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Review

Polymeric micelles drug delivery system in oncology

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ABSTRACT

Polymeric micelles (PM) system, as an efficient drug carrier, has received growing scientific attention in recent years owing to its solubilization, selective targeting, P-glycoprotein inhibition and altered drug internalization route and subcellular localization properties. Seven PM formulations of anti-tumor drugs being evaluated in clinical trials are reviewed in this paper, in terms of formulation study, *in vitro* cytotoxicity, *in vivo* pharmacokinetics, anti-tumor efficacy and safety as well as clinical trials, to shed new light on the discovery of novel PM formulations. In these seven PM formulations, PM system was employed to overcome the issues of low water solubility, high toxicity and (or) multidrug resistance accompanied with the conventional formulation, which greatly hampered their clinical application. Those promising preclinical and clinical results combined with rapid advancement and intense multidisciplinary collaboration enable the extension of the PM system to traditional Chinese medicine, imaging agents, gene and combination agent deliveries as well as some other administration routes, which facilitate the clinical translation of the PM drug delivery system.

Contents

1. Introduction

In the past two decades, new drug exploration in terms of new chemical compounds was more and more challenging. In 2007, Somatuline Depot®, Lanreotide Acetate long-acting injection, was approved

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by the US Food and Drug Administration as a new molecular entity [1], indicating a significant role of the drug delivery system (DDS) in new drug exploration. Poor water solubility accompanied with many potential new drugs due to the required lipophilic groups for receptor recognition and membrane permeability, is rapidly becoming the principal obstacle for their clinical application [2]. Besides, many conventional chemotherapy regimens should be discontinued because of the significant adverse effects resulted from the non-selective targeting, despite their persisting effects. Polymeric micelles (PM) are composed of two separated functional segments: inner core and outer shell. The outer shell controls the *in vivo* pharmacokinetic (PK) behavior, while the inner core is responsible for drug loading capacity, stability and drug release behavior. The suitable PM size, too large for extravasation from normal vessel walls and renal excretion, and too small for extravasation from tumor blood vessels, combined with the pathophysiological characteristics of solid tumor tissues, hypervascularity, incomplete vascular architecture, secretion of vascular permeability factors and absence of effective lymphatic drainage leads to enhanced permeability and retention (EPR) effect of PM in solid tumors [3,4], which warrants the passive targeting of PM, while the passive targeting is the basis of active targeting. Apart from its solubilization, small particle size, long circulation, targeting and easy production properties, PM system can alter the drug internalization route and subcellular localization, lessen the P-glycoprotein (P-gp) efflux effect, consequently, exert a different mechanism of action from the entrapped drugs [5,6]. As well, compared with those more recent nano-DDSs, including liposomes, nanoparticles and dendrimers, PM possesses higher drug loading capacity as well as improved stability. Due to the promising characters, PM received fast-increasing scientific attention as an efficient drug carrier in recent years. In this paper, seven PM formulations of anti-tumor drugs being evaluated in clinical trials are reviewed (Table 1) to shed new light on the discovery of novel PM formulations. The future prospects of PM systems are also discussed.

2. Polymeric micelles formulations of anti-tumor drugs in clinical trials

2.1. Genexol®-PM

Paclitaxel (PTX) is an effective anti-tumor agent by promoting the assembly of microtubules from tubule dimmers and preventing them from depolarizing [27]. Because of its low solubility (0.3 μg/mL), commercially common used formulation Taxol® is formulated in a 50/50 (v/v) mixture of Cremophor EL (CrmEL)/ethanol. However, CrmEL is biologically and pharmacologically active and the amount of CrmEL required is considerably high, which results in significant side effects [28]. Because of the inherent problems associated with CrmEL, some new DDSs for PTX, which have good aqueous solubility and fewer side effects, are under current investigation including emulsion [29], liposomes [30], water-soluble prodrugs [31], nanoparticles [32], polymeric micelles [33] and nanoparticle colloidal suspension [34]. Among these DDSs, the most successful formulations which have been marketed are Genexol®-PM and Abraxane®.

Genexol®-PM is a PM formulation of PTX in monomethoxy poly(ethylene glycol)-*block*-poly(D,L-lactide) (mPEG-PDLLA) which was synthesized by the ring opening polymerization procedure with mPEG molecular weight of 2000 g/mol [7]. In a similar system by Zhang [35], the optimum mPEG-PDLLA diblock copolymer was characterized by mPEG molecular weight and mPEG-PDLLA weight ratio of 2000 g/mol and 60:40, respectively, in terms of the drug loading capacity and stability. In this system, the drug loading was as high as 25% and the reconstitution solution can be stable with the size smaller than 50 nm for 2 months and 1 day at 4 °C and room temperature, respectively. Genexol®-PM was prepared by a solid dispersion technique with a drug loading of 16.7% [7,8].

Table 1
Information of polymeric micelle formulations of anti-tumor drugs in clinical trials.

Formulation	Polymer	Drug	Issues solved	Incorporation mode	Diameter	Drug loading	*In vitro* cytotoxicity (*vs.* free drug)	*In vivo* PK parameters fold change (*vs.* free drug)			MTD (mg/m^2)	Status	Company	Ref.
								$t_{1/2}$	AUC_{blood}	AUC_{tumor}				
Genexol®-PM	mPEG-PDLLA	Paclitaxel	Solubilization	Physical entrapment	<50 nm	16.7%	Comparable	0.62[a1]	0.74[a1]	1.74[a1]	390[b1]	II[c]	Samyang, Korea	[7–12]
NK105	PEG-P(Asp)[d]	Paclitaxel	Solubilization Targeting	Physical entrapment	85 nm	23.0%	Comparable	6.11[a2] 3.71[a3]	86.11[a2] 50.40[a3]	24.00[a2] 24.06[a3]	180[b2]	II	Nanocarrier/Nippon Kayaku, Japan	[13–15]
NC-6004	PEG-P(Glu)(Cisplatin)	Cisplatin	Targeting	Coordinate bonding	30 nm	39.0%	6–15 fold lower	0.19	64.77	3.59	120[b2]	I/II	Nanocarrier, Japan	[16–18]
NC-4016	PEG-P(Glu) (DACHPt)	DACHPt	Targeting	Coordinate bonding	40 nm	N/A	N/A	N/A	N/A	N/A	N/A	I	Nanocarrier, Japan	[19]
NK012	PEG-P(Glu)(SN-38)	SN-38	Solubilization Targeting	Chemical conjugation	20 nm	20.0%	Comparable or a little bit lower	16.41[e]	14.09[e]	9.53[e]	28[b3]	II	Nippon Kayaku, Japan	[20,21]
NK911	PEG-P(Asp)(DOX)	Doxorubicin	Targeting	Physical entrapment	40 nm	N/A	Comparable	2.62	28.88	3.46	67[b4]	II	Nippon Kayaku, Japan	[22,23]
SP1049C	Pluronic L61, F127	Doxorubicin	Anti-MDR	Physical entrapment	30 nm	8.2%	Improved cytotoxicity on DOX-resistant cells	1.38[f1] 1.05[f2]	2.06[f1] 1.20[f2]	1.69	70[b5]	III	Supratek, Canada	[24–26]

Abbreviation: AUC: area under curve; DACHPt: dichloro-(1, 2-diaminocyclohexane) platinum(II); DOX: doxorubicin; MDR: multidrug resistance; MTD: maximum tolerated dose; N/A: Not available; P(Asp): poly(aspartic acid); P(Glu): poly(glutamic acid); PDLLA: poly(D,L-lactide); PEG: poly(ethylene glycol); PK: pharmacokinetic; SN-38: 7-ethyl-10-hydroxy-camptothecin.
[a]Dose. [a1]150 mg/kg dose of Genexol®-PM *vs.* 20 mg/kg dose of Taxol®; [a2]50 mg/kg; [a3]100 mg/kg.
[b]Dosage regimen. [b1]3-h infusion every 3 weeks; [b2]1-h infusion every 3 weeks; [b3]30-min infusion every 3 weeks; [b4]10 mg/min every 3 weeks; [b5]4 mg/min every 3 weeks.
[c]Marketed in South Korea in 2007.
[d]P(Asp) modified with 4-phenyl-1-butanol.
[e]Polymer-unbound SN-38 from NK012 group (30 mg/kg) *vs.* SN-38 from CPT-11 group (66.7 mg/kg).
[f]Mice condition. [f1]Normal mice; [f2]Tumor bearing mice.

The mPEG-PDLLA micelles were shown to be biocompatible and non-toxic in view of the *in vitro* and *in vivo* studies [7,36]. Genexol®-PM as well as its similar system displayed a comparable cytotoxicity with that of Taxol® against different human cancer cells, including breast, colon, ovarian and non-small cell lung cancer (NSCLC) cells [7,37].

PTX was rapidly and evenly distributed in the tissues after Genexol®-PM administration. Besides, in tritiated-PTX-loaded ^{14}C-mPEG-PDLLA micelles treated rats, tritium radioactivity deviated from ^{14}C radioactivity with the observation of urine and feces as the major elimination route for mPEG-PDLLA and PTX, respectively [36]. All those facts combined the comparable cytotoxicity between Genexol®-PM and Taxol® suggested the rapid release of PTX from the micellar formulation and such rapid release was possibly due to the decomposition of micelles by α- and β-globulins [38]. Consequently, unlike the usual PK behavior of micellar formulation, an 82% decrease in plasma area under curve (AUC) of PTX after micellar formulation intravenous (i.v.) treatment was found when compared with equivalent PTX dose of Taxol® treatment [39]. However, it has been reported that the high plasma AUC of Taxol® resulted from CrmEL entrapment, consequently, generates nonlinear PK and narrow distribution, prevents PTX tumor accumulation, increases myelosuppression and hinders the P-gp inhibition effect of CrmEL on tumor cells [28,40]. Meanwhile, despite the plasma AUC of Genexol®-PM (50 mg/kg) being lower than that of Taxol® (20 mg/kg), the organ AUC including the tumor was increased proportionally to the PTX dose [7].

Because of CrmEL-free property, Genexol®-PM possessed a higher maximum tolerated doses (MTD) and median lethal dose than Taxol® [7]. The antitumor efficacy of Genexol®-PM was investigated in SKOV-3 human ovarian cancer and MX-1 human breast cancer cell subcutaneously transplanting nude mice and compared with Taxol® at each MTD dose. A remarkable anti-tumor activity was observed in Genexol®-PM group especially in MX-1 bearing mice, even with some complete responses [7]. Meanwhile, mPEG-PDLLA did not show any tumor growth inhibition [7].

Due to the encouraging anti-tumor activity and safety profile, Genexol®-PM advanced into clinical trials in South Korea in 2001 [8]. Genexol®-PM was administrated i.v. over 3 h every 3 weeks with the dose escalating from 135 to 390 mg/m^2 according to the standard "3 + 3" rule. No hypersensitivity reaction was observed although no premedication was performed. The MTD was defined as 390 mg/m^2, which was higher than that of Abraxane® (300 mg/m^2) [41] a novel PTX albumin-bound nanosuspension, with the dose-limiting toxicities (DLTs) of neutropenia, sensory neuropathy and myalgia, and recommended dose (RD) for phase II was defined to be 300 mg/m^2 as a 3-h infusion every 3 weeks. Despite the disappointing PK behavior like that obtained from animals, the responses can be compromised by the available high dose level. 3 patients (14%), 2 of whom were refractory to prior taxane therapy, responded partially and 6 patients (28%) remained stable. At present, another phase I clinical trial of Genexol®-PM has been conducted in Singapore, with a dosage regimen of 1-h infusion on a weekly basis for 3 weeks followed by a resting week and doses of 80, 100, 120, 140, 160 and 180 mg/m^2 [9]. Although the incidence of neutropenia was similar to that observed in the 3-week regimen, the non-hematologic toxicities were less severe. The MTD and RD for phase II were determined as 200 and 180 mg/m^2 as a 1-h infusion weekly, respectively. 21 out of 24 patients were assessable for response, 5 (21%) of whom had partial response and 9 (38%) of whom had stable disease for 16 weeks. Unlike Taxol®, Genexol®-PM was characterized by linear PK behavior from those two independent phase I trials [8,9].

Up till now, three phase II clinical trials of Genexol®-PM have been conducted in patients with NSCLC [11], metastatic breast cancer [10] and advanced pancreatic cancer [12], respectively. In the first trial for NSCLC, Genexol®-PM (230 mg/m^2) was administrated for 3 h and followed by cisplatin (60 mg/m^2) every 3 weeks. Intrapatient dose adjustment of Genexol®-PM to 300 mg/m^2 was carried out from the second cycle according to the safety profile. Consequently, the mean dose delivered after the first cycle was 84 mg/m^2/week (planned dose 100 mg/m^2/week). The DLTs were similar to the phase I trial. Although Genexol®-PM as a CrmEL-free PTX formulation, the grade 3/4 hypersensitivity reactions occurred in two (13.3%) of the initial 15 patients without premedication, whereas only two (3.7%) of 54 patients after routine premedication. The unexpected hypersensitivity may result from PTX itself and cisplatin. Because histamine release was observed with PTX alone, while no release with CrmEL alone. As well, repeated i.v. injection of mPEG-PDLLA diblock copolymer may be involved in such hypersensitivity and will be discussed later. In this trial, the partial response rate was 38% without complete response. The results of the other two phase II clinical trials of Genexol®-PM were listed in Table 2 and were compared with Taxol® and Abraxane®. For breast cancer, the response rate was comparable to a rate even higher than that of Abraxane® [42,43]. Both Genexol®-PM and Abraxane® showed superior efficacies compared with Taxol® formulation. The tumor accumulation effect as well as the available high dose administrated is likely to account for such a high response. Although the incidence of neutropenia for Genexol®-PM treatment was a little bit higher than that for Abraxane®, no febrile neutropenia of any grade was observed for Genexol®-PM arm while 5% was observed for Abraxane®. For NSCLC, Genexol®-PM combined with cisplatin was more effective than Abraxane® alone [44] and free PTX combined with cisplatin arm [45]. Grade 3/4 sensory neuropathy was more frequent in Genexol®-PM treated metastatic breast cancer arm, consistent with the fact that sensory neuropathy is the major adverse reaction of microtubule-stabilizing agent-based chemotherapy and is controlled by the treatment cycle and dosage regimen. However, the sensory neuropathy was manageable. Due to the CrmEL-free character, high dose is warranted in clinical use, resulting in superior efficacies and inferior adverse reactions as observed in the clinical trials which, consequently, enabled Genexol®-PM to be the first PM formulation commercially available for the treatment of NSCLC, ovarian cancer, breast cancer and gastric cancer.

2.2. NK105

NK105 is a promising artwork of PTX-incorporating PM formulation designed by Nanocarrier® and in clinical trials by Nippon Kayaku. The polymer of NK105 was poly(ethylene glycol)-poly(aspartic acid) (PEG-P(Asp)) modified with 4-phenyl-1-butanol to increase the hydrophobicity. PTX was successfully loaded into the micelle system by physical entrapment. Upon lyophilization, NK105 can be obtained with a drug loading of about 23% (w/w) and it can be readily dissolved in 5% glucose solution for clinical injection with a size of about 85 nm ranging from 20 to 430 nm and more than 1 day stability at room temperature [13].

The *in vitro* cytotoxicity result indicated NK105 and free PTX possess an equivalent cytotoxic activity with a comparable median inhibiting concentration (IC_{50}) at 48 and 72 h on different tumor cell lines from human cancers including lung, gastric, esophagus, colon, breast and ovarian cancers [13].

NK105 was administrated to colon 26 tumor bearing CDF1 mice resulting in a prolonged circulation and tumor accumulation PK profile [13]. The plasma PTX can be detected up to 72 h for NK105, while negligible at 24 h for free PTX. The plasma concentration at 5 min and the AUC of NK105 were 11–20 and 50–86-fold higher than those of free PTX, respectively. As expected of prolonged circulation in the blood by the stealth role of PEG and the EPR effect, the tumor maximum concentration (C_{max}) and AUC of NK105 was approximately 3 and 24 times higher than those of PTX, respectively. What's more, the tumor PTX concentration was higher than 10 μg/g even at 72 h

Table 2
Phase II/III clinical trials of Genexol®-PM, ABI-007 and Cremophor EL based paclitaxel formulation.

Formulation and dosage regimen	Cancer type	Phase in clinical trials	No. of patients	Mean cycles per patient	MTP (mon)	Response (%)				Hematologic toxicities (Grade 3/4) (%)			Non-hematologic toxicities (Grade 3/4) (%)		
						CR	PR	SD	PD	Leucopenia	Neutropenia	Febrile neutropenia[a]	Sensory neuropathy	Myalgia/ arthralgia	Hypersensitivity
Genexol®-PM 230–300 mg/m^2 (3 h) and cisplatin 60 mg/m^2 every 3 weeks [11]	NSCLC	II	69	4.1	5.8	0	38	29	23	6	46	3	13	13	13 (4)[b]
Genexol®-PM 300 mg/m^2 (3 h) every 3 weeks [10][c]	Metastatic breast cancer	II	41	8.1	9.0	12	46	32	5	39	68	0	51	5	5(0)[b]
Genexol®-PM 300–350 mg/m^2 (3 h) every 3 weeks [12]	Advanced pancreatic cancer	II	45	N/A	3.0	2	4	53	N/A	N/A	18	N/A	11	N/A	N/A
ABI-007 300 mg/m^2 (30 min) every 3 weeks [42]	Metastatic breast cancer	II	63	5.1	6.2	3	44	N/A	N/A	24	51	5	11	8	0[d]
ABI-007 260 mg/m^2 (30 min) every 3 weeks [44]	NSCLC	II	43	N/A	6.0	0	16	49	N/A	0	9	N/A	5	9	0[d]
ABI-007 260 mg/m^2 (30 min) every 3 weeks [43]	Breast cancer	III	229	N/A	5.4	42[e]		N/A	N/A	6	30	<2	10	13	0[d]
PTX 175 mg/m^2 (3 h) every 3 weeks [43]	Breast cancer	III	225	N/A	3.9	27[e]		N/A	N/A	7	46	<2	2	5	2[f]
PTX 175 mg/m^2 (3 h) and cisplatin 80 mg/m^2 every 3 weeks [45]	NSCLC	III	202	4.4	4.1	1	23	49	21	N/A	45	4	4	5	<1[f]

Abbreviation: CR: complete response; PR: partial response; PTX: paclitaxel; SD: stable disease; PD: progressive disease; NSCLC: non-small-cell-lung-cancer; MTP: median time to progression; N/A: not available.
[a] The incidence of febrile neutropenia in all grades.
[b] The incidence of hypersensitivity without premedication and with premedication in parenthesis.
[c] The initial dose was 435 mg/m^2 in the first 11 patients, but was reduced to 300 or 350 mg/m^2 in the following 45 patients. The results listed above were derived from the following 45 patients.
[d] The incidence of hypersensitivity without premedication.
[e] The overall response rate for patients who received first-line therapy.
[f] The incidence of hypersensitivity with premedication.

after i.v. injection of NK105, indicating a sustained release behavior inside the tumor following the accumulation.

Accordingly, NK105 exerted superior anti-tumor activity as compared with free PTX in HT-29 colon cancer cells transplanted BALB/c female nude mice although with similar *in vitro* cytotoxic activity [13]. Moreover, the most exciting result is tumor effacement with a single administration of NK105 at the dose of 100 mg/kg and being tumor free henceforth, even without the weight loss at such a high dose. The neurotoxicity of PTX can be attenuated by the micellar encapsulation both electrophysiologically and histopathologically [13]. The synergetic effect of PTX and radiation has been studied on a biological basis [46], and was confirmed by several clinical studies [47,48]. As well it is common sense that lung toxicity is the adverse reaction of thoracic radiation. Therefore, the anti-tumor activity and lung toxicity of NK105 combined with radiation were evaluated on Lewis lung carcinoma bearing mice [14]. The enhanced anti-tumor activity was observed in NK105 combined with the radiation group, which may have resulted from the fact that from flow cytometric analysis more cell cycle arrest at G2/M phase is induced by NK105, the most radiosensitive phase of the cell cycle. There was no significant difference in histopathological changes and immunohistochemical analysis of lung sections among the groups receiving the same dose of radiation but different PTX formulations, indicating that the lung toxicity was induced by radiation rather than PTX.

Nippon Kayaku Co., Ltd., a licensed company in Japan, started the phase I clinical trial of NK105 in May 2004 [15]. Nineteen cancer patients were recruited including pancreatic (n = 11), bile duct (n = 5), gastric (n = 2) and colonic (n = 1) cancers. NK105 was administrated with the dosage regimen of 1-h i.v. infusion every 3 weeks, without antiallergic premedication. The dose began with 10 mg/m^2 and was escalated up to 180 mg/m^2 according to the accelerated titration method. Neutropenia, the only grade 4 toxicity was observed. Most of the nonhematological toxicities were grade 1. Neuropathy, the most common adverse reaction during Taxol treatment, was only grade 1 or 2, despite long term administration. Although without antiallergic premedication, allergic reactions were not observed except one grade 2 hypersensitivity in a patient at 180 mg/m^2. Accordingly, the MTD and RD for phase II were designated as 180 and 150 mg/m^2, respectively. Most importantly, among the nineteen patients, a partial response was observed in a patient with metastatic pancreatic cancer who was treated at 150 mg/m^2. Colon and gastric patients experienced stable disease lasting for ten and seven courses, respectively. For the PK study, the AUC and total clearance rate (CL_{tot}) of NK105 at 150 mg/m^2 were about 32-fold larger and 72-fold lower than that of Genexol®-PM at a dose of 300 mg/m^2 [8], indicating a higher stability of NK105 in blood circulation. The minor cumulative urinary excretion rates and major difference between renal clearance rate (CL_r) and CL_{tot} indicated a non-kidney elimination way. Moreover, like Genexol®-PM, NK105 possessed a linear PK behavior. In terms of these encouraging results, a phase II clinical trial against advanced stomach cancer as a second-line therapy was conducted in 6 institutions in Japan in Nov. 2007 and has been completed in 2010 [49]. However, the update information hasn't been reported up till now. A phase III clinical study is now in preparation.

2.3. NC-6004

Cisplatin (cis-dichlorodiammineplatinum (II), CDDP) is an important anti-tumor agent and is widely used for the treatment of many malignancies. However, CDDP in the conventional formulation was cleared rapidly from circulation and excreted through the glomerular filtration with a high CDDP level in kidney within a few minutes after i.v. injection, resulting in a dose limiting factor nephrotoxicity, which greatly hampered its clinical use [50]. Therefore, the development of DDSs for CDDP with a better selective accumulation into solid tumors and decreased distribution into normal tissue is anticipated. NC-6004

is a CDDP-incorporating PM formulation which consists of PEG and the coordinate complex of poly(glutamic acid) (P(Glu)) and CDDP, a polymer–metal complex-forming chain. A narrowly distributed size of NC-6004 (30 nm) was confirmed by dynamic light-scattering measurement [17]. The drug loading in NC-6004 was as high as 39% (this value was obtained from a slightly modified formulation with the only change of [Glu] in distilled water from 4.7 to 5 mmol/L) [16]. NC-6004 was extremely stable in distilled water even under much diluted condition, with a critical micelle concentration (CMC) less than 5×10^{-7} [17]. Nevertheless, CDDP was sustained released in physiological saline due to an inverse ligand substitution of the Pt (II) atom from a Glu residue of PEG–P(Glu) to chloride [17]. The affinity between the Pt (II) atom and Glu residue is optimum in terms of the anti-tumor effect and toxicity of CDDP because a highly stable complex, such as CDDP-DNA, may weaken and even eliminate the anti-tumor effect of CDDP, while a low affinity complex, such as CDDP-hyaluronic acid, may possess the same toxicity as CDDP [51]. With the CDDP release, NC-6004 may dissociate gradually while maintaining a size of about 25 nm up to 50 h [16], which was longer than PEG–P(Asp)(CDDP) (30 h) [52]. Interestingly, in a similar system to NC-6004, the release of CDDP was accelerated by decreasing the pH from 7.4 to 5.2, suggesting accelerated drug release from NC-6004 in the endosomal lysosomal system when CDDP was transported into the cell with intact micellar form [53].

The *in vitro* cytotoxicity of NC-6004 was tested on 12 human tumor cells derived from bladder, colon, lung, gastric and breast cancers [17]. The IC_{50} values of NC-6004 were 6 to 15 fold higher than those of CDDP. Such a decreased cytotoxicity activity of CDDP after the micellar encapsulation may be attributed to the sustained release of CDDP and this assumption can be verified by the following facts: (1) only 19.6 and 47.8% CDDP release at 24 and 96 h, respectively [17]; (2) significant decrease of IC_{50} of NC-6004 with the increase of incubation time, while slight change of IC_{50} of CDDP [17]; (3) a comparable cytotoxicity between CDDP incorporated micelles upon preincubation in physiological saline for 48 h and free CDDP [54].

In spite of the decreased cytotoxicity *in vitro*, NC-6004 showed a superior anti-tumor activity *in vivo* when compared with that of CDDP in murine colon adenocarcinoma 26 (C26) [16] and human gastric cancer cells MKN-45 [17] bearing mice, even with some complete tumor regression confirmed by dissection. Regarding the time-course of body weight change, a significant body weight loss was only observed in the CDDP group for both of these two tumor bearing animal models. Moreover, NC-6004 displayed a potent anti-tumor activity against HT29 oxaliplatin-resistant bearing mice [55].

For the PK study [17], NC-6004 possessed a prolonged blood circulation property. AUC and C_{max} for NC-6004 were 65 and 8 fold higher, while CL_{tot} and V_{ss} were 19 and 75 fold lower than those for CDDP, respectively. NC-6004 was largely distributed in the liver and spleen, the reticuloendothelial system, whereas in the kidney, the elimination system, for CDDP. Furthermore, the tumor AUC was 3.6 fold higher for NC-6004 than for CDDP. Considering the altered biodistribution, an improved nephrotoxicity of NC-6004 which may enable the out-hospital treatment was expected. Blood urea nitrogen and creatinine of CDDP (10 mg/kg) group were significantly higher than those of the control and NC-6004 (10 mg/kg) group, even the NC-6004 (15 mg/kg) group, coinciding with the kidney histopathological change which was only observed in CDDP group. In addition to the lessened nephrotoxicity, another dose-limiting factor neurotoxicity was also improved both qualitatively and quantitatively. However, a transient and reversible hepatotoxicity was observed after the administration of NC-6004 (10 and 15 mg/kg), which may be induced by the macromolecular property of NC-6004, although preserving a stealth effect through its PEG outer shell, so precaution against hepatic dysfunction was exercised in the following clinical trial of NC-6004.

The phase I clinical trial of NC-6004 has been conducted in two centers in the United Kingdom in May 2006 [18]. NC-6004 was i.v. administered within 1 h every 3 weeks with a dose range of 10 to 120 mg/m^2. Treatment was well tolerated with minor nephrotoxicity which was kept to a minimum by modest hydration and no significant myelosuppression, ototoxicity or neurotoxicity. No DLT except one grade 3 fatigue was observed per protocol. However, hypersensitivity reactions caused by NC-6004 occurred more frequently than those caused by CDDP, regardless of the dose level, although a prophylactic regimen was performed. Regarding the incidence of unexpected hypersensitivity and renal impairment, 120 and 90 mg/m^2 was defined as the MTD and RD for phase II trial, although the definition as the protocol of MTD was not actually reached. The best response in this trial was stable disease in 7 out of 17 patients. The PK behavior of both free and micellar component of Pt was characterized. For the total plasma Pt, NC-6004 was characterized by a prolonged blood circulation time with a linear PK model. In terms of the ultrafilterable Pt (active species including cisplatin) result, a delayed and sustained release of Pt from NC-6004 can be concluded, with a lower C_{max} while higher AUC and half time compared with non-protein-bound cisplatin after the cisplatin injection resulting in an inferior cisplatin-related toxicity as well as a superior anti-tumor efficacy. At present, a phase I/II clinical study of NC-6004 is ongoing in Taiwan and Singapore with advanced or metastatic pancreatic cancer patients to examine the appropriate dose and evaluate safety, tolerability and efficacy in combination with Gemcitabine [56].

2.4. NC-4016

NC-4016 was the third under research PM formulation of Nanocarrier® and was investigated in the cooperation with Debiopharm S.A. The product development code at Debiopharm S.A. is Debio 0507. Like NC-6004, this formulation is composed of PEG as the hydrophobic segment and coordinate complex of poly amino acid and dichloro-(1, 2-diaminocyclohexane) platinum(II) (DACHPt), an active metabolite of oxaliplatin, as the hydrophilic segment. Debiopharm S.A. started the phase I clinical trial of NC-4016 in Mar. 2009. However, no information regarding this formulation is available at present [19,49].

In a similar system to NC-4016 [57,58], the size of PEG–P(Glu)(DACHPt) increased with the increase of [DACHPt]/[Glu], but smaller than 100 nm. As a coordinate complex based PM formulation, PEG–P(Glu)(DACHPt) did not show any DACHPt release in distilled water, while it displayed a similar release profile as NC-6004 in media containing chloride ions and a longer stability than NC-6004 in phosphate buffered saline. Like other PM formulation, the cytotoxicity of PEG–P(Glu)(DACHPt) was lower than oxaliplatin, but higher than NC-6004 because of the higher potency of DACHPt than CDDP. Moreover, PEG–P(Glu)(DACHPt), especially for the formulation prepared from the copolymer with P(Glu) block length of 20 U, displayed a remarkably prolonged blood circulation and a higher relative tumor targeting compared to free oxaliplatin. In addition to the anti-tumor activity against the primary solid tumors, PEG–P(Glu)(DACHPt) possessed a potent anti-tumor activity against metastatic tumors.

2.5. NK012

7-ethyl-10-hydroxy-camptothecin (SN-38) was a biologically active metabolite of irinotecan hydrochloride (CPT-11), and exerted a superior cytotoxicity against various cancer cells *in vitro* than CPT-11, but unavailable in the market because of its poor solubility. Upon chemical conjugation with P(Glu) segment of PEG–P(Glu) block copolymer, SN-38 was covalently bound to PEG–P(Glu) with a phenyl ester bond to increase the hydrophobicity of P(Glu), consequently, NK012 was available by self-assembly of the amphiphilic copolymer PEG–P(Glu)(SN-38) in an aqueous media with a size of about 20 nm [20]. The drug loading of NK012 was about 20% (w/w).

The releasing rates of SN-38 from NK012 in PBS (pH 7.3) at 37 °C were 57% and 74% at 24 and 48 h, respectively, while neglectable in 5% glucose solution (pH 4.6) because of the labile phenyl ester bond under neutral and mild alkaline condition but stable under acidic condition, ensuring the stability after reconstitution for clinical use. Besides, the release behavior of SN-38 from NK012 was a nonenzymatic manner, which is regardless of the interindividual variability of carboxylesterase activity [20].

The IC_{50} of NK012 was 43 to 545-fold lower than CPT-11, while comparable or a little bit higher than SN-38 against a series of human cancer cells [20,59–64]. However, there was no significant difference between the cytotoxicity of NK012 against different vascular endothelial growth factor (VEGF) secreting cells, SBC-3/Neo and SBC-3/VEGF [20], as well as different vascularity cancer cells PSN1 and Capan1 [60], which may be due to the discounting EPR effect *in vitro*.

Like other PM formulation, the PK of NK012 was characteristic of slow clearance and tumor accumulation. The concentrations of free SN-38 were higher in SBC-3/VEGF tumors than those in SBC-3/Neo tumors at any time points after NK012 administration, however, comparable after CPT-11 administration [20]. Moreover, NK012 displayed relatively higher accumulation in organs of reticuloendothelial system [20]. Considering diarrhea the major toxicity of CPT-11, the small intestine distribution was also evaluated by fluorescence microscopy [65]. CPT-11 was strongly distributed in the epithelium of the small intestine whereas NK012 tended to be distributed weakly and uniformly in the mucosal interstitium, consistent with the less intestinal toxicity observed in NK012 group pathologically. A test to clarify the biodistribution of NK012 in liver metastases of human colon cancer and normal liver has been conducted [66]. The results indicated that NK012 is distributed mainly adjacent to tumor vessels after 1 day for a long time for liver metastases, while in Kupffer cells for at least 7 days without any injury of hepatocytes and depletion of Kupffer cells for the normal liver.

NK012 possessed a more potent anti-tumor activity than CPT-11 in the mouse model of hypervascular and hypovascular cancers both orthotopically and subcutaneously, such as glioma [61], colorectal [67], lung [20,62,64,65], renal [59], pancreatic [60] and gastric cancers [68]. In HT-29 bearing nude mice, NK012 displayed a superior anti-tumor activity, while CPT-11 exerted insignificant anti-tumor activity at each MTD (30 *vs.* 66.7 mg/kg) [20]. Compared the *in vivo* anti-tumor activity of each treatment towards SBC-3/Neo and SBC-3/VEGF bearing mice [20], the following results can be obtained: (1) without any treatment, the tumor in SBC-3/VEGF was more aggressive than in SBC-3/Neo group, portending a less potent curative effect upon common anti-tumor preparation; (2) as expected, the anti-tumor activity of CPT-11 in SBC-3/VEGF group was comparable even inferior to that of the SBC-3/Neo group; (3) the most distinguished anti-tumor activity was observed in SBC-3/VEGF bearing mice treated with NK012, that is effacement of SBC-3/VEGF bulky masses in all mice without relapse for 3 months after treatment except one mouse treated with NK012 20 mg/kg. The same anti-tumor activity was observed in combination with cisplatin [65]. The encouraging result can be ascribed to the hypervascularity and hyperpermeability induced by VEGF, resulting in a more notable EPR effect, followed by accumulation of the nano-scale formulation in tumor, which was confirmed by the above tissue distribution of NK012 in SBC-3/VEGF bearing mice. Also, NK012 exerted more positive effect on Renca tumors (more CD34-positive neovessels) than SKRC-49 tumors, despite the comparable cytotoxicity *in vitro* [59]. In addition, the peritoneal metastasis prevention effect was more pronounced in NK012 group. In the case of 44As3Luc bearing mice, the survival rates on day 150 were 80% and 0% for the NK012 and CPT-11 group, respectively [68]. Surprisingly, NK012 possessed an excellent tumor accumulation and anti-tumor activity in not only PSN1 a hypervascular tumor but also Capan1 a hypovascular tumor [60], as well as SUIT-2 transplanted orthotopic pancreatic tumor [63]. Besides, the anti-tumor activity of NK012 against lung cancer xenografts has not been weakened by bevacizumab, an anti-VEGF humanized monoclonal antibody, although the vascular density was decreased significantly by bevacizumab [62]. In the case of hypovascular cancer or cancer with decreased vascularity by bevacizumab, such an effective anti-tumor activity of NK012 may be attributed to its small particle size, flexibility as well as sustained release to fully utilize the time-dependent anti-tumor activity of SN-38. Apart from the superior anti-tumor activity to CPT-11 alone, NK012 exerted more synergetic activity with 5-fluorouracil (5-FU) against HT-29 tumors compared to CPT-11 [67] because NK012 caused a more pronounced and prolonged accumulation of cells in the S phase, which is a specific cell cycle of DNA damaging by 5-FU infusion. Similarly, NK012 in combination with S-1, a dihydropyrimidine dehydrogenase inhibitory fluoropyrimidine, exerted a more potent synergetic anti-tumor effect than CPT-11/S-1 in a mouse model of NSCLC, accompanied with less intestinal damage [64].

Two independent phase I clinical trials of NK012 have been conducted in Japan [21] and the USA [69]. NK012 was infused i.v. for 30 min every 3 weeks upon dilution for a volume of 250 mL with 5% glucose with an SN-38 equivalent dose range of 2–28 and 9–28 mg/m^2 in Japan and the USA, respectively. In these two trials, no DLT was observed until 28 mg/m^2 except one elevated γ-glutamyl transpeptidase (20 mg/m^2) in the Japanese trial. The DLT observed in each trial was mainly neutropenia. Nonhematologic toxicities were minimal and grade 3 or 4 diarrhea was absent. Cholinergic symptoms appeared to occur less frequently than CPT-11 clinical trial [70]. The RD for phase II from each trial was determined to be 28 mg/m^2 with at least a 3-week interval, although MTD had not been reached according to the protocol. In the Japanese trial, NK012 conformed to a linear PK in the dose range of 2–28 mg/m^2. Compared to the phase I trial of CPT-11 [70,71], NK012 exhibited a high systemic exposure and slow elimination, consistent with the preclinical studies. A total of eight partial responses have been reported: 1 patient with esophageal cancer and 1 patient with lung carcinoid cancer in the Japanese trial (2 of 23 patients); 3 patients with triple negative breast cancer, 1 patient with endometrial cancer, 1 patient with pancreatic neuroendocrine cancer and 1 patient with SCLC in the USA trial (6 of 18 patients). Due to the promising preclinical and phase I clinical trials, phase II clinical trials are ongoing in patients with colorectal cancer in Japan and in patients with advanced metastatic triple negative breast cancer and SCLC in the USA [64,72].

2.6. NK911

The research of NK911 started from a PM formulation of doxorubicin (DOX) containing PEG as hydrophilic segment and DOX conjugated P(Asp) (P(Asp)(DOX)) as hydrophobic segment with free DOX and DOX derivatives in this system [73]. This formulation displayed a superior anti-tumor activity *in vivo* as well as an enhanced tumor accumulation and prolonged circulation in blood [74,75]. In order to clarify the anti-tumor activity from which component, the conjugated DOX or the free DOX, a purified PEG–P(Asp)(DOX) without free DOX was prepared, and then free DOX was loaded into PEG–P(Asp)(DOX) micelles [76]. However, the dimmer of DOX molecules was observed in some examples. The *in vitro* cytotoxicity, *in vivo* anti-tumor activity and PK studies suggested that physically entrapped DOX accounts for the anti-tumor activity, whereas DOX dimmer for the stabilization of the micelles, prolonging the release of DOX and a reservoir of DOX. Nevertheless, after long storage even at 5 °C the lyophilized product became insoluble [77,78]. Finally, a new type of PM formulation designated NK911, loading only DOX monomers, was prepared by modulating PEG molecular weight (5000 g/mol), DOX conjugation substitution degree (45%) and DOX entrapment amount [22]. Upon reconstitution, lyophilized product of NK911 had a diameter of about 40 nm. In this system, the drug loading capacity, stability and

DOX release were controlled by DOX conjugation substitution degree of PEG–P(Asp)(DOX). The physically loaded DOX can be gradually released in 8–24 h to exert the anti-tumor activity.

The purified PEG–P(Asp)(DOX) micelles showed insignificant anti-tumor activity both *in vitro* and *in vivo* [76,77] and the IC_{50} of NK911 against P388D1 mouse leukemia cells and mouse colon adenocarcinoma 26 (C26) was comparable with that of free DOX in terms of the dose as only physically loaded DOX, suggesting anti-tumor activity resulted from only the physically loaded DOX and the comparable cytotoxicity between NK911 and free DOX [77,78].

The chemically conjugated DOX was pharmacologically inactive and had an insignificant effect on the plasma DOX concentration after NK911 treatment, so the dose was calculated based on the physically loaded DOX amount alone. NK911 presented a tumor accumulation property with a 3.4-fold increased tumor AUC compared with free DOX. The plasma C_{max} and AUC values of NK911 were 36.4-fold and 28.9-fold larger than those of free DOX, respectively [22]. Furthermore, DOX from NK911 was distributed more efficiently from the tumor vessel to those distant tumor cells than DOX from Doxil, a PEGylated liposomal formulation of DOX, which may be favorable in the case of intractable cancers with a rough tumor vessel network [79]. Consequently, NK911 displayed a stronger anti-tumor activity against mouse C26, M5076 sarcoma, P388 leukemia and human MX-1 breast cancer cell bearing mice with a more lessened toxicity than free DOX [77].

The phase I clinical trial of NK911 began at the National Cancer Center Hospital, Tokyo, Japan, in July 2001 [23]. 23 patients, many of whom had pancreatic ($n=7$) and colorectal cancers ($n=7$), were recruited. After reconstitution in sterile phosphate-buffered saline at a final DOX concentration of 2 mg/mL, NK911 was administrated with a starting dose of 6 mg/m^2 every 3 weeks at a speed of 5 mL/min. The dose was accelerated to 12, 24, 36, 50 and 67 mg/m^2. The toxicity spectrum of NK911 resembled that of free DOX. No grade 3/4 hematological toxicity was observed until 50 mg/m^2 ($n=11$) with 3 patients with grade 3 leucocytopenia, 5 patients with grade 3 neutropenia and 2 patients with grade 4 neutropenia. There was no DLT regarding nonhematological toxicity within this trial. Some common side reactions accompanied with Doxil, such as infusion-related reaction, hand-foot syndrome and cardiac dysfunction [80,81], were rare and mild. The MTD and RD for phase II trial were defined to be 67 and 50 mg/m^2, respectively, with the DLT as grade 4 neutropenia lasting for at least 5 days and neutropenia-associated fever. Although RD for phase II trial of NK911 was the same as DOX liposomal formulation, the infusion rate has been increased significantly. NK911 displayed a linear PK profile. However, the AUC of NK911 was more than 1429-fold lower (3.3 *vs.* 4663.3 μg h/mL) than that of PEGylated liposomal DOX at the same dose of 50 mg/m^2 [80]. These results were consistent with the less stability of NK911 than Doxil because of the removal of DOX dimmer observed in the preclinical study. The antitumor efficacy can be enhanced by its more potent tumor distribution, consequently, NK911 resulted in a partial response in metastatic pancreatic cancer and eight stable diseases in this trial. A phase II trial of NK911 for the treatment of metastatic pancreatic cancer was performed, however, no update data is available.

2.7. SP1049C

Multidrug resistance (MDR) is one of the major significant obstacles for the effectiveness of clinical chemotherapy. It has been reported that nonionic surfactant PEO–PPO–PEO copolymers (Pluronic) can considerably reduce drug resistance. SP1049C is a Pluronic PM formulation of DOX and is prepared by reconstituting DOX with a 0.9% sodium chloride solution containing Pluronic L61 (0.25% w/v) and F127 (2% w/v) to obtain an ultimate DOX concentration of 2.0 mg/mL [25]. In this formulation, Pluronic L61 is reported as the most effective modulator on DOX activity among those studied Pluronic copolymers against a variety of MDR cells [82], while Pluronic F127 provides a significant stabilizing effect without changing of cytotoxicity of Pluronic L61 DOX formulation [24]. With the stabilizing effect of Pluronic F127, the size of DOX-loaded mixed Pluronic copolymer formulation is about 30 nm, otherwise liquid phase separation is inevitable with Pluronic L61 alone even at a relatively low concentration [24].

The cytotoxicity of DOX-L61 was tested against a panel of MDR cells and their corresponding sensitive parental cells [24]. DOX-L61 displayed an improved cytotoxicity effect on those DOX-resistant cells with the L61 concentration lower than CMC, suggesting that unimers rather than micelles are responsible for the hypersensitization effect. Most importantly, those resistant cells became more susceptible to DOX-L61, with a lower IC_{50} of DOX, than their sensitive counterparts, which may be attributive to the enhanced membrane permeability of resistant cells. Besides, the increased drug cellular uptake by L61 was more significant than that by verapamil, a typical P-gp inhibitor, while inhibition efflux effect by L61 was weaker than verapamil, indicating a P-gp independent pathway, such as L61-mediated endocytosis, may be involved. Compared with DOX alone and DOX with verapamil, the subcellular distribution of DOX with L61 vesicles was mainly in the nucleus, which may be due to the decreased high pH gradient between the vesicles and cytosol in MDR cells by L61 and deprotonation of DOX, resulting in the increased ability of diffusing through the vehicular membrane.

In terms of the biodistribution study [24], SP1049C and DOX exhibited similar C_{max} and AUC in the liver, kidney, heart, lung and plasma of both normal and tumor bearing mice. However, an increased AUC was observed in tumor and brain of SP1049C treated animals, suggesting a tumor targeting effect by mixed Pluronic copolymer. Accordingly, in P388, Sp2/0, 3LL-M27, MCF-7, KBV and their corresponding MDR tumor bearing mice model, the tumor inhibition and life-span of mice were considerably increased in SP1049C compared with DOX treated group [24]. The acute and subacute toxicity of SP1049C and its mixed copolymer carrier were evaluated. The acute toxicity of carrier in rodents and non-rodents was established to be circa 12 and 9.6 mL/kg/min with the concentration as clinical use, respectively, which was considerably higher than the dose that would be used in human clinical therapy (planned human dose 0.3 mL/kg/min). The MTD of SP1049C and DOX were the same, being established as 15 mg/kg in mouse and 7.5 mg/kg in rat. The treatment-related toxic effects and vascular irritation of SP1049C and DOX were also the same and were only observed at MTD [24].

Those properties of SP1049C, hypersensitization towards MDR cells, nucleus location, superior anti-tumor activity *in vivo*, tumor targeting and similar toxicity profiles with DOX, enabled its phase I clinical trial in Canada in 1999 [25]. Patients with histologically proven cancer refractory to conventional treatment or for which no suitable conventional therapy existed were considered for entry into this study. SP1049C was administered via a peripheral i.v. cannula with a starting dose of 5 mg/m^2, which was escalated to 90 mg/m^2 with a 3-week interval for a maximum of six cycles. SP1049C showed a similar spectrum of toxicities at a dose of 35 mg/m^2 and above to that observed in conventional DOX with neutropenia as the principal DLT. Hand-foot syndrome observed in Doxil treatment was not observed herein. According to the protocol, the MTD was determined to be 70 mg/m^2. Three patients (11.5%) had a complete or partial response during the treatment and eight patients (30.8%) had stable disease with a median time to progression of 17.5 weeks (range 9–24 weeks). However, all these promising responses weren't kept for a long time. The PK parameters of different doses conformed to a linear PK behavior.

Given the promising preclinical results in MDR tumor settings and potent efficacy in the patients resistant to the conventional treatment in phase I trial, phase II clinical trial in patients with advanced adenocarcinoma of the esophagus and gastroesophageal junction were

performed from February 2002 [26]. A 75 mg/m^2 i.v. 30-minute infusion was given with the frequency of once every 3 weeks for up to six cycles. Thirteen patients (61.9%) experienced grade 3/4 neutropenia and one required granulocyte colony-stimulating factor treatment for grade 4 neutropenia and fever. Regarding the nonhematological toxicities, grade 3/4 mucositis occurred in one patient. Furthermore, gradual absolute decrements in left ventricular injection fraction (LVEF) values were noted with cumulative treatment. Four patients developed an absolute percentage decrement of at least 15% but were asymptomatic and without an LVEF below 45%. Considering the micellar encapsulation effect, these unexpected toxicities were probably associated with micelle degradation upon injection resulting in a release of free drug, which also can be speculated from the similar PK behaviors of SP-1049C and free DOX. In the nineteen evaluable patients, nine patients had a partial response without a complete response and eight patients had a stable disease. The median overall survival and progression-free survival were 10.0 and 6.6 months, respectively, which were longer than free DOX combined with cisplatin and 5-FU in patient with advanced adenocarcinoma of the stomach or esophagus [83].

3. Future prospects of polymeric micelles system in oncology

3.1. Polymeric micelles for oral anti-tumor drug delivery

Oral chemotherapy is one of the most important issues in 21st century medicine. It may radically change the current regimen of chemotherapy in terms of its sustained and mild plasma drug concentration, convenient and flexible administration, low cost and the resulting improved compliance. However, most anti-tumor drugs, especially those with excellent anti-tumor effects such as taxanes (paclitaxel and docetaxel), are not orally bioavailable (*i.e.*, not absorbable/interactive in gastrointestinal tract) [84]. Nanomedicine may radically change the way we make drugs and the way we take drugs, and thus provide an ideal solution for oral chemotherapy [85]. To this day, research on oral anti-tumor PM DDS is still at the exploratory stage with some uncertain fields for investigation, especially the mechanism and PM formulation forms across the gastrointestinal membrane. The absorption improving mechanisms by PM may involve different membrane permeating behavior, inhibition of efflux transports as well as bioadhesive properties.

There are several different opinions concerning the PM formulation forms across the gastrointestinal membrane. Yao [86] reported intact nanomicelles form across the Caco-2 cell monolayer and the everted gut sac with transmission electron microscope. This intact-micelles based mechanism may be mediated by an endocytosis pathway through non-specific interactions between the polymer and the intestinal membrane. Alternatively, amphiphilic unimer based and micelles-reservoir mechanism was concluded by the observation of high apparent permeability coefficient (P_{app}) at low polymer concentration and different P_{app} of polymer and entrapped drug [87–89]. Moreover, the influence of bile acid on micellar size and AUC of entrapped drug enabled bile-facilitated mechanism. AUC in the sham operated rat group was significantly higher than that in the bile duct ligation group (4543 *vs.* 1.64 ng/mL/h) [90].

P-gp is the most common efflux transport in intestine and is well studied. Apart from the above mentioned P-gp inhibitor poloxamer, D-α-tocopheryl PEG succinate (TPGS) is another commonly used P-gp inhibitor in oral DDS [86,91,92]. Some other amphiphilic polymers have been reported as P-gp inhibitors, such as mPEG-block-polycaprolactone [93], PEG-phosphatidylethanolamine [94], PEG-b-PLA [95], mPEG-poly(caprolactone-trimethylene carbonate) [88], N-octyl-O-sulfate chitosan [96] and so on. Bioadhesive polymers, especially positively charged polymers, were preferential to enhance drug absorption by prolonging the residence time at the site of absorption. A functional PTX nanomicelles system was established with the combined absorption-enhancing mechanisms, $TPGS_{1000}$ as the P-gp inhibitor and dequalinium, a cationic amphiphilic compound, as the bioadhesive polymer by the electrostatic interaction with the negative intestinal membrane [86]. The results indicated that P_{app} of PTX transported across the Caco-2 cell monolayer of functional PTX nanomicelles was about 2.1 and 7.4 folds higher than PTX nanomicelles and Taxol® after 3 h incubation, and 1.9 and 11.8 folds higher when across the everted gut sac, respectively. Functional PTX nanomicelles possessed the strongest inhibitory effect on resistant breast cancer MCF-7/Adr cells *in vitro* and *in vivo*. Most importantly, oral administration of functional PTX nanomicelles displayed a comparable anti-tumor efficacy in resistant MCF-7/Adr cells xenografted mice to i.v. administration.

In spite of the above promising results of PM system in oral anti-tumor drug delivery, there are many issues to be resolved before its clinical use, such as the stability in gastrointestinal tract, the possible formation of mixed micelles in the presence of bile acid, the suitable dosage form in terms of the package, storage and patients compliance, the effective manufacturing process and industrialization, and so on. The low CMC may warrant its stability *in vivo*, however, the amphiphilic polymers may be unstable in the acid condition. With the advancement in industrial pharmaceutics, some technology may be helpful, for example enteric coating after solidification of PM formulation.

Currently, some amphiphlic polymers were employed in hot melt extrusion (HME) process, characterized by its shorter processing time, environmental advantage and efficient delivery benefits. Soluplus, a graft copolymer composed of PEG, polyvinylcaprolactam and polyvinylacetate was introduced by BASF. Its unique properties, low hygroscopicity and glass transition temperature of about 70 °C make it suitable for solubilization by HME process [97]. Whether those commonly used amphiphilic polymers in PM system or modified derivatives can be used in HME process to prepare solid dosage form directly? If so, the industrialization of PM for oral anti-tumor drug delivery can speed up in the future.

3.2. Polymeric micelles for anti-tumor traditional Chinese medicine

Many effective chemotherapeutics for cancer are accompanied by toxicities that can reduce patient compliance and quality of life, even hinder their clinical use. Increasing evidence demonstrates that combination regimen in cancer treatment can usually amplify the therapeutic efficacies with minimal toxicities [98]. Traditional Chinese medicine (TCM), a unique medical system advocating the principle of multi-components and multi-targeting, is widely employed in a variety of diseases with an increasing global regulatory process reception [99]. In particular, TCM has a long history of being prescribed for cancer prevention and treatment [100,101]. However, the unclear multi-components and therapeutic mechanism, low water solubility and bioavailability and poor targeting specificity hamper its druggability. Polymeric micelles DDS can solve these problems in terms of its solubilization and targeting properties. However, research on PM formulation of TCM is still at the exploratory stage. At present, the entrapped agents of TCM in PM system, mainly focus on monomer extracted from TCM, such as curcumin, oridonin, gambogic acid and so on, few research covered the effective parts, much less the whole formula. So how to efficiently entrap those components with different physicochemical properties, especially the water solubility, into the PM system to maintain the entirety principle of TCM, is a crucial point. Considering the entirety principle and different physicochemical properties of TCM, oral polymeric micelles DDS may be more suitable for TCM. Upon identification of the essential components followed by preparation PM for the key component in the formula to solve the problems in its clinical use and then mixing with other extracted components, high quality TCM formulation is available with the combination of TCM theories and advanced pharmaceutical principles to speed up the modernization of TCM.

Guiding agent which can guide the principal drug to its site of action, leading to an improved therapeutic efficacy and decreased toxicities, is a common concept in TCM theories [102]. Interestingly, this concept is consistent with the targeting DDS in modern pharmaceutics. So we believe that the guiding agent in TCM can be widely used in targeting DDS with the following methods: (1) coupling to a hydrophobic segment as the hydrophilic segment to obtain the amphiphilic polymer for loading of water insoluble drugs; (2) attaching to the PM system physically or chemically as a "magic bullet" to prepare active targeting PM DDS. In this system, multi-targeting effect is available by the combination of passive targeting from PM system and pathophysiological properties of tumor tissues as well as active targeting from guiding agent.

3.3. Some other considerations on anti-tumor polymeric micelles formulation

3.3.1. How to utilize the EPR effect to a great extent

Considering the great significance of EPR effect on targeting property of nano-DDS, how to utilize the EPR effect more efficient is the main issue in the exploration of nano-DDS. It is universally acknowledged that hypervasculature and defective vascular architectures play an important role in EPR effect, so the accumulation of PM DDS in tumor tissues may be modulated by adjusting the tumor vascular state. The blood-flow volume in tumor tissue can be increased under angiotensin-II (AT-II) induced hypertensive state owing to the lack of smooth-muscle layer, which regulates blood pressure and blood-flow volume, whereas that remains constant in normal tissues with vasoconstriction of the smooth-muscle layer, consequently, the drug extravasation increased at tumor tissues while decreased at the normal tissues. The improved tumor accumulation effect was observed in styrene-maleic acid copolymer-conjugated neocarzinostatin/Lipiodol administration under the AT-II induced hypertensive state in many patients with solid tumors [103,104]. Moreover, EPR effect can be also augmented by some other factors, such as transforming growth factor-β inhibitor [105], prostaglandin I_2 agonist [106], nitroglycerine [107], antiangiogenic agent [108], and so on [109,110].

3.3.2. Whether accelerated blood clearance phenomenon can be induced by polymeric micelles system with PEG as the hydrophilic segment

PEG is a commonly used hydrophilic segment for PM formulation and was considered as biocompatible previously. However, accelerated blood clearance (ABC) phenomenon can be induced by repeated injection of PEGylated liposomes because of anti-PEG IgM antibody production after the first dose followed by complement system activation and mononuclear phagocyte system (MPS) uptake at the second dose [111–113]. Unfortunately, the undesirable decreased therapeutic efficacy and increased side effect will be emerged in clinical use because of the altered PK behavior. As well ABC phenomenon was also triggered by PEG-phospholipid engineered nanomedicines, carbon nanotubes and PEG modified PLA nanoparticles [114,115]. Considering the ABC phenomenon induced by PEGylated stealth nanomedicines and the fact of PEG as the common outer shell of PM, it is of great urgency to know whether the ABC phenomenon can be induced by repeated administration of PM formulation or not. A PM size-dependent ABC phenomenon has been reported by Koide H [116]. The ABC phenomenon was observed in the group by the first dose of PM with the size of 50.2 nm, but not in the groups with the size of 31.5 and 9.7 nm. The relationship between the particle size and ABC extent was consistent with PEG modified PLA nanoparticles [117], but in contrast to PEGylated liposomes [118]. However, an ABC phenomenon absence of Gadolinium-containing PEG-poly(L-Lysine acid) PM with the size of 84.5 nm after the first dose of same PM even empty liposomes were reported by the same research team [119], indicating that other factors such as structure and hydrophobic character may affect the recognition after second dose. Although the answer to "whether ABC phenomenon can be induced by PM with PEG as the hydrophilic segment" is elusive, a promising result that an opposite behavior to the ABC phenomenon in the repeated injection of DOX containing PEGylated liposomes in its clinical application has been reported recently [120]. The opposite phenomenon may be resulted from toxic activity of the encapsulated anti-tumor drug DOX against MPS. Furthermore, all of the drugs entrapped in PM formulations in clinical trials are anti-tumor drugs. Nevertheless, further investigations regarding various factors, such as time interval, dose, PEG content and molecular weight, structure–function relationship, particle size and so on, should be required to clarify the mechanism involved in ABC phenomenon of PM.

4. Conclusion

Nano-technology based drug delivery system has been recognized as a promising strategy for clinical translation of those potential but water insoluble new drugs. As well polymeric micelles DDS has the advantages of solubilization, selective tumor targeting, decreasing systemic side effect and hypersensitization towards MDR cells, which have been verified by those preclinical and clinical trials discussed above. With the rapid advancement in pharmaceutics, molecular biology, bioimaging and oncology, as well as the intense multidisciplinary collaboration, PM system has been extended to imaging agent, gene and combination agent delivery for tumor diagnosis and therapy. In sum, polymeric micelles system has become increasingly important in oncology, and all evidence suggests an increasing hope in cancer therapy.

Abbreviations

5-FU	5-fluorouracil
ABC	accelerated blood clearance
AT-II	angiotensin-II
AUC	area under curve
CDDP	cis-dichlorodiammineplatinum (II)
CL_r	renal clearance rate
CL_{tot}	total clearance rate
C_{max}	maximum concentration
CMC	critical micelle concentration
CPT-11	irinotecan hydrochloride
CrmE	Cremophor EL
DACHPt	dichloro-(1, 2-diaminocyclohexane) platinum(II)
DOX	doxorubicin
DDS	drug delivery system
DLT	dose-limiting toxicity
EPR	enhanced permeability and retention
HME	hot melt extrusion
i.v.	intravenous(ly)
IC_{50}	median inhibiting concentration
LVEF	left ventricular injection fraction
mPEG-PDLLA	monomethoxy poly(ethylene glycol)-block-poly(D,L-lactide)
MDR	multidrug resistance
MPS	mononuclear phagocyte system
MTD	maximum tolerated dose
NSCLC	non-small cell lung cancer
P-gp	P-glycoprotein;
P(Asp)	poly(aspartic acid)
P(Glu)	poly(glutamic acid)
P_{app}	apparent permeability coefficient
PK	pharmacokinetic(s)
PM	Polymeric micelles
PTX	Paclitaxel
RD	recommended dose
SN-38	7-ethyl-10-hydroxy-camptothecin
TCM	traditional Chinese medicine

TPGS D-α-tocopheryl PEG succinate
VEGF vascular endothelial growth factor.

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References

[1] http://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/DrugandBiologicApprovalReports/ucm081690.htm.
[2] C. Lipinski, Poor aqueous solubility — an industry wide problem in drug discovery, Am. Pharm. Rev. 5 (2002) 82–85.
[3] H. Maeda, J. Wu, T. Sawa, Y. Matsumura, K. Hori, Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review, J. Control. Release 65 (2000) 271–284.
[4] V. Torchilin, Tumor delivery of macromolecular drugs based on the EPR effect, Adv. Drug Deliv. Rev. 63 (2011) 131–135.
[5] A.S. Mikhail, C. Allen, Block copolymer micelles for delivery of cancer therapy: transport at the whole body, tissue and cellular levels, J. Control. Release 138 (2009) 214–223.
[6] U. Kedar, P. Phutane, S. Shidhaye, V. Kadam, Advances in polymeric micelles for drug delivery and tumor targeting, Nanomed. Nanotechnol. Biol. Med. 6 (2010) 714–729.
[7] S.C. Kim, D.W. Kim, Y.H. Shim, J.S. Bang, H.S. Oh, S. Wan Kim, M.H. Seo, In vivo evaluation of polymeric micellar paclitaxel formulation: toxicity and efficacy, J. Control. Release 72 (2001) 191–202.
[8] T.Y. Kim, D.W. Kim, J.Y. Chung, S.G. Shin, S.C. Kim, D.S. Heo, N.K. Kim, Y.J. Bang, Phase I and pharmacokinetic study of Genexol-PM, a cremophor-free, polymeric micelle-formulated paclitaxel, in patients with advanced malignancies, Clin. Cancer Res. 10 (2004) 3708–3716.
[9] W.T. Lim, E.H. Tan, C.K. Toh, S.W. Hee, S.S. Leong, P.C. Ang, N.S. Wong, B. Chowbay, Phase I pharmacokinetic study of a weekly liposomal paclitaxel formulation (Genexol-PM) in patients with solid tumors, Ann. Oncol. 21 (2010) 382–388.
[10] K.S. Lee, H.C. Chung, S.A. Im, Y.H. Park, C.S. Kim, S.B. Kim, S.Y. Rha, M.Y. Lee, J. Ro, Multicenter phase II trial of Genexol-PM, a Cremophor-free, polymeric micelle formulation of paclitaxel, in patients with metastatic breast cancer, Breast Cancer Res. Treat. 108 (2008) 241–250.
[11] D.W. Kim, S.Y. Kim, H.K. Kim, S.W. Kim, S.W. Shin, J.S. Kim, K. Park, M.Y. Lee, D.S. Heo, Multicenter phase II trial of Genexol-PM, a novel Cremophor-free, polymeric micelle formulation of paclitaxel, with cisplatin in patients with advanced non-small-cell lung cancer, Ann. Oncol. 18 (2007) 2009–2014.
[12] M.W. Saif, M.S. Rubin, J.A. Figueroa, R.O. Kerr, Multicenter phase II trial of Genexol-PM (GPM), a novel Cremophor-free, polymeric micelle formulation of paclitaxel in patients with advanced pancreatic cancer (APC): Final results, Gastrointestinal Cancers Symposium, 2008, p. 269, Abstr.
[13] T. Hamaguchi, Y. Matsumura, M. Suzuki, K. Shimizu, R. Goda, I. Nakamura, I. Nakatomi, M. Yokoyama, K. Kataoka, T. Kakizoe, NK105, a paclitaxel-incorporating micellar nanoparticle formulation, can extend in vivo antitumour activity and reduce the neurotoxicity of paclitaxel, Br. J. Cancer 92 (2005) 1240–1246.
[14] T. Negishi, F. Koizumi, H. Uchino, J. Kuroda, T. Kawaguchi, S. Naito, Y. Matsumura, NK105, a paclitaxel-incorporating micellar nanoparticle, is a more potent radiosensitising agent compared to free paclitaxel, Br. J. Cancer 95 (2006) 601–606.
[15] T. Hamaguchi, K. Kato, H. Yasui, C. Morizane, M. Ikeda, H. Ueno, K. Muro, Y. Yamada, T. Okusaka, K. Shirao, Y. Shimada, H. Nakahama, Y. Matsumura, A phase I and pharmacokinetic study of NK105, a paclitaxel-incorporating micellar nanoparticle formulation, Br. J. Cancer 97 (2007) 170–176.
[16] N. Nishiyama, S. Okazaki, H. Cabral, M. Miyamoto, Y. Kato, Y. Sugiyama, K. Nishio, Y. Matsumura, K. Kataoka, Novel cisplatin-incorporated polymeric micelles can eradicate solid tumors in mice, Cancer Res. 63 (2003) 8977–8983.
[17] H. Uchino, Y. Matsumura, T. Negishi, F. Koizumi, T. Hayashi, T. Honda, N. Nishiyama, K. Kataoka, S. Naito, T. Kakizoe, Cisplatin-incorporating polymeric micelles (NC-6004) can reduce nephrotoxicity and neurotoxicity of cisplatin in rats, Br. J. Cancer 93 (2005) 678–687.
[18] R. Plummer, R.H. Wilson, H. Calvert, A.V. Boddy, M. Griffin, J. Sludden, M.J. Tilby, M. Eatock, D.G. Pearson, C.J. Ottley, Y. Matsumura, K. Kataoka, T. Nishiya, A Phase I clinical study of cisplatin-incorporated polymeric micelles (NC-6004) in patients with solid tumours, Br. J. Cancer 104 (2011) 593–598.
[19] http://www.nanocarrier.co.jp/en/research/pipeline/03.html.
[20] F. Koizumi, M. Kitagawa, T. Negishi, T. Onda, S. Matsumoto, T. Hamaguchi, Y. Matsumura, Novel SN-38-incorporating polymeric micelles, NK012, eradicate vascular endothelial growth factor-secreting bulky tumors, Cancer Res. 66 (2006) 10048–10056.
[21] T. Hamaguchi, T. Doi, T. Eguchi-Nakajima, K. Kato, Y. Yamada, Y. Shimada, N. Fuse, A. Ohtsu, S. Matsumoto, M. Takanashi, Y. Matsumura, Phase I study of NK012, a novel SN-38-incorporating micellar nanoparticle, in adult patients with solid tumors, Clin. Cancer Res. 16 (2010) 5058–5066.
[22] T. Nakanishi, S. Fukushima, K. Okamoto, M. Suzuki, Y. Matsumura, M. Yokoyama, T. Okano, Y. Sakurai, K. Kataoka, Development of the polymer micelle carrier system for doxorubicin, J. Control. Release 74 (2001) 295–302.
[23] Y. Matsumura, T. Hamaguchi, T. Ura, K. Muro, Y. Yamada, Y. Shimada, K. Shirao, T. Okusaka, H. Ueno, M. Ikeda, N. Watanabe, Phase I clinical trial and pharmacokinetic evaluation of NK911, a micelle-encapsulated doxorubicin, Br. J. Cancer 91 (2004) 1775–1781.
[24] V. Alakhov, E. Klinski, S. Li, G. Pietrzynski, A. Venne, E. Batrakova, T. Bronitch, A. Kabanov, Block copolymer-based formulation of doxorubicin. From cell screen to clinical trials, Colloids Surf., B 16 (1999) 113–134.
[25] S. Danson, D. Ferry, V. Alakhov, J. Margison, D. Kerr, D. Jowle, M. Brampton, G. Halbert, M. Ranson, Phase I dose escalation and pharmacokinetic study of pluronic polymer-bound doxorubicin (SP1049C) in patients with advanced cancer, Br. J. Cancer 90 (2004) 2085–2091.
[26] J. Valle, A. Armstrong, C. Newman, V. Alakhov, G. Pietrzynski, J. Brewer, S. Campbell, P. Corrie, E. Rowinsky, M. Ranson, A phase 2 study of SP1049C, doxorubicin in P-glycoprotein-targeting pluronics, in patients with advanced adenocarcinoma of the esophagus and gastroesophageal junction, Invest. New Drugs 29 (2010) 1029–1037.
[27] C.M. Spencer, D. Faulds, Paclitaxel, A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in the treatment of cancer, Drugs 48 (1994) 794–847.
[28] H. Gelderblom, J. Verweij, K. Nooter, A. Sparreboom, Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation, Eur. J. Cancer 37 (2001) 1590–1598.
[29] L. He, G.L. Wang, Q. Zhang, An alternative paclitaxel microemulsion formulation: hypersensitivity evaluation and pharmacokinetic profile, Int. J. Pharm. 250 (2003) 45–50.
[30] R.R. Sawant, O.S. Vaze, K. Rockwell, V.P. Torchilin, Palmitoyl ascorbate-modified liposomes as nanoparticle platform for ascorbate-mediated cytotoxicity and paclitaxel co-delivery, Eur. J. Pharm. Biopharm. 75 (2010) 321–326.
[31] M. Skwarczynski, M. Noguchi, S. Hirota, Y. Sohma, T. Kimura, Y. Hayashi, Y. Kiso, Development of first photoresponsive prodrug of paclitaxel, Bioorg. Med. Chem. Lett. 16 (2006) 4492–4496.
[32] G.V. Shavi, A.R. Kumar, A. Karthik, M. Naseer, G. Aravind, B.D. Praful, M.S. Reddy, N. Udupa, Novel paclitaxel nanoparticles: development, in vitro anti-tumor activity in BT-549 cells and in vivo evaluation, J. Control. Release 148 (2010) e119–e121.
[33] J. Gong, M. Huo, J. Zhou, Y. Zhang, X. Peng, D. Yu, H. Zhang, J. Li, Synthesis, characterization, drug-loading capacity and safety of novel octyl modified serum albumin micelles, Int. J. Pharm. 376 (2009) 161–168.
[34] A. Sparreboom, C.D. Scripture, V. Trieu, P.J. Williams, T. De, A. Yang, B. Beals, W.D. Figg, M. Hawkins, N. Desai, Comparative preclinical and clinical pharmacokinetics of a cremophor-free, nanoparticle albumin-bound paclitaxel (ABI-007) and paclitaxel formulated in Cremophor (Taxol), Clin. Cancer Res. 11 (2005) 4136–4143.
[35] X. Zhang, J.K. Jackson, H.M. Burt, Development of amphiphilic diblock copolymers as micellar carriers of taxol, Int. J. Pharm. 132 (1996) 195–206.
[36] H.M. Burt, X. Zhang, P. Toleikis, L. Embree, W.L. Hunter, Development of copolymers of poly(D, L-lactide) and methoxypolyethylene glycol as micellar carriers of paclitaxel, Colloids Surf., B 16 (1999) 161–171.
[37] X. Zhang, H.M. Burt, D. Von Hoff, D. Dexter, G. Mangold, D. Degen, A.M. Oktaba, W.L. Hunter, An investigation of the antitumour activity and biodistribution of polymeric micellar paclitaxel, Cancer Chemother. Pharmacol. 40 (1997) 81–86.
[38] H. Chen, S. Kim, W. He, H. Wang, P.S. Low, K. Park, J.-X. Cheng, Fast Release of Lipophilic agents from circulating PEG-PDLLA micelles revealed by in vivo förster resonance energy transfer imaging, Langmuir 24 (2008) 5213–5217.
[39] X. Zhang, H.M. Burt, G. Mangold, D. Dexter, D. Von Hoff, L. Mayer, W.L. Hunter, Anti-tumor efficacy and biodistribution of intravenous polymeric micellar paclitaxel, Anticancer Drugs 8 (1997) 696–701.
[40] A. Sparreboom, J. Verweij, M.E. van der Burg, W.J. Loos, E. Brouwer, L. Vigano, A. Locatelli, A.I. de Vos, K. Nooter, G. Stoter, L. Gianni, Disposition of Cremophor EL in humans limits the potential for modulation of the multidrug resistance phenotype in vivo, Clin. Cancer Res. 4 (1998) 1937–1942.
[41] N.K. Ibrahim, N. Desai, S. Legha, P. Soon-Shiong, R.L. Theriault, E. Rivera, B. Esmaeli, S.E. Ring, A. Bedikian, G.N. Hortobagyi, J.A. Ellerhorst, Phase I and pharmacokinetic study of ABI-007, a cremophor-free, protein-stabilized, nanoparticle formulation of paclitaxel, Clin. Cancer Res. 8 (2002) 1038–1044.
[42] N.K. Ibrahim, B. Samuels, R. Page, D. Doval, K.M. Patel, S.C. Rao, M.K. Nair, P. Bhar, N. Desai, G.N. Hortobagyi, Multicenter phase II trial of ABI-007, an albumin-bound paclitaxel, in women with metastatic breast cancer, J. Clin. Oncol. 23 (2005) 6019–6026.
[43] W.J. Gradishar, S. Tjulandin, N. Davidson, H. Shaw, N. Desai, P. Bhar, M. Hawkins, J. O'Shaughnessy, Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer, J. Clin. Oncol. 23 (2005) 7794–7803.
[44] M.R. Green, G.M. Manikhas, S. Orlov, B. Afanasyev, A.M. Makhson, P. Bhar, M.J. Hawkins, Abraxane, a novel Cremophor-free, albumin-bound particle form of paclitaxel for the treatment of advanced non-small-cell lung cancer, Ann. Oncol. 17 (2006) 1263–1268.
[45] U. Gatzemeier, J. von Pawel, M. Gottfried, G.P. ten Velde, K. Mattson, F. de Marinis, P. Harper, F. Salvati, G. Robinet, A. Lucenti, J. Bogaerts, G. Gallant, Phase III comparative study of high-dose cisplatin versus a combination of paclitaxel and cisplatin in patients with advanced non-small-cell lung cancer, J. Clin. Oncol. 18 (2000) 3390–3399.

[46] C. Hennequin, V. Favaudon, Biological basis for chemo-radiotherapy interactions, Eur. J. Cancer 38 (2002) 223–230.
[47] A.A. Halim, H.A. Wahba, H.A. El-Hadaad, A. Abo-Elyazeed, Concomitant chemoradiotherapy using low-dose weekly gemcitabine versus low-dose weekly paclitaxel in locally advanced head and neck squamous cell carcinoma: a phase III study, Med. Oncol. (2011), doi:10.1007/s12032-010-9811-x.
[48] W.C. Chen, J. Kim, E. Kim, P. Silverman, B. Overmoyer, B.W. Cooper, S. Anthony, R. Shenk, R. Leeming, S.H. Hanks, J.A. Lyons, A Phase II study of radiotherapy and concurrent paclitaxel chemotherapy in breast-conserving treatment for node-positive breast cancer, Int. J. Radiat. Oncol. (2010) 1–7.
[49] http://www.nanocarrier.co.jp/en/company/history.html.
[50] J.T. Hartmann, H.P. Lipp, Toxicity of platinum compounds, Expert. Opin. Pharmacother. 4 (2003) 889–901.
[51] B. Schlechter, A. Neumann, M. Wilchek, R. Arnon, Soluble polymers as carriers of cis-platinum, J. Control. Release 10 (1989) 75–87.
[52] N. Nishiyama, M. Yokoyama, T. Aoyagi, T. Okano, Y. Sakurai, K. Kataoka, Preparation and Characterization of self-assembled polymer–metal complex micelle from cis-Dichlorodiammineplatinum(II) and Poly(ethylene glycol)-Poly(α, β-aspartic acid) block copolymer in an aqueous medium, Langmuir 15 (1998) 377–383.
[53] N. Nishiyama, F. Koizumi, S. Okazaki, Y. Matsumura, K. Nishio, K. Kataoka, Differential gene expression profile between PC-14 cells treated with free cisplatin and cisplatin-incorporated polymeric micelles, Bioconjug. Chem. 14 (2003) 449–457.
[54] N. Nishiyama, Y. Kato, Y. Sugiyama, K. Kataoka, Cisplatin-loaded polymer–metal complex micelle with time-modulated decaying property as a novel drug delivery system, Pharm. Res. 18 (2001) 1035–1041.
[55] N. Alami, K. Banerjee, S. Juste, V. Page, M. Brossard, T. Hayashi, E. Igarashi, B.L. Jones, NC-6004, a novel cisplatin-incorporated polymeric micelle, is highly effective against oxaliplatin-resistant tumor models, Proc. Am. Assoc. Cancer Res. 47 (2006) 562 Abstr.
[56] http://www.nanocarrier.co.jp/en/news/topics/pdf/e20101013.pdf.
[57] H. Cabral, N. Nishiyama, S. Okazaki, H. Koyama, K. Kataoka, Preparation and biological properties of dichloro(1,2-diaminocyclohexane)platinum(II) (DACHPt)-loaded polymeric micelles, J. Control. Release 101 (2005) 223–232.
[58] H. Cabral, N. Nishiyama, K. Kataoka, Optimization of (1,2-diamino-cyclohexane) platinum(II)-loaded polymeric micelles directed to improved tumor targeting and enhanced antitumor activity, J. Control. Release 121 (2007) 146–155.
[59] M. Sumitomo, F. Koizumi, T. Asano, A. Horiguchi, K. Ito, T. Kakizoe, M. Hayakawa, Y. Matsumura, Novel SN-38-incorporated polymeric micelle, NK012, strongly suppresses renal cancer progression, Cancer Res. 68 (2008) 1631–1635.
[60] Y. Saito, M. Yasunaga, J. Kuroda, Y. Koga, Y. Matsumura, Enhanced distribution of NK012, a polymeric micelle-encapsulated SN-38, and sustained release of SN-38 within tumors can beat a hypovascular tumor, Cancer Sci. 99 (2008) 1258–1264.
[61] J. Kuroda, J. Kuratsu, M. Yasunaga, Y. Koga, Y. Saito, Y. Matsumura, Potent antitumor effect of SN-38-incorporating polymeric micelle, NK012, against malignant glioma, Int. J. Cancer 124 (2009) 2505–2511.
[62] H. Kenmotsu, M. Yasunaga, K. Goto, T. Nagano, J. Kuroda, Y. Koga, A. Takahashi, Y. Nishiwaki, Y. Matsumura, The antitumor activity of NK012, an SN-38-incorporating micelle, in combination with bevacizumab against lung cancer xenografts, Cancer 116 (2010) 4597–4604.
[63] Y. Saito, M. Yasunaga, J. Kuroda, Y. Koga, Y. Matsumura, Antitumour activity of NK012, SN-38-incorporating polymeric micelles, in hypovascular orthotopic pancreatic tumour, Eur. J. Cancer 46 (2010) 650–658.
[64] T. Nagano, M. Yasunaga, K. Goto, H. Kenmotsu, Y. Koga, J. Kuroda, Y. Nishimura, T. Sugino, Y. Nishiwaki, Y. Matsumura, Synergistic antitumor activity of the SN-38-incorporating polymeric micelles NK012 with S-1 in a mouse model of non-small cell lung cancer, Int. J. Cancer 127 (2010) 2699–2706.
[65] T. Nagano, M. Yasunaga, K. Goto, H. Kenmotsu, Y. Koga, J. Kuroda, Y. Nishimura, T. Sugino, Y. Nishiwaki, Y. Matsumura, Antitumor activity of NK012 combined with cisplatin against small cell lung cancer and intestinal mucosal changes in tumor-bearing mouse after treatment, Clin. Cancer Res. 15 (2009) 4348–4355.
[66] A. Takahashi, N. Ohkohchi, M. Yasunaga, J. Kuroda, Y. Koga, H. Kenmotsu, T. Kinoshita, Y. Matsumura, Detailed distribution of NK012, an SN-38-incorporating micelle, in the liver and its potent antitumor effects in mice bearing liver metastases, Clin. Cancer Res. 16 (2010) 4822–4831.
[67] T.E. Nakajima, M. Yasunaga, Y. Kano, F. Koizumi, K. Kato, T. Hamaguchi, Y. Yamada, K. Shirao, Y. Shimada, Y. Matsumura, Synergistic antitumor activity of the novel SN-38-incorporating polymeric micelles, NK012, combined with 5-fluorouracil in a mouse model of colorectal cancer, as compared with that of irinotecan plus 5-fluorouracil, Int. J. Cancer 122 (2008) 2148–2153.
[68] T.E. Nakajima, K. Yanagihara, M. Takigahira, M. Yasunaga, K. Kato, T. Hamaguchi, Y. Yamada, Y. Shimada, K. Mihara, T. Ochiya, Y. Matsumura, Antitumor effect of SN-38-releasing polymeric micelles, NK012, on spontaneous peritoneal metastases from orthotopic gastric cancer in mice compared with irinotecan, Cancer Res. 68 (2008) 9318–9322.
[69] H.A. Burris, J.R. Infante, D.R. Spigel, F.A. Greco, D.S. Thompson, S. Matsumoto, S. Kawamura, S.F. Jones, A phase I dose-escalation study of NK012, J. Clin. Oncol. 26 (2008) 2538 Abstr.
[70] H.C. Pitot, R.M. Goldberg, J.M. Reid, J.A. Sloan, P.A. Skaff, C. Erlichman, J. Rubin, P.A. Burch, A.A. Adjei, S.A. Alberts, L.J. Schaaf, G. Elfring, L.L. Miller, Phase I dose-finding and pharmacokinetic trial of irinotecan hydrochloride (CPT-11) using a once-every-three-week dosing schedule for patients with advanced solid tumor malignancy, Clin. Cancer Res. 6 (2000) 2236–2244.
[71] G.G. Chabot, D. Abigerges, G. Catimel, S. Culine, M. de Forni, J.M. Extra, M. Mahjoubi, P. Herait, J.P. Armand, R. Bugat, et al., Population pharmacokinetics and pharmacodynamics of irinotecan (CPT-11) and active metabolite SN-38 during phase I trials, Ann. Oncol. 6 (1995) 141–151.
[72] http://clinicaltrialsfeeds.org/clinical-trials/results/term=nk012.
[73] M. Yokoyama, S. Inoue, K. Kataoka, N. Yui, Y. Sakurai, Preparation of adriamycin-conjugated poly(ethylene glycol)-poly(aspartic acid) block copolymer. A new type of polymeric anticancer agent, Die Makromol. Chem., Rapid Commun. 8 (1987) 431–435.
[74] M. Yokoyama, T. Okano, Y. Sakurai, H. Ekimoto, C. Shibazaki, K. Kataoka, Toxicity and antitumor activity against solid tumors of micelle-forming polymeric anticancer drug and its extremely long circulation in blood, Cancer Res. 51 (1991) 3229–3236.
[75] G. Kwon, S. Suwa, M. Yokoyama, T. Okano, Y. Sakurai, K. Kataoka, Enhanced tumor accumulation and prolonged circulation times of micelle-forming poly (ethylene oxide-aspartate) block copolymer-adriamycin conjugates, J. Control. Release 29 (1994) 17–23.
[76] M. Yokoyama, T. Okano, Y. Sakurai, K. Kataoka, Improved synthesis of adriamycin-conjugated poly (ethylene oxide)-poly (aspartic acid) block copolymer and formation of unimodal micellar structure with controlled amount of physically entrapped adriamycin, J. Control. Release 32 (1994) 269–277.
[77] M. Yokoyama, S. Fukushima, R. Uehara, K. Okamoto, K. Kataoka, Y. Sakurai, T. Okano, Characterization of physical entrapment and chemical conjugation of adriamycin in polymeric micelles and their design for in vivo delivery to a solid tumor, J. Control. Release 50 (1998) 79–92.
[78] S. Fukushima, M. Machida, T. Akutsu, K. Shimizu, S. Tanaka, K. Okamoto, H. Mashiba, M. Yokoyama, T. Okano, Y. Sakurai, K. Kataoka, Roles of adriamycin and adriamycin dimer in antitumor activity of the polymeric micelle carrier system, Colloids Surf., B 16 (1999) 227–236.
[79] Y. Tsukioka, Y. Matsumura, T. Hamaguchi, H. Koike, F. Moriyasu, T. Kakizoe, Pharmaceutical and biomedical differences between micellar doxorubicin (NK911) and liposomal doxorubicin (Doxil), Jpn. J. Cancer Res. 93 (2002) 1145–1153.
[80] Y. Fujisaka, A. Horiike, T. Shimizu, N. Yamamoto, Y. Yamada, T. Tamura, Phase 1 clinical study of pegylated liposomal doxorubicin (JNS002) in Japanese patients with solid tumors, Jpn. J. Clin. Oncol. 36 (2006) 768–774.
[81] R. von Moos, B.J.K. Thuerlimann, M. Aapro, D. Rayson, K. Harrold, J. Sehouli, F. Scotte, D. Lorusso, R. Dummer, M.E. Lacouture, J. Lademann, A. Hauschild, Pegylated liposomal doxorubicin-associated hand-foot syndrome: recommendations of an international panel of experts, Eur. J. Cancer 44 (2008) 781–790.
[82] E.V. Batrakova, T.Y. Dorodnych, E.Y. Klinskii, E.N. Kliushnenkova, O.B. Shemchukova, O.N. Goncharova, S.A. Arjakov, V.Y. Alakhov, A.V. Kabanov, Anthracycline antibiotics non-covalently incorporated into the block copolymer micelles: in vivo evaluation of anti-cancer activity, Br. J. Cancer 74 (1996) 1545–1552.
[83] M.H. Kulke, B. Wu, J.W. Clark, P.C. Enzinger, T.J. Lynch, M. Vincitore, A. Michelini, C.S. Fuchs, A phase II study of doxorubicin, cisplatin, and 5-fluorouracil in patients with advanced adenocarcinoma of the stomach or esophagus, Cancer Invest. 24 (2006) 229–234.
[84] S.S. Feng, L. Zhao, J. Tang, Nanomedicine for oral chemotherapy, Nanomedicine 6 (2011) 407–410.
[85] S.S. Feng, New-concept chemotherapy by nanoparticles of biodegradable polymers: where are we now? Nanomedicine 1 (2006) 297–309.
[86] H.J. Yao, R.J. Ju, X.X. Wang, Y. Zhang, R.J. Li, Y. Yu, L. Zhang, W.L. Lu, The antitumor efficacy of functional paclitaxel nanomicelles in treating resistant breast cancers by oral delivery, Biomaterials 32 (2011) 3285–3302.
[87] F. Mathot, A. Schanck, F. Van Bambeke, A. Ariën, M. Noppe, M. Brewster, V. Préat, Passive diffusion of polymeric surfactants across lipid bilayers, J. Control. Release 120 (2007) 79–87.
[88] F. Mathot, L. van Beijsterveldt, V. Préat, M. Brewster, A. Ariën, Intestinal uptake and biodistribution of novel polymeric micelles after oral administration, J. Control. Release 111 (2006) 47–55.
[89] F. Mathot, A. des Rieux, A. Ariën, Y.J. Schneider, M. Brewster, V. Préat, Transport mechanisms of mmePEG750P(CL-co-TMC) polymeric micelles across the intestinal barrier, J. Control. Release 124 (2007) 134–143.
[90] P.M. van Hasselt, G.E.P.J. Janssens, T.K. Slot, M. van der Ham, T.C. Minderhoud, M. Talelli, L.M. Akkermans, C.J.F. Rijcken, C.F. van Nostrum, The influence of bile acids on the oral bioavailability of vitamin K encapsulated in polymeric micelles, J. Control. Release 133 (2009) 161–168.
[91] Z. Zhang, S.H. Lee, C.W. Gan, S.S. Feng, In vitro and in vivo investigation on PLA-TPGS nanoparticles for controlled and sustained small molecule chemotherapy, Pharm. Res. 25 (2008) 1925–1935.
[92] J.M. Dintaman, J.A. Silverman, Inhibition of P-glycoprotein by D-alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS), Pharm. Res. 16 (1999) 1550–1556.
[93] J. Zastre, J. Jackson, M. Bajwa, R. Liggins, F. Iqbal, H. Burt, Enhanced cellular accumulation of a P-glycoprotein substrate, rhodamine-123, by caco-2 cells using low molecular weight methoxypolyethylene glycol-block-polycaprolactone diblock copolymers, Eur. J. Pharm. Biopharm. 54 (2002) 299–309.
[94] R.D. Dabholkar, R.M. Sawant, D.A. Mongayt, P.V. Devarajan, V.P. Torchilin, Polyethylene glycol-phosphatidylethanolamine conjugate (PEG-PE)-based mixed micelles: Some properties, loading with paclitaxel, and modulation of P-glycoprotein-mediated efflux, Int. J. Pharm. 315 (2006) 148–157.
[95] L. Xiao, X. Xiong, X. Sun, Y. Zhu, H. Yang, H. Chen, L. Gan, H. Xu, X. Yang, Role of cellular uptake in the reversal of multidrug resistance by PEG-b-PLA polymeric micelles, Biomaterials 32 (2011) 5148–5157.
[96] R. Mo, X. Jin, N. Li, C. Ju, M. Sun, C. Zhang, Q. Ping, The mechanism of enhancement on oral absorption of paclitaxel by N-octyl-O-sulfate chitosan micelles, Biomaterials 32 (2011) 4609–4620.
[97] H. Hardung, D. Djuric, S. Ali, Combining HME & solubilization: Soluplus — the solid solution, Drug Deliv. Technol. 10 (2010) 20–27.
[98] B.A. Chabner, T.G. Roberts, Chemotherapy and the war on cancer, Nat. Rev. Cancer 5 (2005) 65–72.

[99] Hard to swallow, Nature 448 (2007) 105–106.
[100] L. Wang, G.B. Zhou, P. Liu, J.H. Song, Y. Liang, X.J. Yan, F. Xu, B.S. Wang, J.H. Mao, Z.X. Shen, S.J. Chen, Z. Chen, Dissection of mechanisms of Chinese medicinal formula Realgar-Indigo naturalis as an effective treatment for promyelocytic leukemia, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 4826–4831.
[101] W. Lam, S. Bussom, F. Guan, Z. Jiang, W. Zhang, E.A. Gullen, S.H. Liu, Y.C. Cheng, The four-herb Chinese medicine PHY906 reduces chemotherapy-induced gastrointestinal toxicity, Sci. Transl. Med. 2 (2010) 45ra59.
[102] D.C. Li, X.K. Zhong, Z.P. Zeng, J.G. Jiang, L. Li, M.M. Zhao, X.Q. Yang, J. Chen, B.S. Zhang, Q.Z. Zhao, M.Y. Xie, H. Xiong, Z.Y. Deng, X.M. Zhang, S.Y. Xu, Y.X. Gao, Application of targeted drug delivery system in Chinese medicine, J. Control. Release 138 (2009) 103–112.
[103] H. Maeda, G.Y. Bharate, J. Daruwalla, Polymeric drugs for efficient tumor-targeted drug delivery based on EPR-effect, Eur. J. Pharm. Biopharm. 71 (2009) 409–419.
[104] H. Maeda, Tumor-selective delivery of macromolecular drugs via the EPR effect: background and future prospects, Bioconjug. Chem. 21 (2010) 797–802.
[105] M.R. Kano, Y. Bae, C. Iwata, Y. Morishita, M. Yashiro, M. Oka, T. Fujii, A. Komuro, K. Kiyono, M. Kaminishi, K. Hirakawa, Y. Ouchi, N. Nishiyama, K. Kataoka, K. Miyazono, Improvement of cancer-targeting therapy, using nanocarriers for intractable solid tumors by inhibition of TGF-beta signaling, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 3460–3465.
[106] S. Tanaka, T. Akaike, J. Wu, J. Fang, T. Sawa, M. Ogawa, T. Beppu, H. Maeda, Modulation of tumor-selective vascular blood flow and extravasation by the stable prostaglandin 12 analogue beraprost sodium, J. Drug Target. 11 (2003) 45–52.
[107] T. Seki, J. Fang, H. Maeda, Enhanced delivery of macromolecular antitumor drugs to tumors by nitroglycerin application, Cancer Sci. 100 (2009) 2426–2430.
[108] G.Y. Lee, K. Park, S.Y. Kim, Y. Byun, MMPs-specific PEGylated peptide-DOX conjugate micelles that can contain free doxorubicin, Eur. J. Pharm. Biopharm. 67 (2007) 646–654.
[109] S.R. Kim, S.K. Bae, H.J. Park, M.K. Kim, K. Kim, S.Y. Park, H.O. Jang, I. Yun, Y.J. Kim, M.A. Yoo, M.K. Bae, Thromboxane A(2) increases endothelial permeability through upregulation of interleukin-8, Biochem. Biophys. Res. Commun. 397 (2010) 413–419.
[110] S.D. Funk, A. Yurdagul Jr., J.M. Green, K.A. Jhaveri, M.A. Schwartz, A.W. Orr, Matrix-specific protein kinase A signaling regulates p21-activated kinase activation by flow in endothelial cells, Circ. Res. 106 (2010) 1394–1403.
[111] T. Ishida, M. Ichihara, X. Wang, H. Kiwada, Spleen plays an important role in the induction of accelerated blood clearance of PEGylated liposomes, J. Control. Release 115 (2006) 243–250.
[112] T. Ishida, H. Kiwada, Accelerated blood clearance (ABC) phenomenon upon repeated injection of PEGylated liposomes, Int. J. Pharm. 354 (2008) 56–62.
[113] X. Wang, T. Ishida, H. Kiwada, Anti-PEG IgM elicited by injection of liposomes is involved in the enhanced blood clearance of a subsequent dose of PEGylated liposomes, J. Control. Release 119 (2007) 236–244.
[114] S.M. Moghimi, A.J. Andersen, S.H. Hashemi, B. Lettiero, D. Ahmadvand, A.C. Hunter, T.L. Andresen, I. Hamad, J. Szebeni, Complement activation cascade triggered by PEG-PL engineered nanomedicines and carbon nanotubes: the challenges ahead, J. Control. Release 146 (2010) 175–181.
[115] H. Xu, K.Q. Wang, Y.H. Deng, W. Chen da, Effects of cleavable PEG-cholesterol derivatives on the accelerated blood clearance of PEGylated liposomes, Biomaterials 31 (2010) 4757–4763.
[116] H. Koide, T. Asai, K. Hatanaka, T. Urakami, T. Ishii, E. Kenjo, M. Nishihara, M. Yokoyama, T. Ishida, H. Kiwada, N. Oku, Particle size-dependent triggering of accelerated blood clearance phenomenon, Int. J. Pharm. 362 (2008) 197–200.
[117] T. Ishihara, M. Takeda, H. Sakamoto, A. Kimoto, C. Kobayashi, N. Takasaki, K. Yuki, K. Tanaka, M. Takenaga, R. Igarashi, T. Maeda, N. Yamakawa, Y. Okamoto, M. Otsuka, T. Ishida, H. Kiwada, Y. Mizushima, T. Mizushima, Accelerated blood clearance phenomenon upon repeated injection of PEG-modified PLA-nanoparticles, Pharm. Res. 26 (2009) 2270–2279.
[118] X.Y. Wang, T. Ishida, M. Ichihara, H. Kiwada, Influence of the physicochemical properties of liposomes on the accelerated blood clearance phenomenon in rats, J. Control. Release 104 (2005) 91–102.
[119] H. Ma, K. Shiraishi, T. Minowa, K. Kawano, M. Yokoyama, Y. Hattori, Y. Maitani, Accelerated blood clearance was not induced for a gadolinium-containing PEG-poly(L-lysine)-based polymeric micelle in mice, Pharm. Res. 27 (2010) 296–302.
[120] A. Gabizon, R. Isacson, O. Rosengarten, D. Tzemach, H. Shmeeda, R. Sapir, An open-label study to evaluate dose and cycle dependence of the pharmacokinetics of pegylated liposomal doxorubicin, Cancer Chemother. Pharmacol. 61 (2008) 695–702.