cavity of polymeric membrane. [7, 13-15] These systems were designed with diverse functionality using several materials and following diverse process or technique. All of these systems have limitations and superiority over each other. Choice of system afoot on desired physicochemical properties and targeted therapeutic objective. [4, 6, 7] Generally biodegradable or biocompatible materials of natural or synthetic origin are used. [1, 20] Table 1 presents glimpse on specialism of nanovesicles.

Adsorbing or grafting of molecules on surface of nanovesicles modifies its surface property that in turn modifies interaction with intestinal mucosa. Ligand molecule like glycoproteins, antibodies or peptides confers targeting [9, 21-24] while that of hydrophilic one like polyethylene glycol (PEG), improves transcytosis. [25] Adsorbing or coating of them with mucoadhesives (viz. Chitosan and its derivatives) improves gastric retention time. [1-3]

Present information will provide prospective action plans on design and manufacture of NvODSM and also underlines balancing their physicochemical properties. This information has applicability in product evolution and will be a helping hand while designing them with excellent feature.

Table 1: Nanovesicles exploited for developing oral delivery system of macromolecules.

Type of Nanovesicles	Composition	Size Range
Liposomes	Natural or synthetic phospholipids	20 nm-10 µm
Solid-lipid nanoparticles	High melting point natural or synthetic fats	50-1000 nm
Micelles	Ionic or non-ionic surfactants	2-20 nm
Lipospheres	High melting lipid, phospholipids	0.2-100 mm
Submicron lipid emulsions	Lipids, hydrophilic liquid, surfactants	1-100 nm

NANOVESICLES

Surfactants and amphiphilic polymers / copolymers form colloidal dispersions of molecular aggregates or vesicles. [14, 16, 26] Lipid-based vesicles termed as solid-lipid nanocarriers (SLN), micelles, lipid microspheres (lipospheres) and liposomes (LIP) while surfactant based called niosomes. [14, 16, 17, 27, 28] Nanovesicles can carry drug in core or on corona and can solubilise poorly soluble drugs and partly protect drug from aqueous environment. [12-14]

SLN usually exhibits beneficiant solid state behaviour (crystallinity, polymorphism and thermal behaviour). Its crystalline natured lipid core contributes additional beneficent features and is not limited to altering pharmacokinetics profiles, comparing others. [15, 27] These are innovative system having expectation for more contributions, particularly, from poorly aqueous soluble macromolecules.

SLN and niosomes are more stable and advantageous comparing LIP, and can incorporate hydrophilic and/or hydrophobic drug. These protects drug (incorporated one) from degradation; are biocompatible, safe, and well-tolerated; offer possibility for controlled and extended release and drug targeting; and so on. [12, 13, 17, 28] LIP is advantageous in terms of biocompatibility, amphiphilic character, and amenability for surface modification.

SLN formulated by melt dispersion method or solvent evaporation method but earlier is advantageous as eliminates use of organic solvents. [29-31] Lipospheres, LIP, and niosomes are prepared by direct dissolution in water or by dissolving drug and polymer in organic solvents before solvent evaporation or dialysis. [14, 26, 32, 33]

Lecithin, spans, tweens, poloxamer, cetyl palmitate, and sodium glycocholate are safe. [28] Copolymer of poly(lactide)-PEG, poly(ε-caprolactone)-PEG, or low molecular weight

polyester-PEG is cheaper and safe but their use limited by involvement of sophisticated technology and machine, and organic solvents. Amphiphilic copolymers render enhanced thermodynamic and kinetic stability comparing surfactants. [12, 13, 17] Copolymer of pluronic-polyacrylic acid and poloxamer results pH-sensitive vesicles. [34, 35] Derivatives of PEGylated α-tocopherol and tocopherol polyethylene glycol succinate suitable for delivering water soluble and poorly water soluble drugs are biocompatible. [12, 14, 17, 28] Poloxamer solubilise drugs and enhance transport of macromolecules across the intestinal barriers. [11, 36] Conjugating chitosan and its derivatives onto surface of nanovesicles confers mucoadhesive, targeting, and tight junctions (TJs) opening property. [37-41]

The polymers or copolymers used for preparing nanovesicles should (i) spontaneously self-assemble in water, (ii) enhance drug solubility by several fold (iii) provide high loading efficiency (iv) remain stable in the gastrointestinal (GI) tract, (v) be biocompatible and non toxic and (vi) easy to synthesise in commercial scale (vii) cheap and easily assessable.

BIODISTRIBUTION OF NANOVESICLES

ATP-binding cassette (ABC) transporters (like P-glycoprotein (P-gp) and multi-drug resistance-associated proteins expressed by intestinal epithelial membrane) and various solute transporters facilitate absorption of nanovesicles. The ABC transporters are ATP dependent thus limits absorption in a dose-dependent, inhibitable and saturable manner and can pump against steep of concentration. [36, 42-45]

Upon crossing mucus the nanovesicles are traversing intestinal epithelium via paracellular pathway and/or transcytosis. Receptor-mediated transcytosis (internalisation through endocytic pathways) is specific to intracellular locations and processes and mediated by enterocytes or M cells. [45] Endocytosis mechanism involves phagocytosis and pinocytosis. Phagocytosis is restricted to M cells and phagocytic immune cells. [42, 46] Pinocytosis occurs by macropinocytosis (a transient process) and micropinocytosis (a constitutive pathway). [3, 36]

After absorption drug loaded nanovesicles can be included in cytoplasmic vesicles or diffuse in cytoplasm and be discharged in serosal spaces for gaining access to mesentheric lymph or blood. [3, 36, 43, 47] These will partly get dissociate and will not completely get absorbed as particle. [10, 12, 17, 28]

The size, composition, surface characteristics and architecture of nanovesicles along with physicochemical properties of polymer (viz. molecular weight, conformation, hydrophobicity, and so on) will monitor their absorption. [9, 10, 42] Hydrophobic drugs forming core of vesicles are more likely to be transported by efflux pumps. [3, 10, 11] Nanovesicles taken up by absorptive enterocytes mainly delivered in blood. While those taken up by M cells will transcytosis close to immune cells and are likely be delivered to the gut-associated lymphoid tissue and lymphoid cells. [3, 9, 10]

BIOAVAILABILITY IMPROVEMENT TECHNIQUES

Junction-proteins present at tight or adherens junction restrains passage of macromolecules or aggregates with size more than one nm, through paracellular route. Consensus on nanovesicles is, generally they do not transverse intestinal barrier by paracellular route. [21, 43, 47] Internalisation of nanovesicles takes place via clathrin-mediated endocytosis (ClME). Endocytic translocation of small particles (with size 50-100 nm) mediated by enterocytes whilst larger particles mediated by M cells. [10, 36, 45, 46] In the transcellular route major limiting factor is intestinal mucosa which is exploited for improving bioavailability of orally delivered macromolecules. [3, 8, 18, 21, 36, 42, 45, 48]

Improvement in delivery of macromolecules, by paracellular route, can be achievable by grafting modulators of junctional proteins on the surface of nanovesicles. [3, 21] Transcellular translocation of nanovesicles can be improved by manipulating their fate within enterocytes, M cells or efflux pumps. Which seem to influencing by several physicochemical parameters including surface hydrophobicity, polymer nature and particle size of nanovesicles. [8, 18, 36, 42, 45]

Bioavailability or performance of nanovesicles can be improved through protecting macromolecules from detrimental GI tract environment, prolonging their GI-residence time, their endocytosis, and permeabilising effect of polymer. [1-3, 8, 34, 42]

Endocytosis

Internalisation of nanocarriers through endocytosis pathway depends on their size and physicochemical characteristics, and cell type. Their GI-translocation can be enhanced by targeting receptors (mediating endocytosis) through grafting or coating them with ligand, having affinity for receptors. [8, 21, 36, 42-45] Several non-exhaustive receptors are there whose targeting can be done to increase GI-translocation of nanovesicles; mediated by intestinal cells, globet cells or M cells. [9, 23, 42, 44, 46, 49, 50] Pattern recognition receptors such as toll-like

receptor-4, platelet-activating factor receptor and α5β1 integrin present on the surface of M cells considered important in antigen transcytosis. [51]

Targeting

Strategy for targeting the delivery devices includes exploitation of biologic affinity interactions and physical/chemical properties, signal provided by an external operator, or by up-regulating expression of some receptors using an appropriate stimulant. [4, 6, 9, 18, 40, 44, 52, 53] Signals of physical (a magnetic field) or biochemical (an enzyme transforming therapeutic to an active form) nature controls the assembly or activity of devices at particular sites or at particular times. [40, 44] Stimulation of receptors with ligands, lipopolysaccharide or cytokines increases uptake of nanovesicles by human follicle-associated epithelium cells. [3, 8, 9, 52, 53]

A few specific ligands of human M cells have been identified. [4, 9, 22] Some lectins can result targeting of enterocytes and M cell membranes. Integrins can be targeted with Arg-Gly-Asp (RGD) and aspartic-acid-based ligands. Amongst them RGD peptides showed high binding affinity and selectivity for integrin. [50] RGD peptides exploited are cyclic peptides like c(RGDfK) and c(RGDyK), RGD4C, and RGD10. Vitamins, carbohydrates, and adhesive factors (flagellin and invasins) derived from microorganisms are exploited as ligands. [1-3, 54-56]

Grafting of ligand on the surface of nanovesicles is particularly attractive for oral vaccine delivery. [9, 22, 53] Conjugating lectins to nanovesicles increases their transport across the intestinal mucosa while grafting them with vitamin B_{12} allows targeting to intrinsic factor specific receptor. [10, 45, 56, 57] Grafting of their surface with M cell homing peptides (CKSTHPLSC (CKS9), CSKSSDYQC (CSC)) result in targeting of M cells. [22, 52, 53] Nanovesicles mimicking structure of pathogen or having affinity for M cells is suitable for oral immunisation. [51]

Nanovesicles coated or grafted with chitosan and poly(lactide-co-glycolide) have enhanced GI-translocation, via ClME. [39] While that upon coating or grafting with ligands like folic acid, albumin and cholesterol, gets internalised by caveolae-mediated endocytosis. [46] Those coated with thiamine captured by peyer's patches (PPs) [52, 54] while that with pluronic 85 enters epithelial cells through caveolae-dependent and caveolae-independent pathways. [58] Targeting of globet cell, [49] PPs, [32, 52] dendritic cells (DCs) of PPs [59] done by grafting ligands, having homing property or affinity for them.

Mucoadhesion

Prolonging GI-residence time of nanovesicles will improve bioavailability trough improvement of their efficiency in transversing intestinal epithelium. [34, 47, 54] This is improved by decreasing their size below 200 nm. Mucoadhesion translates to cumulative release and absorption of macromolecules. [34, 55, 60]

STRATEGY AND PROSPECTIVE ACTION PLAN FOR DEVELOPMENT

In present days NvODSM considered as promising alternative to parenteral one. Untargeted, or targeted (with or without grafting of ligand) NvODSM had been exploited in most cases. [1-3, 18, 38, 61] But future of their marketing remains uncertain due to following facts.

(i) High cost of synthesising polymer, manufacturing nanovesicles and scaling-up,

(ii) Improvement of bioavailability to stable and efficient therapeutic level,

(iii)High inter- and intra-individual variations in pharmacokinetics of macromolecules, and

(iv)Optimising polymeric composition of nanovesicles for specific macromolecules.

Selection of nanovesicular system remains controversial for delivering macromolecules. Micelles considered viable approach for delivering poorly soluble macromolecules comparing lipid-based systems, [14, 16, 26, 28] while that of pH-sensitive one minimises initial burst release in stomach but releases them at pH 5. [35] LIP and lipospheres have lower toxicity and higher tolerability, and suitable for poorly soluble macromolecules. [32, 33] SLN comprises physiological and well-tolerated lipids. They offer controlled release and targeting, provide protection against degradation, and advantageous over other systems. [15, 29, 30, 62, 63]

Size of nanovesicles should be essentially within 10-200 nm. Said size promotes their diffusion in mucus and uptake by intestinal cells, and decreases their uptake by reticulo-endothelial system (RES). [18] Their surface modification with PEG will prevent their uptake by RES and prolong circulation half-life. [24, 25] Coupling their surface with mucoadhesion property prolongs their residence time without impeding diffusion in mucus. [34, 54, 55] Their surface charge has to be positive for favouring interaction with mucus and cell membrane but be neutral to decrease RES clearance. [34, 47, 60]

After oral administration, nanovesicle degrades in presence of enzymes or bile salts and variation in pH. They should be stable in GI tract and be releasing drug at desired site with predetermined rate. Their composition strongly influences their stability in GI tract. [7, 16] Their targeting potentialities for M cells or immunocompetent cells of GI tract will beneficial. [9, 28, 42, 45]

Nanovesicles of poly(allylamine) protects macromolecules from degradation by pepsin, trypsin and chymotrypsin. Simultaneously they improve paracellular and transcellular transport while former resulted through reversible disruption of TJs. [64, 65] SLN of cetyl palmitate sustain release while that of wheat germ agglutinin (WGA)-N-glutaryl phosphatidylethanolamine exhibits protection against enzymatic degradation. [11, 15, 31, 63, 66] Phosphatidylcholine, dipalmitoyl-phosphatidylcholine (DPPC), egg, or phosphatidylinositol-cholesterol based LIP used. DPPC offers protection of drug against degradation in GI tract and their entry in bloodstream, intact. [32, 33]

Vesicles of poly(lactide)-Pluronic block copolymer passes through cell membranes and attracted to small intestine. [58] While that prepared with copolymer of pluronic- poly(acrylic acid) and poloxamer minimise initial burst release of drug, in the acidic stomach, but releases drug, in a molecularly dispersed form, at pH five. Targeting and mucoadhesion property of these synergises efficiency and efficacy. [34] Vesicles of monomethyl PEG750-poly(caprolactone-co-trimethyl carbonate), copolymer, confers 1 to 3 fold increase in solubility of poorly soluble macromolecules and bioavailability. [13, 17]

Surface conjugation or coating of nanovesicles with mucin, PEG, WGA, tomato lectin (TL) or Ulex europaeus agglutinin 1 (UA1) imparts targeting potentiality. Mucin-LIP and PEG-LIP results sustained release and provides protection against bile salts. [1, 32, 33] LIP-WGA is taken up by endocytosis, LIP-TL exhibits resistance to enzymatic action in the intestine and mucoadhesion, LIP-UA1 have targeting specificity to M cells of PPs. [9, 32, 52, 53] Conjugation of their surface with CKS9, CSC, diethylenetriamine pentaacetic acid (DPC) enhances absorption. [22, 49, 52, 53] CSC result targeting of goblet cells while DPC disrupts TJs. [67] PEG-grafting on their surface results their rapid taken up by peritoneal macrophages. [39]

Coating of their surface with Eudragit® RS [68] and chitosan or derivatives of chitosan [37, 40, 41, 55, 69, 70] improves gastro retention and able to cause reversible opening of TJs but same with PEG results fast-diffusing nanovesicles. However improvement in paracellular permeability by chitosan or its derivatives did not results an increases in permeability of endotoxins and lipopolysaccharide thus is safe. [71]

N-trimethylated chitosan (NTC), thiolated chitosan (TC), [69] N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan (NTAC), [70] are important derivatives of chitosan [37] that preserves loss in mucoadhesive and TJ opening properties of it at pH 7.4. [37, 41, 72] These

categorised as pH-sensitive polymers and used for improving bioavailability alone or in combination with other. [5, 67] Among them some are biodegradable. [35] TC improves mucoadhesion and absorption [69] whereas NTAC exhibit higher cationic charge density, increased mucoadhesion, TJ opening ability and are non-cytotoxic. [70] PEGylated chitosan have enhanced permeability and lower toxicity. [24] Gama-polyglutamic acid (γPGA)-NTC allows absorption of macromolecules throughout the intestinal tract. [67, 73] Chitosan-γPGA increases their absorption in intestine and sustains release.

Co-encapsulation of permeation enhancers (e.g. sodium taurocholate, dimethyl palmitoyl ammonio propanesulfonate) enhances bioavailability and paracellular translocation, through disrupting TJ. [74] Co-encapsulating pluronics and poloxamers inhibits P-gp and enhance net macromolecule transport through intestinal barrier. [13, 17, 36, 58] Poloxamers improves solubility and transport of macromolecules. [8, 36, 43]

Averting drug release in GI tract has perquisite that, concentration of micelle (i.e., vesicles) to be above critical micelle concentration (CMC) and it shall expose to an ionic strength below their flocculation point. [75, 76] The inhibition of P-gp by poly(ε-caprolactone)-PEG is above CMC whereas that by pluronics is highest just below CMC. [13, 17] Micelles of poorly soluble macromolecules could be passively target by enhanced permeabilisation-retention effect. [17] Poly-ion complex vesicle enhances transport across intestinal epithelial barrier. [65, 76]
Lipid-based systems (lipospheres, micelles, and LIP) prepared with several lipids well tolerated in living systems, with inclusion or exclusion of emulsifying agents. These did not exhibit cytotoxic effects, up to total of 2.5% lipid content. Considering tolerability, surfactant with GRAS status used with preference, comparing lipids. Acylglycerols comprising fatty acids and lecithins accepted as safe. [14, 28]

Surface charges or chemistry of nanovesicles monitor their fate. Their fate can be modulated through surface engineering. Coating of their surface with PEG transforms them to fast-diffusing one. Dense coating of same effectively minimises adhesive interactions of them with mucins, allowing penetration. [25] Their gastric residence time can be improved with cationic polymers or coating them with cationic groups or with thiol groups (that binds to mucin). [25, 77] Negatively charged one display higher transport rates referring near neutral, or positively charged one. [3, 60] However, a balance between mucoadhesion and mucus penetration is important. [3]

Their size must be small enough to avoid significant steric inhibition by the fibre mesh and should avoid adhesion to mucin fibres; and to allow diffusion and uptake, but large enough to carry a favourable amount of macromolecules. [43] They should be stable, bioabsorbable, non-toxic, non-thrombogenic, non-immunogenic, non-inflammatory, and avoid uptake by RES and have versatile applicability. [1, 19, 20] Their cytotoxicity is an essential product parameter of in vivo tolerance evaluation in humans and/or animals. [14, 19, 28] Stability of them should be assessed involving approved method and accordingly shelf-life be assigned. [34]

Targeting of nanovesicles to globet cell, [49, 50] PPs, [9, 32, 52] and DCs of PPs [53, 59] can be achieved with grafting of ligands. Optimisation of ligand density on their surface must allow tissue penetration and cellular uptake a prerequisite for resulting optimal therapeutic efficacy. Redressing issue of variation in M cells populations and receptors is necessitated; those vary with species, anatomical location, age, sex and exogenous factors. [1, 9, 10]

Selection of in vitro models used for understanding fate of nanovesicles within intestinal epithelium is critical for their development. Combining both quantitative analysis method (for studying their transport) and confocal microscopy (for locating them) improve efficiency of study. [2, 34, 78] Their cellular tracking can be done with dynamic colocalization microscopy and quantum dots technique. [2, 34, 57, 78] Quantitative and qualitative information such as pore size, velocity, diffusivity, viscoelasticity, directionality and transport mode of macomolecules can be determined from particle trajectories. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays can provide cytotoxic report of nanovesicles. [2, 70] Association of nanovesicles with DCs and their effect on their maturation can be determined with flow-cytometry. [79]

Novel NvODSM can be developed through. [1, 2]

(i) Development of biocompatible polymers with tailored attributes,
(ii) Understanding the mechanisms of their cellular uptake and fate,
(iii) Devising novel methods and techniques for studying their fate including its components, and
(iv) Identifying ligands for conferring targeting attributes.

CONCLUSION

NvODSM will be a promising platform for delivering macromolecules. In vivo experimental results of animal models need to be interpreted and correlated with care, linked with

existence of differences in anatomy and physiology of laboratory animals and humans. In order to have successful NvODSM, bioavailability of them to reach therapeutic level with minimal inter and intra individual variation and toxicity had to be looked at. Greater stability along with a financially feasible manufacturing process for mass production is prerequisite. Future will evidence utility of NvODSM in devising projected benefit in a cost-effective way.

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