

Expert Opinion

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Polymeric micelles as a new drug carrier system and their required considerations for clinical trials

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Importance of the field: A polymeric micelle is a macromolecular assembly composed of an inner core and an outer shell, and most typically is formed from block copolymers. In the last two decades, polymeric micelles have been actively studied as a new type of drug carrier system, in particular for drug targeting of anticancer drugs to solid tumors.

Areas covered in this review: In this review, polymeric micelle drug carrier systems are discussed with a focus on toxicities of the polymeric micelle carrier systems and on pharmacological activities of the block copolymers. In the first section, the importance of the above-mentioned evaluation of these properties is explained, as this importance does not seem to be well recognized compared with the importance of targeting and enhanced pharmacological activity of drugs, particularly in the basic studies. Then, designs, types and classifications of the polymeric micelle system are briefly summarized and explained, followed by a detailed discussion regarding several examples of polymeric micelle carrier systems.

What the reader will gain: Readers will gain a strategy of drug delivery with polymeric carriers as well as recent progress of the polymeric micelle carrier systems in their basic studies and clinical trials.

Take home message: The purpose of this review is to achieve tight connections between the basic studies and clinical trials.

Keywords: anticancer drug, block copolymer, EPR effect, polymeric micelle, targeting, toxicity

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1. Introduction

Polymeric micelles have lately appeared as a new type of drug carrier; the preceding drug carriers were micro(nano)particles, liposomes and non-micelle-forming polymeric carriers. The study of polymeric micelle drug carriers started in the 1980s [1-4], and these carrier systems were recognized as one of the most potent drug carrier types in the 1990s [5-13]. Then, in the 2000s, several significant related clinical trials [14-23] got underway, while more and more R&D projects were conducted.

A polymeric micelle is a macromolecular assembly composed of an inner core and an outer shell. The polymeric micelles can have a spherical or a cylindrical shape, depending on the chemical structure and chain length of the macromolecules. For the purpose of drug targeting, most polymeric micelle studies have dealt with the spherical shape, whereas a very limited number of the filamentous shape systems have been studied [24,25]. Therefore, this review deals only with the spherical polymeric micelles. A spherical polymeric micelle structure forms from block copolymers or graft copolymers [26]. Figure 1 illustrates the formation of a polymeric micelle structure resting on an AB-type block copolymer in which two polymer chains are of a tandem-connected form. Drug molecules are incorporated into the inner core of the micelle through both chemical conjugation and physical

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Article highlights.

- In this review, I discuss toxicities of the polymeric micelle carrier systems as well as pharmacological activities of the block copolymers insofar as these subjects have not been well discussed irrespective of their importance in clinical applications.
- There are two types of drug incorporation into the inner core of the polymeric micelles, namely chemical conjugation and physical entrapment.
- Other than targeting, three functions can be obtained with the polymeric micelles.
- In the system in Kabanov's report, the block copolymer worked as a biologically active agent rather than as a carrier to specific sites.
- In the limited situation, the research team reported on three important observations, which merit an explanation and a discussion here.
- There are six widely known clinical trials regarding polymeric micelle drug carrier systems.

This box summarises key points contained in the article.

entrapment. Such a micellar structure forms if one segment of the block copolymer can provide enough interchain cohesive interactions in a selective solvent. For the cohesive interactions, hydrophobic interactions have been used most because most drug molecules possess a hydrophobic character. Typically, the polymeric studies on drug carriers have involved AB- or ABA-type block copolymers because the close relationship between micelle-forming behavior and the structure of polymers can be evaluated more easily with these types of block copolymer than with the other types.

In this review, toxicities of the polymeric micelle carrier systems are discussed as well as pharmacological activities of the block copolymers insofar as these subjects have not been well discussed irrespective of their importance in clinical applications. (Please see other excellent reviews [27-32] for more general information on the polymeric micelle drug carrier systems.) In this explanation of these toxicity and activity subjects, anticancer targeting systems are referenced because most studies on polymeric micelle drug carrier systems have concerned anticancer targeting to solid tumors. Figure 2 summarizes the developmental process of drug targeting systems, which has comprised the basic study stage, the preclinical study stage and the clinical trial stage. The basic study includes syntheses of polymers, preparation of polymeric micelles containing drugs, *in vitro* cytotoxicity tests, *in vivo* anticancer activity tests and *in vivo* pharmacokinetic analyses. The basic study includes *in vivo* toxicity tests, but these tests span a narrower examination range than do preclinical tests. In the preclinical tests, anticancer activity is examined with reference to a greater variety of cancer models, and pharmacokinetic analyses are carried out in greater detail than is the case of the basic study. Furthermore, in the preclinical stage, toxicities undergo detailed examination relative to various kinds of animals. Clinical trials are carried out in three phases (from Phase I to Phase III). In Phase I trials, the two

chief objectives concern toxicity evaluation and determination of a recommended dose for the Phase II trials.

I would argue that, among these three stages, there are slight but significant differences in the importance of evaluations. As illustrated in Figure 2, the importance of anticancer activity tests is greater in the basic study than in the preclinical test. On the other hand, the importance of toxicity tests is lower in the basic study than in the preclinical and clinical tests. These differences reflect the purposes and functions of each stage, but the importance of the toxicity evaluations in the basic studies should be stressed, in particular for researchers in the basic sciences. The two overall reasons for emphasizing this point are as follows.

- (1) The critical-factor difference between the basic study and the clinical trial. In basic studies, *in vivo* toxicity is assayed at least in anticancer activity tests because high anticancer activity is obtained at the maximum tolerated dose that does not provide lethal toxicity to experimental animals. In this circumstance, one or only a few types of toxicity are critical owing to homogeneity of the experimental animals. Inhibition of the critical toxicity(ies) can be a good strategy for the anticancer activity enhancement. Therefore, basic study researchers tend to pay attention only to one or a very small number of toxicities that are correlated to the maximum tolerated dose. By contrast, many more types of toxicity must be seriously considered and observed in the clinical trials because of the heterogeneity of human patients. A certain type of toxicity that is very mild in the basic study can be critically toxic to some patients owing to genetic characteristics or to a slight disorder in some functions of the patients as well as to physiological differences between the experimental animals and the human beings. If a proportion of the patients receiving the critical (lethal) toxicity is high (e.g., 10%), a drug is dropped from the clinical test, even for anticancer drug cases.
- (2) A drug carrier system shows unexpected toxicity in the clinical tests. The unexpected toxicity of an approved drug carrier system is a well-known phenomenon. A liposomal anticancer drug targeting system containing doxorubicin [33,34] was approved in 1995 against Kaposi's sarcoma, followed by FDA approval for treating ovarian cancer. This liposomal system can be targeted to solid tumors owing to its successful poly(ethylene glycol) surface modification, which allows this liposome to use the enhanced permeability and retention effect (EPR effect) [35-37]. The EPR effect is a passive targeting mechanism based on hyperpermeability of the tumor vasculature. One of the most serious adverse effects of this liposome in human clinics is hand-foot syndrome, in which there are observable developed palmar-plantar skin eruptions on the hands and the feet [38,39]. This hand-foot syndrome is never observed with doxorubicin alone or with this liposome carrier alone. This correlation means that the

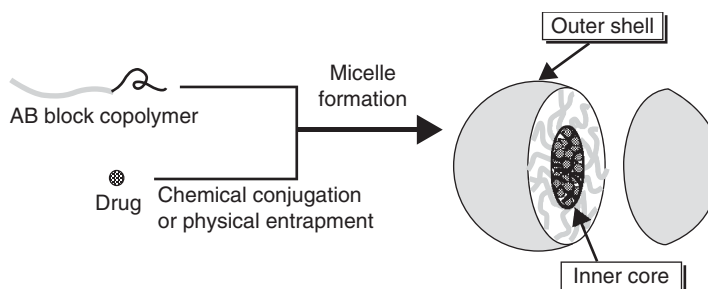


Figure 1. Design of polymeric micelle drug carrier system.

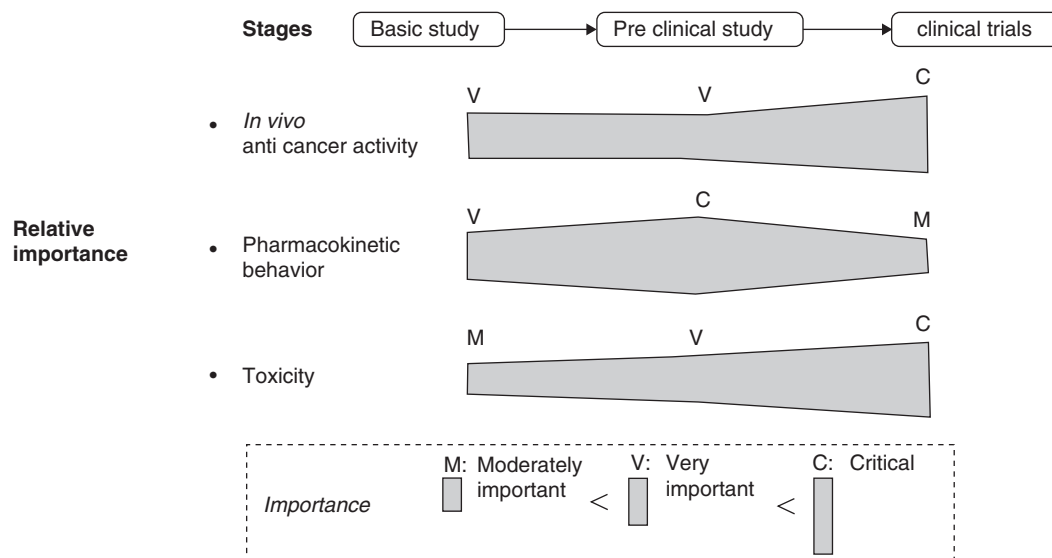


Figure 2. Importance of evaluations on each developmental stage.

adverse effect is observed only in this liposomal doxorubicin carrier system. Furthermore, it is generally believed that delivery to skin tissue is well inhibited for the carriers showing the EPR effect. This hand-foot syndrome was not reported in basic studies of this liposomal doxorubicin; at least, the hand-foot syndrome was not a dose-limiting toxic side effect in mouse anticancer activity assays. Therefore, the appearance of the hand-foot syndrome as a severe side effect in clinical trials was totally unexpected. If skin toxicity had undergone a detailed examination, the hand-foot syndrome could have been reported even in basic studies using mouse models. However, such detailed examinations cannot be conducted in either the basic studies or the preclinical studies for less important side effects surfacing in the mouse models unless some special information related to this toxic effect is present.

This story about the liposomal doxorubicin system does not lead to a necessary conclusion that there should be detailed examinations for all types of toxicity, including unexpected

ones. A powerful lesson from the liposomal doxorubicin story is that the system constitutes a good strategy by which basic researchers can pay attention to mild but characteristic side effects in the basic studies.

Drawing on the above-mentioned facts and viewpoints, in this review toxicities of the polymeric micelle targeting systems in animal experiments are summarized and discussed, particularly non-dose-limiting toxicities (i.e., mild toxicities) both of the carrier systems and of the carrier block copolymers.

2. Characterizations and classifications of polymeric micelle drug carrier systems

Before proceeding to the topics in Section 3, which presents some examples of polymeric micelle drug carrier systems, the polymeric micelle systems will be classified depending on their purposes and functions relative to drug delivery system drug delivery system (DDS). This classification should facilitate readers' grasp of the materials in later chapters. Furthermore, the advantages and characteristics of the polymeric micelle are briefly summarized.

2.1 Types of drug incorporation

There are two types of drug incorporation into the inner core of the polymeric micelles, namely chemical conjugation and physical entrapment. Physical entrapment using hydrophobic interactions can be applied to many drugs [40-42], as most drug molecules possess a hydrophobic moiety(ies) (even in the case of water-soluble drugs), and because functional groups that are required for chemical conjugation are not necessary for physical entrapment. In this type of polymeric micelle system, toxicities of carrier block copolymers need not be considered seriously because block copolymers without chemically conjugated drug molecules are expected to be biologically inactive or much less active than the incorporated drug. This type of system can be applied well, particularly to anticancer drug carrier systems because toxicities or pharmacological activities of non-drug-binding block copolymers are believed to be much less intense than those of highly cytotoxic anticancer drugs.

The second type of drug incorporation, chemical conjugation, occurs through chemical bond formation between a drug molecule and the inner-core-forming block of the block copolymer [43-53]. In this type, drug release by cleavage of a chemical bond is an important issue in most systems. One common reaction for this cleavage is hydrolysis. Owing to the phase separation of the inner core from both the outer shell and an outer environment, the drug's access to water molecules, hydrogen ions, hydroxyl ions and hydrolytic enzymes is considerably inhibited. Therefore, the cleavage rate is expected to be much lower than occurs with conventional polymer-drug conjugates. In turn, micelle structures can facilitate the quick release of a drug if the hydrophobicity of the bound drug contributes to inner core association for micelle formation. As the drug molecules are released, the drug release can be accelerated owing to a decrease in inner core hydrophobicity. From this point of view, Li and Kwon [50] have designed methotrexate-conjugated block copolymer micelles, in which the drug release rate can be controlled with conjugated drug amounts. The other possible strategy for chemical conjugation is an alternative action mechanism for the drugs. In a physically entrapped system, only released drugs are expected to express pharmacological activity, even though the delivery systems change both the whole body's distribution and the intracellular distribution. In the case of chemical conjugation, a drug may show activity in both a released form and in a conjugated form. If the conjugated form can express activity, the chemical conjugation systems may overcome the multi-drug resistance induced by P-glycoprotein, as the P-glycoprotein is not expected to result in an efflux of the polymer-drug conjugates to the cells' exteriors. Regarding toxicity concerns, the chemically conjugated drug may pose a risk of a new type of toxicity that was not observed in the corresponding free drug, as the former drug's pharmacokinetic and pharmacodynamic behaviors can be very different from those of the free drug.

2.2 Functions of drug carriers

One important function of the polymeric micelle drug carriers is targeting. As described in Section 2.3, the polymeric micelles possess an inherent ability to target solid tumors owing to the micelles' appropriate size for the EPR effect, which is a passive targeting mechanism to solid tumors.

Other than targeting, three functions can be obtained with the polymeric micelles. The first function is controlled release of a drug. Timing and duration of drug actions can be controlled by controlling drug-release rates from the micelles [51,52,54,55]. This function is not distinctly recognized because both targeting and controlled release functions are achieved in one polymeric micelle system in many cases. In these cases, it is more difficult to prove the contribution of the controlled release for better therapeutic effects than to prove the targeting contribution.

The second function is pharmacological activities of the carrier polymers. In most polymeric micelle systems, block copolymers work only as carriers without showing pharmacological activities. Alternatively, some block copolymers show pharmacological activities. In the former case, toxicity concerns of the carrier block copolymers are not as serious as in the latter case.

There are two types of pharmacologically active copolymer. The first type is the case where block copolymers have a biological activity. Kabanov *et al.* reported that poly(propylene oxide)-*b*-poly(ethylene oxide) block copolymers had an inhibitory activity on P-glycoproteins that play an important role in multi-drug resistance [8,9,19,68,79,70]. Section 3.1 introduces this topic in detail. The second type is a case where drug molecules are attached to the block copolymers. This type of system may have an action mechanism completely different from that of the corresponding free drug (e.g., a cytotoxic action in the conjugate form without a need for the drug release). Such an unusual action mechanism could help result in better pharmacological effects in such matters as circumvention of P-glycoprotein-driven multi-drug resistance. In turn, the new action mechanism has another potential: to cause new toxic side effects that are not found in the corresponding free drug.

The third function of the polymeric micelle systems is solubilization of water-insoluble drugs. Water-insoluble or hardly soluble drugs cannot be safely injected into the bloodstream. One method to overcome this problem is the use of solubilizers that transform these insoluble drugs into a pseudo-soluble status. Water-miscible organic solvents and low-molecular-mass surfactants are representative solubilizers for this purpose. However, these solubilizers are frequently observed showing very high toxicity. Therefore, a DDS carrier that simply solubilizes a water-insoluble drug without any other function is of great value in chemotherapy. Several studies reported that polymeric micelle carriers showed high functions for the solubilization [13,56-62]. This desired function is discussed in Section 2.3, and examples of the solubilization are explained in Sections 3.2 and 3.3.

2.3 Advantages and disadvantages of polymeric micelle carrier systems

Advantages and disadvantages of the polymeric micelle systems as drug carriers are summarized and discussed briefly in this section. Table 1 summarizes the advantages.

The first advantage is the very small size of polymeric micelles. Polymeric micelles are formed typically in a diameter range from 10 to 100 nm, with a substantial narrow distribution. This size range of particles is considered preferable for the attainment of stable, long-term circulation of the carrier system in the bloodstream because the larger particles are actively captured in the reticuloendothelial system and because the smaller particles are rapidly excreted from the kidneys. Alternatively, the small size of polymeric micelles is a great benefit in the sterilization processes associated with pharmaceutical production. Polymeric micelles are easily and inexpensively sterilized by filtration, itself resting on the use of typical sterilization filters with 0.45 or 0.22 μm pores.

The second advantage is the high structural stability of polymeric micelles as compared with the micelles forming from low-molecular-mass surfactants. The high structural stability of polymeric micelles stated above is an important key to *in vivo* delivery in micellar forms (not in single polymer chain form).

The third advantage is the high water solubility of the polymeric micelle drug carrier system incorporating a large amount of hydrophobic drugs [63]. Generally, in conventional polymer-drug conjugate systems, a loss of the water solubility of the polymeric carrier resulting from the introduction of a hydrophobic drug creates a serious problem. Several research groups reported this problem of the drug-polymer conjugates in syntheses [64-66] and in their intravenous injections [67]. Polymeric micelles can incorporate a large number of hydrophobic drug molecules in the large volume of the micelles' inner core, and simultaneously the micelles can maintain their water solubility by inhibiting the intermicellar aggregation of the hydrophobic cores and by using a hydrophilic outer shell layer that works as a barrier against the intermicellar aggregation.

The beneficial character of low toxicity may be described as the fourth advantage. Generally, polymeric surfactants are known to be less toxic than low-molecular-mass surfactants such as sodium dodecyl sulfate. Furthermore, in theory polymeric micelles are considered to be very safe in relation to chronic toxicity. Possessing a much larger size than critical filtration values in the kidney, polymeric micelles can evade renal filtration, even if the molecular mass of the constituting block copolymer is lower than the critical molecular mass for renal filtration. Of course, potential toxicities of polymers and degradation products of polymers (for biodegradable polymers, for example, inflammation induction due to acidic products from polyesters) must be considered. On the other hand, all polymer chains can be released (as single polymer chains) from the micelles during a long time period. This phenomenon results in the complete excretion of the block copolymers from the renal route if the polymer chains are

designed with a lower molecular mass than the critical value for renal filtration. Such a result constitutes an advantage of polymeric micelles over the conventional (non-micelle forming) and non-biodegradable polymeric drug carrier systems.

The fifth advantage is separated functionality. Polymeric micelles are composed of two phases: inner core and outer shell. Various functions required for drug delivery systems can be shared by these structurally separated phases. Each phase can play different roles in drug delivery. The outer shell is responsible for interactions with biocomponents such as proteins and cells. These interactions determine pharmacokinetic behavior and the biodistribution of drugs; therefore, the *in vivo* delivery of drugs may be controlled by the outer shell segment independently of the inner core, which is responsible for pharmacological activities through drug loading and release. This heterogeneous structure is more favorable in the construction of highly functionalized carrier systems than in the conventional (non-micelle-forming) polymeric carrier systems, as properties of both phases are freely and independently controlled through a selection of the polymer chains that are appropriate for each segment of block copolymers.

Here, the disadvantages of the polymeric micelle systems will be explained. One disadvantage that cannot easily be recognized in scientific papers is a fact that relatively high levels of polymer chemistry are needed in the polymeric micelle studies. As illustrated in Figure 1, an AB type of block copolymer is one of the most favorable structures for the formation of polymeric micelles possessing well-defined shape, size and diameter distribution. The architecture of the AB block copolymer is very simple (two polymer blocks are connected in a tandem manner), but in general its synthesis is more difficult than that of random polymers, where different units are aligned on a polymer chain in a random manner. (As a complete explanation of the difficulties and the limitations of block copolymer syntheses would require more space than is available for this paper, please see other references or textbooks on polymer chemistry.) As a result of the synthesis-related difficulty, choices of monomer units and their combinations are considerably limited. In some cases where laboratories can synthesize a desired block copolymer, it is difficult to synthesize the block copolymer on a large industrial scale in a highly reproducible manner. (In contrast to this general situation, a limited number of block copolymers for drug carriers are produced on an industrial scale and are commercially available. The typical example is Pluronic, poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) block copolymers.) In addition to the above-mentioned problem, some studies have reported cases in which substantial optimization relative to the chemical structures and the chain lengths of the block copolymers was essential for successful drug targeting [61,62,63,68]. This finding indicates that high levels of polymer chemistry are critical for the syntheses of the controlled chemical structures and chain lengths.

Table 1. Advantages of polymeric micelle drug carrier systems.

1. Small diameter with narrow distribution (10 – 100 nm)
2. High structural stability
3. High water solubility
4. Low toxicity
5. Separated functionality

The other disadvantage of the polymeric micelle systems is the immature technology for drug incorporation in a physical manner. Yokoyama *et al.* reported that physical incorporation efficiencies were dependent on drug incorporation methods [61]. At present, there seems to be no universal incorporation method applicable to any polymer. Therefore, researchers must find an appropriate incorporation method for each drug through trial and error. Furthermore, in some methods the drug incorporation may be difficult on a large industrial scale, but easy and efficient on a small laboratory scale. The scale problem is more serious than the polymer synthesis matter because physical factors (e.g., diffusion, solvent exchange rate) are influenced very strongly by the scales in the drug incorporation processes, such as solvent exchange through dialysis membrane. Therefore, more scientific and engineering studies are necessary for significant development on the incorporation technology.

Also, it is worthwhile mentioning that the control of micelle dissociation and drug release rate is essential for drug targeting, and that this control of these matters is sometimes technically difficult to optimize for the targeting, although this is not a disadvantage of the polymeric micelle systems.

3. Reports concerning toxicities of the polymeric micelle systems and biological activities of carrier block copolymers

This section introduces reports concerning toxicities of the polymeric micelle systems and biological (pharmacological) activities of carrier block copolymers.

3.1 Kabanov's report

Kabanov and his colleagues used a poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) block copolymer (ABA type, commercial name Pluronic®) for their polymeric micelle drug delivery system [8,9,19,69-71]. In this system, the block copolymer worked as a biologically active agent rather than as a carrier to specific sites. They reported that Pluronic polymers specifically inhibited the ATP production in mitochondria. P-glycoprotein, which plays an important role in multi-drug resistant cancer cells, shows the efflux action of anticancer drug in an ATP-dependent manner. Consequently, the drug efflux action is inhibited by a Pluronic polymer through the inhibition of ATP production. By using this block

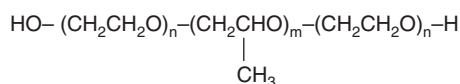
copolymer, they reported successful circumvention of the multi-drug resistance both *in vitro* and *in vivo*. This is an innovative application of a synthetic polymer to cancer chemotherapy.

This activity of the block copolymer is very interesting and rather unexpected, as Pluronic polymers lack the functional groups (e.g., charged groups such as carboxyl and amine groups and bulky hydrophobic groups such as long acyl groups) that are expected to have strong interactions with proteins. Kabanov *et al.* showed that occurrences of this activity featured Pluronic polymers possessing an appropriate hydrophilic/hydrophobic balance and appropriate chain lengths. Furthermore, research has not shown any other synthetic polymer that shows P-glycoprotein inhibitory activity. Consequently, it seems that this very interesting and useful activity for cancer chemotherapy is obtained with a very limited number of polymers. However, researchers must pay attention to biological activities of carrier polymers because the mitochondrion and P-glycoprotein inhibitory activities run the risk of raising the levels of toxic side effects in the normal organs and tissues irrespective of the success that Kabanov *et al.*'s animal cancer model had in obtaining enhanced *in vivo* anticancer activity. The presence of this activity can be found relatively easily in the *in vitro* cytotoxic assays (e.g., through determination of drug influx and efflux amounts into/from cells in the presence/absence of polymers). If researchers observe that an anticancer drug's *in vitro* activity increases in strength owing to the drug's combinatory use with a polymer, an advisable subsequent step is to examine the P-glycoprotein in greater detail.

3.2 Yokoyama's report

Yokoyama *et al.* have reported several polymeric micelle anticancer drug targeting systems since the late 1980s, and most of this research team's reports involved the basic study stage. Therefore, the main focus of these reports was on enhanced anticancer activity, improved biodistribution and pharmacokinetic behavior, and related physicochemical characterizations of the carrier systems, whereas descriptions of the carrier systems' toxicities and of the carrier polymers' biological activities were relatively limited. In this limited situation, the research team reported on three important observations, which merit an explanation and a discussion here.

In a series of papers, Yokoyama and co-workers reported enhanced *in vivo* anticancer activity and tumor-specific drug delivery of an anticancer drug, doxorubicin, with drug incorporation into a polymeric micelle carrier [72-78]. These reports focus mainly on *in vivo* anticancer activity and on tumor targeting; however, the reports treat some important information about toxicity. At this point, some details should be noted concerning the chemical structure of the research team's doxorubicin-micelle system because the polymer structure is correlated with toxicity concerns. Figure 3B shows the block copolymer structure. Doxorubicin (DOX) was chemically conjugated to aspartic acid residues of the poly(ethylene

A. Pluronic® (PEG-*b*-PPO-*b*-PEG)

B. Doxorubicin-conjugated block copolymer

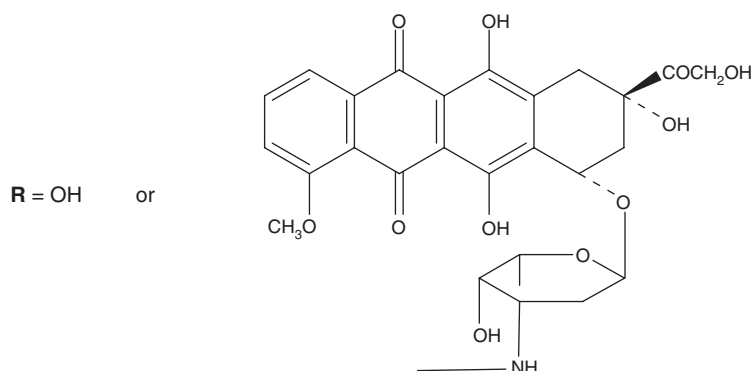
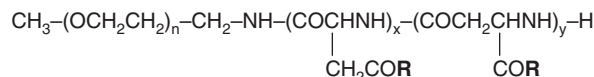
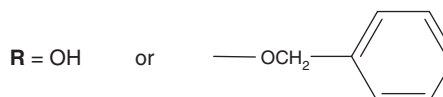
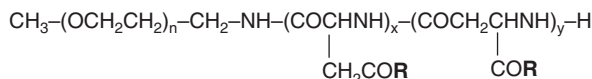
C. A PEG-*b*-poly (amino acid) block copolymer without conjugate drugs

Figure 3. Chemical structures of block copolymer used as polymeric micelle carriers.

glycol)-*b*-poly(aspartic acid) block copolymer through amide bond formation. The poly(ethylene glycol) polymer block was hydrophilic, whereas the DOX-substituted poly(aspartic acid) chain was hydrophobic. Therefore, the obtained drug-block copolymer conjugate (PEG-P(Asp(DOX))) formed micellar structures owing to its amphiphilic character. Doxorubicin was further incorporated into the inner core by means of physical entrapment using hydrophobic and π - π interactions with the chemically conjugated DOX molecules. As a result, polymeric micelles containing both the chemically conjugated and the physically entrapped DOX in the inner core were obtained with the PEG outer shell. Yokoyama *et al.* reported that the carrier polymer PEG-P(Asp(DOX)) did not show any *in vitro* or *in vivo* activities, and that the cytotoxic and anticancer activities were obtained with physically entrapped DOX [78]. The inactivity of the chemically conjugated DOX resulted from the fact that DOX molecules were directly conjugated, without any spacer, to the aspartic acid residues of the block copolymer through amide bonds. These 'direct' amide bonds were considered to be too stable for its cleavage,

which provides pharmacologically active free DOX. Therefore, for the purpose of anticancer activity assays, the block polymer can be considered only a carrier, not a biologically active species. On the other hand, attention must be paid to the *in vivo* toxicities attributable to the polymers conjugating drug molecules, particularly in chronic toxic side effects that are not examined in simple toxicity assays done in the basic studies focusing on anticancer activity evaluations.

In another report [76], Yokoyama *et al.* analyzed pharmacokinetic behaviors of the DOX polymeric micelle system as well as enhanced *in vivo* antitumor activity. In this analysis, they showed tumor-selective delivery; the accumulated DOX physically entrapped in the micelle was an amount approximately ninefold larger than the amount of free DOX, whereas the accumulation of the micellar DOX (physically entrapped) in normal organs and tissues was smaller than or the same as the accumulation of free DOX. This report identifies an interesting pharmacokinetic behavior of the micellar DOX. Within 1 h of intravenous (i.v.) injection, accumulated amounts of the micellar DOX in liver were smaller than those

of free DOX. This inequality indicates that the targeting strategy based on the EPR effect was effective in this targeting system even for liver that is known to possess pores large enough for micelles' extravasation in the liver's vasculature. By contrast, 4 h and later, after i.v. injection, this situation was reversed. The micellar DOX showed larger accumulation amounts in liver than free DOX. This inequality resulted from rapid clearance of free DOX in liver through the liver's metabolic activity for drugs, whereas the micellar DOX concentration in liver did not undergo a significant drop, probably because the physically entrapped DOX in the micelle core was largely protected from the metabolic activity. Consequently, the concentration of the micellar DOX was several-fold larger than that of the free DOX. This fact implies that the nonspecific distribution (e.g., at liver) may be an important concern even for successful targeting systems. In another report [74], Yokoyama *et al.* examined toxic side effects of the DOX polymeric micelle system in a composition similar to that of the former one. For liver toxicity, this research team examined pathological observations as well as alanine aminotransferase (ALT)- and aspartate transaminase (AST)-level measurements in blood on day 10 after the first drug injection. The conclusion of this toxicity evaluation was that the toxic side effects of the DOX polymeric micelle were shown in the same pattern as that of free DOX. This means that the liver toxicity was not especially higher than the other side effects for the DOX polymeric micelle. However, considerable attention must be paid to chronic liver toxicity that could not be evaluated in that report up to day 10.

In this DOX-containing polymeric micelle system, the liver toxicity does not seem to be a serious problem because neither unusual nor unexpected toxic side effects were observed in its Phase I clinical trial [14,79,80]. The appearance of the side effects showed the same pattern as that of free DOX in this human clinical trial, where patients underwent longer observations (e.g., several weeks to several months) than was the case in the report's basic study stage.

As explained above, chronic liver toxicity was not serious for the DOX-containing polymeric micelle system. Most scientists in this field posit that the considerable slowness of the metabolism of the incorporated drug compared with the free drug is a universal phenomenon of polymeric micelle drug carrier systems owing to the isolated drug-containing inner core from the outer aqueous environment, where a liver's metabolic enzymes work. Therefore, chronic liver toxicity merits careful examination not only for the polymeric micelle systems, but also for all nano-sized drug carrier systems such as PEG-coated liposomes.

The second important study is Yokoyama and co-worker's report concerning toxicity evaluation of a polymeric micelle carrier lacking drug incorporation [81]. A micelle-forming block copolymer whose chemical structure is shown in Figure 3C had been used as a carrier for an anticancer compound camptothecin [61,62,68,82-85] and synthetic retinoids [86-90] such as all-*trans* retinoic acid. The research team analyzed

toxicities by conducting pathological examinations that used rats. No pathological abnormality was found for a considerably high dose (200 mg/kg \times 5). The team, however, observed significant activation of the mononuclear phagocytic system (MPS) in several organs such as the spleen and liver. Selective accumulation of the polymeric micelle at the MPS was confirmed in an immunohistochemical analysis. These results were obtained with a drug-free polymeric micelle. By contrast, the team concluded that the MPS suffered considerable damage from incorporated cytotoxic drugs in anticancer drug-carrying polymeric micelles. That is why the MPS activation phenomenon seems less important than the other side effects for cytotoxic anticancer drug-carrying systems. However, this MPS-related phenomenon may be important if the polymeric micelle carrier systems are applied to delivering drugs that are much less toxic than typical anticancer drugs.

The third relevant study for biological activity of polymeric micelle carriers is one reported recently concerning the accelerated blood clearance (ABC) phenomenon [91]. ABC is a phenomenon where clearance rates of carrier systems from the bloodstream are raised substantially at repeated injections [92-95]. This ABC phenomenon has been well studied with PEG-coated liposomes that have long-circulating characters at the first injection. A PEG-coated liposome is injected (the first dose) intravenously at an appropriate dose, and then the same liposome is injected (the second dose) according to an appropriate interval (5 – 7 days). This phenomenon occurs owing to immunological activity induced at the first dose, and the change in the clearance is considerable; for example, a case could arise where 90% of the injected dose is circulating 2 h after the first injection and where < 5% of the injected dose is circulating in blood 2 h after the second injection. As polymeric micelles with PEG outer shells have the same profile as that of the PEG-coated liposomes in terms of the PEG outer layer, it is of great interest to know whether these polymeric micelles induce the ABC phenomenon or not. Yokoyama and Oku *et al.* reported the first observation of polymeric micelles' display of the ABC phenomenon [91]. These researchers injected three kinds of polymeric micelle at the first dose, followed by an injection of a PEG-coated liposome at the second injection. The ABC phenomenon was observed only for one polymeric micelle, it was not observed for the other two polymeric micelles. This indicates that some polymeric micelles can induce the ABC phenomenon. At present, the essential properties of the micelle for ABC induction are unknown and will be elucidated in future studies.

The ABC phenomenon is related to the above-mentioned uptake of carrier systems at the MPS and activation of the MPS in the following two aspects.

- (1) The existing research suggests that ABC induction develops at the liver [92,93] or the spleen [94,95], both of which are typical organs relative to the MPS. Therefore, the control of uptake extent at the MPS can be a key factor in ABC induction.

- (2) It was reported that the ABC phenomenon ceased when the PEG-coated liposomes contained a cytotoxic drug at animal experimental and human clinical levels. Therefore, the importance of the ABC phenomenon becomes greater when drug carriers are applied to pertinent drugs that are much less cytotoxic than typical anticancer drugs. The application to the less cytotoxic drug is a new and important direction of DDS.

3.3 Clinical trials

There are six widely known clinical trials regarding polymeric micelle drug carrier systems. Table 2 summarizes these trials. The objectives for the use of the polymeric micelles are different among these six examples: tumor targeting is the objective for four examples (a – d) [96,97], solubilization of water-insoluble drugs is the objective for one other example (e), and circumvention of multi-drug resistance (MDR) is the objective for the remaining example (f).

Kabanov *et al.* designed and formulated SP-1049C for MDR circumvention on the basis of this research team's basic studies ([8,9,19,69-71]; see Section 3.1). In this system, an anticancer drug, doxorubicin, was physically encapsulated in the hydrophobic inner core of the Pluronic micelles. After being intravenously injected, however, doxorubicin is very rapidly released from the micelle. Owing to this rapid release, concentrations in blood and pharmacokinetic behaviors of the SP-1049C's doxorubicin were almost the same 5 min post-injection as those of the free doxorubicin injection case. I consider that this rapid release stemmed from two factors: the Pluronic micelle inner cores were of relatively low hydrophobicity and of the fluidic state. In the SP-1049C system, the anticancer drug and the biologically active block copolymer are delivered separately to solid tumor cells, and then the MDR circumvention effect will take place there.

Genexol-PM incorporates paclitaxel in a micelle formed from the poly(ethylene glycol)-*b*-poly(DL-lactide) block copolymer [20-23,42]. This micelle possesses a glassy inner core composed of the poly(DL-lactide) block, but drug release in blood occurred very rapidly after intravenous injection, in almost the same manner as that of SP-1049C. Consequently, the pharmacokinetic behavior of the incorporated drug paclitaxel is almost the same as that of the conventional formulation (Taxol). However, Genexol-PM can be superior to Taxol in terms of safe solubilization of water-insoluble drugs. The conventional formulation, Taxol, contains a large amount of surfactant called Cremophor EL. This surfactant has the function of making paclitaxel soluble in water, but accompanying this function is a set of substantial toxic side effects resulting from this surfactant's toxicities. The poly(ethylene glycol)-*b*-poly(DL-lactide) block copolymer is practically a non-toxic substance and successfully plays a role in the solubilization. Therefore, the solubilization is of great value to cancer chemotherapy. As a large incorporation capacity for hydrophobic drugs is a strong advantage of polymeric micelle

carriers, more and more drugs may be applied to the polymeric micelle systems for the drug solubilization purposes.

In clinical trials, Japanese, US and British teams examined the remaining four polymeric micelles in relation to solid-tumor targeting. Chemical structures of the inner-core-forming polymer blocks vary depending on the incorporated drug, whereas the poly(ethylene glycol) chain is used for the outer shell in all cases. Tumor targeting is the primary objective of these carrier systems. However, these systems possess another objective: the system has a function to solubilize a water-insoluble drug in the same manner as does Genexol-PM. Matsumura and co-workers reported that NK-105 incorporating paclitaxel showed highly tumor-selective delivery in murine tumor models [14,15,18,40,41]. In clinical stages, NK-105 can have two solubilization-related advantages over the conventional paclitaxel formulation, Taxol. The first advantage attributable to NK-105 is its relatively low toxic side effects, which reflect the fact that the block copolymer is much less toxic than Cremophor EL used in Taxol. The second advantage attributable to NK-105 is that it does not need the premedication that Taxol requires for reducing its own side effects.

We must wait for the final results of Phase II and III trials to answer the question 'Are the polymeric micelle systems valuable in cancer chemotherapy?' While waiting for the answer, we can take comfort in the fact that Phase I clinical studies have already yielded important information concerning toxic side effects. Even for targeted drugs, serious side effects arise because doses are escalated until dose-limiting toxicities become observable. The important obtained information revealed the toxicity profiles of the polymeric micelle drugs, which turned out to be the same as those of the corresponding free drugs. Most of the toxic side effects of the polymeric micelle drugs appear to result from the carriers' release of the drug in the bloodstream. The absence of uncommon and unexpected types of toxicity is a very meaningful fact that can contribute to the safety of clinical use. Not enough clinical results have been obtained to draw a general conclusion that synthetic block copolymers can be safely used in clinical stages, at least for cancer chemotherapy. However, basic study researchers and clinicians must develop their studies while keeping in mind this potential clinical advantage of a drug carrier.

4. Conclusion

In drug targeting with polymeric micelle drug carrier systems, research has reported less information concerning the toxicities of this carrier system than concerning either the systems' therapeutic activities or the systems' pharmacokinetic behaviors. However, even in this limited information, valuable characteristics of the polymeric micelle (no incidence of unexpected or serious toxicities such as hand-and-foot syndrome and toxicities of Cremophor EL) can be known, and these characteristics must be used for further development of

Table 2. Polymeric micelle anticancer drug carrier systems in clinical trials.

Code	Trade name	Primary objective	Incorporated drug	Progress	Company	Refs
a	NK-911	Targeting	Doxorubicin	Phase II	Nippon Kayaku Co., Japan	[14,15,17]
b	NK-105	Targeting	Paclitaxel	Phase II	Nippon Kayaku Co., Japan	[14,15,18,40,41]
c	NC-6004	Targeting	Cisplatin	Phase I	Nanocarrier Co., Japan	[14,15,96,97]
d	NK-012	Targeting	SN-38	Phase I	Nippon Kayaku Co., Japan	[14,15,44-49]
e	Genexol-PM	Solubilization	Paclitaxel	Phase II	Samyang Corp., Korea	[20-23,42]
f	SP-1049C	Anti-MDR effect	Doxorubicin	Phase II	Supratek Pharma, Inc., Canada	[8,9,19,68-70]

the carrier system. Further, some micelle-forming block copolymers show unique pharmacological activities, which perhaps can serve as a basis for new and innovative chemotherapeutic strategies.

5. Expert opinion

In this section, important points are summarized.

- (1) Purpose of carrier use. Most pertinent studies state that the use of polymeric micelle carriers serves a drug-targeting purpose, although a considerable proportion of clinical trials are aimed at the other purposes describe below. The drug targeting with polymeric micelle carriers is of high potential owing to the carriers' several physicochemical advantages, such as very small diameters. Two more purposes, solubilization of water-insoluble drugs and circumvention of multi-drug resistance, are unique and valuable regardless of whether or not they accompany the targeting purpose.
- (2) Pharmacological activities of block copolymers. Researchers must check whether or not micelle-forming block copolymers alone show any pharmacological activity in the basic study stage, although this type of activity-positive case is not so common according to previous reports. This activity can be evaluated by means of *in vitro* cell culture examinations because drug incorporation generally lowers *in vitro* drug activities for the activity-negative cases. If enhanced or considerably high activities

are found, researchers are encouraged to conduct not only further *in vitro* studies to elucidate mechanisms of the activity, but also *in vivo* examinations.

- (3) *In vivo* toxicities of the polymeric micelle drug carrier systems. *In vivo* toxicities and their profiles of the polymeric micelle drug carrier systems must be carefully observed in the basic study stage even if some toxicities are so mild that they do not qualify as dose-limiting toxicities. The information concerning the toxicities is valuable in developments for the clinical trial stage even though the information is such that the toxicity profile is the same as the profile of the corresponding free drug.
- (4) Some features of the polymeric micelle drug carrier systems in clinical trials. At present, clinical results of the polymeric micelle drug carrier systems are obtained in a very limited manner as compared with liposomal drug carrier systems. Even in this situation, the fact that neither unexpected nor very different profiles of toxicities have been observed is a very valuable one. This is a potentially great advantage of the polymeric micelle system, but many more clinical and basic examinations are required for satisfactory proof of this advantage.

Declaration of interest

M Yokoyama may receive financial benefits from patents NK-911, NK-105 and NC-6004 (summarized in Table 2) when these pharmaceuticals go to market after their approval.

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