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Cholesterol: enhancing stability and efficacy in lipid-based drug delivery

Lipid-based drug delivery systems (LBDDS) have gained significant attention in pharmaceutical research due to their versatility and numerous benefits. LBDDS can overcome formulation challenges of poorly water-soluble drugs by improving their solubility and bioavailability while offering controlled and targeted drug release, high drug content loading, and stability. They can be used for various administration routes and can be tailored to meet specific requirements. Cholesterol, a key component in LBDDS, provides structural support and stability, enhances the efficiency of intracellular delivery and gene transfection, and plays a crucial role in the formulation of immune stimulating complexes (ISCOMs) and liposomal adjuvant systems. Additionally, plant-based cholesterol offers a few benefits as an alternative to egg- or animal-based cholesterol for pharmaceutical applications.

The versatility and benefits of lipid-based drug delivery

Throughout the past decade pharmaceutical research has focused on development of novel drug delivery systems such as lipid-based drug delivery systems (LBDDS), improving the bioavailability of existing drugs while reducing their toxicity. LBDDS can address common formulation challenges of poorly water-soluble drugs e.g., solubility and bioavailability, while providing a range of benefits including controlled and targeted drug release, high drug content loading (compared to other carriers), pharmaceutical stability and excipient versatility. Lipid formulations can be used to formulate pharmaceuticals across a range of dosage routes including oral, parenteral, ocular, dermal, transdermal, and vaginal. Further illustrating their versatility, LBDDS can be tailored to meet requirements set by disease indication, administration route, cost, active pharmaceutical ingredient (API) stability, toxicity and efficacy[1].

LBDDS can be broadly grouped into solid lipid particulate dosage forms, emulsion-based systems, solid lipid tablets and vesicular systems.

Modifications to these groups include, but are not limited to lipospheres, lipid nanoparticles (LNPs), liposomes, and self-emulsifying formulations (SEFs)[2].

Lipids form self-assembled nanostructures such as liposomes or LNPs to deliver small molecules and nucleic acids. Phospholipids are water-insoluble and form a phospholipid bilayer when mixed with water due to their amphipathic properties; however, the lipid bilayers they form are unstable and prone to fall apart. This instability can be addressed by cholesterol, a lipid component that can be used to provide structural support and stabilise nanoparticles.

Enhancing stability and functionality in synthetic and natural membranes

From a chemical point of view, cholesterol is an amphipathic lipid belonging to the class of sterols and consisting of four hydrophobic rings and a hydrophilic hydroxyl moiety. Cholesterol occurs naturally in both plants and animals – for example in animals it is a precursor for the biosynthesis of steroid hormones, bile acid and vitamin D. It's also an essential structural component of animal cell membranes composing about 30% of them. In addition to providing strength and stability in animal cell membranes cholesterol also reduces permeability of the membrane[3]. Since natural sterols play such a crucial role in stabilising biological membranes, it's not surprising that they play a similarly important role in synthetic systems as well. Just as it stabilises biological membranes, the presence of cholesterol also fills gaps between phospholipids to stabilise synthetic vesicle membranes.

Cholesterol is also effective at broadening the liquid crystalline phase transition temperature range of a lipid mixture. Whether or not the cholesterol will lower or increase the phase transition temperature of a specific lipid mixture depends on several variables: the acyl chain length of the lipids, the degree of unsaturation of the acyl chains, and the cholesterol concentration. Cholesterol can broaden the phase transition temperature of lipid mixtures, which is especially useful when using saturated lipids like distearoylphosphatidylcholine (DSPC) that have higher phase transition temperatures. The use of cholesterol results in a vesicle that is more likely to retain its cargo and is less "leaky". Cholesterol is used in many lipid systems from liposomes to LNPs.

Cholesterol is a major component of many LBDDS. Cholesterol inserts itself between phospholipids in lipid formulations. Its polar hydroxyl group aligns with the polar headgroup of the phospholipid, and its highly non-polar part is deeply immersed in the phospholipid vesicle. Cholesterol's planar steroid ring generates a conformationally rigid structure, and when it is present in large amounts in the lipid vesicle, cholesterol introduces conformational ordering of the lipid chains acting as a permeability barrier. This ordering effect of cholesterol not only reduces the dynamics of the individual lipids in the vesicle but also influences the thickness, compressibility, water penetration and intrinsic curvature, increasing the membrane's mechanical stiffness while maintaining its fluidity[4]. Overall cholesterol can provide structural support and stability for LBDDS.

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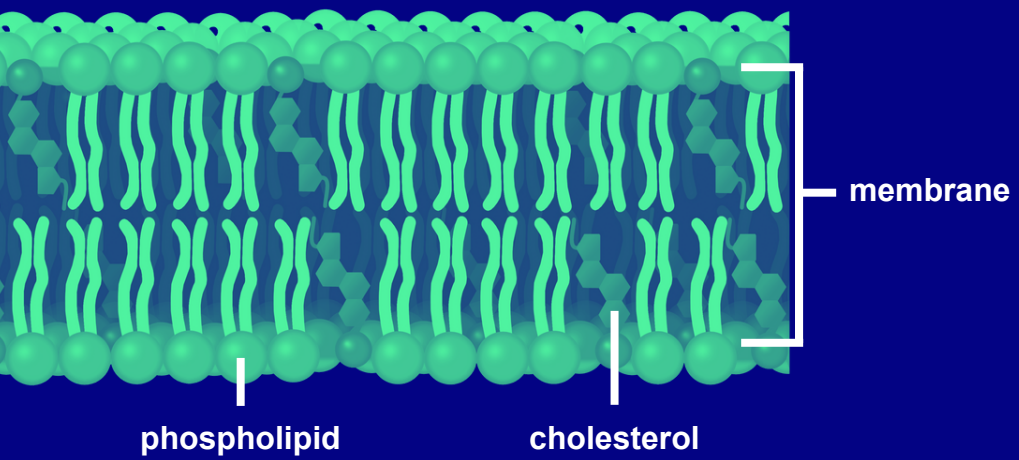
Cholesterol is primarily vital for maintaining the integrity of lipid-delivery systems. However, cholesterol has also been shown to play a crucial role in the efficient intracellular delivery of LNPs and improved gene transfection. One factor in cholesterol's ability to enhance transfection efficiency is its ability to promote membrane fusion. Cholesterol-rich domains near the surface of an LNP can interact with cholesterol-rich domains on cell membranes. This can lead to the formation of lipid rafts and facilitate the fusion of the lipid-delivery system with a target cell. This in turn enhances the internalisation of the therapeutic payload into the cell where it is released.

The cholesterol-rich domains not only assist the internalisation of the payload, but also the payload's endosomal escape once the lipid delivery system is internalized. Cholesterol-rich domains have been shown to assist the endosomal escape of genetic material into the cytoplasm which improves the overall therapeutic efficacy. Cholesterol plays a vital role in most LBDDS by enhancing the overall stability of the formulation and transfection efficacy[5,6].

Cholesterol in synthetic and natural membranes



Deemed to be designed as the ultimate adjuvant formulation when it comes to efficacy, ISCOMs offer immunological benefits over existing adjuvants.

Cholesterol-saponin affinity: unlocking the potential of ISCOMs for enhanced vaccine efficacy

Cholesterol doesn't elicit an immune response in itself; however, it's used to stabilise certain liposomal adjuvant systems and immune stimulating complexes (ISCOMs). Saponins - such as Quil-A and QS-21 for example - have a strong affinity for cholesterol and this affinity creates an interesting situation: If used alone as adjuvants in a vaccine, they bind to the biological cholesterol of the cell membranes creating pores and this results in undesired reactogenicity at the site of the injection. However, the same cholesterol affinity of saponins enabled scientists to develop both the mentioned ISCOMs and liposomal adjuvant systems such as AS01.

ISCOMs are spherical open cage-like adjuvant systems that are formed by adjuvant-active saponin, cholesterol, phospholipid, and antigen. The ISCOM matrix consists of the same composition, shape, and morphology as the ISCOM complex, minus the incorporated antigen. Demonstrated in animal models ISCOMs enhance the antigen targeting, uptake and activity of antigen presenting cells including dendritic cells, B cells and macrophages. This results in the production of proinflammatory cytokines, namely interleukin IL-1, IL-6 and IL-12 in addition to enhanced expression of costimulatory molecules major histocompatibility complex (MHC) class II, B7.1 and B7.2[7].

Deemed to be designed as the ultimate adjuvant formulation when it comes to efficacy, ISCOMs expand the immune response. Currently most adjuvants only activate the humoral immune response; whereas ISCOMs are able to induce strong activation of both the cell mediated and humoral arms of the immune system, generating long-lasting biologically active antibodies and a strong cellular response [8,9] . In addition to the immunological benefits ISCOMs technology can provide commercial benefits including great flexibility in vaccine design due to the ability to mix the ISCOMs matrix with the required antigen at the post-manufacturing stage.

One of the most successful ISCOMs is the AS01b formulation used in the Zoster vaccine (Shingrix®) which has been proven to be effective at preventing Shingles in adults. This system is formulated with a combination of monophosphoryl lipid A (MPLA) and QS-21 adjuvants, as well as cholesterol and a phospholipid. AS01b is also being tested in clinical trials as a component of Mosquirix (the first approved malaria vaccine) and HIV vaccines. Cholesterol is an important component in many ISCOMs including AS01b and is being investigated in next-generation AS01b-like systems.

Plant-based cholesterol

Currently, most cholesterol used in industry is sourced from animal materials and extracted from either lanolin or animal tissues. Plant-based cholesterol eliminates the impurities and potential contamination associated with animal-based cholesterol and can address lipid shortages in vaccine production. Avanti, now part of Croda Pharma, supplies pure, plant-based parenteral cholesterol that meets USP-NF, Ph. Eur. (2397) and JP pharmaceutical specifications. Our parenteral grade plant-based cholesterol can be used as a high-purity alternative to traditional egg- and animal-based cholesterol. Acting as a stability enhancer and assisting in the transfection of nucleic acids, it is available for both R&D work and parenteral applications.

Consistent quality is a must when evaluating a partner for cGMP manufacturing. Ensuring patient safety and regulatory compliance are paramount to providing the best treatments available. That's why you should trust Croda Pharma's Avanti® plant-based cholesterol. Who better to rely on for consistent quality than a brand built on the highest quality lipids for over 50 years? Contact a representative today to discuss using plant-based cholesterol in your drug delivery project!

A long-term lipid innovations partner

Croda Pharma offers its innovative Avanti® lipids portfolio in both research and GMP grades. This expanded capability follows Croda's acquisition of Avanti Polar Lipids, a leading partner for the development and formulation of lipid technologies to address key formulation challenges. Croda's high-purity lipids and excipients can be utilised in delivery systems for complex therapeutic drugs and in next-generation mRNA vaccines, supported by a vast portfolio of phospholipids, cationic lipids, PEG lipids, sterols, and more. Working collaboratively with researchers and formulators, Croda uses Smart science to improve lives™.

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