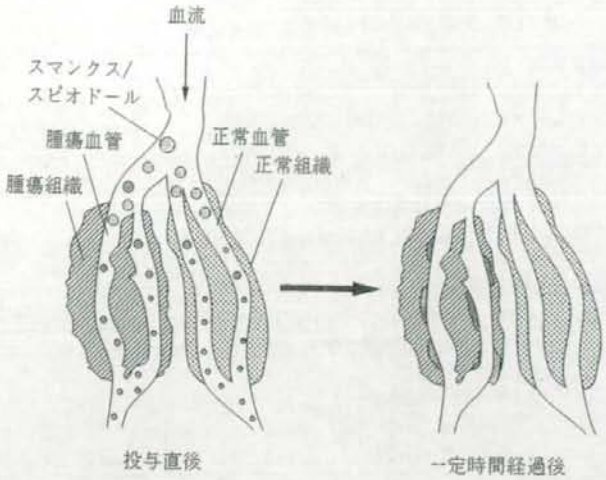


図 11.11　スマンクスのターゲティング

によっている。つまり，図 11.11 に示すように高分子の EPR 効果ではなく，油のがん組織への高い分配におもに基づいている。

〔3〕 **抗体-抗がん剤複合体**　モノクローナル抗体作成技術の発展に伴い，1980 年ごろから数多くの研究例，臨床例が報告された。現在，認可されたのは下記の Mylotarg® の一例である[21]。Mylotarg® は，2000 年に急性骨髄性白血病に対して米国での使用認可が得られている。

抗体はその機能と性質が明らかになるにつれ，アクティブターゲティングのキャリヤーとして理想的と考えられたのであるが，期待されたほどには成功していないのが現状である。この理由として第 1 に挙げられるのは，抗体自身の抗原性である。特に，初期に用いられた抗体はマウスで作成したものであるために，ヒトの体内ではマウスの抗体は異物，すなわち抗原となり，これに対する抗体が産生されて，マウス抗体のキャリヤーとしてのはたらきを中和してしまう。この点を改良するために，遺伝子工学の手法を用いてマウスとヒトのキメラ抗体や完全なヒト化抗体を用いることが 1990 年代後半から行われ，表 11.3 に示すように，抗体単独でのがん治療薬として，すでに五つが使用認可を受けている[22]。抗原性の問題を解決した抗体をキャリヤーとして用いた開発が今後期待される。

第 2 の問題点は，抗体自身の毒性である。抗体は天然のタンパク質だからといって副作用がないわけではなく，多くの場合 infusion-related reaction（発熱，寒気など）などの重篤な副作用がみられる。その意味で，現在唯一の認可例である Mylotarg® では，用いている抗がん剤の calicheamicin 誘導体は，通常の抗がん剤に比べて殺細胞活性が 100～1 000 倍程度ときわめて高いために，投与する抗体-抗がん剤複合体量がきわめて低く抑えられている（9 mg/m²。ちなみに，表 11.3 に抗体単独の抗がん剤として記されている Rituxan® は

表 11.3　許可されたがんに対する抗体医薬，ターゲティングシステム

抗体単独：5
- Rituxan®　マウスとヒトのキメラ抗体，non-Hodgikin lymphoma に対して
- ハーセプチン（Herceptin®）　乳がんに対して Taxol とともに用いる
- Campath®　ヒト化 IgG1κ，B-cell choronic lymphocytic leukemia に対して
- Avastin®　ヒト化抗体，大腸がん，直腸がんに対して 5-FU ベースの化学療法とともに用いる
- Erbitux®　キメラ抗体，大腸がんに対してイリノテカンとともに用いる

抗体-抗がん剤複合体：1
- Mylotarg®　ヒト化 IgG4　anti-CD33 抗体-NAc γ calicheamicin　dimethyl hydrazide 複合体 Acute Myeloblastic leukemia に対して

抗体-ラジオアイソトープ複合体：1
- Zevalin®　murine anti-CD20 monoclonal antibody-^{90}Y あるいは ^{111}I 複合体 non-Hodgikin lymphoma に対して Rituxan とともに用いる

375 mg/m² である。mg/m² は体表面積当りで表した投与量である）。

第 3 の問題点は，その組成にあると考えられる。抗体分子に薬物を結合していくと，その薬物導入量が増加するにつれて抗体のキャリヤーとしての性質は失われていく。抗原結合部位が薬物の結合で修飾されてしまうことによる抗原結合能の消失はもちろんであるが，薬物導入による物理化学的性質の変化も重要である。抗体の特異的結合能を活用するためには，血液中から効率よく標的組織に移行していかなければならないが，薬物によって疎水性などの物性が顕著となると，肝臓などの異物処理作用に捕捉されやすくなって，標的組織近傍へ到達する効率が著しく減少する事態が考えられる。この抗体-薬物複合体の物理化学的性質と体内でのデリバリー効率との相関を系統的に解析した研究は行われていないが，これまでに動物での最も高い抗がん活性を出した抗体-抗がん剤複合体[23]では，抗がん剤の結合量が低く抑えられている事実は，この相関の重要性を強く示唆する。

〔4〕 ポリ（2-ヒドロキシプロピル）メタクリルアミド用いた合成高分子システム

Kopecek（米国，Utah 大学）と Duncan（イギリス，Carfdiff 大学）は，ポリ(2-ヒドロキシプロピル）メタクリルアミド用いたシステムを開発した[24,25]。図 11.12 に示すように水溶性の高分子ポリ(2-ヒドロキシプロピル）メタクリルアミドを主鎖とし，四つアミノ酸より

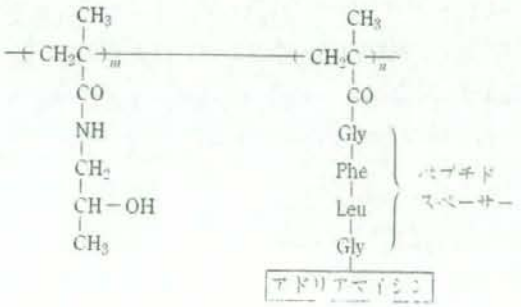


図 11.12　PHPMA-抗がん剤複合体の構造

なるオリゴペプチドスペーサーを介して薬物をつなげることにより，血液中では安定で標的細胞に取り込まれたのち，リソゾーム酵素によってこのオリゴペプチドスペーサーが開裂して薬物を放出するシステムを実現した。これは，11.1.3項で述べたde Duveのlysosomotropic agentとRingsdorfの高分子医薬のモデルを実現させたものである。

薬物として抗がん剤アドリアマイシンを用いたシステムで，イギリスにおいて臨床試験(Phase Ⅱ)を終了している。これは，EPR効果に基づいた固形がんへのパッシブターゲティングである。このPHPMAに抗がん剤のアドリアマイシンを結合させたシステムの設計上の特徴は，薬物導入量と分子量にある。アドリアマイシンの結合量を8 wt.%というかなり小さい値に抑えて，ポリマーによるEPR効果に基づいた固形がん組織へのターゲティング能をアドリマイシンの疎水性が妨害しないようにしている。また，この高分子は生体内非分解性であるため，その分子量を約3万に抑えて，腎臓からの排出を確保している[26),27)]。このPHPMAシステムの非生体内分解性である短所を改良すべく，各種生体内分解ポリマーを用いた研究が盛んに行われている[28)~30)]。

〔5〕 **リポソーム** 脂質二分子膜構造からなるリポソームも古典的な組成では，肝臓や脾臓といった細網内皮系に捕捉される割合が圧倒的で，ターゲティングの目的には向いていなかった。しかし，1980年代後半から，細網内皮系による捕捉を免れ得る，いわゆる「ステルスリポソーム」が開発されるに及び，状況は一変した。最初はGM_1という糖脂質をリポソームの成分に入れることでステルス性を獲得したが[31)]，より手軽に合成的に得られるポリエチレングリコールを結合した脂質にとって代わった[32)]。ポリエチレングリコール結合脂質を用いたステルスリポソームの模式図を**図11.13**に示す。ステルスリポソームの一つである商品名Doxil®は血液中を長時間にわたって安定に循環し，固形がん組織に集積する。すでにカポシ肉腫と卵巣がんへの使用が米国で認可されており，そのほかのがんへの適用を広げるべく検討が続いている[33)]。この例のように，血液中を安定に循環させるのに必要な用件は，脂質の固体性，ポリエチレングリコールなどによるステルス性発現のための表面修飾，

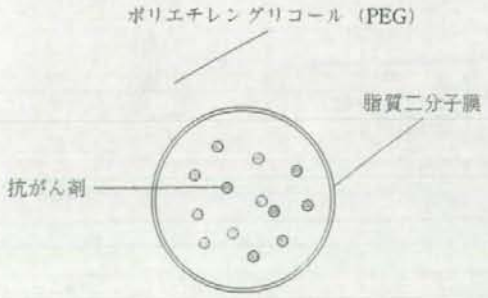


図11.13 Doxil®（PEG修飾リポソーム）の構造

適切な大きさ（約 200 nm 以下）である[32]。また，リポソームの内水相の pH を下げることで外側との pH 勾配を作って，アドリアマイシンを高濃度で高効率に封入することも大切な技術的支柱である。副作用として顕著に現れるのは，意外なことに手足での皮膚毒性であった。この皮膚毒性の発現メカニズムは解明されていないが，血液中の滞留性が高くなりすぎて，血液から組織への移行が遅い皮膚でも無視できない量の蓄積となった，というのが一つの仮説である。

以上の例にも示されたリポソームの大きさを，ターゲティングに好ましい 100 nm にそろえることを可能にしたのは，extrusion という均一な大きさの穴を有する膜を圧力で透過させる技術である。この技術により，直径 60 nm のポリエチレングリコールでコートしたリポソームも得られる[34]。

〔6〕 **高分子ミセル**　高分子ミセル型ドラッグキャリヤーシステムとは，**図 11.14** に示すように，A 鎖と B 鎖が直列につながったブロックコポリマーのような不均質な構造を有するものの，一成分のみに選択的に薬物を化学的結合あるいは物理的吸着により導入することにより，薬物を導入した部分の疎水性と，薬物を有しない部分の親水性からなる両親媒性により，高分子ミセル構造を形成させるものである[35)~39)]。高分子ミセル構造をドラッグキャリヤーとしての応用を初めて試みたのは Ringsdorf[40] で，薬物の徐放を目的とした研究がなされた。生体内のターゲティングに高分子ミセル構造を活用する研究は，1989 年ごろに日本の横山，片岡ら[41),42)] とロシアの Kabanov[43] らがそれぞれ独立に発表した論文から始まる。前者の研究はあとで紹介することとして，Kabanov らの研究は高分子ミセルをドラッグキャリヤーとして活用するよりは，ブロックコポリマーを高分子界面活性剤として血液から組織への薬物移行過程を改善するために役立てる側面が強く，のちにそれを目的とした興味ある研究結果を発表している[44)~46)]。

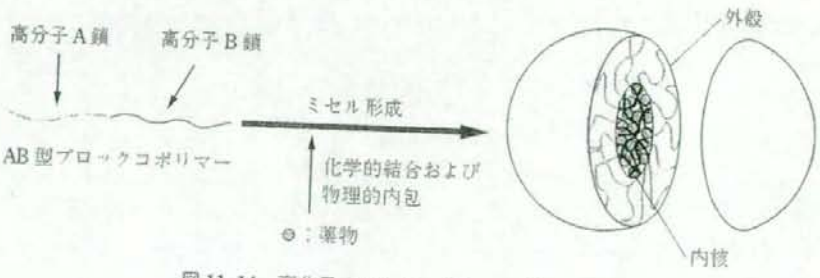


図 11.14　高分子ミセルターゲティングシステム

高分子ミセルドラッグキャリヤーシステムは，パッシブな固形がんへのターゲティングに必要とされる性質を本来的に有することが大きな特徴となっている。すなわち，200 nm 以下の微小粒径と細胞との相互作用の少なさである。この二つの性質は血液中を長時間循環す

るシステムに必要な用件である。高分子ミセルは通常，20～100 nm の直径を有するので，分離手段や特殊な技術を使用することなく，これらの用件を満たしている。また，高分子ミセルの外側部分（外殻）を形成する高分子鎖を，親水性で非荷電（あるいは弱く負荷電）のものに選択することで，細胞との相互作用を少なくしてEPR効果を実現できる。さらによいことに，高分子ミセルは抗がん剤を保持する内核と外殻が明確に分離した構造になっているために，抗がん剤を大量に内包させた場合でも，その抗がん剤の性質に左右されることなく，外殻構成高分子鎖の好ましい性質をターゲティングに十分活用することが可能である。

すでに，抗がん剤アドリアマイシンを内包した高分子ミセルを用いて，著しい *in vivo* 抗がん活性の増進を得て，がん組織への選択性と高い集積性が確認された[47)~49)]。これは，抗がん剤アドリアマイシンをポリエチレングリコール-ポリアスパラギン酸ブロックコポリマーのアスパラギン酸部分に化学的に結合させて，ポリエチレングリコール鎖を外殻とする高分子ミセルを形成させ，さらにその内核にアドリアマイシンを物理的に吸着させたものである。抗がん活性を示すのは物理的に吸着したアドリアマイシンで，**図 11.15** に示すように，この物理吸着アドリアマイシンを高分子ミセルに封入することで，薬物単独に比べ約9倍もの量がC 26 固形がんに集積していた。さらに特徴的なのは，時間が経過するに従って（24時間まで）分布量が増加していることで，この特徴はEPR効果による集積機構を支持する。

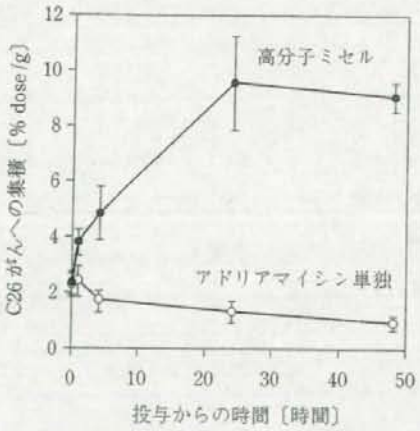


図 11.15 高分子ミセルによるC 26 固形がんへのターゲティング

このシステムは，パッシブターゲティングによる抗がん活性の著しい向上が得られたうえに，製剤的に製造・保存に適していることから，現在，臨床試験を目前にした開発が進行中である。これらは現在，国立がんセンターでの臨床第Ⅱ相試験中である。また，抗がん剤タキソールを内包した高分子ミセルシステムも同所で臨床第Ⅰ相試験中である。

この高分子ミセルのシステムはシスプラチン[50)]やほかの難水溶性抗がん剤[51),52)]にも容易に適用が可能なことから，汎用性の大きい抗がん剤ターゲティングシステムであるといえる。

11.2 安　定　化

11.2.1 安定化とは

ドラッグデリバリーシステム（DDS）は，通常，コントロールドリリース，吸収改善，ターゲティングの三つの領域からなり，「安定化」という概念や方法がDDSで議論されることは少ない。本節で，あえてこの「安定化」技術について述べるのは，以下の二つの理由による。

・後述するPEG修飾インターフェロンのように，「安定化」と呼ぶのが最も適切で理解しやすい認可例が出現し，医療で大きなインパクトをもちつつあるため。

・再生医療で必要とされるサイトカインや成長因子は，その分子量や生体内での挙動が上記のインターフェロンに似た部分が多く，生理活性を高く発現させるためには「安定化」の技術を使用する意義が高いと考えられるため。

ここで，本節で述べる「安定化」を定義する。「安定化」とは，「生理環境下での薬理活性をより長期間にわたって発揮するために，血液中や組織中での循環・滞留時間を延長したり，不活性化反応を抑制すること」とここでは定義する。11.2.2項において，安定化の方法論とその実例をまとめる。

11.2.2 安定化の方法論と実例

〔1〕 **血液循環性の増大**　血液中で，その薬効を示すタンパク質や生理活性物質の血液中の循環性を高めて（血液中の半減期を増大させて），より長時間薬理作用を得ようとするものである。代表的な例は，比較分子量の小さなタンパク質のポリエチレングリコール（PEG）による修飾である。血液中に投与されたタンパク質は，さまざまな代謝・排出作用を受けるが，その分子量が約30 000以下のタンパク質では，腎臓のろ過作用による排出が血液から消失するおもな経路となる。

各種インターロイキン，インターフェロン，増殖因子は分子量が約10 000～30 000程度の比較的小さなもので，血液からの消失が速い。この消失が速いことは，これらの生理活性タンパク質の非常に高い活性をその分泌部位に活性を局限し，作用時間を限定することで，生体内で有害な副作用が起きないことに寄与している。しかし，治療目的で用いる場合には，この短い血中循環性が欠点となる。

治療目的で用いるタンパク質の血中半減期が短い（タンパク質によってその半減期は数十分～数時間とさまざまではあるが）ことによる治療上不利な点は

・治療に有効なタンパク質の血液中濃度を維持する時間が短い。つまり有効な薬効を示す

時間が短い。

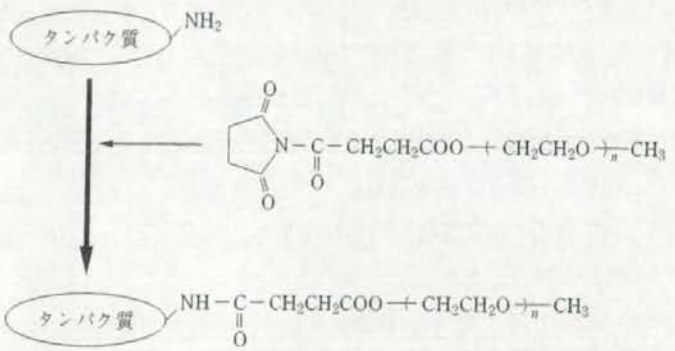
・有効な薬効を示す時間をなるべく長くすべく投与量を上げると，投与初期の高い濃度が毒性を示す濃度を超えて副作用を示す。

・有効な薬効を示す時間をなるべく長くすべく頻回投与を強いられる。例えば，C型肝炎治療ではインターフェロンは1日1回の投与を数週間続ける必要がある。患者にとって連日の投与は苦痛であり，入院あるいは毎日の通院は患者の社会生活に大きな負担を与える。

生理活性タンパク質の修飾に使われる，ポリエチレングリコール（PEG）の構造式を図11.16（a）に示す。PEGは非抗原性で，生体成分（細胞やタンパク質）との相互作用が小さな水溶性高分子として知られており，これによってタンパク質を修飾することで，血液循環性の増大，抗原性の減少などの効果を得る。

$$HO\text{-}(CH_2CH_2O)_n\text{-}H$$

（a）ポリエチレングリコール（PEG）の構造



（b）タンパクへの結合法の例

図11.16　ポリエチレングリコール（PEG）の構造とタンパクへの結合法の例

血中半減期の増大は，結合したPEGによって分子量が大きくなって腎臓からの排出が抑制されることによる。タンパク質を形成するポリペプチド鎖は球状に折り畳まれているのに対し，PEG鎖は血液中でも比較的伸びきった形状をしているため，見かけ上のタンパクの分子量を増加させる効果は大きい。例えば，分子量5 000のPEG鎖を2本結合させるとタンパク質の分子量は1万増加するが，血中半減期を増大させる効果はポリペプチド鎖に換算すると数万の分子量増加に匹敵する効果がある。

また，抗体や免疫関連のレセプターがタンパク質を認識・結合することをPEG鎖は阻害するので，タンパク質の抗原性（免疫原性）を減少させることができる。患者に望ましくな

い免疫応答を抑制することは，安全な治療のために重要な事柄である。また，この抗原性（免疫原性）の減少は，抗体による中和を通してタンパク質が血中より取り除かれることを抑制するので，結果的に血中半減期の増大につながる。

図 11.16（b）に示すように，片末端を活性化した PEG をタンパク質のアミノ基と反応させて（もう一方の末端は反応性のないメトキシ基となっている），アミド結合によって修飾をするのが最も一般的な方法である。

このような PEG によるタンパク質の化学修飾は，Abuchowski ら[53]の血清アルブミンを PEG で修飾する研究から始まり，すでに 1990 年代前半にアデノシンデアミナーゼ，アスパラギナーゼを修飾したものが使用認可されている。ただし，これら二つのタンパク薬剤は適用可能な症例が少ないために，薬物治療でのあまり大きなインパクトを与えるものではなかった。2001 年に α-インターフェロンを PEG 修飾したものが認可され，さらなる臨床試験が活発に進められている。この製剤は，患者数の多い C 型肝炎治療を対象としていることから，PEG 修飾タンパクがきわめて大きなインパクトをもちつつある。2005 年現在では，日本でも 2 種類の PEG 修飾インターフェロンが C 型肝炎に保険適用になっている。また，PEG によるタンパク修飾は，抗 TNF（tumor necrosis factor）抗体によるリウマチ治療などにも応用されつつある。

〔2〕 **不活性化の抑制**　薬物は，血液中などの生体内で酵素の作用や pH の影響でさまざまな不活性化反応を受ける場合がある。この不活性化を抑制して，薬物の薬効持続時間を長くすることが安定化の第 2 番目の方法論である。この方法の例としては，タンパク質の薬物と低分子の薬物の場合がある。

まず，タンパク質の例は 11.1.5 項で前述したスマンクスがある。スマンクスの活性部位は非ペプチドであるクロモフォアであるが，血液中ではこのクロモフォアが加水分解酵素のはたらきでペプチドから脱離して殺細胞活性を失う。この不活性化はたいへん速く，10～15 分で活性は 1/10 に低下する。SMA ポリマーを結合してスマンクスとすることで，活性が落ちるまでの時間を 5 倍程度延長することが可能となった[54),55)]。

低分子の薬物の場合には，薬物キャリヤーに結合・封入することで一般に安定化が得られる。薬物の不活性化反応を起こす酵素や低分子（OH^- や H^+ など）の薬物への接近をキャリヤーが阻害するからである。この阻害効果は，エマルション，リポソーム，高分子ミセルなど薬物を内部に封入するタイプのキャリヤーではもちろんのこと，水溶性高分子キャリヤーに結合する場合でも得られる。それは，水溶性高分子が酵素の接近を立体的に阻害する効果と，結合した疎水性薬物分子どうしが会合して[56]疎水的な環境を作り出して酵素や低分子の接近を阻害する効果とによる。図 11.17 に高分子ミセルの例を示す。この例の薬物アドリアマイシンは pH 7.4 の緩衝液中でも，OH^- の作用でその化学構造が分解し，その結果，

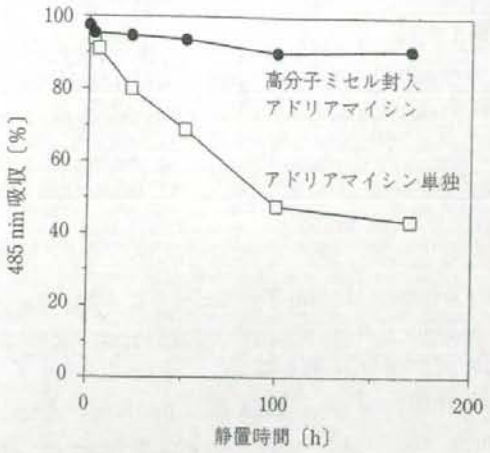


図 11.17 高分子ミセル封入による薬物の安定化

赤い色の減少（485 nm の吸光度の減少）がみられるが，高分子ミセル内部の疎水的な環境に内包することで，その吸光度の減少が著しく抑制された。

この薬物の安定化を一歩進めた形で，薬物の存在形態を制御する研究が報告されている。Kwon らは，抗真菌剤アンホテリシン B を疎水性の内核に封入することにより，通常の存在形態である四量体ではなく，より毒性の少ない単量体の形態を優先的にとらせることに成功した[57)~59)]。これによって，アンホテリシン B の毒性である溶血活性を抑えて，抗菌活性を得ることができた。

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CHAPTER 2

Polymeric Micelles as Nanosized Drug Carrier Systems

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CONTENTS

1. POLYMERIC MICELLES AS DRUG CARRIERS

1.1. What Are Polymeric Micelles for Drug Delivery?

A polymeric micelle is a macromolecular assembly composed of a spherical inner core and an outer shell. Typically, a polymeric micelle structure forms from block copolymers or graft copolymers [1]. In Figure 1 formation of a polymeric micelle structure is illustrated using an AB type block copolymer in which two polymer chains are tandem connected. Such a micellar structure forms if one segment of the block copolymer can provide enough interchain cohesive interactions in a selective solvent. Most studies of polymeric micelles both in basic and applied aspects have been done with 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 AB- or ABA-type block copolymers than with graft or multisegmented block copolymers.

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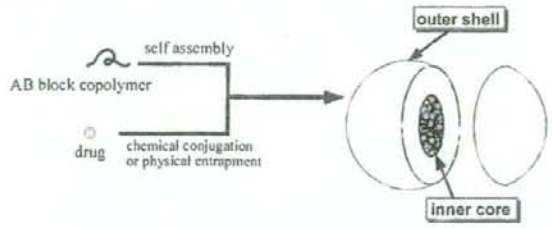

Figure 1. Polymeric micelles for drug carriers.

The cohesive interactions in the inner core utilized as the driving force of micelle formation include hydrophobic, electrostatic, π–π interactions, and hydrogen bonding. Because of frequent use of hydrophobic drug molecules as incorporated drugs, hydrophobic interactions are used most commonly for drug carrier systems. Actually, most reported examples of polymeric micelle drug carrier systems were done by this combination [2–11] with a few exceptions [11, 12]. The second interaction, electrostatic interaction, may be applied to macromolecules with electric charges at a high density. DNA and RNA are appropriate candidates for this because of their anionic phosphodiester bonds that exist per each repeating unit [13]. Proteins with a large number of charged groups such as aspartic acid and lysine residues are also included for this combination [14]. Hydrogen bonding and π–π interactions may work cooperatively with other cohesive interactions. For drugs possessing aromatic rings in their chemical structures, π–π interactions are considered to work cooperatively with hydrophobic interactions.

Drugs can be incorporated into polymeric micelles both by chemical conjugation and physical entrapment. Drugs can be incorporated into both the inner core and the outer shell; however, the inner core is considered an appropriate site for the drug incorporation due to the following two reasons. First, possible interactions between the incorporated drug molecules and the outer shell segment of the block copolymer may lead to intermicellar aggregation. Intermicellar aggregation should be avoided when the goal is to achieve polymeric micelle delivery, not the delivery of aggregates. The second reason concerns a shielding function of the outer shell for drug targeting. The minimization of hydrophobic interactions between drug carriers and biocomponents such as proteins and cells is an important key for targeting, especially for passive tumor targeting. By incorporating hydrophobic drugs in the inner core, hydrophobic interactions arising from the incorporated drugs can be effectively inhibited owing to the shielding effects of the hydrophilic outer shell.

1.2. Advantages of Polymeric Micelle Drug Carriers

Polymeric micelles possess strong and unique advantages as drug carriers. Table 1 summarizes these advantages. The first

Table 1. Advantages of polymeric micelles as drug carriers.

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

advantage is their small size. Polymeric micelles are formed typically in a diameter range from 10 to 100 nm with a substantial narrow size distribution. This size range is considered ideal for evasion of the reticuloendothelial system's uptake and renal excretion. As a result of this evasion, polymeric micelle drug carriers can attain stable and long-term circulation in the bloodstream. On the other hand, the small size of polymeric micelles is a big benefit in the sterilization processes associated with pharmaceutical production. Polymeric micelles are easily and inexpensively sterilized by filtration using typical sterilization filters with 0.45 or 0.22 μm pores.

The second advantage of the polymeric micelle drug carriers is high structural stability. It is known that polymeric micelles possess high structural stability provided by the entanglement of polymer chains in the inner core. This stability has two aspects: static [15, 16] and dynamic [17]. Static stability is described by a critical micelle concentration (cmc). Generally, polymeric micelles show very low cmc values in a range from1 mg/ml to 10 μg/ml. These values are much smaller than typical cmc values of micelles forming from low molecular weight surfactants. The second aspect, dynamic stability, is described by the low dissociation rates of micelles, and this aspect may be more important than the former for *in vivo* drug delivery in physiological environments that in nonequilibrium conditions. The high structural stability of polymeric micelles stated above is an important key to *in vivo* delivery in micellar forms and simultaneously eliminates substantial contribution of single polymer chains to drug delivery.

The third advantage is the high water solubility of the polymeric micelle drug carrier system when this drug carrier incorporates hydrophobic drugs. Generally, in conventional polymeric drug carrier systems, a loss of the water solubility of the polymeric carrier resulting from the introduction of a hydrophobic drug creates a serious problem. The conjugation of drugs to a homopolymer easily leads to precipitation because of the high, localized concentration of hydrophobic drug molecules bound along the polymer chain. Several research groups reported this problem of the drug–polymer conjugates in syntheses [18, 19] and in their intravenous injections [20]. Therefore, conventional drug–polymer conjugates must be designed with a considerably low drug content so that the risk of precipitation is avoided or lessened. As one example, the maximum weight contents of an anticancer drug such as adriamycin or daunomycin (an adriamycin derivative) was reported to range from 10 to 35 wt % (18, 21, 22). On the other

hand, Kopecek and Duncan et al. controlled the adriamycin weight content at a substantially lower value (7 wt %) in their poly(2-hydroxypropyl)methacrylamide carrier system [23, 24] than those reported contents (10 to 35 wt %). This may be due to their strategic design to minimize unfavorable hydrophobic interactions of the conjugated drug molecules with proteins and cells in living bodies as well as to evade the precipitation problem.

Alternatively, polymeric micelles can maintain their water solubility by inhibiting the intermicellar aggregation of the hydrophobic cores that possess a hydrophilic outer shell layer. In fact, it was reported that micellar systems exhibited a much larger hydrophobic drug content than did the conventional polymeric carrier systems. A polymeric micelle system was reported to contain 60 wt % adriamycin [25] that was much larger than those of the conventional polymeric systems [18, 21, 22]. Furthermore, it was reported that polymeric micelles incorporated large amounts of water-insoluble drugs such as KRN-5500 [26, 27, 28], taxol [29, 30], and camptothecin [31–33]. In the field of cancer chemotherapy, a strong demand for the solubilization of water-insoluble drugs has been voiced, particularly because many newly developed and very potent anticancer drugs are water-insoluble or barely water-soluble.

Low toxicity as drug carriers may be described as the fourth advantage. Generally, polymeric surfactants are known to be less toxic than low molecular weight surfactants such as sodium dodecyl sulfate. In fact, some pluronic block copolymers (poly(propylene oxide)–poly(ethylene oxide)–poly(propylene oxide)) have been approved for intravenous injection. Clinical trials of poly(ethylene glycol)-*block*-poly(aspartic acid) block copolymer-based systems are now in progress for delivery of adriamycin and taxol, and their results will be reported in the near future.

Furthermore, polymeric micelles are considered very safe in relation to chronic toxicity. Possessing a much larger size than the filtration systems at the kidney, polymeric micelles can evade renal filtration, even if the molecular weight of the constituting block copolymer is lower than the critical molecular weight for renal filtration. On the other hand, polymeric micelles are formed by intermolecular noncovalent interactions and, therefore, all polymer chains can be released (as single polymer chains) from the micelles during a long timeperiod. This phenomenon may result in the complete excretion of polymeric micelles from the renal route if the polymer chains are designed with a lower molecular weight than the critical value for the renal filtration. Such a result constitutes an advantage of polymeric micelles over the conventional (non-micelle forming) and nonbiodegradable polymeric drug carrier systems.

The fifth advantage is separated functionality. Polymeric micelles are composed of two phases: the inner core and the outer shell. Various functions required for targeting systems can be shared by these structurally separated phases. Each phase can play different roles in drug delivery. As shown in Figure 2, the outer shell is responsible for interactions with

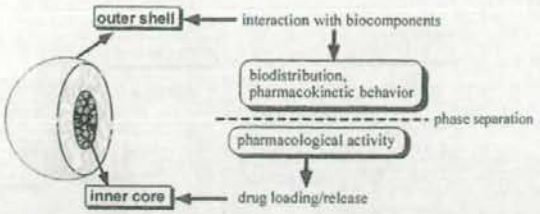

Figure 2. Separated functionality of polymeric micelles as drug carriers.

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 constructing of highly functionalized carrier systems than in the conventional (non-micelle-forming) polymeric carrier systemsbecause 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.

2. STRATEGIES FOR TUMOR TARGETING USING POLYMERIC MICELLES

2.1. Passive Targeting

2.1.1. Classification of Targeting Methodology

Drug targeting may be classified into two general methodologies: active and passive targeting [34–36]. Active targeting aims to increase delivery of drugs to the target by utilizing specific interactions at the target sites where drug pharmacological activities are required. These interactions include antigen–antibody and ligand–receptor binding. Alternatively, physical signals such as magnetic field and temperature that are externally applied to the target sites are utilized for the active targeting. Examples of carriers in this methodology are antibodies, transferrin, ferrite-containing liposomes, and thermoresponsive polymeric micelles that are described in the next section .

On the other hand, passive targeting is defined as a methodology to increase the target/nontarget ratio of delivered drugs by adjusting physical and chemical properties of carriers to physiological and histological characteristics of the target and non-target tissues, organs, and cells. Carriers in this methodology include synthetic polymers and some natural polymers such as albumin, liposomes, micro(nano)particles, and polymeric micelles. Influential characteristics for the passive targeting are chemical factors such as hydrophilicity/hydrophobicity and positive/negative charge and physical factors such as size and mass. Passive targeting can be achieved by minimizing nonspecific interactions and delivery with/to nontarget organs, tissues, and cells as well as by maximizing

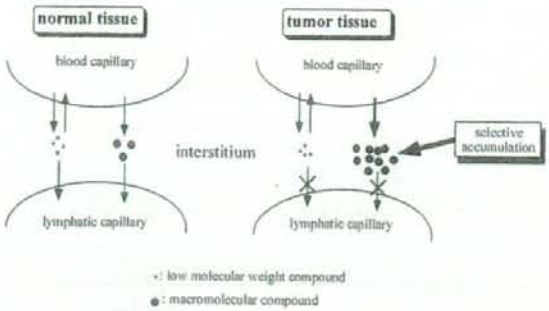

Figure 3. Passive targeting of macromolecules to solid tumors by enhanced permeability and retention effect (EPR effect).

delivery to the target.

2.1.2. EPR Effect

A fundamental concept for drug targeting to solid tumors by a passive targeting mechanism was presented by Maeda and Matsumura in 1986 [37, 38]. This is the enhanced permeability and retention effect (EPR effect) of macromolecules at solid tumor tissues. As illustrated in Figure 3, vascular permeability of tumor tissues is enhanced by the action of secreted biological factors such as kinin. As a result of this increased vascular permeability, macromolecules significantly increase their transport from blood vessels to the interstitium of the tumor tissues. Different from low molecular weight compounds that are very diffusible out of the tumor interstitium into blood circulation again, macromolecules cannot go back easily to the bloodstream due to their low diffusion coefficients. Furthermore, the lymphatic drainage system does not operate effectively in the tumor tissues. Therefore, enhanced accumulation at the tumor interstitium is prominent only for macromolecules. Maeda and Matsumura showed the EPR effect first with Evans blue-modified albumin [37]. Then, it was reported that the EPR effect was also applicable to synthetic polymers [24, 39], liposomes [40, 41], and polymeric micelles [42, 43].

In the theory of the EPR effect, specific targeting moieties such as antibodies are not necessary for highly selective delivery to tumors. Furthermore, any synthetic and natural macromolecule can selectively accumulate at solid tumor sites. However, carrier polymers must fulfill the following two requirements to avoid nonspecific capture at nontumor sites: (1) to possess appropriate size or molecular weight, i.e., the diameter of carriers must be smaller than about 200 nm to evade the reticuloendothelial system's uptake [44, 45]. Additionally, molecular weights larger than a critical value (about 40,000) are favorable to evade renal filtration, and (2) not to possess a character of strong interactions with or uptake by normal organs (especially the reticuloendothelial systems). This character is typically seen for cationic [46] and hydrophobic polymers [47]. Therefore, carrier polymers must be hydrophilic and neutral or weakly negative in their charge without other biologically recognizable chemical structures by normal tissues. These requirements are effectively achieved in polymeric micelle carrier systems as described in section 1.2.

2.2. Thermoresponsive Polymeric Micelles

For the passive targeting of polymeric micelles to solid tumors, selective cytotoxic activity of the drug to tumor cells was obtained by a slow drug release rate that matched well to a time period required for delivery to the solid tumor sites through the bloodstream. [43] Thus controlled drug release rate can avoid a considerable amount of drug release during circulation in the bloodstream. This drug release, however, was not site specific at the solid tumor sites. If an anticancer drug is released selectively at solid tumor sites from the polymeric micelle carriers, the therapeutic index must be further raised by decreasing the unfavorable drug release in the bloodstream as well as by increasing drug release rates at the tumors. From this perspective, Yokoyama and Okano et al. [48–53] designed thermoresponsive polymeric micelles whose drug release was enhanced at an elevated temperature. After the polymeric micelles are delivered to tumor sites, local hyperthermia is applied. At the elevated temperature, anticancer drug encapsulated in the polymeric micelles is released by this temperature stimuli to express cytotoxic action to tumor cells. As shown in Figure 4, a thermoresponsive polymeric micelle is formed from block copolymers composed of a thermoresponsive block and a hydrophobic block. The thermoresponsive block forms the outer shell, while the hydrophobic block forms the inner core that incorporates drugs. A thermoresponsive polymer poly(*N*-isopropylacrylamide) chain is used as the block for the outer shell. Poly(*N*-isopropylacrylamide) (PIPAAm) is known to exhibit phase transition at 32 °C and has been utilized for controlled release and intelligent materials. The phase transition temperature can be adjusted to an appropriate temperature (around 39 °C) for this multitargeting system by the introduction of hydrophilic comonomers such as *N,N*-dimethylacrylamide. The inner core of the polymeric micelle is made of hydrophobic polymer blocks. Poly(butyl methacrylate) is used [48, 49] for the inner core hydrophobic block because this polymer is considered to possess both hydrophobicity enough to incorporate hydrophobic drug and chain flexibility enough to exhibit substantial structural change when the outer thermoresponsive block shows phase transition. Figure 5 shows drug release behaviors of the polymeric micelles forming from poly(*N*-isopropylacrylamide)-

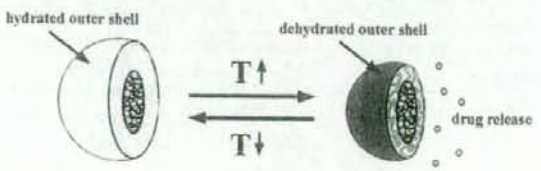

Figure 4. Thermoresponsive polymeric micelles.

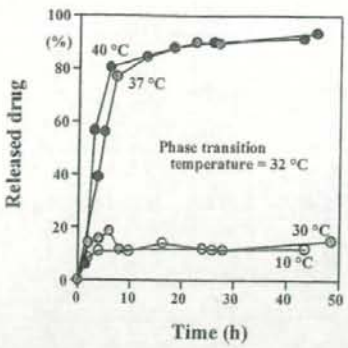

Figure 5. Thermoresponsive drug release from polymeric micelle possessing LCST at 32 °C. Reprinted with permission from [51]. F. Kohori et al., *Colloids Surf., B: Biointerfaces* 16, 195 (1999) Copyright © 2005, Elsevier.

block-poly(butyl methacrylate) block copolymers. The encapsulated drug (adrimycin) in the micelle inner core was observed to be released only at the elevated temperature above the phase transition temperature of the poly(*N*-isopropylacrylamide) chain even though an initial burst drug release accompanied it. Alternatively, biodegradable poly(D,L-lactide) was used [50, 52] as the hydrophobic block for the inner core.

When the temperature is raised above the transition temperature of the outer shell block, the outer shell is dehydrated. It is thought that drug is released upon this dehydration of the outer shell due to the phase mixing between the inner core and the outer shell or due to penetration of water into the inner core that is induced by mechanical distortion at the interface between the inner core and the outer shell. Detailed mechanical consideration was made in a previous review [54].

Nakayama et al. further developed this type of thermoresponsive polymeric micelles using well-controlled block lengths that were obtained by a living radical polymerization [55].

Feijen et al. studied another type of thermoresponsive polymeric micelle carrier system using poly(ethylene glycol)–poly(*N*-isopropylacrylamide) block copolymers [56, 57]. In this type, copolymers are composed of a hydrophilic block such as poly(ethylene glycol) and a thermoresponsive block. Upon a raise in temperature above the LCST, the thermoresponsive block undergoes the phase transition and become water insoluble to associate with each other polymer chain. As a result of this association, the block copolymers form a micellar structure with a hydrophobic inner core. At a lower temperature than the LCST, drug is released upon dissociation of the micellar structure due to hydration of the inner core. An extension of this study was carried out by Hennink et al. They synthesized poly(ethylene glycol)-*block*-poly(*N*-isopropylacrylamide-*co*-[poly(lactic acid) grafted-*N*-(2-hydroxypropyl) methacrylate] block copolymer. This block copolymer possessed the LCST below 32 °C due to an effect of the hydrophobic poly(lactic acid) chain and, therefore, formed polymeric micelles at 37 °C. After hydrolysis of the poly(lactic acid) chain, the resulting block copolymer (poly(ethylene glycol)-*b*-poly(*N*-isopropylacrylamide-*co*-*N*-(2-hydroxypropyl) methacrylate) could not form the micelle structures at 37 °C because the LCST was raised above 37 °C due to the contribution of the hydrophilic *N*-(2-hydroxypropyl) methacrylate units.

As stated in this section, drug release control by temperature stimuli from the polymeric micelle carriers was achieved *in vitro*. The *in vivo* drug release control is the next challenging subject. For a success of this *in vivo* control, block copolymer structures must be designed to exhibit the thermoresponsive property for the drug release control with maintaining targeting ability to solid tumor sites as described in section 2.1.2.

3. EXAMPLES OF DRUG TARGETING WITH POLYMERIC MICELLES

3.1. Adriamycin (Doxorubicin)

Adriamycin is the most widely used drug in the drug targeting research, and it is the first drug with which drug targeting to solid tumors using polymeric micelles was proved. Yokoyama and colleagues succeeded in tumor targeting of anticancer drug adriamycin (ADR) (= doxorubicin) using a polymeric micelle system [58, 59]. The molecular design of their system is shown in Figure 6. Adriamycin (ADR) was chemically conjugated to aspartic acid residues of poly(ethylene glycol)–poly(aspartic acid) block copolymers (PEG-P(Asp)) by amide bond formation. The poly(ethylene glycol) (PEG) segment was hydrophilic, whereas the ADR-substituted poly(aspartic acid) chain was hydrophobic. Therefore, the obtained drug–block copolymer conjugate (PEG–P(Asp(ADR))) formed micellar structures due to its amphiphilic character. In the second step, ADR was incorporated into the inner core by physical entrapment utilizing hydrophobic and π–π interactions

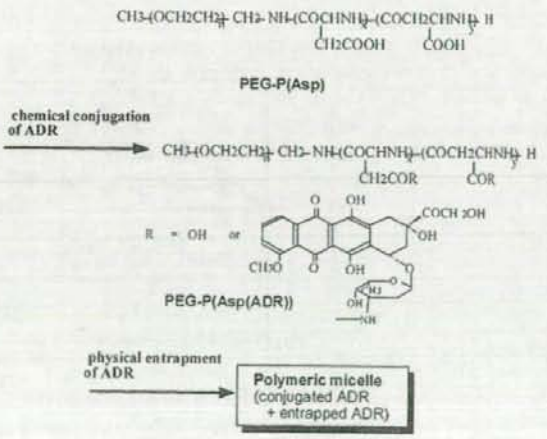

Figure 6. Chemical structure of ADR-incorporated polymeric micelle.

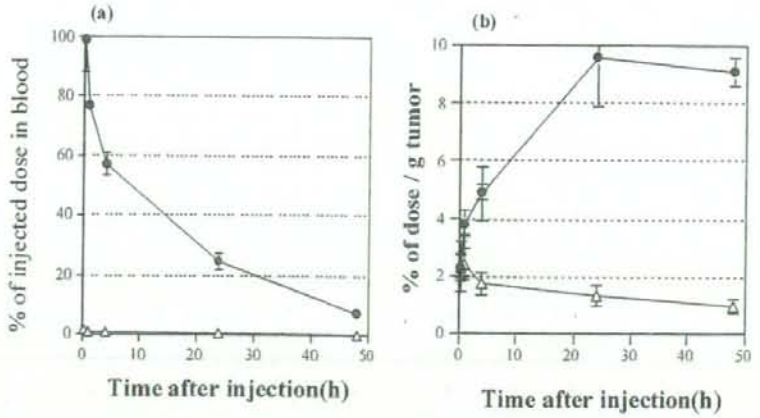

Figure 7. Concentrations in blood (a) and C26 tumor (b) after intravenous injection. ADR (△) and polymeric micelles (●).

with the chemically conjugated ADR molecules. As a result, polymeric micelles containing both the chemically conjugated and the physically entrapped ADR in the inner core were obtained with the PEG outer shell. In this system, strong intermolecular interactions of ariamycin molecules were effectively utilized for stable drug incorporation as well as for stable micelle formation.

As shown in Figure 7, the physically entrapped ADR circulated in the bloodstream for a long time period (Fig. 7a) and was delivered to the solid tumor site at much higher concentrations than free ADR (Fig. 7b) [31]. Furthermore, the observed time profile with a peak concentration at 24 h postintravenous injection, as well as retention of this high concentration in longer time period postinjection, matched well to passive delivery by the EPR effect [37, 38]. On the other hand, accumulation of the physically entrapped ADR in the polymeric micelles in normal organs and tissues was at the same or lower levels than free ADR.

In accordance with this highly selective delivery to solid tumor sites, a dramatic enhancement of antitumor activity was observed [31]. Figure 8 shows *in vivo* antitumor activity against murine colon adenocarcinoma 26. For free ADR, only the maximum tolerated dose (10 mg/kg of body weight) provided considerable inhibition effects on tumor growth; however, a decrease in tumor volume was never seen from the day of the first injection. For the polymeric micelles, the tumor completely disappeared in two doses (20 and 10 mg of physically entrapped ADR/kg of body weight). All these results clearly demonstrate the successful passive targeting of an anticancer drug using the polymeric micelle carrier system to solid tumors.

3.2. KRN-5500

Although adriamycin shows some hydrophobic properties, it is still water soluble. In the field of cancer chemotherapy, a

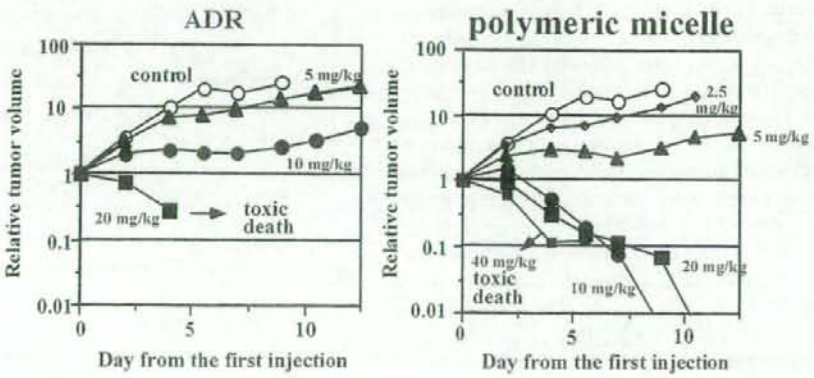

Figure 8. *In vivo* antitumor activity of free adriamycin (ADR) and polymeric micelle containing ADR.

strong demand for the solubilization of water-insoluble drugs has been voiced, particularly because many newly developed and very potent anticancer drugs such as camptothecin and taxol are water insoluble or barely water-soluble. KRN-5500 (KRN) was the first drug incorporated into polymeric micelles among water insoluble drugs [62–64]. By an appropriate choice of a block copolymer, the water insoluble KRN was successfully incorporated into polymeric micelles [62]. This polymeric micelle showed higher antitumor activity than KRN injected into a conventional formulation. This polymeric micelle did not exhibit the severe vascular nor pulmonary toxic side effects that surfaced in the KRN in the conventional formulation that shows toxicities of the organic solvents and the surfactants used to dissolve KRN [63, 64]. This fact indicates that polymeric micelle carrier systems show not only a strong benefit of drug targeting, but also very high potency in dissolving water-insoluble drugs for safe intravenous injection.

3.3. Cisplatin

Cisplatin is a platinum chelate complex with two chloride and two ammonium ligands. This compound is water soluble, but not highly water-soluble. Nishiyama and colleagues introduced the cisplatin into the aspartic acid residues of poly(ethylene glycol)–poly(aspartic acid) block copolymers by a ligand exchange reaction between chloride and carboxylate ions [65, 66]. Polymeric micelles were formed by cross-linking between the poly(aspartic acid) blocks through platinum atoms. Later, they improved targeting efficiency using poly(ethylene glycol)–poly(glutamic acid) block copolymers on behalf of poly(ethylene glycol)–poly(aspartic acid) block copolymers [67]. In physiological saline, cisplatin could be released from the micelles by a ligand exchange reaction between chloride and carboxylate ions and, simultaneously, micelle structures would be broken. This cisplatin delivery system is currently in a clinical stage.

3.4. Taxol

Taxol is a water-insoluble anticancer drug recognized as one of the most potent anticancer drugs among those approved in the 1990s. For intravenous injection, an organic surfactant cremophor EL and ethanol are used to solubilize taxol. Because cremophor EL is considerably toxic, injection without the use of cremophor EL is wanted. Samyoung Company developed PEG-PLA based polymeric micelles containing taxol. By this incorporation, water solubility was significantly increased as compared with the conventional cremophor EL formulation, and toxicity of cremophor EL was successfully diminished. This system is now in a phase I clinical trial, however, it does not show targeting effects because all of the incorporated drug is released immediately after the intravenous injection. Alternatively, Hamaguchi et al. [29] studied a taxol-containing polymeric micelle system that possessed targeting ability to solid tumors by optimizing block copolymer structures. The phase I clinical trial of this targeting system is currently (2006) ongoing.

3.5. Amphotericin B

Amphotericin B is a potent antibiotic widely used in the treatment of systemic fungal infections. This drug is barely water-soluble and, therefore, its intravenous injection is not possible. Kwon and colleagues successfully incorporated amphotericin B into polymeric micelles formed from poly(ethylene glycol)–poly(*N*-hexylaspartamide)-based block copolymers [68–70]. They obtained enhancement of watersolubility and decreased toxic side effects. It is known that amphotericin B exists in an intact (monomer) form and an aggregated form in physiological conditions, and that the aggregated form is mainly responsible for the toxic side effects. They speculated that the sustained release of amphotericin B and a larger proportion of monomers, which form due to encapsulation in the hydrophobic environment, were the reasons for this decreased toxicity. If the latter reason is true, this is the first case in which polymeric micelle carriers raised drug efficacy by changing the drug's existing forms due to the hydrophobic environment for drug storage.

3.6. Methotrexate

Methotrexate (MTX) is a widely used anticancer drug, and recently it has also been used to treat rheumatoid arthritis. Kwon and associates conjugated MTX to a block copolymer and obtained polymeric micelles, with the hydrophobicity of the conjugated MTX serving as the driving force behind the micelle formation [71]. By controlling the amount of the conjugated MTX, micelle stability was successfully controlled. Micelle stability (that is, the equilibrium between the micelle form and the single polymer chain) is correlated with the drug release rate because hydrolysis between the polymer and MTX can proceed more rapidly in a single polymer chain form due to both its easier access to water and other small molecules responsible for hydrolysis. This is the first example of a release control of chemically conjugated drugs from the polymeric micelle systems.

3.7. Camptothecin

Camptothecin is an agent showing high anticancer activity in mice models, and its clinical application was examined. This agent was not approved as an anticancer drug; however, it is a mother compound of topotecan and CPT-11, which are approved as anticancer drugs. Due to a unique action mechanism (topoisomerase I inhibition) of the camptothecin (and its analogues) and to high effectiveness of topotecan and CPT-11 in clinical results, drug targeting of camptothcin is an interesting subject. Camptothecin is a water-insoluble compound, therefore its incorporation into polymeric micelles is meaningful for solubilization of this drug as well as targeting. The