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Review Article

Theme: Translational Application of Nano Delivery Systems: Emerging Cancer Therapy
Guest Editors: Mahavir B. Chougule and Chalet Tan

Nanocarrier for Poorly Water-Soluble Anticancer Drugs—Barriers of Translation and Solutions

Mayuri Narvekar,[1] Hui Yi Xue,[1] June Young Eoh,[1] and Ho Lun Wong[1,2]

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Abstract. Many existing chemotherapeutic drugs, repurposed drugs and newly developed small-molecule anticancer compounds have high lipophilicity and low water-solubility. Currently, these poorly water-soluble anticancer drugs (PWSAD) are generally solubilized using high concentrations of surfactants and co-solvents, which frequently lead to adverse side effects. In recent years, researchers have been actively exploring the use of nanotechnology as an alternative to the solvent-based drug solubilization approach. Several classes of nanocarrier systems (lipid-based, polymer-based and albumin-based) are widely studied for encapsulation and delivery of the existing and new PWSAD. These nanocarriers were also shown to offer several additional advantages such as enhanced tumour accumulation, reduced systemic toxicity and improved therapeutic effectiveness. In this article, the recent nanotechnological advances in PWSAD delivery will be reviewed. The barriers commonly encountered in the development of PWSAD nanoformulations (*e.g.* formulation issues and nanotoxicity issues) and the strategies to overcome these barriers will also be discussed. It is our goal to provide the pharmaceutical scientists and clinicians with more in-depth information about the nanodelivery approach, thus, more efficacious and safe PWSAD nanoformulations can be developed with improved translational success.

KEY WORDS: anti-cancer drug; drug delivery; nanocarrier; poorly water-soluble drugs; translation.

INTRODUCTION

In the past few decades, many anticancer drug compounds were discovered and developed, most notably the newer chemotherapeutic agents or "chemo-drugs" such as taxanes (e.g. paclitaxel, docetaxel) and platinum-based drugs. These agents are often referred as cytotoxic drugs because of their strong cancer cell-killing activities. Recently, a new generation of small-molecule drugs has been developed for targeting the molecular pathways in cancer progression. In addition, some existing drugs originally indicated for non-cancer diseases have been "repurposed" for cancer treatment. Some of these less cytotoxic newly developed or repurposed compounds have already entered clinical trials in the hope for successful translation (1–3).

The lack of good aqueous solubility has been frequently identified as a key obstacle of the development and clinical use of these anticancer compounds. According to the Biopharmaceutics Classification System (BCS), a drug is considered to be poorly water-soluble if its highest dose strength is not soluble in 250 mL or less of aqueous media over the pH range of 1 to 7.5 (2). Unfortunately, many cytotoxic drugs including paclitaxel, etoposide and docetaxel fall in this category owing to their bulky polycyclic nature (4), which contributes to the high lattice energy for the drug to dissolve and the inability of the drug molecules to form hydrogen bonds with water. A similar problem is observed with the new small-molecule anticancer drugs. Since the 1990s, the newer drug pipeline tends to have lower solubility resulting in an increase in poorly water-soluble BCS Class 2 compounds from ~30 to 50–60% and the corresponding decrease in water-soluble BCS Class 1 compounds from ~40 to 10–20% (5). The aqueous solubilities of recently developed anticancer compounds are typically in the microgram per milliliter range.

Table I lists the solubility and partition coefficients of some of these poorly water-soluble anticancer drugs (PWSAD). The solubility issue is further magnified considering the strong need for administering them by intravenous (IV) infusion. Anticancer drugs are by nature toxic compounds, so IV infusion is often needed to achieve more predictable pharmacokinetics and reduced gastrointestinal toxicity. A drug with limited aqueous solubility is simply not suitable for this route of administration. These PWSAD clearly deserve more attention in order to turn them into safe and effective clinical therapy.

Common drug solubilization strategies used in pharmaceutical industry include prodrug formation, complexation with cyclodextrins, use of co-solvents and/or surfactants and encapsulation into nanodelivery systems (nanocarriers) (6–8). Among these strategies, the use of high concentrations of co-solvents and/or surfactants has been the current standard for

[1] School of Pharmacy, Temple University, 3307 North Broad Street, Philadelphia, Pennsylvania 19140, USA.
[2] To whom correspondence should be addressed. (e-mail: ho-lun.wong@temple.edu; holunwong2011@yahoo.com)

Table I. Solubility and Partition Coefficient Data of Selected Anticancer Drug

Drug	Aqueous solubility (25°C)	Partition coefficient
Chemotherapeutic drugs		
Paclitaxel	<0.3 μg/mL	3.5
Docetaxel	4.93 μg/mL	2.92
Campothecin	50 μg/mL	1.48
Etoposide	200 μg/mL	0.6
Repurposed drugs		
Curcumin	0.011 μg/mL	3
All-*trans*-retinoic acid	29 μg/mL	6.3
Luteolin	5.72 μg/mL	7.1
Small molecule anticancer drugs		
VEGFR inhibitors (*e.g.* Cabozantinib, Nintedanib)	All <1 mg/mL	Not known
Wnt/β-catenin modulators (*e.g.* XAV-939, ICG-001)		
Hedgehog inhibitors (*e.g.* SANT75, HPI1)		
PI3K/Akt/mTOR modulators (*e.g.* rapamycin, Buparlisib)		

PWSAD. However, many of the co-solvents/surfactants used for solubilization are responsible for adverse side effects. Cremophor EL used in the commercial formulation of paclitaxel is a classic example of a surfactant triggering severe hypersensitivity reactions in patients. It has been reported that 30–40% of the patients receiving Taxol suffer from severe hypersensitivity reactions such as hyperlipidemia, abnormal lipoprotein patterns, aggregation of erythrocytes and peripheral neuropathy (7–9). In addition, Cremophor EL also interacts with the plastic components of polyvinyl chloride bags and infusion lines to cause toxicity (10). Similarly, the ethanol and Tween 80 used for docetaxel solubilization also lead to adverse effects (11). It is clear that a better drug solubilization strategy is greatly needed, and nanotechnology has provided a highly promising means to address this unmet need.

In this review, the authors will

- Examine the benefits of using nanocarriers for PWSAD solubilization
- Review the commonly used nanocarriers for PWSAD solubilization and delivery
- Provide an update on the advances in nanodelivery of the new and repurposed small molecule anticancer drugs
- Discuss the barriers commonly encountered in PWSAD nanoformulation development and the strategies to overcome these barriers

Considering that low aqueous solubility of drug is likely a problem that will persist in future, it is our goal to provide researchers and clinicians with a better understanding of the nanodelivery approach so safer and more efficacious PWSAD products can be developed.

BENEFITS OF NANODELIVERY OF PWSAD

Nanocarriers are sub-micron particles with diameter between 1 and 100 nm in any dimension. For biomedical use, they can be larger but should be at least <200 nm in diameter to avoid occlusion of capillaries and achieve higher tumour accumulation by the enhanced permeation and retention effects. To date, numerous studies have already demonstrated the ability of nanocarriers to efficiently encapsulate and deliver PWSAD.

PWSAD generally has a tendency to distribute and accumulate in the peripheral tissues. For instance, the mean apparent volume of distribution at steady state of paclitaxel ranged from 227 to 688 L/m^2, indicating extensive extracellular distribution (12). Considering the toxic nature of most anticancer drugs, this will translate into significant systemic toxicity. In addition to simply solubilizing a drug by the co-solvents/surfactants, studies showed that, once a chemotherapeutic drug is encapsulated inside a nanocarrier, its pharmacokinetics and biodistribution will be dictated by the properties of the nanocarriers rather than the drug molecules themselves (10). This opens up a variety of new ways to make a PWSAD work better without the need to modify it. As summarized in Table II, a well-designed nanocarrier can lead to several additional benefits including (1) improved circulation half-life, (2) active and passive targeting to the cancer cells leading to improved tumour levels, (3) reduced systemic side effects as a result, (4) allowing co-delivery of multiple drug combinations using a single nanocarrier, leading to enhanced anticancer effects, (5) overcoming clinical resistance conferred by drug efflux transporters (e.g. P-glycoprotein). Encapsulation in a nanocarrier also protects a labile drug from quick degradation. The nanotechnology approach is therefore a promising alternative for PWSAD delivery.

NANOCARRIERS FOR DELIVERY OF PWSAD

Most PWSAD are lipophilic molecules, which means that only nanocarriers with high enough lipophilicity can efficiently encapsulate this type of compounds. Lipid- or phospholipid-based nanocarriers are natural candidates for this purpose. As polymers and dendrimers can be easily engineered to the desirable lipophilicity, polymeric or dendrimeric nanocarriers of PWSAD are common. In addition, albumin was also studied for this purpose with clinical success. Table III lists some of the nanodelivery systems studied for PWSAD delivery.

Lipid- or Phospholipid-Based Nanocarriers

Liposomes

Liposomes were the first phospholipid vesicular systems developed back in 1960s. They consist of phospholipid

Table II. Summary of Advantages of Nanosystems for PWSAD Delivery

Advantages of Nanosystem for PWSAD delivery
• Higher tumor accumulation by passive and active targeting
• Improved aqueous solubility
• Protection from rapid degradation
• Controlled and sustained delivery
• Improved circulation half -life
• Reduced nonspecific toxicity
• Co-delivery of multiple chemotherapeutic agents

PWSAD poorly water-soluble anticancer drugs

Table III. Nanodelivery Systems Studied for PWSAD

Type	Characteristics	Drug delivered
Liposomes	Amphiphilic, biocompatible	Paclitaxel
	Easy modification	ATRA
	Targeting potential	Docetaxel
	Improve solubility	Etoposide
	Stability	Campothecin
		Curcumin
Solid-lipid nanoparticles (SLN)	Easy scale-up	Paclitaxel
	High lipid content, good encapsulation	Docetaxel
	Nontoxic organic solvent	Curcumin
		Campothecin
Nanostructured lipid carriers	Higher drug loading than SLN	Docetaxel
	Prevent water loss (good for skin hydration)	Paclitaxel
	Cosmetic and oral delivery	ATRA
		Curcumin
Nanoemulsion	Oil/water emulsion	Curcumin
	Kinetically stable	Docetaxel
	Used in parenteral delivery	ATRA
Polymeric nanoparticles	Water-soluble, nontoxic, biodegradable	Paclitaxel
	Surface modification	Docetaxel
	Specific targeting of cancer cells	ATRA
	Ease of reproduction	Etoposide
Polymeric micelles	Suitable for water-insoluble drug	Paclitaxel
	Biocompatible, self-assembling, biodegradable	Docetaxel
	Easy functional modification	Campothecin
	Targeting potential	
Dendrimers	Highly stable	Campothecin
	Size easily controlled	Paclitaxel
	Easy functional modification	Docetaxel
Albumin	Biocompatible, biodegradable	Paclitaxel
	Favourable biodistribution	
	May enhance tumor uptake of other chemo-drugs	

ATRA all-trans retinoic acid, *NLC* nanostructured lipid carriers, *SLN* solid lipid nanoparticles

bilayers which resemble human plasma membrane and therefore exhibit very high level of biocompatibility, and can also aid the diffusion of drug across plasma membrane (13). Owing to their amphiphilic nature, they can accommodate and stabilize hydrophilic drugs in the aqueous core and lipophilic drugs in their lipid bilayers (Fig. 1).

Since Doxil® was approved by FDA in 1995 as the first long circulating formulation for cancer treatment, many other chemo-drugs have been successfully encapsulated in liposomes. However, liposomal encapsulation of hydrophilic drugs is relatively easy compared with lipophilic drugs. Achieving high drug loading for hydrophobic drugs is quite challenging as the space within the lipid bilayer is limited compared with the aqueous core (Fig. 1). It is important to achieve a delicate balance between high drug loading without disturbing the stability of the lipid bilayers that maintain the integrity of the liposomal system. A variety of factors such as bilayer composition, physicochemical properties of the drug entity and method of preparation need to be well optimized to achieve efficient lipophilic drug loading (14).

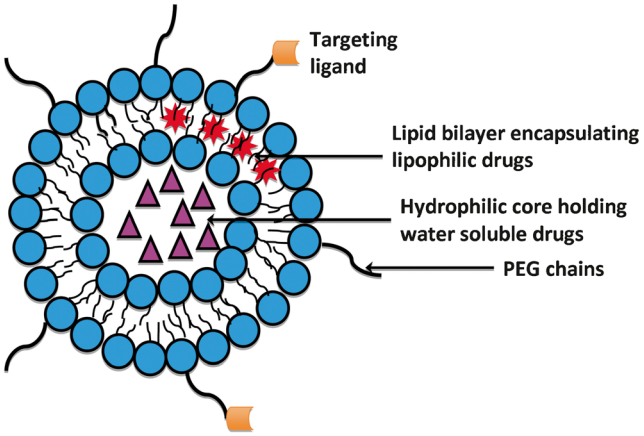

Fig. 1. Structures of a liposome and the manner it encapsulates poorly water-soluble lipophilic drug molecules

In spite of this limitation, several liposomal formulations have been developed for lipophilic, poorly water-soluble chemo-drugs. Due to the deficiency of solvent-based formulation, paclitaxel in particular has drawn most interest, and some liposome systems have demonstrated good clinical potential. For instance, Zhang *et al.* have developed a sterile lyophilizable liposomal paclitaxel formulation (15). They found that the liposomal formulation of paclitaxel was well-tolerated for doses up to 325 mg/m^2 which are much higher than the recommended dose of 175 mg/m^2.

Docetaxel, a semi-synthetic analogue of paclitaxel, and etoposide, a topoisomerase inhibitor, are other examples of PWSAD studied for liposomal delivery. Muthu and coworkers developed targeted theranostic liposomes encapsulating therapeutic (docetaxel) and imaging agent (quantum dots) (13). It is not only the drug was well-solubilized: The targeted liposomes also showed significantly higher cytotoxic effect compared with the commercial docetaxel (Taxotere) preparation in MCF-7 cells (IC_{50}=0.23 for liposomes *versus* 9.54 for Taxotere). It was observed that the addition of cholesterol to the phosphatidylcholine results in improved loading of lipophilic drugs due to increased hydrophobicity of lipid bilayer.

For etoposide, unilammellar liposomes with positive charges were synthesized to increase the antitumour efficacy and reduce the adverse effects commonly associated with etoposide (e.g. myelosuppression) (16). Encapsulation in these cationic liposomes increased the area under the concentration (AUC) of etoposide from 24.18 to 42.98 μg h ml^{-1} and extended the half-life from 58.6 to 186 min. Interestingly, similar to the above docetaxel formulation, incorporation of cholesterol also progressively stabilized the formulation. The benefits in pharmacokinetics contributed by liposomes were also demonstrated in other etoposide-loaded liposomes. For example, the liposomes developed by Sistla *et al.* showed a 60% increase in AUC with a 35% decrease in clearance ($p<0.05$) (17).

Recently, liposomal formulations delivering both docetaxel and etoposide were studied for drug powder inhalation. The cationic liposomes were combined with p53 gene therapy to form lipoplex, and it was shown that this multiple drug formulation was able to achieve synergism. The gene therapy sensitized the cancer cells to enhance the apoptotic effects of the chemo-drugs (18). This study has demonstrated the high versatility of liposomes for PWSAD delivery.

Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLN) were developed in 1990s to combine the advantages of polymer nanoparticles (e.g. controlled drug delivery, efficient encapsulation) and lipid emulsion (e.g. biocompatibility, improved bioavailability) (19,20). They are characterized by the inclusion of lipids that remain solid state at body temperature. A variety of physiological or biocompatible lipids such as triglycerides, fatty acids, steroids and waxes are frequently used in SLN formulation.

What makes SLN attractive from the perspective of pharmaceutical development is that they can be prepared with a variety of techniques including hot or cold homogenization, which means easy scale-up in production, preparation with high reproducibility and products free of toxic organic solvents (19,21). From a drug delivery perspective, because of the high lipid content, by nature, they encapsulate lipophilic PWSAD efficiently (22). Solid lipids are also known for their low intrinsic toxicity even when compared with poly (lactic-co-glycolic acid) (PLGA) (23). In addition, solid lipid-based nanosystems were shown to have inherent abilities to carry anticancer compounds into cancer cells, bypassing the drug efflux transporters, *e.g.* P-glycoprotein, by enhanced endocytosis (24). This means that SLN of PWSAD may also be more effective for drug-resistant cancer treatment.

A number of researchers have already studied SLN for PWSAD delivery. Yang *et al*. showed that, when campothecin was encapsulated in SLN, higher therapeutic efficiency was achieved after oral administration (25). SLN have also been used extensively for the delivery of taxanes such as paclitaxel and docetaxel (10,26). Yuan and coworkers reported a lower IC_{50} value with folate receptor targeted SLN compared with the free solution of PAX in A549 cells. SLN have also shown great potential in improving delivery of ATRA, a drug with cancer differentiation activity, to cancer cells *in vitro* and reducing its hemolytic potential when compared with the free drug (27).

As shown in Fig. 2a, the key limitation of SLN is that they are prone to issues such as drug expulsion and high burst release as result of the compact arrangement of solid lipid molecules. It was found that addition of lipids of different lengths to create a mixture may help reduce the drug expulsion, probably by introducing more room in the lipid structure for drug encapsulation (19). This concept was adopted in the design of nanostructured lipid carriers (NLC).

Nanostructured Lipid Carriers (NLC)

NLC are a modified version of SLN to reduce the burst release effect of SLN and further improve the drug loading. There are different variations of NLC (Fig. 2b), but nowadays the most common NLC systems involve solid lipids and liquid lipids (oil) mixing together to achieve reduced burst release effect (28,29). A higher drug loading compared with SLN was achieved as drugs possess higher solubility in oils compared with the solid lipids (20). Even though NLC have shown positive results, it is difficult to surface functionalize these carriers (30). It was reported that PEGylation to improve the circulation half-life of NLC resulted in reduced drug loading and faster drug release (20). Till recent years, NLC were more popular for cosmetic and oral drug delivery (20,28,31). However, with better understanding of this design, this trend has been changing, and nowadays, many researchers are considering NLC for systemic delivery of PWSAD that include docetaxel, paclitaxel, ATRA, curcumin and tamibarotene (32–37). These NLC devices generally shared the advantages of conventional SLN but achieved better encapsulation of PWSAD and more controlled release kinetics as anticipated. For instance, the NLC of the new synthetic retinoid tamibarotene was able to achieve average drug encapsulation efficiency and loading capacity as high as 90.85±1.03% and 9.08±0.10%, respectively, and demonstrated sustained release behaviour (37). This system also showed a longer retention time and higher AUC in mice when compared with free tamibarotene solution. NLC are clearly a highly promising class of nanodelivery systems for PWSAD.

Nanoemulsions

Nanoemulsions are nanosized oil-in-water emulsion systems formed by using high energy such as sonication or homogenization. Digestible oils such as soybean oil, sesame seed oil, cottonseed oil and safflower oil are often used to dissolve the lipophilic drugs (38). Nanoemulsions are kinetically stable and suitable for parenteral delivery of PWSAD (39). In comparison to other nanocarriers, nanoemulsion is easy to prepare and does not necessarily require organic solvents/co-solvents. The risk of carrier toxicity of food oil is low. As a result, several nanoemulsion formulations of PWSAD have been developed and studied. These include paclitaxel, docetaxel, curcumin and all-*trans* retinoic acid (ATRA) (40–44). Most of these formulations are still at development stage.

Nanoemulsions have their inherent limitations. These are illustrated by the example of Tocosol, a vitamin E-based nanoemulsion of paclitaxel (45). Tocosol had shown very promising results treating breast cancer in Phase II trial. However, in Phase III studies, this nanoemulsion failed to demonstrate significant advantage over Taxol (46). Tocosol has no specific mechanism to enhance tumour penetration or transport. Besides, it showed significantly higher rates of neutropenia compared with Taxol probably due to the higher dose of paclitaxel administered in the Tocosol group. The issues faced by Tocosol are difficult to address. As a liquid-based system, most of the surface-engineering techniques (e.g. active-targeting) that can be applied to other nanodelivery systems to increase their cancer specificity to reduce systemic toxicity may not be applicable. At this moment, the role of nanoemulsion for PWSAD remains as a reliable solubilization strategy.

Polymer- or Dendrimer-Based Nanocarriers

Polymeric Nanoparticles

Polymeric nanoparticles were invented in the 1970s to overcome the limitations associated with liposomes. They are considered to be more stable, and it is relatively easy to reproduce their physicochemical properties. They also offer more controlled release properties compared with liposomes and can be easily surface-modified (47). Polymeric nanoparticles can be divided into two categories: nanospheres and nanocapsules. Nanospheres are “matrix-type”, in which the

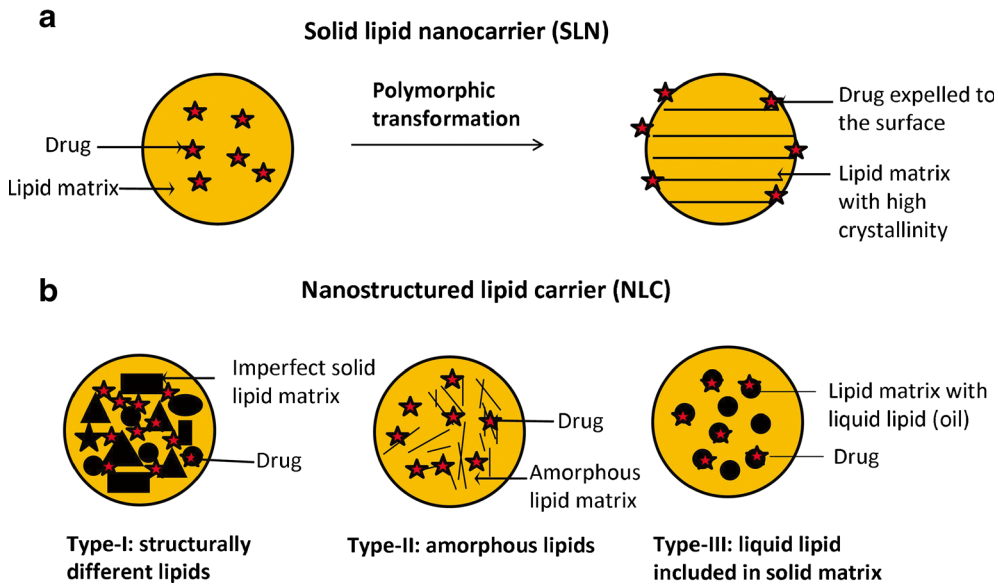

Fig. 2. Structures of **a** solid lipid nanoparticles (SLN); **b** three different versions of nanostructured carriers (NLC) and how these nanocarriers encapsulate poorly water-soluble anticancer drug molecules. The *top panel* shows the manner how the drug molecules are expelled to the nanoparticles surface due to polymorphic transformation in a suboptimal SLN system

drug is dispersed throughout the matrix while nanocapsules are "reservoir-type", in which the drug is present in the core surrounded by a polymeric shell. In recent years, biodegradable nanoparticles have shown a lot of promise as drug delivery vehicles because of their biocompatibility. They are generally made from biodegradable polymer such as PLGA, polylactic acid and polycaprolactone most of which have really good track record in the pharmaceutical industry.

In spite of being in the development for last three to four decades, polymer nanoparticles of PWSAD are still in the development or preclinical stage. They generally suffer from poor drug encapsulation efficiency, and the large molecular weight of polymers makes them more prone to triggering immunogenic responses (48). In order to make the nanoparticle clinically adaptable, many research groups have come up with polymer–lipid hybrid nanoparticles (24,49–51). This approach will be discussed in depth in the later sections.

Polymeric Micelles

Polymeric micelles are formed by spontaneous self-assembly of amphiphilic polymers (often block co-polymers), typically with a hydrophobic core and hydrophilic shell. Polymeric micelles were first reported in 1980s. Since then, they have made tremendous progress, and many are currently in the clinical trials. Till recently, there are around six polymeric micelle-based formulations in clinical trials (52).

This class of nanocarriers are particularly appealing devices for PWSAD delivery. Their size is usually in the range of 50–100 nm, which are small enough for extravasation into tumours by passive targeting (3). When compared with liposomes, they do not have a water-rich core; instead, they possess a lipidic central compartment which is suitable for holding lipophilic drugs. They generally have high thermodynamic stability (3), so they do not tend to release drug prematurely. In addition, they can be conveniently surface-modified (53).

The surface-modification can be as simple as just PEGlyation to extend the circulation time (*i.e.* addition of polyethylene glycol (PEG)). PEG-phosphoethanolamine lipid micelles were shown capable of accumulating in subcutaneous Lewis lung tumours and EL4 T-lymphoma tumours in mice after IV administration (54). The circulation time was extended, and the micelles were found physically intact after prolonged exposure to serum. Since then, several other chemo-drug-loaded polymeric micelles have been developed (55–60). NK105 is another paclitaxel-loaded micelle that has shown encouraging results (56) When compared with solvent-based paclitaxel in rodent tumour models, NK105 led to 90-fold higher plasma AUC, 25-fold higher accumulation in tumour tissue, 100-fold reduction in steady-state volume of distribution (i.e. less peripheral distribution) and hence reduced toxicity (56). Currently, NK105 is in phase III trial for the treatment of metastatic and recurrent breast cancer in Japan (61). Meanwhile, another polymeric micelle Genexol-PM for paclitaxel solubilization and delivery has also shown promise. Like NK105, Genexol-PM has shown superior chemotherapeutic activity and less toxicity than the free drug (57) and consequently been approved for metastatic breast cancer in Korea and is under clinical evaluation (recruiting stage) in the US (62).

Dendrimers

Dendrimers are highly branched polymer molecules typically 5–20 nm in size (up to 100 nm) formed by a central core to which the branches are attached. As a single macromolecule, dendrimeric nanoparticles are very stable. Dendrimers are highly versatile nanocarriers (63). Their size can be easily adjusted and surfaces conveniently functionalized due to the availability of multiple reactive functional groups. It was suggested that dendrimers can improve the solubility of PWSAD by various types of interactions including ionic interaction,

hydrogen bonding or hydrophobic interaction. In addition, they possess several end sites for conjugation of multiple drugs and targeting moieties (64).

As a relatively new class of nanodelivery systems, the only dendrimer-based product currently in the clinical stage is VivaGel for prevention of HIV infection (65), but because of the favorable physicochemical properties, there have been increasing attempts to use dendrimers for PWSAD delivery. Dendrimeric nanoformulations for the delivery of chemo-drugs such as campothecin (66), paclitaxel (64) and docetaxel (67) have been studied. Recently, Gajbhiye and Jain compared Tween-80 (P80) anchored poly(propyleneimine) (PPI) dendrimer formulation of docetaxel with docetaxel-PPI and free docetaxel for brain tumour treatment (67). The *in vivo* study revealed that P80-PPI significantly reduced the tumour volume ($p<0.0001$) and extended the median survival time for the tumour-bearing rats from 23 days (docetaxel-PPI), 15 days (receptor blocked group) and 18 days (free docetaxel) to 42 days. This example demonstrated the efficiency of dendrimer for delivery of PWSAD and their potential to be surface-engineered for targeted therapy.

Albumin-Based Nanoformulation

As lipophilic drugs tend to have strong binding affinity for serum albumin, it is a logical choice to study the use of albumin for PWSAD delivery. The most successful albumin-based system is definitely Abraxane, a protein-bound paclitaxel formulation. Abraxane was initially approved by the FDA in 2005 for breast cancer that is chemotherapy-refractory or has relapsed, and was later approved for non-small-cell lung cancer treatment in 2012 and metastatic pancreatic cancer in 2013 (68).

Abraxane® enjoys such a clinical success mainly because it does demonstrate superior efficacy over the solvent-based paclitaxel formulation. Multiple factors may contribute to this advantage (69). First, Abraxane leads to an advantageous pharmacokinetic profile, resulting in a 33% higher tumour uptake relative to solvent-based paclitaxel. The albumin component of Abraxane may also be able to bind to secreted protein acidic and rich in cysteine which enhances active drug transport and accumulation in the tumour. This albumin-based drug may also enhance tumour accumulation of other chemo-drugs such as gemcitabine. It was suggested that Abraxane appears to interact with tumour in several ways in a manner still not fully understood (68). Further studies are required to allow full leverage of these mechanisms for optimal treatment outcomes.

AN UPDATE ON NANODELIVERY OF NEW OR REPURPOSED ANTICANCER DRUGS THAT ARE POORLY WATER-SOLUBLE

Most of the PWSAD nanoformulations developed so far are for delivery of conventional cytotoxic chemo-drugs. In the last decade, several less cytotoxic drugs are developed for targeting molecular pathways that contribute to the cancer growth, progression and spreading, *e.g.* inhibitors of VEGF receptors, PI3K/Akt/mTOR, sonic hedgehog and Wnt/β-catenin pathways (2). In addition, there are also existing non-cytotoxic compounds such as curcumin and resveratrol that have been "repurposed" for cancer prevention and treatment (3). Given their promising *in vitro* data, many of these drugs have aqueous solubilities below 1 mg/ml and require organic solvent for solubilization (see Table I), so it is difficult to efficiently administer them in clinical setting. Researchers are therefore beginning to develop nanocarriers for these new or repurposed PWSAD.

For instance, Yuan *et al*. developed PEGylated liposome to encapsulate SANT75 (70). SANT75 is a compound practically water-insoluble and has potent inhibitory effects on the hedgehog pathway. This SANT75-liposomal formulation was shown to have similar potency as free drug when tested in transgenic zebrafish and improved the bioavailability of the drug and extended the survival time in tumour-bearing mice without obvious systemic toxicity. Another group encapsulated another hedgehog inhibitor HPI-1 with polymeric nanoparticles (71). This nanoformulation, named "NanoHHI", was shown to bypass the drug resistance in cancer cells and potently suppress *in vivo* tumour growth of liver cancer xenografts. NanoHHI was also found to be superior to the free drug in attenuating the systemic metastases in the orthotopic setting. A number of nanoformulations were also developed for the poorly water-soluble repurposed compounds. Curcumin is a phenol compound with anticancer and antioxidant properties but has low solubility and oral bioavailability. This issue was addressed by delivering it with different types of nanocarriers (72–75). All studies showed improved biodistribution and efficacy. In brief, these early works suggest that nanotechnology is a valuable tool to improve the delivery of the newly developed or repurposed PWSAD.

Because these newer PWSAD are less cytotoxic, researchers began to combine them with the cytotoxic chemo-drugs in nanocarriers for improved anticancer effects. Cho *et al*. developed PEG-block-poly(−caprolactone) micelles for co-encapsulation and simultaneous delivery of paclitaxel (cytotoxic agent), cyclopamine (hedgehog inhibitor) and gossypol (Bcl-2 inhibitor) (76). Although this three-drug micelle did not show better efficacy in the traditional two-dimensional cancer cell culture, it was significantly more effective in three-dimensional cell spheroids and in xenograft models, resulting in tumour growth inhibition and prolonged survival over paclitaxel alone. Hasenstein *et al*. studied the co-delivery of paclitaxel (cytotoxic agent), rapamycin (mTOR inhibitor) and 17-AAG (Hsp90 inhibitor) with a similar micelle system named Triolimus (77). This three-drug Triolimus system was tested in a MDA-MB-231 breast cancer model. Tumour growth delays resulted from a doubling in tumour cell apoptosis, and reduction in tumour cell proliferation were observed comparing with paclitaxel-only micelles. These studies demonstrated the potential of developing nanocarrier-based multi-PWSAD therapy to achieve more efficacious anticancer treatment.

BARRIERS TO CLINICAL TRANSLATION OF PWSAD NANOFORMULATIONS

Table IV summarizes the barriers limiting the clinical translation of nanodelivery systems of PWSAD. These barriers are discussed in more details as follows.

Table IV. Summary of Barriers Limiting the Clinical Translation of Nanodelivery Systems of PWSAD

Barriers limiting the clinical translation of nanodelivery systems
Formulation issues
• Low/poor encapsulation efficiency
• Poor drug release kinetics: burst release, no sustained release effect
• Stability: drug leakage, aggregation, drug expulsion
Biological issues
• Poor biodistribution
• Nanotoxicity
Other issues
• Scalability, lack of good manufacturing practice, *etc.*

Formulation Issues

Inefficient Encapsulation of the Drug

For any nanodelivery system, higher encapsulation efficiency and drug loading are preferred because this means less carrier materials and excipients will enter the patient's body, which translate into lower risk of toxicity and immunogenicity. This issue may be significant for certain polymeric systems. First, some synthetic polymers may have slow biodegradation rate or toxic degradants, and this can lead to local toxicity if used at higher concentrations (19). Moreover, a poorly designed polymeric nanosystem may have inadequate interactions with PWSAD molecules, which means less amount of drug per unit weight of polymer matrix (78). In general, poor PWSAD encapsulation can be contributed by several factors such as matrix polymorphism, method of preparation, surfactants or solvents used to dissolve the drug. It has been reported that during the emulsification step, poorly water-soluble compounds tend to precipitate out and remain outside the nanocarriers leading to low encapsulation efficiency (79). In addition, the drug loading in nanocarriers is generally influenced by the relative distribution of the drug between the polymeric phase and the aqueous phase during the preparation process (80), and this distribution is largely determined by the drug's solid-state solubility in the nanocarrier matrix.

Unfavorable Drug Release Kinetics

Burst release effect is referred to the phenomenon describing the fast, uncontrolled drug release from a drug carrier when it is first exposed to the external medium. This is a particularly common phenomenon in nanocarriers as a result of their large surface area. Even though burst release is desirable in a few exceptional cases (e.g. local wound treatment or pulsatile release), it is often an unpredictable, difficult to control and thus an unwanted process (81), not to mention, this process is also economically wasteful.

Burst release is particularly undesirable in the context of cancer drug therapy as many chemo-drugs have high toxicity. The quick dumping of these drugs from the carriers in the initial minutes or hours at the site of administration of circulation will likely cause high local or systemic toxicity. From a therapeutic perspective, this also means a loss of a significant portion of the drugs before they can reach the tumour tissues to achieve the intended anticancer effects. Sustained drug release from the nanocarriers for prolonged treatment also becomes less achievable (78,81).

The origin of burst release effects is related to the nanocarrier preparation process. During the emulsification process, nanoparticles tend to shrink and the loaded drug molecules will migrate to and deposit on or near the surface of the nanocarriers (82). These deposited drug particles give rise to a biphasic drug release profile, where a large amount of drug is released in the initial hours followed by a sustained drug release. This phenomenon is observed in both polymeric and solid lipid nanoparticles.

Stability Issues

Nanocarriers ideally should have a shelf life of 2 years or longer. Within the shelf life, the nanocarrier should be able to protect the therapeutic activity of the drug as well as maintain the physical integrity of the matrix that holds the drug and colloidal stability (constant size). This goal is not easy to achieve. For instance, in the case of SLN, the lipids can be converted into a more stable and well-organized β form during storage (29) which leads to drug expulsion and consequently diminished drug loading and burst release. In addition, the expelled drug also becomes unprotected and may lose its potency.

Many nanoformulations were prepared by emulsification in aqueous environment so they tend to contain a lot of water. Many components such as lipids may undergo chemical changes such as hydrolysis/oxidation after long-term storage, which consequently may compromise the stability of the entrapped drug (83). To improve their stability and handling, freeze drying is essential. However, during freeze-drying, the size of nanocarriers generally increases and freeze-drying stresses can also rupture the fragile membrane of nanocapsules. Such rupture can lead to drug leakage from the nanocapsules. Similarly, liposomes can undergo aggregation due to fusion by the stresses (83).

Scale-Up Issues

The newer generation of PWSAD nanoformulations often includes multiple components to achieve several functions (*e.g.* targeting, imaging, long-circulation). Their scale-up production thus becomes more costly and technically difficult. Studies to optimize this process will eventually add to the final cost of the product. Technically speaking, many commonly used laboratory techniques such as sonication are difficult to implement at production scale (78). It is also quite challenging to achieve nanoparticles with same size in a larger batch. For polymeric nanoparticles that frequently involve organic solvents in their preparation, issues associated with the evaporation of large quantity of solvents during their industrial production and their toxicity are also concerning.

Biological Issues

Unfavorable Biodistribution

From the previously discussed case of Tocosol, it is clear that to improve the therapeutic efficacy of PWSAD

nanoformulations, favourable biodistribution of the nanocarrier is essential. The biodistribution of nanoparticle in the body is dependent on its size, surface chemistry (presence of PEG or other ligands) and surface charge of the nanoparticles (84).

Although nanocarriers smaller than 100 nm in diameter are generally preferable, to date, there is still no real consensus about what particle size will lead to most favourable *in vivo* and clinical biodistribution. In fact, over-zealous size reduction could be counter-productive. It was shown that nanoparticles less than 50 nm resulted in higher uptake in liver and spleen and could lead to liver toxicity (85), while nanoparticle less than 5 nm in size are removed by kidney (21).

In addition to size, surface properties are key factors affecting biodistribution. Unmodified nanocarriers with hydrophobic surface are readily removed from the circulation by opsonization and taken to the liver. After clinical approval of the PEGylated liposomes, this issue appeared resolved, and a tremendous interest was generated in the development of PEGylated nanocarriers. However, it should be noted that, even though PEGylation is necessary to improve the residence time in circulation, excessively high PEG density can also render a nanocarrier too hydrophilic which may reduce the extravasation into tumour.

Nanotoxicity

To achieve clinical translation, the safety of nanocarriers becomes a significant concern. Learning from the inorganic nanomatters (*e.g.* carbon, silica, iron oxide) (86), an apparently inert material can become toxic after downsizing to submicron size range. This so-called nanotoxicity is likely caused by a dramatic increase in surface area for this material to directly react with the biological components (*e.g.* cell membrane, key proteins) or trigger formation of harmful chemicals (*e.g.* reactive oxidative species). Thorough studies to evaluate the toxicity of PWSAD nanoformulations are thus essential.

Currently, there has been a general lack of awareness of the potential negative impacts coming from nanocarriers, and this issue is particularly alarming for PWSAD nanotherapies. Because these therapies are developed to exert toxic effects on cancer cells, researchers thus tend to overlook the distinction between their efficacy and nanotoxicity. It should be noted that a PWSAD nanotherapy is supposed to be toxic only on cancer cells (efficacy) but not non-cancer cells (toxicity). Besides, it is generally desirable that the cancer-killing effects are derived mainly from the drug itself and not the nanocarrier, as the cytotoxicity mechanism of nanocarrier is typically less established and less predictable.

To better understand the nanotoxicity, a thorough study of the chemical and physical properties (size, charge, shape) of the nanomaterials and their interaction with biological components is essential (87). However, most of the time the data extracted from *in vitro* cytotoxicological evaluation may not be applicable to *in vivo* systems (78,87), not to mention in clinical situations. In addition, the "tools" used for nanotoxicity evaluation nowadays are far from optimal. There has been a lack of well-agreed standard for nanotoxicity assessment. It is almost impossible to objectively compare the nanotoxicity data from different studies when different tools and standards were used.

STRATEGIES TO OVERCOME BARRIERS

Screening for Optimal Drug/Nanomaterial Miscibility

The higher the miscibility of the drug with nanomaterials, the more likely a high drug loading can be achieved (80). Whether the drug of interest will be well-miscible with the nanocarrier matrix should be carefully evaluated in the preformulation stage. Screening and optimization should not be only based on the dissolved state parameters (*e.g.* partition coefficient); information about the solid-state solubility is also necessary. Use of techniques such as differential scanning calorimetry, X-ray crystallography and microscopy to examine the polymer-drug product will be helpful. In addition, polymer end-groups can also play a vital role in improving the encapsulation efficiency of a polymeric delivery system (88). This also needs to be well screened for and optimized during preformulation.

Hybrid Nanosystems

In recent years, there has been an increase in the development of hybrid nanocarriers (49–51,89). Hybrid nanocarriers are made of two or more classes of nanomaterials, typically polymer and lipid (*i.e.* polymer-lipid hybrid nanocarriers). There are also reports of polymer-silica and polymer-metal nanoparticles, but these are seldom used for drug delivery (90,91).

Zhang *et al*. formulated a lipid—polymer hybrid nanoparticle to combine the benefits of polymeric nanoparticles and liposomes. This hybrid system had a lipid monolayer encapsulating polymeric core to hold the PWSAD docetaxel (51). This system offered higher encapsulation efficiency (59%) than polymer-only nanoparticles (37%). In addition, with the lipid coating on the surface to serve as controlled release barrier, the nanoparticles offered a sustained drug released effect which lasted for 120 h. The burst release was also suppressed, with only 50% of drug released within first 20 h comparing to 7 h for the polymer-only nanoparticles.

Our group has developed a polymer-oil hybrid nanocarrier (PONC) for the encapsulation of all-trans-retinoic acid (ATRA). ATRA is a highly lipophilic compound (log p= 6.3) that is prone to precipitation. With the incorporation of oil in PLGA polymeric matrix of PONC, significantly higher encapsulation efficiency and reduced burst release effect were achieved. These improvements were particularly obvious at higher drug loading (*e.g.* 5%). PONC demonstrated over 202% increase in the EE over the standard PLGA nanoparticles (49). It was suggested that the oil not only contributed to drug solubilization, it also introduce amorphosity to the PLGA matrix to provide more room to accommodate the higher drug content in the manner similar to NLC. Overall, these hybrid nanosystems are promising, but considering that they have more carrier components, more extensive evaluation of their toxicity and immunogenicity will be needed for their successful translation.

Active Targeting

The active targeting approach is becoming increasingly popular for PWSAD delivery. By putting a targeting moiety that have high specificity and affinity for a target at or near the

tumour on a nanocarrier, increased tumour accumulation can be achieved. The target is typically a receptor that is highly expressed on the surface of cancer cells (or sometimes cells in the neighborhood) and expressed at substantially lower levels in non-cancer tissues. The targeting also allows dose-sparing which is important for the highly toxic chemo-drugs. In addition, active-targeting frequently increases the cellular uptake of the nanocarrier and its loaded drug by enhanced endocytosis, thereby improving the anticancer efficacy as well.

When choosing targeting ligand, in addition to cancer specificity, the negative impacts should also be considered as the targeting ligand itself could lead to immune response and phagocytosis (92). Monoclonal antibodies are often chosen due to its high specificity and affinity for the overly expressed receptors. Although it is possible to directly conjugate the antibody to the drug, these antibody–drug conjugates do not offer any controlled drug release effect like nanocarriers (93), so there are advantages of using antibody-coated nanocarriers. A number of monoclonal antibody-based nanoparticles, or "immuno-nanoparticles", have therefore been developed for PWSAD delivery (93,94). In addition to antibodies, antibody fragments, peptides and receptor substrates are also feasible choices as targeting ligands.

Regarding the targets, folate receptor is commonly used as it is overexpressed in a variety of cancers as a result of increased demand for folic acid for DNA synthesis in cancer cells (92). Folate is also less expensive, non-immunogenic and easy to conjugate (92). Up to date, there is enough evidence to support that folate-conjugated nanoparticles show improved accumulation in malignant cells (50,95). Transferrin receptor is also commonly exploited due to its higher expression on cancer cells (almost 100-fold higher than normal cells) (96). Other common targets used include human epidermal growth factor receptor 2 (HER2) (94) and epidermal growth factor receptor (EGFR) (97). HER2 is over-expressed in many cancer types, *e.g.* breast cancer and ovarian cancer. Cirstoiu-Hapca *et al*. coated nanoparticles with anti-HER2 monoclonal antibodies (Herceptin) to create immuno-nanoparticles for paclitaxel delivery (94). They showed significantly higher tumour accumulation of paclitaxel in a disseminated ovarian cancer model when compared with free paclitaxel. However, the difference was considered not significant comparing the immuno-nanoparticles and non-targeted nanoparticles. This indicates that, while active targeting generally leads to promising results *in vitro*, it does not always translate into superior performance *in vivo* or in clinical studies.

Emphasis on Nanotoxicity Evaluation

Nanotoxicity is a major concern, and in coming years, it will play a major role in deciding the fate of nanomedicine. Hence, a more standardized approach for evaluation of nanotoxicity has been taken up by the Nanotechnology Characterization Lab (NCL). NCL has streamlined the process of preclinical evaluation of nanoscale delivery platforms to speed up its clinical translation and regulatory approval. The assay cascade involves a standardized in depth *in vitro* physicochemical evaluation of nanoparticles, followed by *in vitro* and *in vivo* assays to establish its biocompatibility and efficacy (available at http://ncl.cancer.gov/working_assay-cascade.asp). Such initiative by the NCL will definitely help in redefining the future of nanomedicine.

In addition to the standards and tools, the experimental design also needs to include more elements for evaluation of the potential nanotoxicity coming from the carrier itself. Better controls, *e.g.* inclusion of drug-free nanocarrier and non-cancer cells in the studies, are essential for differentiating between the true anticancer efficacy (specific for killing/suppressing cancer cells) and nanotoxicity (non-specific toxicity on healthy cells and tissues from the nanocarrier). The long-term nanotoxicity or some subtle forms of toxicity should also be aware. Nanocarriers such as dendrimers have been shown to alter the expression of multiple genes without directly killing the cells, but, in a long run, this effect can eventually cause cell mortality and tissue damages (98).

CONCLUSION

Poor aqueous solubility of drugs has been a common but serious issue in clinical application and development of anticancer compounds. Many highly active and promising new molecules are regularly rejected because of their low solubility. Higher dosing of many current chemo-drugs is also limited by the toxicity of high concentrations of surfactants/co-solvents used to solubilize the drugs. Nanodelivery systems not only help solubilize many existing and developing PWSAD; they offer many additional advantages such as improved biodistribution, reduced systemic toxicity and enhanced therapeutic effectiveness. In near future, it is foreseeable that more advanced nanocarriers such as hybrid systems will be developed for PWSAD delivery, more extensive and optimized use of active-targeting strategies will be emphasized, and there will be higher awareness of the nanotoxicity issues. These advances will improve the translational success of nanotechnology-based PWSAD products and help bring more of them into the market.

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