Review Article

An Overview of Dual Targeting Nanostructured Lipid Carriers for the Treatment of Ovarian Cancer

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ABSTRACT

Purpose: To summarize main findings from research on oral delivery of Nanostructured lipid carriers targeting ovarian cancer. **Methods:** A narrative review of all the relevant papers known to author was conducted. **Results:** Ovarian cancer is one of the most common gynaecologic cancer, the frontline treatments for which are surgical approach followed by radiotherapy or chemotherapy. Chemotherapy has its own limitations; consequently, there is a need to develop a targeted drug delivery system with high efficacy. Preclinical evidence suggests that combination therapies are likely to be effective in ovarian cancer. However, from clinical reports, it is evident that chemotherapeutic drugs have lower therapeutic value for the treatment of ovarian cancer. We propose that these limitations can be overcome by a novel formulation consisting of nanostructured lipid carriers. Recent pharmacological research has successfully potentiated the effects of combination therapy in acute animal preparations by inhibiting proteins that are involved in different physiological pathways of cancer cells. **Conclusion:** We have reviewed the scope of nanostructured lipid carriers concerning chemotherapy of ovarian cancer. The advantages of oral administration of these lipid carriers are discussed. Also, the various nano-lipid formulations and combination therapies reported along with their therapeutic outcomes have been reviewed. In light of this, nanostructured lipid carriers containing two different active ingredients for oral administration could be a potential approach for the effective treatment of ovarian cancer.

Key words: Ovarian cancer, Nanostructured lipid carriers, Dual targeting, Chemotherapy, Oral delivery.

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INTRODUCTION

One of the lethal and second most common gynecologic malignancies in the world is ovarian cancer (OC) which has a high mortality rate. Around 70% of cases are diagnosed at an advanced stage. This is due to the secret growth of the tumour, delayed onset of symptoms, and lack of proper screening.[1,2] OC treatment employs invasive surgery for the removal of affected ovaries, fallopian tubes, and cervix. This is followed by radiotherapy or chemotherapy, based on the stage of OC identified. Intravenous cisplatin and paclitaxel (PTX) are conventional drugs used for the treatment of OC. However, conventional therapy has its own limitations and drawbacks that include subsequent disease relapse, development of drug resistance, and toxicities. Several side effects including nausea, alopecia, and declination in plasma counts are associated with chemotherapy treatment when administered to patients having OC. The combination of PTX and Methotrexate results in decreased bioavailability, increased toxicity, and drug incompatibility thereby reducing cancer targeting. To surpass treatment drawbacks of conventional therapeutics,

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various targeting drug delivery systems are developed. These delivery systems direct anticancer therapeutics to tumour sites specifically. Also, advanced polymeric nanotechnology provides better alternatives for treating OC, thereby minimizing systemic toxicities linked with the administration of the chemotherapeutic drug.[3]

Also, a combination of chemotherapeutics can be used to improve anticancer efficacy against OC. This depends on the theory that one drug-resistant tumour cells will be sensitive to another drug with a different mechanism of action. Research has witnessed exceptional growth over the past two decades in using nanotechnology for therapeutic applications, particularly in oncology. Nanotechnology increases drug-tumour accumulation and reduces off-target toxicity, consequently achieving higher treatment efficiency.[4] Nanostructured lipid carriers (NLCs) have shown to be a reliable platform for the delivery of anticancer drugs in the treatment of OC. NLCs have distinct advantages that make them be considered as promising nano-systems. Oral administration of these NLCs can be regarded as a better approach for the treatment of chronic diseases like diabetes, hypertension, and cancer. All these advantages are discussed in the following sections. Combination therapy for dual targeting can be adopted with NLCs via oral administration for effective treatment of OC. This reduces adverse effects, sustains the release of drugs thereby enhancing the anticancer efficacy with longer shelf storage stability. Systemic toxicity can be decreased and since these are administered orally, patient compliance and ease of administration are attained. Dual targeting can be achieved by loading two drugs with a different mechanism of action like Paclitaxel and Hydroxyurea into an NLC. Here, Paclitaxel acts on TUBB that is involved in cell division, and Hydroxyurea acts on RRM2 which is responsible for purine, pyrimidine, and glutathione metabolism. Figure 1 describes the NLC loaded with these two drugs.

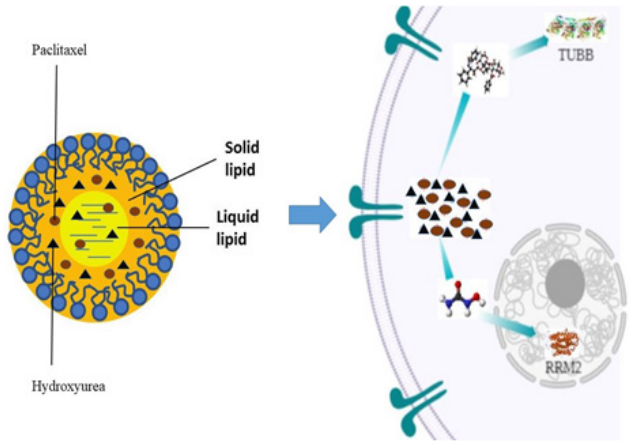

Figure 1: NLC loaded with two drugs targeting cancer cells. Paclitaxel acts on TUBB and Hydroxyurea act on RRM2.

Scope of NLCs in chemotherapy

It is evident from recent studies that NLCs enhance efficacy, stability, and reduces the side effects of cytotoxic drugs. Various nano-systems carrying anticancer drugs have been developed like albumin-paclitaxel nanoparticles for breast cancer, which was approved for chemotherapy in early 2005. For lung carcinoma cells etoposide NLCs were found to be cytotoxic. Topotecan NLCs were developed which were stable and prolonged the release of drugs for treating ovarian and small-cell lung cancer. These NLCs have various advantages such as high drug loading efficacy, prolonged-release characteristics, improved stability, increased cytotoxicity when incorporated with anticancer drugs. Some potential problems associated with SLN that include drug leakage and decreased loading capacity can be avoided by these NLCs. They prolong the exposure of tumour cells to chemotherapeutic agents and enhance permeability and retention, which thereby increases therapeutic index. These NLCs avoid some potential problems associated with SLN, such as drug leakage during storage and decreased loading capacity. They act by prolonging the exposure of tumour cells to anti-tumour drugs and enhancing permeability and retention effect to further increase the therapeutic effect.[5,6]

NLCs were developed by Muller *et al.* They used mixtures of solid lipids and liquid lipids which at both room and body temperature form an amorphous solid matrix. The fundamental step is the incorporation of liquid lipids in the matrix that significantly enhances formulation properties when compared to SLNs. Liquid lipids create an amorphous lattice with considerable imperfections in the crystalline solid matrix by which higher drug loading can be attained. For various therapeutic and cosmetic applications, NLCs were studied as delivery platforms. They have significantly higher encapsulation efficiency, bioavailability, controlled release, biocompatibility, and safety profiles.[7-9]

A tumour is affiliated with defective and leaky vascular. This is due to a poorly regulated tumour angiogenesis environment. Within the tumour, the interstitial fluid is insufficiently drained by a poorly formed lymphatic system. As an outcome, a submicron-sized particulate issue may preferentially extravasate into the tumour and be kept there. This is often mentioned as the "enhanced permeability and keeping" (EPR) effect.[10] The EPR effect can be fruitful by the proper design of nanoparticles like NLCs for passive tumour targeting. This partially solves the poor specificity problems.

Also, surface engineering of these NLCs can alter their biodistribution properties. Surface engineering involves the modification of external physicochemical properties of NLCs, which directs them to the selected tissue. This enhances the target specificity of the formulation and reduces systemic toxicity.[8,10] Thus, it is convincible that anticancer drugs administered to treat OC can be loaded into NLCs so that tumour targeting is achieved and thereby reducing systemic adverse effects.

NLCs targeting ovarian cancer

Various NLCs loaded with anticancer drugs for the treatment of OC have been reported. Few of them have been reviewed in the following.

Liping Wang *et al.* reviewed that ovarian tumours express CD44 family of cell surface proteoglycans whose natural ligand is hyaluronic acid (HA). They aimed to develop HA-based paclitaxel (PTX) loaded NLCs to improve OC therapeutic outcomes. They prepare cationic PTX-NLCs. By electrostatic absorption technique, they coated HA to these NLCs to obtain HA-PTX-NLCs. *In vitro* and *in vivo* studies revealed that there was an enhanced reduction in tumour growth when administered with these NLCs compared to PTX injection. They demonstrated that significant results were due to the usage of novel HA-PTX-NLCs.[11]

Pooja Mittal *et al.* focused on the formulation and characterization of genistein NLCs for sustained drug release for OC treatment. Genistein is a natural flavonoid that has anticancer activity. Its drawbacks include poor solubility and low oral bioavailability. To overcome these they developed genistein NLCs by solvent emulsification and evaporation technique. Pharmacokinetic and biodistribution studies of these NLCs revealed that plasma drug concentration for a longer time period and improved drug distribution in OC tissues was achieved. They concluded that genistein NLCs seem to be an alternative for higher entrapment and excellent stability.[12]

Maria Luisa Bondi *et al.* studied curcumin that is a natural molecule possessing an anti-cancer effect. Its clinical use has been limited as its bioavailability is poor. They believed that nanocarrier drug delivery systems would overcome the limitations of curcumin. They aimed to increase the bioavailability of curcumin by loading into NLCs. They prepared compritol NLCs considering its slower drug release characteristics. Their *in vitro* results showed that these NLCs had better anticancer efficacy than free curcumin. This shows that NLCs loaded with curcumin have potential drug delivery profiles in the treatment of OC.[13]

Ki Hyun Bang *et al.* reviewed that NLCs are emerging tools that improve tumour treatment outcomes. They designed PTX NLCs with platelet membrane proteins as these proteins are involved with the angiogenesis and interaction of circulating tumour cells. They coated PTX NLCs with platelet membrane proteins isolated from blood by the gravity-gradient method. Transmission electron microscopy, western blot, and ELISA confirmed this coating process. *In vitro* cell studies displayed that these NLCs had improved antitumor effect towards SK-OV-3 cells. They summarized that these PTX NLCs had an affinity and targeting ability for OC cells.[14]

NLCs for oral delivery

For treating various deadly diseases, the oral route of administration of drugs is a valuable option. It has several advantages which makes it to be the most commonly accepted route of administration. These advantages include patient compliance, cost-effectiveness, and ease of administration. While administering chronic agents such as antidiabetic, antihypertensive, and anticancer agents this route is highly preferred.[15] More than 40% of drugs emerging from the drug discovery process are unfortunately not suitable for oral delivery because of their hydrophobicity and poor oral bioavailability. First pass metabolism, drug expulsion via P-glycoprotein, and food effects are other barriers that are encountered with this route. This shows that there is a high necessity for the development of oral drug delivery systems which overcome the aforementioned factors for desired therapeutic activities.[15,16]

Various attempts and approaches are being designed in research to overcome these challenges and bioavailability enhancement with respect to oral drug delivery platforms. Prodrug strategies, slat formation, nano-encapsulation of drugs via polymeric micelles, emulsions, liposomes, etc. are a few methods adopted for this purpose.[17] Over the past few decades, lipid drug delivery systems showed better effects on drug absorption. Reports suggest that liposomes, micelles, emulsion, and other conventional lipid systems are susceptible to degradation during storage and in the acidic environment of the stomach. NLCs have been consistently reported for increased entrapment efficiency.[17] This is attributed to the structural parity of two lipids in NLCs resulting in structural imperfections which while solidification gives more space for drugs.[18] The drugs also possess more solubility in liquid lipids than solid lipids. These NLCs have longer storage stability. All these properties make NLCs to be considered as advanced carriers when compared to conventional lipid-based drug delivery systems.[19] NLCs can be incorporated with lipophilic and

hydrophilic drugs. They also provide sustained release of drugs and site-specific targeting ability. From various studies, it is evident that this lipid-based drug delivery system has improved the oral bioavailability of drugs through better intestinal absorption. Thus, NLCs can be considered as a light of hope in the treatment of various chronic diseases including OC for their advantages when administered orally.

Beneficial aspects of dual targeting over single targeting

Dual targeting is significantly beneficial over single protein targeting, as there are various research studies carried out in this area proving to be effective.

Ni W *et al.* worked on 5-fluorouracil and curcumin loaded nanoparticles for enhanced treatment of hepatocellular carcinoma. Their formulation design was based on the fact that multidrug combination therapy along with targeting techniques will result in increased anticancer effects. In clinical applications, these strategies have become significantly important. They concluded that these dual drug-loaded NPs are a promising tool for cellular targeting and synergistic anticancer efficacy.[20]

Wu S *et al.* explored that dual drug nanohybrids with the specific targeting capability and high drug loading have gained more importance in cancer therapy. Their formulation was composed of 10-hydroxycamptothecine and methotrexate. They designed a green approach that develops emerging drug delivery systems for cancer treatment.[21]

Jose A *et al.* developed imatinib and tamoxifen-loaded temperature-sensitive liposomes to target breast cancer. They reported that co-delivery of dual or multiple chemotherapeutic agents using nanocarriers enhances antitumor efficacy, which can be adopted as a potential strategy against breast cancer.[22]

Serri C *et al.* studied combination chemotherapy that employs two or more drugs, which is prone to suppress the inception of multidrug resistance, exploiting the fact that diverse drugs act in different points of the cellular cycle that amplifies cancer cells. Their results proved that gemcitabine (GMC) and quercetin (QCT) combination showed a synergistic effect in the inhibition of pancreatic cancer cells migration. GMC and QCT were loaded into biodegradable NPs based on poly(lactic-co-glycolic acid), which were externally decorated with hyaluronic acid (HA). HA specifically interacts with the CD44 receptor, for which it plays a major role in tumor targeting. The GMC and QCT loaded NPS decorated with HA showed improved cellular uptake and cytotoxicity.[23]

Table 1: Various drug and their targets approved for OC.

SL NO.	Drugs	Target	Mechanism
1.	Cyclophosphamide	DNA	Alkylating agent
2.	Melphalan	DNA	Alkylating agent
3.	Thiotepa	DNA	Alkylating agent
4.	Gemcitabine	RRM1	Antimetabolite
5.	Paclitaxel	TUBB	Tubulin depolymerization inhibitor
6.	Doxorubicin	TOP2	Topoisomerase inhibitor
7.	Cisplatin	DNA	DNA alkylator
8.	Carboplatin	DNA	DNA alkylator
9.	Bevacizumab	VEGFA	Monoclonal Antibody
10.	Altretamine	DNA	Alkylating agent
11.	Hydroxyurea	RRM2	Antimetabolite
12.	Topotecan	TOP1	Topoisomerase inhibitor
13.	Niraparib	PARP 1, 2	PARP inhibitor
14.	Olaparib	PARP 1, 2, 3	PARP inhibitor
15.	Rucaparib	PARP 1, 2, 3	PARP inhibitor

Various targets in OC

The following table (Table 1) consists of the various FDA approved drugs available for the treatment of OC. The targets of these drugs are mentioned along with their mechanism of inhibition. This data compilation can serve as a tool for the design and selection of dual or multiple targeting strategies for enhanced efficacy against OC cells.[24]

CONCLUSION

We conclude that NLCs have a potent scope in the area of chemotherapy. They are advantageous over SLNs and other lipid-based conventional drug delivery systems. Various NLCs loaded with drugs used for OC treatment have shown a path for further development in advanced strategies that increase therapeutic efficacy and safety.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

OC: Ovarian cancer; **PTX:** Paclitaxel; **NLC:** Nanostructured lipid carriers; **SLN:** Solid Lipid Nanoparticle; **HA:** Hyaluronic acid; **GMC:** Gemcitabine; **QCT:** Quercetin.

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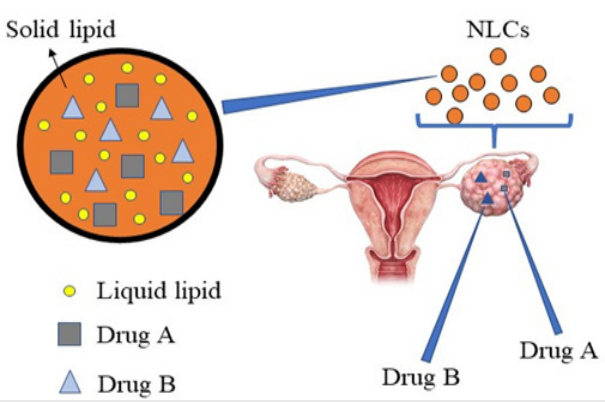
PICTORIAL ABSTRACT



Dual targeting approach for ovarian cancer

SUMMARY

One such strategy is oral administration of anticancer agents via NLCs, through which better absorption of drugs and their sustained release can be achieved. Besides this, there is a lack of attention given to multiple proteins targeting in various formulations developed against OC. Those formulations that have dual drug combinations, that is, targeting more than one protein involved in OC pathophysiology as mentioned above have declared better results. Also, various other targets available for ovarian cancer and drugs targeting these were summarised. Further steps include the development of formulation in light of the proposed strategy, *in vitro* and *in vivo* characterization to demonstrate the efficacy of the formulation.

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