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Pharmaceutical Development of (Investigational) Anticancer Agents for Parenteral Use—A Review

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ABSTRACT

Due to the high mortality rate, the therapy of cancer has been and still is under vigorous investigation. Traditional approaches to new drug development have led to the discovery of several chemotherapeutic agents, which are now routinely used in the clinic. The objective of this review is to evaluate the current status of the pharmaceutical formulation of antineoplastic drugs from 1986 onwards. Among the aspects of the pharmaceutical development of antineoplastic drugs discussed are the following: formulation development process, solubilization techniques, the application of several colloidal systems, the use of prodrugs, and lyophilized (freeze-dried) formulations. Formulation procedures, the advantages and disadvantages of the different strategies, and the toxicity risk are described and illustrated with examples from the literature.

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INTRODUCTION

Cancer is the second leading cause of death in Europe and North America. Due to the high mortality rate, the therapy of cancer has been and still is under vigorous investigation. However, advances in the treatment of (disseminated) malignant diseases have been limited by failure to identify unique biochemical and/or biological properties which are able to clearly distinguish cancer cells from normal cell population (1). The limitations of the current drug development program are highlighted by the fact that of more than 800,000 compounds screened so far, only 40 agents have been licensed and are now used routinely in the clinic (2). Traditional approaches to new drug development have led to the discovery of alkylating agents and other compounds that interfere with the functioning of DNA. It is now clear that other, mechanism-based approaches defined by tumor biochemistry and biology must be followed as it is unlikely that the classic empirical strategies will lead to further dramatic improvements in cancer therapy (3). Computer-aided drug design is a new approach to obtain more insight into the molecular interaction between an anticancer drug and its target, with the aim of designing drugs with an increased selectivity for a particular oncogene or other protein targets. Today, the majority of therapeutic drugs used for cancer treatment are only moderately cytotoxic and drug resistance often arises. It is, therefore, of the utmost importance that the research for new anticancer drugs continues with combined forces.

The development of a new anticancer agent starts with the acquisition of the chemical entity. Subsequently, the new drug substance (NDS) will be developed according to the scheme in Table 1 (4).

Table 1

The Main Steps in Anticancer Drug Development

1.	Acquisition
2.	Screening and pharmacology
3.	Synthesis on a larger scale
4.	Pharmaceutical formulation
5.	Animal toxicology
6.	Preclinical pharmacokinetics
7.	Phase I studies
8.	Early phase II studies
9.	Phase III studies

The main sources of compounds for drug development originate from natural sources (2,5) or from new synthetic routes (6), or are analogues of known agents (7,8). The chemical drug entity is then screened for cytotoxic activity in vivo in L1210 or P388 leukemia and in several murine and human xenograft tumors, or in vitro in several tumor lines derived from solid-tumor malignancies (2). Once the screening process has been successfully completed, the compounds selected based on established criteria must be produced in sufficient quantities and should be properly pharmaceutically characterized and formulated before animal toxicology and clinical phase I and II studies begin.

It is rare for a drug substance to be administered as the pure chemical compound itself. Drugs are almost always administered in some kind of formulation. *Formulation* can be defined as the process in which raw material of a compound is transformed into a pharmaceutical product which is suitable for the intended use in humans. On the whole, antineoplastic agents possess poor aqueous solubility and stability characteristics. The major obstacle in anticancer drug development has often been the difficulty of finding a stable formulation, which is well tolerated without formulation-related unacceptable, irreversible toxicities. For some compounds, for example, combretastatin A4 and pancratistatin, a suitable formulation has not been developed to date. There are, however, a few compounds which are formulated in simple aqueous solutions such as 0.9% sodium chloride infusion fluid or a buffer solution. For example, the investigational platinum analogues tertraplatin and CHIP (*cis*-dichloro, *trans*-dihydroxybis-isopropylamine platinum IV) are available as 2-mg/ml solutions in normal saline (9). Furthermore, a phase I study was recently completed with the topoisomerase I inhibitor topotecan (SK&F 104,864), where the drug was formulated as a 5-mg/ml base solution in 0.1 M gluconate buffer at pH 3.0 (10). The pH of the formulation was acidic in order to prevent hydrolysis of the lactone ring of topotecan, forming the inactive carboxylate form. More often, however, formulations are complex mixtures containing the drug compound, or a derivative of the drug compound, together with excipients, diluents, stabilizers, or preservatives. This is the case for most antineoplastic agents, since they often lack adequate aqueous solubility and stability (6,11,12).

The development of dosage forms for anticancer agents is, in principle, not different from the procedures employed for other drugs. Since any component of the formulation may contribute to or modify the toxicity, it

should be the formulator's goal to keep the formulation as simple as possible. It is also imperative to evaluate the formulation, and not just the drug. Sometimes formulation excipients are not biologically inert and can, for example, influence drug uptake (13). In comparison with most other drug classes, antitumor agents usually exhibit a narrow therapeutic index and are often administered at dosages that produce significant toxicity (14). It is clear that in those cases it is of even greater importance that the formulation as such is nontoxic.

Most chemotherapy agents are formulated as sterile dosage forms intended for intravenous (IV) use. The IV route of administration is preferred for several reasons. Firstly, IV administration leads to immediate bioavailability and consequently to accurate dosing. This is important because many products are not therapeutically active after oral (PO) administration. Secondly, the rate of administration can be easily modified. Thirdly, IV administration is preferred when nausea and vomiting are anticipated due to gastrointestinal irritation. Fourthly, administration of drug can be immediately halted if an acute toxic response is observed. This is not always possible by other routes of administration. There are also disadvantages to IV administration; many antineoplastic agents are chemically reactive and irritating to tissues, which may lead to discomfort for the patient, with sometimes devastating reactions after extravasation (15). Furthermore, the occurrence of sepsis and thrombosis or problems resulting from particulate matter in infusions are not uncommon for the IV route of drug delivery (16). On the whole, in oncology an IV formulation is preferred in the early development stage.

Chen and coworkers have written an excellent review article about the possible strategies for the formulation of antineoplastic drugs (17). Formulation scientists of the Joint Formulation Working Party of the Cancer Research Campaign (CRC)/European Organization for Research and Treatment of Cancer (EORTC)/United States National Cancer Institute (NCI) have summarized and discussed their experiences in the formulation of about 20 investigational cytotoxic drugs during the past 5 years (18). The aim of this review is to evaluate the current status of the pharmaceutical formulation of antineoplastic drugs from 1986 onwards.

FORMULATION DEVELOPMENT PROCESS

After the NDS has been screened for cytotoxic activity, yielding promising results, it will enter the formulation stage. Although regulatory requirements may vary from country to country, the approach to formulation development described here is representative of the methods and procedures applied worldwide. At our formulation laboratory, the NDS will be pharmaceutically developed according to the scheme in Table 2.

After the acquisition and screening of the NDS, the bulk drug should be fully structured and analytically characterized. Structural characterization is performed by nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), and infrared (IR) spectroscopy; elemental analysis is considered in some cases. Analytical characterization and purity determinations are performed by high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC) and ultraviolet/visible (UV/VIS) spectrophotometry. The latter method is less selective than the former two methods. It is of the utmost importance that the HPLC method has been proven to be capable of indicating stability so that the parent compound can be separated from the possible degradation products. The method should also be capable of separating parent compound from synthetic intermediates, starting materials, or related materials and contaminants if the NDS is a natural product. An array of additional methods, appropriate to the structure of the NDS, can be applied to further characterize the bulk drug. For example, the Karl Fischer titration method to determine the moisture content in the bulk drug, differential scanning calorimetry (DSC), differential thermal analysis (DTA), and x-ray diffraction to determine phase diagrams and polymorphy, melting point and optical rotation tests, and chemical stability studies. If the presence of organic volatile impurities, which may have arisen from the synthesis, are anticipated, the residual levels should be determined by, for example, head space gas chromatography (GC). It is, therefore, of major importance to be familiar with the route of synthesis of the NDS. The European Pharmacopoeia (EP) has proposed limits for the amounts of residual acetonitrile, chloroform, benzene, 1,4-dioxan, 2-methylpropanol, methylene chloride, di-

Table 2

Pharmaceutical Development of the NDS

1. Quality control of the bulk drug: structural and analytical characterization, and purity determination
2. Quality control of the excipients
3. Formulation and manufacturing
4. Quality control of the formulated NDS
5. Shelf-life surveillance

methylformamide, 2-methoxyethanol, methanol, toluene, formamide, ethylene oxide, and trichloroethylene which may be present in the bulk drug (19). In addition, the residual level of ethanol is often quantitated. The possible presence of intermediates, heavy metals such as lead, and catalysts of the synthesis can also be derived from the route of synthesis. If present, these should be identified and quantitated.

The quality of registered (cytotoxic) drugs is often warranted by a pharmacopoeia. The drug has a monograph, describing the tests and specifications to which it has to conform. In general, the regulatory agencies require more information than is specified in a pharmacopoeia monograph, especially with regard to the presence of impurities. Investigational drugs intended for research and development trials are at present not subject either to marketing or manufacturing European Community legislation. Thus, an investigational cytotoxic drug does not have a monograph in a pharmacopoeia. Presently, the active ingredient manufacturer (AIM) often summarizes the chemical and analytical information, and the proposed specifications of the NDS in a drug master file (DMF). The DMF can be used as the basis for developing a pharmacopoeia monograph. Each subsequent new batch of bulk drug should conform to these specifications. The quality of the investigational bulk drug has to be ensured in order to manufacture a drug product of a guaranteed quality.

The development of a drug delivery system is dependent upon the physicochemical properties of the drug, intended dose, and other biological properties of the drug and patient/disease factors (17). For example, if the chemical entity contains ionizable groups, aqueous solubility may be increased through salt formation. The most important properties of the drug to be considered are the aqueous solubility, stability, and rate of dissolution. The aqueous solubility rate is, among others, influenced by the particle size, salt, ester or other chemical form, the pH of the solution, polymorphism, and hydrate formation. Manipulation of these factors may increase the solubility. The solubility study should include equilibrium solubility determinations in water, acid, base, and organic solvents such as ethanol, dimethylsulfoxide (DMSO), *N,N*-dimethylacetamide (DMA), and other potential pharmaceutical vehicles. The required solubility is dependent upon the expected phase I starting dose and the estimated human maximum tolerated dose (MTD) (20). Once an acceptable dosage form has been developed, a pilot batch should be prepared to investigate the stability and compatibility of the product with equipment and conditions of manufacture. The stability of the formulated drug product should be established as a function of temperature, light conditions, and humidity. Additives—such as antioxidants, antibacterial agents, bulking materials, and chelating agents, inert gases and solubilizing agents—are often incorporated into the formulation in order to increase the solubility and/or stability of the drug. The effects of these additives should be thoroughly investigated in order to guarantee maximal safety and minimal toxicity of the formulated drug.

After having fully characterized the NDS and having developed a pharmaceutical formulation, a protocol should be prepared, describing all the steps of the manufacturing process in order to ensure the quality of the formulated drug product intended for clinical use. This protocol should contain the following sections: (a) quality control of the bulk drug, (b) quality control of the excipients, (c) formulation and manufacturing, including inprocess control steps, (d) quality control of the formulated product, and (e) shelf-life surveillance.

a. Quality control of the bulk drug. The bulk drug has to be fully characterized. The specifications to which each new batch of bulk drug has to conform and all the validated methods of analysis are described in this part.

b. Quality control of the excipients. If excipients are used in the formulation of the NDS, the quality of these substances has to tested. In general, the excipients should conform to the EP and the supplier should provide a certificate of analysis with each lot of excipient. For safety reasons, the identity, and in some cases the content and purity, of the excipients is double-checked in our own laboratory. The specifications and methods of analysis used to reconfirm the quality of the excipients which are used in the formulation are listed in the second part of the protocol.

c. Formulation/manufacturing. The formulation/manufacturing process, including the in-process control steps, have to be described as completely as possible, preferably in a stepwise manner. Furthermore, all of the equipment that is used in the manufacturing of the final product has to be listed; for example, type of glass vials, ampoules, stoppers, seals, glassware, etc. In this third section, the preparation of all the equipment has to be described, such as cleaning, sterilization, and packing procedures. In addition, the manufacturing area has to be described in terms of air class (since most cytotoxic drugs are prepared aseptically), cleaning, and spill procedures.

d. Quality control of the formulated product. In the last section of the protocol, the quality of the formulated product is tested. The methods used for the quality con-

trol of the formulated product depend on the nature of the compound. The identity of the active ingredient is often reconfirmed by the HPLC analysis, UV/VIS spectrophotometry, or another suitable method. Other tests to be carried out are appearance, foreign insoluble matter and color (by visual inspection), content determination (by HPLC analysis, UV/VIS spectrophotometry, or another suitable method), purity determination (by HPLC or HPTLC analysis), pyrogen test (by Limulus Amoebocyte Lysate test), sterility test, weight variation, reconstitution, pH after reconstitution, and residual moisture content (if the product is a dry powder or freeze- or vacuum-dried product). All of the specifications of the formulated product should be listed, and the methods of analysis should be described in detail and validated. If the final product conforms to all the specifications which were drawn up for this particular drug, a certificate of analysis should be prepared. This certificate guarantees the quality of the formulated product and provides information about the storage conditions and, for example, stability characteristics after reconstitution and dilution.

e. Shelf-life surveillance. Following the release of a manufactured formulated batch of the NDS, sufficient vials or ampoules should be set aside for long-term shelf-life evaluation of the product.

It should be noted that the tests listed for the quality control of both the bulk drug and the formulated product are sufficient for the preparation of supplies for toxicology (preclinical supplies) and phase I clinical studies. However, if the compound is pursued for further clinical studies and ultimately presented to the registration authorities to obtain a commercial status, more tests are required to ensure a product of guaranteed quality.

CLASSICAL SOLUBILITY APPROACHES: SOLUBILIZATION

There are several techniques to solubilize poor water-soluble drugs, namely, solubilization in surfactant solutions, the use of cosolvent systems, and altering solubility through complexation.

Surfactant Systems

Solubilizing is generally considered to be the uptake of solute molecules (e.g., the drug) into isotropic solutions of surfactants, which in general leads to a higher solubility. The mechanism of action of surfactants is based upon the wetting process principle. Wetting is a process by which the wetting agent (surfactant) comes into contact with the solid particle to form a solid–liquid interface, which leads to a reduced solid–liquid interfacial tension (21,22). The surfactant concentration at which the interfacial tension is reduced to a maximum is called the critical micelle concentration (CMC). At (and above) the CMC, the surfactant molecules will form micelles (aggregates) in the aqueous solution. In addition, the fact that the drug might be "protected" from attacking species such as protons or hydroxide ions gives rise to stabilization of the drug (23). A higher dissolution rate is often achieved due to a decrease of the surface tension and an increase of the wettability. Surfactants can be classified into (a) amphoteric compounds such as lecithin and gelatin; (b) nonionic surfactants such as Brij 30, polysorbate 80 (Tween 80), and Cremophor EL®; and (c) ionic (positively or negatively charged) surfactants such as sodium palmitate and sodium taurocholate. The nonionic surfactants are important in the formulation of pharmaceutical compounds, because in general they are less toxic, are less likely to cause hemolysis, are less irritating to the skin, and in solution produce near neutral pH values. The intrinsic toxicity of surfactants is caused by their high affinity for biological membranes. They frequently adsorb to and penetrate the membranes, and thereby change their fluidity and barrier properties (23).

Nonionic surfactants have been used in the formulation of many antitumor agents. The commercial formulation of etoposide (Vepesid®) contains per milliliter of formulation solution, 20 mg active drug substance, 2 mg citric acid (for pH adjustment), 80 mg Tween 80 (surfactant), 30 mg benzyl alcohol (preservative), 30.5% (v/v) ethanol (cosolvent), and 650 mg polyoxyethylene glycol 300 (cosolvent) (24). Cook and coworkers investigated the uptake of etoposide from the Vepesid formulation by tumor spheroids and activity in the presence of the nonionic surfactant Brij 30 and a hydrotropic agent, sodium salicylate, as compared to nonformulated etoposide (13). Both the Vepesid formulation and the formulation containing sodium salicylate showed enhanced drug penetration. The formulation containing Brij 30 was very toxic to the cells in the tissue culture.

Teniposide is commercially available in 5 ml ampoules (Vumon®) and the formulation consists of 300 mg DMA, 2.5 g Cremophor EL, maleic acid q.s. pH 5.1, and absolute alcohol q.s. 4.715 g per 50 mg of teniposide (25). The solubility of teniposide is very high (> 50 mg/ml) in this formulation. However, in high-dose teniposide therapy, complications can be caused by

ethanol intoxication and Cremophor EL toxicity (26).

Paclitaxel is a natural compound originating from the bark of the Pacific yew tree and it exhibits poor solubility in water. The current clinical formulation (Taxol®) is a 6 mg/ml solution in 50% polyoxyethylated castor oil (Cremophor EL) and 50% dehydrated alcohol (27). Cremophor EL is probably the most widely used nonionic surfactant in IV formulations and it has a CMC of 0.009% (w/v) in water. The amount of Cremophor EL that can be used in IV formulations is, however, limited since it has been shown to cause serious or fatal hypersensitivity reactions in both laboratory animals (28,29) and humans (30). These anaphylactoid reactions are characterized by vasodilation, dyspnea, and hypotension. The MTD for paclitaxel single-dose schedules (infusion time 1–24 hr) is in the range of 250–275 mg/m^2, which implicates that about 40 g of Cremophor EL has been given to patients (31). The incidence of the hypersensitivity reactions during paclitaxel therapy is less than 2% in patients who are premedicated with antihistamines and corticosteroids (32). Recently, the mechanism of action of Cremophor EL has become under vigorous investigation. Cremophor EL is not physiologically inert, but it lacks direct antitumor activity (33). It has been speculated that this excipient might have a positive effect on overcoming multidrug resistance (MDR) to paclitaxel, since it has been shown that Cremophor EL blocks the proline-glycoprotein (P-glycoprotein) drug efflux pump which is held responsible for the MDR phenotype (34–36). It has recently been shown that the plasma concentration of Cremophor EL achieved after a standard dose of Taxol is greater than or equal to the concentration required to reverse MDR in vitro (34). Thus, the presence of Cremophor EL in a formulation might have, besides increasing the solubility of a drug in an infusion solution, the additional advantage of overcoming MDR.

Carzelesin, an investigational cytotoxic drug and cyclopropylpyrroloindole (CPI) analogue of the potent antibiotic CC-1065, is formulated in polyethylene glycol 400 (PEG 400), absolute ethanol and Tween 80 (6:3:1, v/v/v; PET formulation) (37). The formulation of carzelesin was based on the formulation of two other very potent CPI analogues, adozelesin (38) and bizelesin (39). Both analogues are formulated in PET and the formulation of bizelesin is stabilized with citric acid. Carzelesin in PET showed increased solubility and stability in the infusion solution as compared with the nonformulated drug. The PET formulation cannot be injected as such and needs to be diluted with an infusion fluid before IV administration. The CMC of Tween 80 is 0.1% (w/v) in water. Carzelesin is administered to patients with a two-pump infusion system. The drug solution and infusion solution are administered simultaneously, and just before entering the patient come in contact with each other for only several seconds, which excludes the possibility of crystallization and thus enhances the safety of this formulation. In the bloodstream it can be anticipated that the extent of protein binding will help to keep the drug in solution. So far no adverse reactions that could be related to in vivo drug crystallization have been noted in the phase I trials with carzelesin.

The use of surfactants in the solubilization of antineoplastic drugs is a commonly used formulation approach. The uptake of the drug into the micellar surfactant solution often leads to an increase in solubility and stability. One of the major disadvantages of the use of surfactants is that they are not always biologically inert and often contribute to the overall toxicity of the drug. Moreover, a drug solubilized in a surfactant solution cannot as such be administered to patients and has to be diluted with, for example, normal saline before administration. Caution has to be taken with the dilution, always keeping the concentration of surfactant in solution above the CMC, thereby minimizing the risk of precipitation of the drug.

Cosolvent Systems

The addition of cosolvents such as ethanol, propylene glycol, polyethylene glycols, and glycerol is probably the most widely used solubilizing technique (17). The desired solubility of a drug in a pharmaceutical formulation depends on the expected dosage needed in clinical trials. In some cases, the use of an appropriate cosolvent can increase the aqueous solubility of a drug by several orders of magnitude. The effect of adding a cosolvent to an aqueous solution of a drug depends primarily upon the polarity of the drug with respect to the solvent (water) and the cosolvent (40). In general, drugs which are solubilized in a cosolvent cannot be directly administered to patients, because these vehicles (as also holds for surfactant systems) often cause irritation and hemolysis. The vehicle should, therefore, first be diluted with normal saline or another infusion fluid (e.g., 5% dextrose) and infused as the diluted solution. The reconstituted drug solution should be used preferentially within 24 hr due to sterility considerations. Moreover, it is important to consider the possible precipitation upon dilution, when the final concentration of the drug is higher than the drug's inherent water solubility

(27,41,42). Use of an in-line filter is, therefore, recommended when administering reconstituted cosolvent systems, to prevent administering precipitated drug particles. Precipitation upon injection can also result in erratic or reduced drug bioavailability, pain on injection, and/or thrombophlebitis (40). Furthermore, the compatibility and adsorption of the drug to the administration set (and when relevant the adsorption to the in-line filter) should be investigated.

The compounds busulfan, an antimetabolite (43), and the Fujisawa Agent (NSC-630176D), an antitumor cyclic peptide (44), are both administered to rats and mice as aqueous solutions of 40% PEG 400 in saline. In this formulation, the solubility of busulfan is 2.5 mg/ml and remains stable for at least 8 hr at room temperature (43).

The anticancer agent 2-amino-5-bromo-6-phenyl-4(3)-pyrimidone (ABPP) is formulated as a 20 mg/ml solution in 0.2 M Na_2CO_3 containing 10% v/v DMA at pH 10.4 (45). The aqueous solubility of ABPP is highly dependent upon the pH and cosolvent concentration in the formulation. When administering the cytotoxic drug amsacrine for the treatment of acute myelogenous leukemia, amounts of up to 64 mg DMA/kg/day for 5 days have been administered (46). DMA itself has also been investigated as a potential cytotoxic agent. In a phase I study, doses of 400 mg/kg caused hallucinations, disorientation, and lethargy (47). Doses of 100 and 200 mg/kg, however, did not produce these effects. The use of DMA should, therefore, be limited to 10% solutions, because at higher concentrations effects on the central nervous system (CNS) may occur (14).

2-Chloro-2′-3′-dideoxyadenosine (2-CIDDA), is formulated as a 10 mg/ml solution in 60% propylene glycol, 10% ethanol, and 30% 0.05 M aqueous phosphate buffer (pH 8.0) (48). Propylene glycol is hemolytic in vitro (14). However, this effect is greatly minimized by the use of the 40% concentrations or less in normal saline. Furthermore, it also depends on the total (absolute) amount of propylene glycol administered. CNS depression and convulsions were observed after infusion of large amounts (300 ml) of propylene glycol in humans (49). The alkylating agent melphalan is formulated for IV use in 60% propylene glycol and 5% ethanol (50). This solution must be further diluted before use to prevent toxicity induced by propylene glycol.

Several nonaqueous vehicles and formulation approaches have been evaluated to facilitate the IV delivery of the macrocyclic lactone bryostatin 1 (51). Phlebitis was observed when bryostatin 1 was administered intravenously in 60% ethanol in clinical trials. The absolute amount of ethanol that can be infused per hour is dependent upon the rate of metabolism of ethanol. Patients become intoxicated when the rate of infusion exceeds the rate of metabolism. The maximum rate of metabolism of ethanol is approximately 10 g/hr (52).

Porphyrin drugs are photosensitizing compounds that are used in the photodynamic tumor therapy. In general, these agents are no stable in aqueous solution and are often susceptible for addition and oxidation reactions (53). The formulation of the porphyrin hemin (25 mg/ml) contains 4 molar equivalents of arginine in propylene glycol:ethanol:water (4:1:5, v/v/v) and is stable for at least 2 years (54). This formulation has already been used in the clinic (55). Apart from one case of mild venous irritation, no other side effects were seen.

Cosolvents have become the most used means of solubilizing drugs for IV administration, because of their relatively low toxicity and their ability to solubilize many nonpolar drugs. The major disadvantage of the cosolvent approach is the possible precipitation upon dilution in the infusion bottle and possibly in the bloodstream. It is advisable to use those solvents that have already been used in other (commercially available) formulations, and that the clinic has sufficient experience with.

Complex Formation

Complexation is nowadays more widely used, firstly, in order to increase the aqueous solubility and stability of a drug, and secondly, to target cytotoxic compounds more specifically to tumor cells. In the context of this paper, only the use of complexation with respect to solubility and stability enhancement is discussed. Complexation may be described as the reversible association of a substrate molecule S with n molecules of a ligand species L to form a new species S_1L_n (56). Complexation is carried out to alter the physicochemical properties of a drug in such a manner that the solubility and stability of the complex are higher as compared with those of the parent compound. However, caution should be taken to ensure that the change in physicochemical characteristics of the drug by complex formation does not alter the activity or mechanism of action of the agent. Moreover, the reversibility of the complexation might lead to direct precipitation upon dilution. Therefore, it is important to study the solubility of the complex at various concentrations. Complexation may be of pharmaceutical use in the enhancement of the equilibrium solubility and the dissolution rate of a drug, even though the degree of solubilization that can be expected

following complexation is normally less than 1 order of magnitude.

In aqueous solutions, the *N*-nitrosourea anticancer agents form a complex with tris(hydroxymethyl)aminoethane (Tris), and the rate of degradation of the drugs in the complex is slower than that of the free drugs (57). Thus, under certain conditions, it is possible to increase the shelf life of these drugs by including Tris in their formulations. On the other hand, when a commercially available solution of 5-fluorouracil in a Tris buffer was IV administered to patients, serious (sometimes lethal) cardiotoxicity was observed (58). It was shown that this cardiotoxicity was caused by the presence of adducts of two degradation products of 5-fluorouracil with Tris.

Complexation with cyclodextrins is receiving more and more attention both in pharmaceutical formulation (59) and as drug carrier systems (60). Cyclodextrins are oligomers of dextrose which are produced by enzymatic (cyclodextrin transglycosylase) degradation of starch. Cyclodextrins possess the ability to form inclusion complexes with many drugs and they are relatively nontoxic (61,62). They can substantially increase the aqueous solubility of pharmaceuticals. Liquid, unstable, or volatile drug substances can be transformed into solid, stable crystalline forms by cyclodextrin complexation. The antitumor agent benzaldehyde is a highly volatile substance, which presents a serious problem in administering it during clinical trials. A stable inclusion complex of β-cyclodextrin with benzaldehyde in solution was prepared (63). The inclusion complex retained the antitumor activity of benzaldehyde against several human tumor cell lines (e.g., colon adenocarcinoma). Cyclodextrins are also used to form complexes with lipophilic drugs. Erbuzole, a new synthetic microtubule inhibitor, was reversibly complexated with hydroxypropyl-β-cyclodextrin for clinical use (64). In laboratory animals, it was shown that a fourfold dose reduction of erbuzole in cyclodextrins could be achieved, as compared to erbuzole in water.

Another class of complexing agents are the hydrotropic compounds. Hydrotropic solubilizing agents are applied to increase the aqueous solubility of poorly water-soluble drugs. The plane-to-plane orientation of the planar water-insoluble drug and the hydrotropic molecules, brought together by electrostatic and hydrophobic interactions, is thought to be responsible for the mechanism of hydrotropic solubilization of hydroxybenzoates (parabens) (65). Other examples of hydrotropic agents are salicylates, sodium salts of several benzoic acids, and fatty acids. Etoposide is commercially available in a nonaqueous IV formulation (Vepesid). However, the risk of precipitation of the drug as microcrystals upon dilution in blood is present, when Vepesid is administered to patients. Etoposide was, therefore, formulated as a 20 mg/ml solution in a 2 M sodium salicylate solution (66). The latter and the Vepesid formulations were diluted with human plasma and it was shown that whatever the dilution factor, Vepesid resulted in needlelike precipitates, while no precipitation was noted during the first hour in any dilutions of the etoposide–sodium salicylate formulation.

The anthracyclines doxorubicin and epirubicin have the tendency to form dimeric or polymeric aggregates, which lowers the wettability of the drug in the lyophilized state and consequently decreases the dissolution rate (67). Parabens are often used as preservatives; however, it has been observed that they may be lost during freeze drying unless other materials are present to complex with parabens. Doxorubicin apparently forms such complexes (68). The application of this observation in new formulations of doxorubicin and epirubicin, where parabens are used because of their antiaggregant action, leads to instantaneous complete reconstitution of doxorubicin and epirubicin. In addition, the complete and fast dissolution is time saving and reduces the potential hazards for the personnel involved in handling the drug product since the risk of spillage increases with the hand-shaking time.

Much attention has been given to the complexation of cytotoxic drugs with a target-specific carrier to deliver the drug to its tumor target. However, the utilization of complexation in altering apparent solubility properties as well as increasing the therapeutic index of a drug has a place in pharmaceutical formulation. The increase in solubility that can be obtained is, in general, not as significant as can be obtained with the use of, for example, cosolvents. One of the major advantages of complexation in pharmaceutical formulation is the reversibility of the reaction; for example, dissociation of the complex to the individual reactants occurs when the complex enters the body. However, care should be taken that the reversibility of the complex does not cause precipitation upon dilution.

COLLOIDAL SYSTEMS

In addition to the classical solubilizing systems, the colloidal systems have gained importance. Colloidal systems such as liposomes, microcapsules, microspheres, nanoparticles, and macromolecule complexes may enhance the solubility and stability of a drug due to the

changes in molecular environment of the drug (17). Encapsulation of biologically labile materials within a colloidal system may also protect them from premature inactivation or degradation within the circulation. The safety of a drug carrier system must be evaluated from two standpoints: the toxicity of the carrier itself and the risk of the novel, drug-induced toxicities arising from differences in the disposition, pharmacokinetics, and metabolism of carrier-associated drug as compared with conventional drug formulations (69).

Liposomes

Liposomes are microscopic vesicles (from 250 Å to >20 μm in diameter) composed of one or more lipid membranes surrounding discrete aqueous compartments (70). These vesicles can encapsulate water-soluble drugs in the aqueous compartments and lipid-soluble drugs within the membrane itself. Thus, liposome entrapment offers the possibility of formulating highly promising lipophilic drugs that could not be developed for intravenous use because of solubility problems. In general, liposome-encapsulated drugs have a longer serum half-life than free drugs. The half-life is dependent upon the size, the amount of lipid used in the preparation of the liposomes, and whether the liposomes are charged or uncharged (70,71). A major potential problem of using lipsomes as pharmaceuticals concerns their stability during extended storage. Liposomal solutions are, therefore, often lyophilized to enhance their long-term stability. Even then the liposomal size may change during storage. Furthermore, the large-scale manufacturing of sterile liposomes is often still a problem. Membrane filtration (0.22 μm) is not applicable to large liposomes (> 0.2 μm), and chemical sterilization with ethylene oxide carries the risk of contamination by the residues of ethylene oxide (72). Sterilization of liposomes by autoclaving or irradiation is not always possible due to chemical and/or physical instability.

Liposomes are formed when certain phospholipids are dispersed in excess water (73). The lipid molecules arrange themselves in bilayers that, above the main transition temperature of the phospholipids, spontaneously vesiculate and enclose an aqueous core. Depending on the preparation method used and selected bilayer composition, liposomes can vary considerably in size, size distribution, and the number of bilayers per liposome (74). Dispersions that have different physical characteristics can facilitate differences in liposomal behavior in vivo. The stability of drug-loaded liposome dispersions preferably should meet the standards of conventional pharmaceutical products (75). Both the chemical stability (the occurrence of ester hydrolysis and oxidation) and physical stability (leakage of the drug from the liposomes and aggregation and/or fusion of liposomes) should be thoroughly investigated.

Liposomes administered intravenously to humans concentrate mainly in the organs with fenestrated capillaries such as liver, spleen, and bone marrow (76). The tendency of IV-administered liposomes of the micrometer range to localize in phagocytic cells of the mononuclear phagocyte system makes them an attractive vehicle for delivery of antineoplastic agents to these cells. Liposomes are also applied as site-specific carriers to deliver the drug to the pharmacological site of action in a protected form. Immunoliposomes combine the specificity of an antibody with the capacity of a liposome to deliver a large amount of antineoplastic drug to the tumor cell.

In the context of this paper, most of the liposomal applications discussed relate to the pharmaceutical development of cytotoxic drugs. Several liposomal formulations have been described for the anthracyclines such as doxorubicin (77,78), epirubicin, the 4′ epimere of doxorubicin (79), daunorubicin (DaunoXome®) (80), and annamycin (81). Doxorubicin is one of the most widely used anticancer drugs and it is commercially available as a stable lyophilized formulation, as a "ready-to-use" injection and as a liposomal formulation. The latter formulation has been developed to improve the therapeutic index of doxorubicin by reducing the occurrence of serious cardiotoxicity. Several clinical studies have been performed with liposome-encapsulated doxorubicin and showed that the disposition of liposomal doxorubicin changed, leading to a decreased occurrence of doxorubicin-related cardiotoxicity (82–85).

Several other cytotoxic drugs have been successfully formulated into liposomal solutions. *cis*-Bis-neodecanoate-*trans*-*R*,*R*-1,2-diaminocyclohexane platinum (NDDP) is a lipophilic analogue of cisplatin and it is completely insoluble in water. It has, therefore, been manufactured as a lyophilized powder, which forms multilamellar vesicles (MLVs) upon reconstitution with normal saline (86). Clinical studies with the liposomal formulation are ongoing.

Camptothecin (CPT) and 9-aminocamptothecin (9-ACPT) are topoisomerase-I inhibitors with cytotoxic activity. CPT base has been found to have 10 times the activity of its sodium salt; however, its poor solubility in aqueous media has precluded its clinical administration by the intravenous route. To enhance solubility, CPT has been formulated into liposomes (87). It was

shown that the liposomal CPT maintained activity comparable to that of the free drug. CPT and 9-ACPT have also been successfully incorporated into liposomes compared of dimyristoyl phosphatidylcholine (PC)/cholesterol/phosphatidylserine (PS) (88). The amount of incorporation was found to be pH dependent.

Mitoxantrone, a synthetic analogue of doxorubicin, is commercially available as an aqueous solution for injection (Novantrone®). An attempt was made to improve the pharmacokinetics of mitoxantrone by incorporating the drug into unilamellar liposomes (89). Preliminary pharmacokinetics showed higher values for the area under the concentration–time curve (AUC) as well as a longer elimination half-life for liposomal mitoxantrone as compared with free mitoxantrone.

Paclitaxel is commercially available in a surfactant formulation (Taxol). This vehicle has, however, been shown to sometimes produce serious hypersensitivity reactions in laboratory animals (28) and humans (30). Although premedication with corticosteroids and antihistamines is now being used successfully to reduce the intensity and incidence of reactions associated with Taxol administration, the search for new formulations with fewer side effects is still ongoing. In two studies performed by Sharma and collegues, paclitaxel was reformulated in liposomes, prepared from phosphatidylglycerol (PG) and PC (90,91). The liposomal paclitaxel formulation was stable and demonstrated lower acute and chronic toxicity in tumor-bearing mice than the commercially available formulation (Taxol).

Several other antitumor drugs have been formulated in liposomal solutions, such as 5-fluorouracil, which is one of the most widely used pyrimidine antimetabolites (92), and interleukin-2, which is an effective agent for a minority of patients with renal cell cancer and melanoma (93). Liposome encapsulation of interleukin-2 appeared to decrease overall toxicity, while maintaining immunomodulatory activity (93).

Photochemotherapeutic agents, such as Photofrin II® (94) and tin protoporphyrin (SnPP) (95) have also been successfully encapsulated into liposomes. Intravenous administration of liposomal SnPP to rats led to a dramatic increase in the amount of drug taken up by the spleen, due to the high concentration of reticuloendothelial cells in that organ.

Nonionic surfactant vesicles (niosomes) are similar to liposomes and can be prepared with cholesterol, a surfactant such as polysorbates (Tweens) or sorbitan esters of fatty acids (Spans), and water. In general, niosomes are capable of releasing the entrapped drug slowly. Methotrexate has been incorporated into niosomes and the nature of the surfactant used seemed to affect the amount of methotrexate entrapped into the niosomes (96). The Spans showed a higher percentage of entrapment than the Tweens. Furthermore, the pharmacokinetics of the methotrexate-loaded niosomes in sarcoma S-180 bearing mice were comparable to those of the free drug. The methotrexate plasma levels were higher and the rate of elimination from plasma was slower when the methotrexate-loaded niosomes were administered to tumor-bearing mice as compared with the free drug.

In conclusion, several cytotoxic agents, which had never been formulated before due to their low aqueous solubility, have been successfully incorporated into liposomes. In addition, several drugs have been reformulated into liposomes, thereby decreasing the toxicity of the former formulation or of the drug itself. However, more research has to be performed to develop liposomes with higher chemical and physical stability to prevent degradation during extended storage.

Microencapsulation Systems

Microencapsulation involves the application of a thin film of material around micronized solid or liquid to produce discrete units ranging in size from less than 1 μm to several millimeters (17). In the pharmaceutical industry, microencapsulation processes have been used to formulate poorly water-soluble drugs, to alter the physical and chemical stability and the physiological effects of drugs. In oncology, because of the relatively low immunogeity, microencapsulation has mainly been used as a drug carrier system to target cytotoxic drugs more specifically to the tumor. The products of microencapsulation can be classified into nanoparticles (200–500 nm), microdispersions (0.5–1 μm), microspheres (1–100 μm), and microcapsules (> 100 μm). Numerous methods have been used to prepare microencapsulated systems; these include pan coating, fluidized bed, spray drying, solvent evaporation, interfacial polymerization, and coacervation techniques.

Polyalkyl-2-cyanoacrylate (PACA) nanoparticles are polymeric nanoparticles with promising uses in cancer chemotherapy. PACA nanoparticles are biodegradable ultrafine particles, which are able to carry anticancer compounds and to modify their distribution profile in vivo considerably. In the preparation of PACA nanoparticles, often stabilizers such as dextrans, the nonionic surfactants poloxamers or Tweens, or β-cyclodextrin are used. Several cytotoxic agents such as actinomycin D, 5-fluorouracil, and doxorubicin have been successfully incorporated into these PACA nanoparticles (97). A

higher uptake into organs such as spleen and liver, and altered biodistribution were seen. Several PACA nanoparticle systems loaded with antineoplastic drugs such as doxorubicin,vinblastine, and methotrexate have been described (98). The uptake values in the organs liver, spleen, and lungs and the intratumoral (IT) uptake of PACA nanoparticles were increased in tumor-bearing mice.

Another class of nanoparticles are the polyacrylamide (PAA) and polymethylmetacrylate (PMMA) nanoparticles. These types of nanoparticles are relatively stable; they were not observed to degrade in vivo (98). Thus, the risk of polymeric tissue or cellular overloading restricted their possible clinical use.

Mitoxantrone was incorporated into poly(butylcyanoacrylate) (PBCA) nanoparticles, and the efficacy and toxicity of this formulation was compared with the free drug and with a liposomal mitoxantrone formulation (SUVs with a negative surface charge) (99). In murine tumor models, mitoxantrone-loaded PBCA nanoparticles led to a significant tumor volume reduction, whereas the liposomal formulation prolonged the survival time in P388 leukemia. Neither the nanoparticle nor the liposome formulation was able to reduce the toxic side effects, namely, leukopenia, caused by mitoxantrone.

Biodegradable poly(3-hydroxybutyrate) (PHB) microspheres containing acylglycerols and the anticancer drug lastet were prepared by the solvent-evaporation process (100). The release rate was determined in vitro and it was shown that lastet was not released from the PHB microspheres in the absence of an acylglycerol, such as glycerol monostearate (25 wt%) or glycerol tristearate (25 w%).

Microdispersion technology permits the formulation of water-insoluble drugs as uniformly dispersed suspensions of spherical microparticles having uniform diameters. For example, merbarone(NSC-336628), a poorly water-soluble cytotoxic drug, was initially formulated as the *N*-methylglucamine salt at pH 10 (101). Phase I clinical testing in the United Stated showed that this formulation induced severe injection-site vasculitis. Therefore, merbarone was reformulated as a microdispersion of nanoparticles at neutral pH. In BDF1 mice carrying L1210 subcutaneously, the injection-site vasculitis and necrosis disappeared completely by the use of the microsuspension formulation as compared with the original formulation solution while the antitumor activity remained unchanged. Recently, a microdispersion formulation of paclitaxel has been described, which demonstrated equivalent antitumor activity to the clinical formulation against a murine tumor (102). Attempts to formulate the anticancer agent pancratistatin as a microsuspension, based on the method by Violante (103), were unsuccessful. Mitomycin C has been incorporated into dextran microspheres (104). The average diameter of the microspheres was 75 μm and the content was 5% of mitomycin C. In vitro studies showed that the microspheres had sustained-release properties.

In two studies performed by Goedemoed and coworkers, the release profiles of the alkylating agents melphalan and melphalan methyl ester from polyphosphazene microspheres were studied in vitro (105,106). The melphalan methyl ester loaded microspheres showed gradual and sustained release as compared with the melphalan microspheres. Thus, the chemical nature of the compound is important for both the incorporation of the drugs into microspheres and the release characteristics from the microspheres. Furthermore, an increased median survival time was obtained with the melphalan methyl ester loaded microspheres in intraperitoneal (IP) leukemia L1210 tumor model in mice (105).

Lipid microspheres of 0.2 μm diameter have properties similar to liposomes in terms of tissue and cellular distribution, and are suitable candidates for the incorporation of lipophilic drugs. Unlike liposomes, lipid microspheres can be produced on a large scale and are stable for 2 years at room temperature. The prostaglandin 9-oxo-15-hydroxy-$\delta^{7,10,13}$-prostatrienoic acid methyl ester (δ^7-PGA) was incorporated into lipid microspheres and the antitumor effect was investigated in vivo in tumor-bearing mice (107). Incorporated δ^7-PGA was shown to exert a significantly greater antitumor activity in several tumor systems as compared with free δ^7-PGA.

The preparation of gelatin microspheres and their use for the controlled release of the anticancer agents bleomycin (108) and methotrexate (109) have been reported. In the latter study, methotrexate was conjugated with gelatin prior to the incorporation in the gelatin microspheres. It was shown that the gelatin–methotrexate conjugate loaded microspheres possess promising potential as a delivery system for methotrexate, thus avoiding the renal toxicity associated with the free drug.

Microencapsulation appears to be a useful manner for the formulation of several antineoplastic agents. The use of biomaterials to increase the specificity of the delivery system represents an interesting approach. There are, however, limitations to the use of microencapsulated systems such as high-temperature involvement during manufacturing, which makes the method unsuitable for thermolabile drugs. In general, higher uptake in organs such as liver, spleen, and lungs was seen. Moreover, lower systemic exposure was observed,

thereby increasing the therapeutic index, as compared with those obtained using conventional parenteral drug formulations (110). These results indicate the importance of further research of microencapsulated systems, since the therapy of solid tumors such as lung and liver has no been very successful up to now. In addition, microencapsulated systems often possess controlled-release characteristics and less toxicity as compared to the free drug.

Fat Emulsions

A substantial increase in the use of parenteral emulsions has occurred with the development of fat emulsions for parenteral nutrition. An emulsion is a heterogeneous mixture of two or more immiscible liquids, with a third component (emulsifier) used to stabilize the dispersed droplets (111). Typical emulsifiers are Tween 80, Span 80 (sorbitan mono-oleate), phosphatides obtained from egg, and lecithin. Unfortunately, emulsifiers are often toxic as a result of hemolytic reactions (112). When the carrier system contains emulsified lipids, typical production steps may encompass premixing of raw materials including the drug, high-energy homogenization, filtration, and filling of bottles followed by heat filtration (113). This procedure is defined as drug incorporation de novo, as compared to extemporaneous addition of the drug to a preformed emulsion. Emulsions can be placed into two categories, namely, oil-in-water emulsions, where a drop of organic liquid is immersed in an aqueous solution; and water-in-oil emulsion, where a drop of water is immersed into an organic solution. Parenteral emulsions can be used as a drug administration vehicle for the following reasons: solubilization and stabilization of poorly water-soluble drugs (114), and reduction of irritation or toxicity of IV administered drugs (115).

There are several commercially available fat emulsions such as Intralipid® 10%and 20% (Kabi Pharmacia), Lipofundin® and Lipofundin S® (Braun), and Liposyn® II (Abbott), which are all essentially nontoxic and suitable for IV use (112). Intralipid has an osmolarity of 280 mOsm/liter, approximately the tonicity of the blood, and complications due to thrombophlebitis are seldom encountered (116). Typical adverse reactions are fever, shivering, chest or back pain, anorexia, and vomiting (117).The particle size of IV emulsions should be below 1 μm as larger particles (4–6 μm) are known to cause embolism (118). Particle size reduction can be achieved by adding glycerol and propylene glycol to the emulsion (119). This study also showed that polyhydroxy alcohols may be acting as cosurfactants and have a stabilizing effect on the emulsion.

Fat emulsions, composed of the commercially available parenteral emulsions, were suitable for formulation of lipophilic cytotoxic drugs, such as the investigational drug rhizoxin (114) and the *S*-triazine agent hexamethylmelamine (HMM) (120,121). HMM has very limited solubility in water and in nontoxic solvents, and has therefore been evaluated only following PO administration. However, HMM has poor bioavailability due to extensive first-pass metabolism rather than poor absorption from the gut. An IV formulation was developed, where HMM was dissolved in ethanol or DMA and subsequently diluted in Intralipid 20% (121). This formulation was optimized by dissolving HMM in dilute hydrochloric acid, adding Intralipid 10%, and adjusting the pH with sodium bicarbonate. Two phase I clinical trials were conducted and showed that the parenteral emulsion formulation was well tolerated by all patients.

Perilla ketone is an investigational lipophilic cytotoxic agent formulated for potential IV administration in 5% dextrose (122). However, between 20% and 60% of the drug is lost due to high affinity for the polyvinylchloride (PVC) administration set. Extemporaneous formulation of perilla ketone into lipid emulsion seemed to eliminate this problem. A 5 mg/ml formulation was developed in 10% ethanol, 40% propyleneglycol, and 50% water, which was diluted 50 times with Intralipid 10%.

Penclomedine is another practically water-insoluble antineoplastic drug. The cosolvent approach was unsuccessful due to very low solubility. The optimal formulation of penclomedine seems to be a lecithin-stabilized 10% soybean emulsion (10 mg/ml), which shows adequate solubility and physical and chemical stability at room temperature during 2 years (6). This formulation has been modified slightly for scale-up for clinical use (123).

Pharmaceutical interest in multiple emulsions, especially water-in-oil-in-water emulsions, arises from the possibility of presenting water-soluble drugs in a liposoluble vehicle in which the oil membrane acts as a barrier to control release. Omotosho and coworkers prepared water-in-oil-in-water (2/1/1, w/o/w) emulsions containing the cytotoxic drug methotrexate (124). The internal aqueous phase consisted of 1 mg/ml methotrexate in 0.2% bovine serum albumin and the oil phase of 2.5% Span 80 in octane, dodecane, hexadecane, octadecane, or isopropyl myristate (IPM). The w/o/w emulsion was formed by reemulsifying the primary w/o emulsion with an equal volume of a hydrophilic surfac-

tant solution (1% Tween 80). The release rate was significantly affected by the nature of the oil phase.

Epirubicin has been shown to be an effective antitumor agent; however, using the conventional routes of administration, the systemic toxicity is quite significant, especially at high doses. Lipiodol is an iodized oil, which is preferentially taken up by hepatoma. A lipiodol–epirubicin emulsion was, therefore, developed and the acute toxicity of this formulation was studied in rabbits (125). A selective uptake of the emulsion into the liver was seen using x-ray films, which suggests a targeting effect for epirubicin to the liver.

The use of fat emulsions seems to be a suitable approach for the pharmaceutical formulation of antineoplastic drugs. In general, the solubility and long-term stability of these compounds were increased, and sometimes the toxicity was decreased. In addition, fat emulsions can be used as more specific drug delivery systems.

Lipoproteins

Lipoproteins are endogenous particulate carriers for the transport of cholesterol and other lipids in the blood circulation. They are not immunogenic and escape recognition by the reticuloendothelial system (126). Lipoproteins can, therefore, be considered as potential vehicles for lipophilic drugs. Lipoproteins are divided into four classes: chylomicrons, very low density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL).

In most studies, the suitability of LDL carrier systems for antineoplastic agents is investigated as opposed to the other lipoproteins. One of the major advantages of using LDL as a carrier system for cytotoxic drugs is the fact that a number of tumor cell lines are highly active in the uptake for LDL (126). Furthermore, the oily core provides a domain for lipophilic (pro)drugs. Disadvantages of all four classes of lipoproteins as drug carriers include their complex and unstable nature, and their potential cytotoxicity to normal tissues (127).

One LDL particle contains about 1500 cholesteryl ester molecules surrounded by a polar shell containing apoprotein B (apo B). After binding to the specific LDL apo B receptor, LDL is internalized and degraded in the lysosomal compartment. Tumor cells require large amounts of cholesterol for their replication, and uptake of LDL from the circulation may meet these requirements. Tumor cells sometimes lack the regulatory mechanism of the LDL receptor as compared to normal cells. The tumor cells express a high number of LDL receptors under conditions where receptors in normal tissues are downregulated, for example, under the influence of dietary fat (128). LDL loaded with an antineoplastic agent, in combination with a special diet, may, therefore, be selectively delivered to tumor cells, whereas normal cells are protected.

When using LDL as a drug carrier to malignant cells, it is crucial to incorporate a cytotoxic drug without interfering with the LDL receptor-recognizing properties. Masquelier and coworkers performed a study with two derivatives of doxorubicin, namely, *N*-trifluoroacetyladriamycin-14-valerate (AD-32) and *N*-trifluoroaceyladriamycin-14-*O*-hemiadipate (AD-143) (129). AD-32, dissolved in anhydrous diethylether, was added to lyophilized LDL and gave an AD-32–LDL complex containing about 100 drug molecules per LDL particle. In mice, the disappearance of the AD-32–LDL complex from plasma and accumulation in organs was similar to that of native LDL. Thus, it seems possible to incorporate or attach drugs to LDL without altering its in vivo fate.

Often lipophilic prodrugs of cytotoxic agents are prepared in order to facilitate incorporation into LDL. Methotrexate and 5-fluorodeoxyuridine were derivatized with oleic acid, in order to study the nondestructive incorporation into LDL (130). It was shown that the two hydrophilic antineoplastic agents were successfully modified in order to achieve association with LDL.

The lipophilic anticancer drug prednimustine was incorporated into model LDL (m-LDL) (131). A microemulsion of the drug was prepared which formed a complex with the apo B receptor of the LDL particle. The cellular binding, uptake, and metabolism of the complex were found to be similar to those of native LDL. The cytotoxic activity of prednimustine–m-LDL complex against T-47D breast cancer cells was found to be nearly 50% higher than that of the free drug (dissolved in a DMSO–ethanol mixture).

The 2-(aminomethyl)acrylophenones (AMA) possess an in vitro antileukemic activity but are devoid of any in vivo antineoplastic activity, because the compounds are actively captured by proteins in the blood (132). It was demonstrated that AMA could be incorporated into LDL, and that in vitro LDL is a potential carrier for AMA.

The cytotoxic steroid mustard carbamate compound 25 was successfully incorporated into the core of reconstituted LDL and was found to be stable in serum in vitro (133). Both the structure and cellular uptake of the drug–lipoprotein complex were similar to those of native LDL.

Recently, the distribution of ^{99m}technetium-LDL has been studied in humans with myeloproliferative disease (134). A clinical study in patients with ovarian or endometrial cancer showed no increase in toxicity for a LDL–vincristine preparation (135). Further, the authors observed that the vincristine-associated neurotoxicity seemed to be reduced. However, the scope of the study did not allow for an assessment of clinical activity (135).

One of the major advantages of using LDL particles as carriers of antineoplastic drugs is the fact that they are endogenous and will not be recognized and eliminated by the reticuloendothelial system of the body.

PRODRUGS

The solubility of a drug in water is an extremely important determinant of its ultimate potency in a biological system. Improving the solubility of a cytotoxic drug and the delivery to the site of action frequently requires the design of derivatives of the drug, which are called prodrugs. The derivatization approach may increase the solubility of a drug by several orders of magnitude when the active compound is derivatized to an inactive HCl salt. Derivatization is also successful when a free hydroxyl, carboxyl, or amino functional group is available. Once within the body, the inactive prodrug can be converted either by spontaneous hydrolysis or by enzymatic degradation into the active form. Disadvantages of the use of prodrugs are that derivatization is, in general, a costly and difficult process, and the pharmacokinetic and pharmacodynamic properties of the parent compound may be altered. The optimal balance between the hydrophilic and lipophilic properties of the prodrug have to be determined in order to achieve optimal solubility enhancement and efficient delivery, and retain the pharmacokinetic and pharmacodynamic properties of the parent compound.

Prodrugs of 5-fluoro-2′-deoxyuridine with different physicochemical properties were synthesized by esterification with aliphatic acids, for example, propionate, *n*-butyrate, and *n*-pentanoate (136). Microspheres were prepared containing these ester prodrugs, and a single IP injection of the *n*-butyrate and *n*-pentanoate prodrug loaded microspheres resulted in higher antitumor effects against P388 leukemia in mice than did free prodrugs.

The formulation of paclitaxel has been difficult due to an extremely low aqueous solubility and lack of functional groups that would allow salt formation. Due to hypersensitivity reactions induced by the vehicle of the commercially available formulation (Taxol), attempts were made to synthesize 2′-monoderivative salt prodrugs (5). Aqueous solubility of some of the prodrugs increased up to 1%. The 2′-monogluturate–sodium salt and the triethanolamine salt prodrug were extremely potent, and the yield of the synthesis was high. In another study, water-soluble prodrugs of paclitaxel were prepared containing a sulfonate group as the water-solubilizing group (137). The resulting prodrugs were quite stable. Other workers have prepared amino acid esters of paclitaxel at the 2′ and 7′ site. Some of the 2′ esters show equivalent activity to Taxol against murine tumors and appear to act as prodrugs (138).

A new series of lipophilic prodrugs of 1-β-D-arabinofuranosylcytosine (ara-C) were synthesized by covalently conjugating ara-C with phosphatidic acid (139). Most of the ara-C conjugate prodrugs produced significant increases in life span and long-term survivors in mice with L1210 and P388 leukemia. The conjugate was suspended in sterile water (20 mg/ml) and sonicated for 5 min, resulting in a micellar solution.

A prodrug form of the cytostatic chlorambucil was prepared by coupling the drug to α,β-poly(*N*-hydroxyethyl-DL-aspartamide) via an ester link with carbonyldiimidazole (140). The conjugate was soluble in water and the in vitro drug release was sustained both at pH 1.1 and in the range 6.8–7.4. The conjugate is thus suitable for both oral and parenteral application.

Macromolecular conjugates between *N*-succinyl-chitosan and mitomycin C (MMC) were prepared (suc-chitosan–MMC), and the complex was evaluated for acute toxicity using normal mice and for antitumor activity using mice bearing P388 leukemia (141). The therapeutic index as well as the efficacy were increased using the suc-chitosan–MMC complex as compared with mitomycin C alone.

Deacetylvinblastine (DAVBL; a metabolite of vinblastine) has been chemically linked to KS1/4 antibody to make KS1/4-DAVBL conjugate (142). The resulting conjugate was shown to have a therapeutic index greater than that of either DAVBL or KS1/4 alone. The KS1/4–DAVBL conjugate was successfully formulated as a 5 mg/ml solution in phosphate-buffered saline at pH 4.5–5.5 for initial clinical trials. In another study, the KS1/4–DAVBL conjugate was formulated in a phosphate buffer at near neutral pH in the presence of glycine and mannitol, and was subsequently lyophilized (143).

Smancs is the conjugate of the proteinaceous anticancer agent neocarzinostatin (NCS) and a hydrophobic synthetic polymer (styrene–comaleic acid). Smancs was dissolved in saline to a final concentration of 1 mg/ml

and was IV administered to rats (144). The solubility and stability of the conjugated drug were sufficiently high to induce significant antitumor effect in vivo as compared to the parent drug NSC. Often, several different formulation approaches are used to formulate a drug.

The application of prodrugs of known cytotoxic drugs has been investigated, in general, with the intent of increasing the aqueous solubility and the selective delivery of the parent compound. After having synthesized the prodrug, it still has to be pharmaceutically formulated. In vivo the inactive prodrug has to be transformed into the parent compound. It is, however, of extreme importance that the prodrugs have the same pharmacokinetic and pharmacodynamic properties as the parent compound.

LYOPHILIZED FORMULATIONS

Despite both the high initial capital equipment costs and the high running costs of the method, lyophilization has become the most important method for the stabilization of labile pharmaceutical products with limited shelf lives in dilute solution (145). The lyophilization process consists of three stages: freezing, primary drying, and secondary drying. In the freezing stage, the solution is cooled until it is completely frozen. This process separates the water from the solute(s). The primary drying phase involves the sublimation of ice from the product—usually accomplished by reducing the pressure in the freeze-drying chamber and providing heat to the product. During the secondary drying phase, adsorbed water is removed from the product (146). Some of the important advantages of freeze drying are: (a) in general the lower the water content, the more stable the product; (b) oxidative denaturation or decomposition is greatly reduced, since the product is usually sealed in vacuo or in inert gas; and (c) a freeze-dried product will, in general, reconstitute more rapidly and completely than a sterile, dry-filled powder, and it will contain less particulate matter as a consequence of membrane filtration during the formulation process. The use of lyophilized formulations has, however, generated some technical problems since the handling of such preparations exposes pharmacists, medical staff, and nurses to significant risks of contamination (e.g., possible spillage and spray formation) because of the reconstitution process. Furthermore, during the freezing stage of the lyophilization process, the physicochemical characteristics of a drug may be affected due to a change in pH of the solution or the formation of a concentration gradient, which may influence the degradation rate of the drug (147).

A properly freeze-dried product is a dry powder in the form of a cake of essentially the same shape as the frozen liquid. Bulking agents are used to enhance the appearance of the cake when the drug alone does not exhibit good freeze-drying properties and/or when the total drug content per vial is very small (14). Bulking agents can provide either a crystalline (e.g., mannitol or sodium phosphate buffer) or an amorphous (e.g., dextran, sucrose, 2-hydroxypropyl-β-cyclodextrin) matrix in which the drug is freeze dried (148). However, the effect of the bulking agent on the physiological toxicity of the final product has to be considered in order to allow IV administration. A drug has to possess some aqueous solubility in order to prepare the formulation solution. *t*-Butanol and *t*-butanol/water mixtures have been suggested as acceptable solvents for freeze drying of those agents exhibiting inadequate water solubility (149). It is, however, quite difficult to remove the solvent completely during the lyophilization process, and therefore the residual content of the solvent in the product must be detected and carefully quantitated, which can be performed by gas chromatography (150).

Examples of stable, lyophilized drugs are: mitomycin C, which contains either mannitol or sodium chloride as a bulking agent (151); trimelamol, a synthetic analogue of pentamethylmelamine and hexamethylmelamine, which is formulated in a 50% PEG 3400–aqueous vehicle and subsequently freeze dried (12); vinzolidine, a semisynthetic derivative of the vinca alkaloid vinblastine, of which the sulfate form is lyophilized (152); pentostatin, to which are added mannitol and sodium hydroxide for pH adjustment (153); and hepsulfam, which is freeze dried from 3-butyl alcohol (7).

EO9, an investigational indoloquinone antitumor agent and analogue of mitomycin C, is unstable in aqueous solution and was, therefore, freeze dried with lactose and sodium hydroxide for pH adjustment (154). Sterilization was performed by membrane filtration, which has the additional advantage of removing unsolved particles. Freeze-dried EO9 was stable for at least 12 months when stored at 4°C in a dark environment.

m-AMSA is an acridine derivative with DNA-intercalating properties. A water-soluble formulation consisting of lyophilized *m*-AMSA-lactate has been developed. In a clinical study, 70 patients suffering from malignant lymphoma, were treated with either *m*-AMSA or *m*-AMSA-lactate (155). The two formulations showed

comparable therapeutic activity and toxicity (e.g., vomiting and myelosuppression). In another study, *m*-AMSA was formulated in DMA and lactic acid (11). It has been suggested that some of the clinical toxicity (e.g., neurotoxicity and phlebitis) was due to the DMA vehicle. It appears that there are difficulties in developing a well-tolerated formulation for *m*-AMSA.

AG-331, a novel thymidilate synthase (TS) inhibitor, has potent antitumor activity in vivo in tumor-bearing mice. AG-331 is water soluble and is lyophilized in the presence of mannitol to enhance the stability of the final product (156).

Recombinant tumor necrosis factor-alpha (TNF-α), an investigational biological response modifier, is a protein and is susceptible to particulate generation and dimer formulation during handling in aqueous solutions. An attempt was, therefore, made to develop a stable freeze-dried formation of TNF-α (148). The prototype contained the amorphous buffer sodium citrate, mannitol to enhance the freeze-drying properties of TNF-α, combined with an amorphous protectant such as dextran, sucrose, or 2-hydroxypropyl-β-cyclodextrin to stabilize the solution. The solutions were stable for at least 9 months, even before they were lyophilized.

A parenteral freeze-dried formulation of the 16-membered antifungal macrocyclic lactone rhizoxin, a poorly water-soluble and chemically unstable drug, requiring a concentration of at least 1 mg/ml, was developed (149). Rhizoxin and mannitol were dissolved in 40% *t*-butanol, because of insufficient solubility in water, and subsequently freeze dried. The content of residual *t*-butanol in the vials was less than 1%.

Earlier attempts to formulate bryostatin 1 in 60% absolute ethanol led to unacceptable toxicity in patients. A two-part formulation was, therefore, developed which includes a vial containing 0.1 mg of bryostatin 1 and 5 mg of povidone, lyophilized from 40% *t*-butanol. The dry powder was then reconstituted with 1 ml of sterile PET diluent and further diluted with normal saline (51).

Freeze drying of pharmaceutical products is a very important method for the stabilization of labile drugs with limited shelf lives in dilute solution. Several drugs, with adequate aqueous solubility but lack of aqueous stability, have been successfully developed into stable, parenteral formulations suitable for human use.

CONCLUSIONS

This paper selectively overviews the pharmaceutical development of antineoplastic drugs for parenteral use from 1986 onwards. Several approaches for designing drug delivery systems for cytotoxic drugs have been discussed. The suitability, advantages, and disadvantages of each approach have been illustrated by examples from the literature.

In general, the classical solubilization approaches—for example, solubilization in surfactant solutions, the use of cosolvent systems, or altering solubility through complexation—are less complex to carry out as compared to the use of colloidal systems, and have led to the successful formulation of several cytotoxic drugs, for example, paclitaxel (Taxol) and etoposide (Vepesid). The major disadvantages of the solubilization approaches are the nonspecificity (high systemic toxicity), the contribution of the excipients to the overall toxicity of the drug, and the possible precipitation upon dilution.

In addition to the classical solubilizing systems there are the colloidal systems, such as liposomes, microcapsules, microspheres, nanoparticles, and macromolecule complexes. The solubility and stability of a drug may be enhanced due to the changes in molecular environment of the drug. Colloidal systems provide, in general, a more specific drug delivery system that can be achieved with the classical solubilization techniques. Encapsulation of biologically labile materials within a colloidal system may protect them from premature inactivation or degradation within the circulation. In many cases, the therapeutic index of the cytotoxic drug (e.g., doxorubicin) was increased. The safety of a drug carrier system must be evaluated from two standpoints: the toxicity of the carrier itself and the risk of the novel, drug-induced toxicities arising from differences in the disposition, pharmacokinetics, and metabolism of carrier-associated drug as compared with conventional drug formulations.

Even though the classical solubilization techniques are often coupled with high systemic toxicity, future perspectives for the pharmaceutical development of antineoplastic drugs are still the use of cosolvent and surfactant systems, in addition to the use of liposomes. Several new approaches are tested to control the systemic toxicity related to chemotherapy,such as rescue therapies with growth factors, bone marrow transplantations, and the simultaneous administration of more powerful antiemetics. In contrast, the colloidal system approach may lead to more specific and, therefore, less toxic drug delivery systems. However, to date, the only commercially available colloidal drug delivery systems are two liposomal formulations of doxorubicin (Doxyl) and daunorubicin (DaunoXome).

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